Table 3. Cases who developed ATL from HTLV-I carrier status

	Demographic characteristics						Baseline clinical and biologic values					ATL development	
Case no.	Sex	Age, y	Place of birth	First opportunity for HTLV-1 testing	Family history of HTLV-1–related disease	Comorbidity at enrollment	HTLV-1 VL, copies/100 PBMCs	sIL-2R, U/mL	Abnormal lymphocytes, percentage	LDH, IU/L	WBC, $\times 10^3/mm^3$	Clinical type	Duration from enrollment, mo
Progression to aggressive type of ATL													
1	M	79.9	Southern	ATL family	ATL	None	5.47	479	2	157	4200	Acute	7.4
2	F	70.3	Southern	ATL family	ATL	None	4.73	904	0	365	9130	Acute	38.6
3	M	71.9	Southern	Other disease	None	Skin disease	4.17	1450	0	351	5140	Lymph	4.6
4	F	75.8	Southern	Unknown	Unknown	None	10.52	2080	3	308	3600	Lymph	30.6
Progression to indolent type of ATL													
5	F	60.0	Southern	Other disease	Unknown	None	9.12	340	14	192	5100	Sm	6.0
6	F	71.9	Southern	Multiphasic screening	None	None	10.60	1320	2	199	4000	Sm	29.8
7	F	59.5	Southern	Multiphasic screening	None	None	21.90	635	4.5	188	4100	Sm	12.0
8	F	74.0	Southern	Other disease	Unknown	Gallbladder cancer	10.11	1110	2	240	2700	Sm	26.8
9	F	54.1	Southern	Other disease	Unknown	None	18.85	971	2	198	5660	Sm	29.0
10	F	43.3	Southern	Pregnancy	ATL	None	13.90	372	1	ND	5400	Sm	64.4
11	F	62.2	Southern	Other disease	Unknown	Eye disease	6.86	1560	ND	508	12100	Sm	6.0
12	M	57.6	Southern	Other disease	Unknown	None	7.67	ND	2	234	5500	Sm	15.4
13	F	41.0	Metropolitan	Pregnancy	None	None	12.14	349	2.5	189	7690	Sm	12.2
14	M	66.1	Southern	Other disease	None	Prostatitis	28.58	2660	0	158	8500	Sm	2.8

ATL indicates adult T-cell leukemia; HTLV-1, human T-cell leukemia virus type 1;VL, HTLV-1 proviral load; PBMCs, peripheral blood mononuclear cells; sIL-2R, soluble interleukin-2 receptor; LDH, lactate dehydrogenase; WBC, white blood cell count; Sm, smoldering type; and ND, not done.

significant ($P = .07$; supplemental Table 1). It is also possible that effects of some of the risk factors are weighted because of only 1 patient with an event because only 14 were analyzed as events in the multivariate analyses. To check the possibility, we performed 14 leave-one-out analyses, omitting 1 of 14 cases at a time from the original dataset. The Jackknifed coefficient of each parameter revealed the stability, which indicated that none of 14 cases affected the original model (data not shown).

Discussion

Previous studies reported no significant differences in the HTLV-1 proviral load by sex and age in asymptomatic HTLV-1 carriers.[21,22,24,33] In the present study, however, we found that there were significant differences in the proviral load by sex and age (Table 2). The median HTLV-1 proviral load was significantly higher in males than females. The median HTLV-1 proviral load for those 40 to 49 and 50 to 59 years of age was significantly higher than for those less than or equal to 40 years. The discrepancy between results of previous studies and those of the present study may be primarily explained by the differences in study population characteristics. We also found sex differences in age distributions of HTLV-1 proviral load; in male subjects, the median proviral load level was the highest at 50 to 59 years of age, whereas in female subjects it was highest at 40 to 49 years of age, although there were no statistical differences. These distribution characteristics of HTLV-1 proviral load are of interest when we consider the differences in sex and age at onset between ATL and HAM/TSP. ATL occurs predominantly in older males (~ 60 years), whereas HAM/TSP occurs predominantly in middle-aged females (~ 45-55 years). Thus, the proviral load levels of asymptomatic HTLV-1 carriers might be the highest in the age groups approximately 5 to 10 years before the average age at onset of ATL and HAM/TSP. These distribution characteristics may be related to differences in host immune responses to HTLV-1 and other unknown host factors.[34]

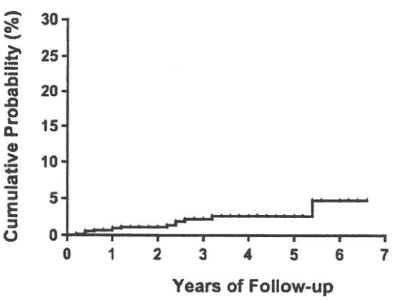

Figure 2. Probability of progression to ATL among 1218 asymptomatic HTLV-1 carriers.

The present study revealed that the median proviral load level of those with a family history of ATL or HAM/TSP was significantly higher than for those with no family history (Table 2). These results support previous studies indicating that HTLV-1–infected blood donors and asymptomatic carriers with familial HAM/TSP or ATL tend to have a higher HTLV-1 proviral load than those without family history.[21,33] In the present study, the proviral loads were also higher in those with a family history of leukemia or lymphoma than those without such history. We assume that the family history of leukemia or lymphoma may have included some ATL cases because some participants provided a diagnosis as just unknown leukemia or lymphoma. Although the present study was a large cohort, data collection regarding family history of HTLV-1–associated diseases was insufficient because one-half of the participants did not know their family HTLV-1 status. Further detailed data collection is needed to confirm the characteristics of HTLV-1 proviral load levels by family histories among asymptomatic HTLV-1 carriers, as this is necessary to determine genetic determinants of HTLV-1–associated diseases.

HTLV-1 carriers have various comorbidities, such as infectious, autoimmune, and malignant diseases.[4,25,35-38] In the present study, 45 participants had various infectious diseases at enrollment (Table

Table 4. Frequency of subjects who developed ATL by demographic characteristics and by VL level

Demographic characteristics	No. of subjects	No. of ATLs (% of subjects)	Frequency of ATL by VL level, n (% of subjects in each quartile in Table 2)		
			Quartiles 1 and 2 (VL: < 1.60)*	Quartile 3 (VL: 1.60-4.54)	Quartile 4 (VL: ≥ 4.54)
Total	1218	14 (1.1)	0	1 (0.3)	13 (4.3)
Sex					
Male	426	4 (0.9)	0	1 (1.1)	3 (2.0)
Female	792	10 (1.3)	0	0	10 (6.4)
Age, y					
Younger than 40	167	0	—	—	—
40-49	200	2 (1.0)	0	0	2 (3.7)
50-59	273	3 (1.1)	0	0	3 (3.7)
60-69	260	3 (1.2)	0	0	3 (4.5)
70 or older	318	6 (1.9)	0	1 (1.3)	5 (6.5)
First opportunity for HTLV-1 testing					
Screening	661	4 (0.6)	0	0	4 (2.8)
Revelation of HTLV-1-positive family	134	2 (1.5)	0	0	2 (8.3)
During treatment for other diseases	265	7 (2.6)	0	1 (1.8)	6 (7.0)
Unknown	158	1 (0.6)	0	0	1 (2.0)
Family history of HTLV-1–related diseases					
Absence or carrier/HU/HAU only	385	5 (1.3)	0	1 (1.0)	4 (5.0)
HAM/TSP	9	0	—	—	—
ATL	107	3 (2.8)	0	0	3 (9.7)
Leukemia or lymphoma	36	0	0	0	0
Unknown family history	681	6 (0.9)	0	0	6 (3.4)
Comorbidity					
Absence	961	10 (1.0)	0	0	10 (4.1)
Infectious diseases	45	1 (2.2)	0	0	1 (5.9)
Autoimmune diseases	29	0	—	—	—
Malignant diseases	80	1 (1.3)	0	0	1 (5.9)
Skin diseases	16	1 (6.3)	0	1 (33.3)	0
Other disease	87	1 (1.1)	0	0	1 (5.3)

ATL indicates adult T-cell leukemia; HTLV-1, human T-cell leukemia virus type 1; VL, HTLV-1 proviral load; HU, HTLV-1 uveitis; HAU, HTLV-1–associated uveitis; HAM, HTLV-1 myelopathy; TSP, tropical spastic paraparesis; and —, not applicable.

*The VL was categorized based on quartile cutoff points (the 25th, 50th, and 75th percentiles of the VL distribution) in 1218 HTLV-1 carriers. The unit of VL was copies/100 PBMCs.

1). We found that the median proviral load of these participants was significantly higher than that of those with no comorbidity (Table 2). The results of the present study support previous reports indicating higher HTLV-1 proviral loads in HTLV-1 carriers with comorbid *Strongyloides stercoralis* or bladder and kidney infections than those without such infections.[25,35,36] HTLV-1 carriers with rheumatoid arthritis or connective tissue disease and those with myelodysplastic syndromes carrying HLA-A26 were also reported to have higher HTLV-1 proviral loads compared with the median proviral load of those without such diseases.[37,38] In the present study, however, the median proviral load was not significantly high in those with autoimmune and malignant diseases. Further studies are required to find other predisposing factors affecting the proviral load level in each person.

A high HTLV-1 proviral load is currently considered as one of the main indicators for the progression to ATL.[20,28] In the present study, 14 participants of asymptomatic HTLV-1 carriers progressed to overt ATL as of 2009, all of whose baseline proviral load levels were high (range, 4.17-28.58 copies/100 PBMCs; Table 3). Therefore, we suggest that those with a high proviral load level (~ > 4 copies/100 PBMCs) are in a high-risk group for developing ATL (this group accounted for ~ 29% of the cohort). Multivariate Cox analyses confirmed that a higher proviral load level was a strong factor in the development of ATL (Table 5). This result strongly supports previous small-scale studies.[20,28] However, the role of the high proviral load level still remains unclear because the majority of asymptomatic carriers with a high HTLV-1 proviral load level in the present study remain carrier status. In the present study, male gender was not a significant risk factor for ATL, even though the median proviral load was significantly higher in males than in females. A high HTLV-1 proviral load is also reported to be associated with HAM/TSP.[20,21,27] These findings suggest that a high

Table 5. Cox proportional hazards modeling of risk factors for ATL development

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Male sex (vs female)	0.74(0.23-2.37)	.61	0.38(0.12-1.18)	.09
Square-root transformed VL per unit increase	2.55(1.91-3.41)	< .001	3.57(2.25-5.68)	< .001
Age per 5-year increase from 40 y	1.20(0.94-1.53)	.15	1.67(1.12-2.50)	.012
Family history of ATL (vs others)	2.68(0.80-8.98)	.11	12.1 (2.26-64.7)	.004
First opportunity for HTLV-1 testing during treatment of other diseases (vs others)	3.40(1.12-10.28)	.03	4.16(1.37-12.6)	.012

Analyses were performed using robust sandwich variance estimates.

ATL indicates adult T-cell leukemia; HTLV-1, human T-cell leukemia virus type 1; VL, HTLV-1 proviral load; HR, hazard ratio; and CI, confidence interval.

proviral load alone is not a unique predictive marker for ATL. In addition, the present study showed that the median proviral load level at enrollment was lower in those who developed aggressive types of ATL (5.1 copies/100 PBMCs) than that in those who developed smoldering types of ATL (11.4 copies/100 PBMCs; $P = .02$). This also suggests that a high proviral load alone is not a predictive marker for aggressive types of ATL.

In the present study, multivariate Cox analysis indicated that increased age, family history of ATL, and first opportunity to learn of HTLV-1 infection during treatment of other diseases were also independent risk factors for the development of ATL, after adjusting for proviral load (Table 5). This suggests that multiple risk factors (including unknown factors) are related to the progression from HTLV-1 carrier status to ATL. The reason why "opportunity to learn of HTLV-1 infection during treatment of other diseases" was an independent risk factor is unknown. The findings that more advanced states of HTLV-1 carriers (ie, an intermediate state[6] and a preleukemic state[13]) tend to be complicated by various comorbid diseases and that HTLV-1 carriers with various comorbid diseases had higher HTLV-1 proviral loads[25,35-38] could in part explain the reason.

Some prospective studies serially evaluated HTLV-1 proviral loads in HTLV-1 carriers and reported that their proviral load level was relatively stable over time with a certain level of fluctuations for persons.[25,26,28] Taylor et al reported that proviral loads of 20 HTLV-1 carriers were stable over a mean of 27 months, even though 9 carriers with various comorbidities showed high proviral load levels.[25] Meanwhile, an increasing proviral load was observed before progression to HAM/TSP and ATL.[27,28] However, there remain more questions how much of the fluctuations in proviral load over time could predict disease progression over the natural fluctuations within persons. Factors other than the proviral load level might be influencing the development of HTLV-1–associated diseases. Future studies should perform serial evaluations of HTLV-1 proviral loads by considering risk factors that have been confirmed in the present study.

The present study has several limitations. The number of ATL events was very small to obtain a conclusive result. However, we have a confidence for our results because we used a robust variance estimate in the multivariate analysis and because 2 validity analyses confirmed the original results. Data collection was insufficient for some items in the questionnaire. To resolve this issue, we will need to administer the questionnaire repeatedly. Our study design did not include enough information for evaluating the development of HAM/TSP. The follow-up duration is too short with regard to the natural history of ATL that has a long latency. Further follow-up of this cohort and similar prospective investigations should provide data needed to support more detailed conclusions. We did not compare the proviral loads by place of enrollment because we realized that many HTLV-1 carriers have migrated from the southern area to the metropolitan area.[39] The migration of HTLV-1 carriers has raised some public health issues in Japan. Screening for HTLV-1 in pregnant women and prevention programs for mother-to-child transmission of HTLV-1 are conducted in endemic areas[40,41] but not in metropolitan areas, which could introduce a higher chance of new HTLV-1 infections in the metropolitan area. To date, there is no nationwide program for preventing new HTLV-1 infections in Japan. Further nationwide studies are needed to determine the precise numbers of HTLV-1 carriers and to prevent HTLV-1 infection.

In conclusion, the present cohort study of 1218 asymptomatic HTLV-1 carriers provided detailed distributions for HTLV-1 proviral loads regarding the host-specific characteristics and the associations with the development of ATL. We confirmed that a higher proviral load levels (especially $\sim > 4$ copies/100 PBMCs), advanced age, family history of ATL, and having the first opportunity to learn of HTLV-1 infection during treatment of other diseases were independent risk factors for progression from carrier status to ATL. Further large-scale epidemiologic studies are needed to clearly identify the determinants of ATL for early detection and rapid cure for HTLV-1–associated diseases.

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Authorship

Contribution: M.I. managed the study database, analyzed data, and wrote the manuscript; T.W. organized the study and managed processing of the samples and measurement of proviral loads; A.U., A.O., K. Uchimaru, K.-R.K., M.O., H.K., K. Uozumi, M.M., K.T., Y. Saburi, M.Y., J.T., and Y.M. were responsible for participant enrollment and data collection; Y. Sagara managed the biomaterial bank; S.H. organized the study and managed the database; S.K. and K.Y. established the study; and all authors critically reviewed the article and approved the final version.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

A complete list of JSPFAD participants is available online in the supplemental Appendix.

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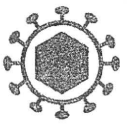

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High Human T Cell Leukemia Virus Type-1(HTLV-1) Provirus Load in Patients with HTLV-1 Carriers Complicated with HTLV-1-unrelated disorders

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Abstract

Background: To address the clinical and virological significance of a high HTLV-1 proviral load (VL) in practical blood samples from asymptomatic and symptomatic carriers, we simultaneously examined VL and clonal expansion status using polymerase chain reaction (PCR) quantification (infected cell % of peripheral mononuclear cells) and Southern blotting hybridization (SBH) methods.

Results: The present study disclosed extremely high VL with highly dense smears with or without oligoclonal bands in SBH. A high VL of 10% or more was observed in 16 (43.2%) of a total of 33 samples (one of 13 asymptomatic carriers, 8 of 12 symptomatic carriers, and 7 of 8 patients with lymphoma-type ATL without circulating ATL cells). In particular, an extremely high VL of 50% or more was limited to symptomatic carriers whose band findings always contained at least dense smears derived from polyclonally expanded cells infected with HTLV-1. Sequential samples revealed that the VL value was synchronized with the presence or absence of dense smears, and declined at the same time as disappearing dense smears. Dense smears transiently emerged at the active stage of the underlying disease. After disappearance of the smears, several clonal bands became visible and were persistently retained, explaining the process by which the clonality of HTLV-1-infected cells is established. The cases with only oligoclonal bands tended to maintain a stable VL of around 20% for a long time. Two of such cases developed ATL 4 and 3.5 years later, suggesting that a high VL with oligoclonal bands may be a predisposing risk to ATL.

Conclusion: The main contributor to extremely high VL seems to be transient emergence of dense smears detected by the sensitivity level of SBH, corresponding to polyclonal expansion of HTLV-1-infected cells including abundant small clones. Major clones retained after disappearance of dense smears stably persist and acquire various malignant characteristics step by step.

Background

Human T-cell leukemia virus type-1 (HTLV-1) is thought to infect mainly CD4 T-cells, and to cause T-cell malignancy adult T-cell leukemia (ATL) after a long latency, a degenerative nervous disorder of HTLV-1-associated myelopathy (HAM), and so on [1,2]. During the clinically asymptomatic period preceding the diseases, the HTLV-1-infected cell number is low, at about less than 2 - 3% per 100 blood mononuclear cells (MNC). Therefore, infected cells in asymptomatic (healthy) carriers are considered to proliferate polyclonally because the provirus integrates at a random site [3]. Recent work using real-time polymerase chain reaction (PCR) quantification for HTLV-1 provirus (proviral load: VL) and inverse PCR indicates that clonal expansion of HTLV-1-infected cells is important for the maintenance of infection [4-6]. Interestingly, the proviral integration sites in genomic DNA in asymptomatic and symptomatic carriers without ATL is either random or constant, implying the difference in clonality detected by Southern blotting hybridization (SBH) [7,8]. Thus, high VL corresponding to an increased number of polyclonal or monoclonal infected cells is one of the key

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events in HTLV-1-associated pathology. Therefore, a high VL with clonal expansion has potential as a biomarker to predict patients predisposed to ATL or HAM [9,10]. On the other hand, HTLV-1-infected individuals who are complicated by opportunistic infections, such as parasites, mycosis, viruses and some bacteria, and abnormal immunity due to aging are known to show an increased proviral load with polyclonal expansion [11-13]. This condition associated with polyclonal expansion of the infected cells was considered to be the intermediate state prior to progression to ATL [14], but the pathological and clinical correlation between clonality and level of VL is not fully understood. Recently, we have had frequent opportunities to see unusual or indeterminate ATL patients or carriers with high VL with discrete band(s) in SBH, but no circulating ATL cells, especially among the elderly.

Accordingly, to address what kind of clonal infected cells contribute to high VL, and clarify unusual ATL or carrier states, we simultaneously analyzed HTLV-1 proviral load and SBH status using the same blood samples. In contrast to the maintenance of stable VL in asymptomatic carriers with no-bands or only faint discrete bands, the VL in symptomatic carriers with complications unrelated HTLV-1 tended to have high VL with dense smears with/without discrete band(s), consisting of mainly polyclonal expansion and partial oligoclonal expansion of the infected cells.

Results

Sample features and SBH band status

The median age of the 29 subjects who donated peripheral blood was 66 years old (range, 49-81). No circulating ATL cells were found morphologically or immunophenotyically in any samples, including 8 samples of lymphoma-type ATL employed as a control. Subsequently, all 33 samples were divided into 3 groups; 13 asymptomatic healthy carriers (median age, 60), 12 symptomatic carriers (median age, 68) with complications unrelated to HTLV-1 such as infectious diseases (Strongyloides, hepatic disorders due to HBV and HCV, chronic pneumonitis or bronchitis) and immune-disorders (Crow-Fukase syndrome, RA, and chronic eczema, and reactive unknown adenitis) and 8 patients with lymphoma-type ATL. The distribution of SBH band patterns in each group is summarized in Table 1 and the median proviral loads of the no-band, dense smears and clonal band groups were 2.0% (range, 0.1 - 9.0), 27.9% (5.0-97.4), and 20.1% (8.3-74.3), respectively, as shown in Table 1 and Figure 1.

Characteristic band patterns in samples with high VL

Although SBH in asymptomatic carriers gave no clonal band with or without very faint Smears, SBH in some symptomatic carriers gave characteristic band patterns, as shown in Figure 2. Those were mainly a mixture type of dense smears and discrete oligoclonal bands in symptomatic carriers with high VL, such as cases 1 (VL, 97%), 3 (74%), 4 (57%), and 5 (21%). On the other hand, in samples from lymphoma-type ATL, the mixture type was detected in only case 10 and the clonal band type was detected in case 10 to 15. For all sample tested, the relationship between VL and band status is depicted in Figure 3, showing no-band or vague smears in all but one of the healthy carriers, either dense smears or a mixture of dense smears and oligoclonal bands (open circle+S:O+S) in symptomatic carriers and mainly discrete clonal band in patients with lymphoma-type ATL. In particular, an extremely high VL of 50% or more was limited to symptomatic carriers whose band findings always contained at least dense smears. Moreover, as shown in Figure 4, sequential samples disclosed that a higher VL value was synchronized with the transient emergence of dense smears, and declined at the same time as disappearing dense smears. After that, several discrete bands became visible and were persistently retained.

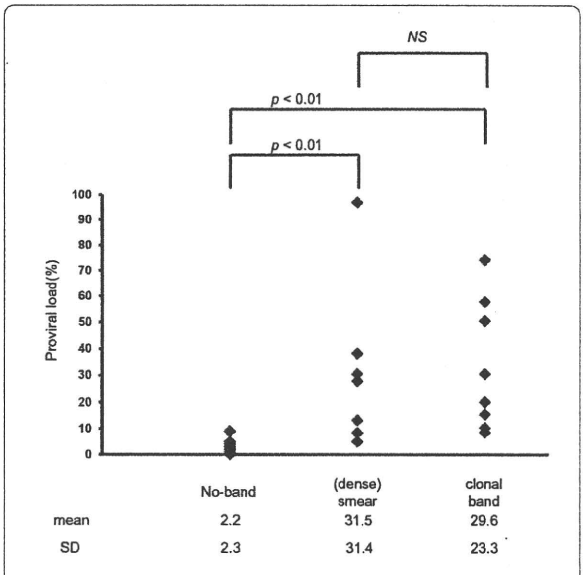

Figure 1 The distribution of HTLV-1 proviral load (VL) per 100 blood mononuclear cells (MNC) in each sample among the no-band, dense smears and clonal band(s) groups classified according to Southern blotting (SBH) status. Short bar; median VL.

Clinical features in symptomatic carriers and patients with lymphoma-type ATL

Clinico-hematological features in 15 cases with a high VL of 10% or more and distinctive band patterns are summarized in Additional file 1. Of 8 symptomatic carriers, complicated disorders were mainly associated with abnormal immunity or non-bacterial pathogens. Two of 8

Table 1: The distribution of HTLV-1 SBH band status in samples without circulating ATL cells among three HTLV-1-seropsitive groups, asymptomatic (healthy), symptomatic carriers with HTLV-1-unrelated disorders and patients with lymphoma type ATL.

HTLV-1 seropositive persons	No.	SBH			
		no-band*	dense smears	clonal band(s)	total
asymptomatic (healthy)	13	11 (84.6%)	2 (15.4%)	0 (0%)	100%
symptomatic, (complication unrelated to HTLV-1)	12	4 (33.3%)	2 (16.7%)	6(4)**(50.0%)	100%
patients with lym. type ATL***	8	1 (12.5%)	3 (37.5%)	4 (1)**(50.0%)	100%
total	33	16 (48.5%)	7(21.2%)	10 (5)**(30.3%)	100%

*: no-band with or without vague smears
**: aberrant bands showing broad bands
***: patients with lym. Type ATL was defined as cases with no morphological and immunophenotypical abnormal lymphocytes
(): the number of cases with co-existence of clonal band(s) and dense smear bands

symptomatic carriers developed ATL, case 3 in 4 years later and case 5 in 3.5 years later, respectively. For lymphoma-type ATL, SBH for Lymph node (LN) suspension cells gave positive results in 5 of 6 samples tested. The band size was different in case 11 and was accordant in case 14 between PB and LN, while the other band profiles were very similar to those of symptomatic carriers; they were like a relic of the symptomatic carriers' past.

Discussion

Recent studies including our previous studies [13,15] suggest that VL in asymptomatic carriers may be approximately one copy per 25 to 1000 MNC. Even in patients with HAM whose VL are known to be high, the VL may be as high as one copy per 10 to 100 MNC. Therefore, we defined a VL of 10% or more per 100 MNC as unusually high.

In the present study, we found that the results of VL and SBH status in healthy carriers were the same as those of the past reports, while there was an extremely high VL with a characteristic band status of high dense smears with or without clonal bands in elderly symptomatic carriers. A VL of 10% or more (range, 10 to 97.4%) was detected in 16 (43.5%) of all samples, 1 (6.3%)/16 asymptomatic healthy carriers, 8 (61.5%)/13 symptomatic carriers unrelated to HTLV-1 and 7(87.5%)/8 patients with lymphoma-type ATL without circulating ATL cells. On the other hand, in SBH analysis, no visible aberrant bands were detectable in low VL samples with less than 10%. All but one of the asymptomatic carriers (mean age; 60) were of this pattern. In contrast, the high VL samples with 10% or more displayed distinctive band patterns accompanied by dense smears with or without discrete clonal band(s), indicating that an increase in polyclonally infected-cells corresponding to dense smears contributed to a high VL. As triggering factors for HTLV-1-infected cells, various microbes and abnormal immunity due to aging in symptomatic carriers were suspected. Furthermore, the observations from sequential samples also support the contribution of dense smears to the elevation of VL. This helps explain the process by which the clonality of HTLV-1-infected cells is established after the disappearance of dense smears.

It is now recognized that clonal expansion of HTLV-1-infected cells is the norm in nonmalignant disease [11]. In the present study, of 13 asymptomatic and 12 symptomatic carriers, the incidence of clonality was 24.0% (6/25 cases), of which 4 cases were accompanied by dense smears and maintained a higher VL. In other word, this suggests that polyclonal expansion, rather than oligoclonal expansion, contributes to a high VL. The contribution of clonal expansion to the elevation of VL in carriers is thought to be small because VL in HAM patients is generally reported to be around 10% on average. In fact, Furukawa et al. [8] reported a high frequency of clonality of 20% in HAM patients and 16% in carriers in families of HAM patients, while the VL was at most 10 to 20% in general. On the other hand, patients co-infected with

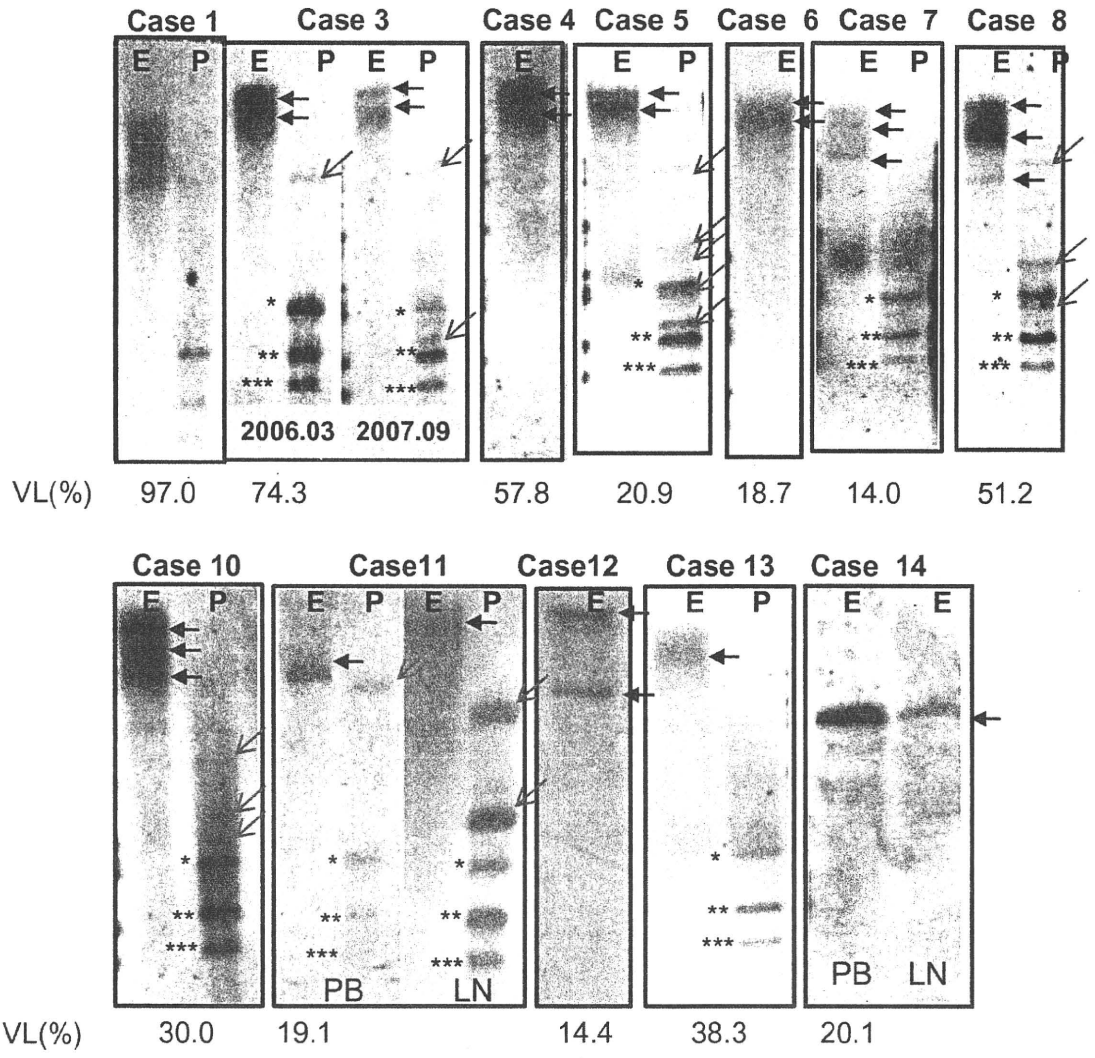

Figure 2 Representative band patterns by SBH analysis in high VL carriers with dense smears, aberrant-bands with/without faint sharp band(s). E & P; EcoR1 and Pst-1 digestion, *, **, ***; internal bands after Pst-1 digestion, ; clonal band(s), PB; peripheral blood, LN; lymph node. Case 1: Typical dense smeared band, case 3: probably two bands within dense smeared bands in March, 2006, and clear multi-bands in September, 2007. Cases 4, 5, 6, 7, 8, and 10; two or more vague clonal band(s). Case 11; different band sizes between peripheral blood (PB) and lymph nodes (LN), Cases 12 and 13; two clonal bands and an atypical broad band, Case 14; the same band size for LN and blood.

strongyloidosis and HTLV-1 have been reported to harbor a higher VL of around 50% with a high incidence (39%) of clonality [16,17]. The relation between VL and clonality is controversial [11,12] because the decision regarding clonality depends on the sensitivity of the method.

Taken together, our study supports the idea that extremely high VL mainly results from polyclonal expansion of HTLV-1 infected cells at the sensitive level of SBH.

In samples from patients with lymphoma-type ATL without ATL cells, a clonal band(s) was demonstrated in 4 of the 8 patients. Band size between blood and lymph nodes was the same in two of the 4 cases, but no circulating ATL was found. Other aberrant bands, as summarized in Additional file 1, were also observed. Such a band profile in ATL (lymphoma-type) appears to be looks a relic of a symptomatic carriers' past, indicating that high VL with aberrant bands could become a biomarker to

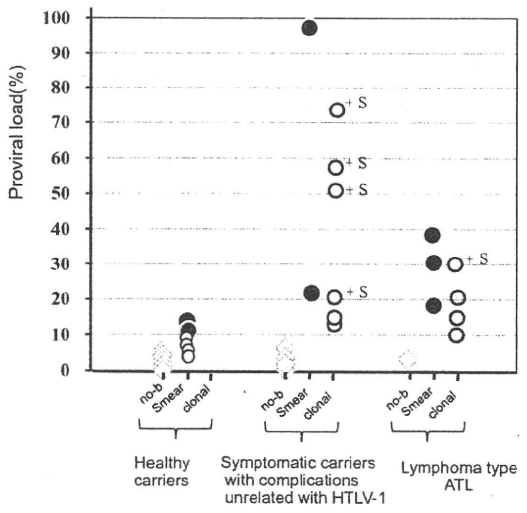

Figure 3 A comparison between the level of VL and SBH status among healthy asymptomatic carriers, symptomatic carriers with complications unrelated to HTLV-1, and patients with lymphoma type ATL. Gray diamond; no-band, gray circle; vague to faint smears, solid circle; dense smears, open circle; clonal band, +S: mixture band of dense smears and conal bands.

predict the development of ATL. Indeed, cases 3 and 5 developed smoldering ATL after 4 years and acute ATL after 3 years, respectively. A conceptual scheme is presented in Figure 5, which shows the biological significance of the fluctuation in a high VL with either dense smears or oligoclonal bands durig multi-step leukomogenesis as a stone corner of dense smear emergence.

Conclusion

Focusing on not only discrete clonal band(s), but also aberrant smears, it is noteworthy that the emergence of dense smears equivalent to polyclonal expansion of infected cells mainly contributed to a high level VL. However, it is reasonable that a high VL does not consist of only polyclonally expanded infected cells, but included abundant small clones in the cell population, because of sensitivity in SBH. SBH for HTLV-1 could evaluate or monitor the clinical clonal status of HTLV-1-infected cells. Clinically, the distinctive profile of high VL and aberrant band status is expected to monitor or predict some events caused by HTLV-1.

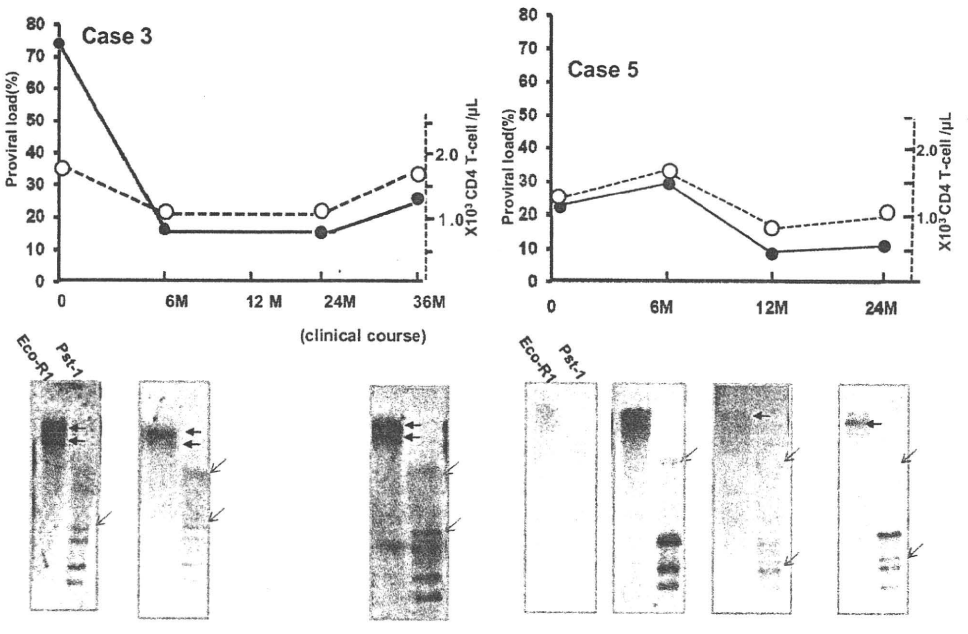

Figure 4 Upper panel shows the sequential measurement of VL (%; solid line) and CD4T-cell number/μL (broken line) in symptomatic cases 3 and 5. Lower panel shows SBH status at the same time as VL assays. In case 3, the first SBH gave dense smears (black arrow, Eco-R1 digestion) and an indeterminate clonal band (red arrow, Pst1 digestion), and then dense smears disappeared followed by clear visualization of a clonal band. In case 5, when dense smears emerged, the VL was the highest, and after disappearance, the clonal band and level of VL stably persisted.

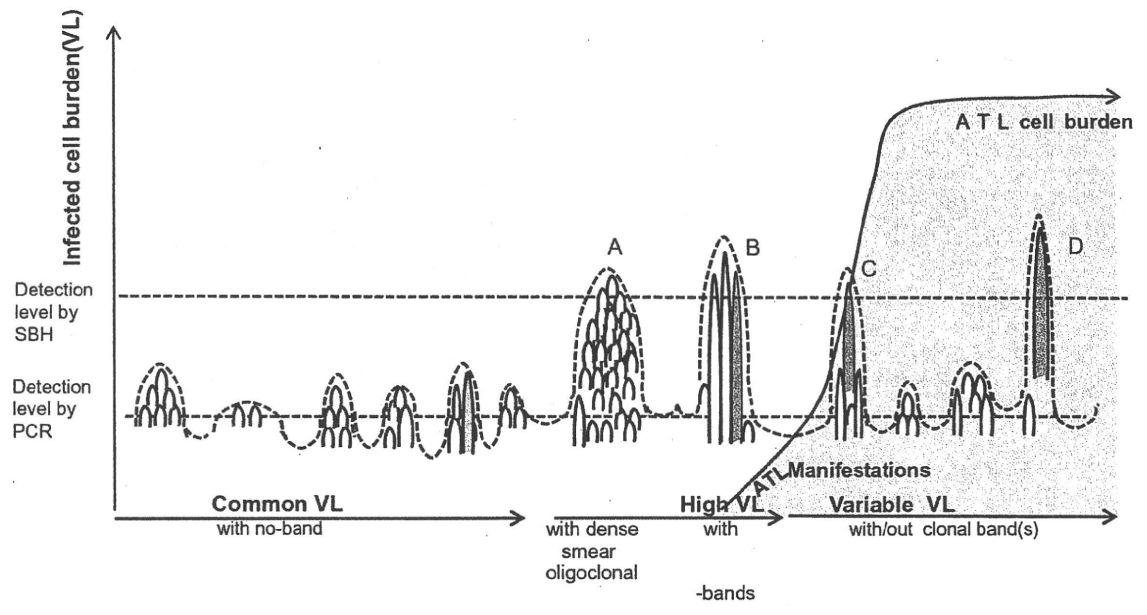

Figure 5 Conceptual scheme of the fluctuations in the total proviral load (VL) in peripheral blood with no circulating abnormal cells during multi-step leukemogenesis. VL is low in the early phase of the latent period, during which infected cells consist of clones with a small population. At some time point, some clones may become large enough to be detected as bands by Southern blotting. Peak A corresponds to smears detected as polyclonal expansion by SBH, and then peaks B, C and D appear with oligoclonal bands.

Materials and methods

Samples

Samples were collected from our ATL clinical laboratory, consisting of 16 asymptomatic and 13 symptomatic carriers and 8 patients with lymphoma-type ATL as a control because this type of ATL has no circulating ATL cells. A total of 37 samples were seropositive for HTLV-1 and undetectable morphologically and immunophenotypically for ATL cells, indicating all samples had no evidence of circulating ATL cells.

Serologic and genomic assays for HTLV-1

Anti-HTLV-1 antibodies were detected by chemiluminescent enzyme immunoassay (Fuji Rebio, Tokyo, Japan). High-molecular-weight DNA was extracted from blood mononuclear cells (MNCs) using a QIAmp DNA Blood Mini kit (Qiagen GmbH, Hilden, Germany). VL was quantified by LightCycler Technology (Roche Diagnostics KK, Tokyo, Japan) using hydro-probes and previously described primers [15], with β-globin as an internal control. The PCR methodology was monitored by determining the amount of β-globin DNA required to generate 10,000 copies per 5,000 MNC. We assumed that one infected cell harbored one provirus, and the number of infected cells was therefore estimated to be the same as the proviral copy number and was expressed per 100 MNC (% or load).

Clonal assay by SBH

SBH analysis was performed as described previously [18,19], using mixture probes covering the total region of the provirus digoxigenined and the restriction enzymes *EcoR1* and *Pst-1*. There are four *Pst-1* sites but no *EcoR1* site within the proviral sequence. Accordingly, if *EcoR1*-digestion gave no-band or faint smears and *Pst-1*-digestion gave only three internal bands, the sample was considered to be negative for clonal expansion. When discernible discrete band(s) in the *EcoR1*-digestion membrane or one or two external band(s) in addition to three internal bands in the *Pst-1*-digestion membrane were visible, the sample was considered to harbor clonal integrated provirus, implying that the infected cells had expanded clonally. The results of SBH analysis were classified into three patterns; no-band, dense smears and one or more discrete band(s). "Dense" smears were assumed to be distinct from those of common carriers (3 fold< smear density relative to background lane densitiy). The detection sensitivity for clonally infected cells in the SBH assay was 3-5% [18]. The sensitivity was monitored in each blotting membrane using 3-5% clonal cells from the ST1 ATL cell line.

Statistics

Data were analyzed using Mann-Whitney or Chi-squared tests. Statistical significance was set at $p < 0.05$.

Additional material

Additional file 1 Summary of the main clinical and laboratory data in seropsitive individuals with high VL and aberrant band patterns in SBH, and outcome in Dec 2008. Two cases (#3 and 5) among 8 advanced carriers (cases 1 to 8) developed ATL 4 and 3 years later. Cases 1, 2 and 15: High VL carriers with polyclonal expansion. Cases 3-14; aberrant bands mainly with faint multiple clonal bands, Final diagnosis was based on the integrated findings of an LN SBH test and clinico-pathological examinations. ALCL; anaplastic large cell lymphoma, DLBCL; diffuse large cell B-cell lymphoma, (-) or (+); negative or positive clonal band, S; smear, B; band, NT: not tested, Dx: diagnosis, *: indeterminate for clonal band, **: pathological diagnosis was indeterminate. For the other abbreviations; refer to the context.

Abbreviations

HTLV-1: human T-cell leukemia virus type-1; ATL: adult T-cell Leukemia; VL: HTLV-1 proviral load; SBH: Southern blot hybridization; PCR: polymerase chain reaction.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SK, DS, and TW conceived this study and provided funding. DS, HH, KY, YY and KT collected samples and carried out the molecular genetic studies. MI, TW, AO and SK analyzed the data and discussed the results. SK organized the study and wrote the manuscript. All authors read and approved the final manuscript.

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Long-term study of indolent adult T-cell leukemia-lymphoma

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The long-term prognosis of indolent adult T-cell leukemia-lymphoma (ATL) is not clearly elucidated. From 1974 to 2003, newly diagnosed indolent ATL in 90 patients (65 chronic type and 25 smoldering type) was analyzed. The median survival time was 4.1 years; 12 patients remained alive for more than 10 years, 44 progressed to acute ATL, and 63 patients died. The estimated 5-, 10-, and 15-year survival rates were 47.2%, 25.4%, and 14.1%, respectively, with no plateau in the survival curve. Although most patients were treated with watchful waiting, 12 patients were treated with chemotherapy. Kaplan-Meier analyses showed that advanced performance status (PS), neutrophilia, high concentration of lactate dehydrogenase, more than 3 extranodal lesions, more than 4 total involved lesions, and receiving chemotherapy were unfavorable prognostic factors for survival. Multivariate Cox analysis showed that advanced PS was a borderline significant independent factor in poor survival (hazard ratio, 2.1, 95% confidence interval, 1.0-4.6; $P = .06$), but it was not a factor when analysis was limited to patients who had not received chemotherapy. The prognosis of indolent ATL in this study was poorer than expected. These findings suggest that even patients with indolent ATL should be carefully observed in clinical practice. Further studies are required to develop treatments for indolent ATL. (*Blood.* 2010;115(22):4337-4343)

Introduction

Adult T-cell leukemia-lymphoma (ATL) is a peripheral T-lymphocytic malignancy associated with human T-cell lymphotropic virus type 1 (HTLV-1).[1] ATL has been classified into 4 clinical subtypes: acute, lymphoma, chronic, and smoldering.[2] In general, acute and lymphoma types of ATL have a extremely poor prognosis despite advances in chemotherapy and allogenic hematopoietic stem cell transplantation[3-5] because of multidrug resistance, a large tumor burden with multiorgan failure, hypercalcemia, and/or frequent infectious complications associated with a T-cell immunodeficiency. A previous study, in which Japanese patients with ATL were followed for a maximum duration of 7 years, reported that the 4-year survival rates for acute, lymphoma, chronic, and smoldering type were 5.0%, 5.7%, 26.9%, and 62.8%, respectively, with the median survival time (MST) of 6.2 months, 10.2 months, 24.3 months, and not yet reached, respectively.[2] Therefore, the chronic and smoldering subtypes of ATL are considered indolent and are usually managed with watchful waiting until disease progression to acute crisis, similar to the management of chronic lymphoid leukemia or smoldering myeloma. However, the follow-up duration of the previous Japanese study was too short for indolent ATL to evaluate the overall risk of progression to acute or lymphoma types (ie, aggressive ATL). A recent Brazilian study, in which patients with ATL were followed for a maximum duration of 14 years, reported that the MST of chronic and smoldering types were 18 months and 58 months, respectively, and the overall survival (OS) rates were less than 20% in both types.[6] Their results suggest that the long-term prognosis of indolent ATL might be worse than expected.

The long-term prognosis of Japanese patients with indolent ATL has not been well evaluated so far. Prognostic factors for patients with indolent ATL are also unclear. In the present study, we investigated the long-term outcome of 90 patients with indolent ATL. The purposes of this study were to estimate the 5-, 10-, and 15-year survival rates for indolent ATL and to evaluate the prognostic factors.

Methods

Patients

We evaluated a total of 90 patients with indolent ATL (25 smoldering type and 65 chronic type) who were newly diagnosed at the Nagasaki University Hospital between July 1974 and December 2003. The distribution of patients by year of diagnosis in decades (1974-1983, 1984-1993, and 1994-2003) are presented in Table 1. The cutoff date for analysis was December 2008. The diagnosis of ATL was based on clinical features, histologically and/or cytologically proven mature T-cell malignancy, the presence of anti–HTLV-1 antibody, and monoclonal integration of HTLV-1 proviral DNA into tumor cells as described previously.[2,7-9] The subtypes of ATL were classified according to criteria established by the Lymphoma Study Group of Japan Clinical Oncology Group.[2] Clinical data included date of diagnosis, complications at diagnosis, therapy regimens if applicable, date of death, cause of death, and date of latest contact. This retrospective, nonrandomized, observational study that used existing data

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Table 1. Distribution of patients in 3 decades from 1974 to 2003

Year	Total no. of patients	No. of smoldering type (% of total)	No. of chronic type (% of total)
1974-1983	19	2 (10.5)	17 (89.5)
1984-1993	35	7 (20.0)	28 (80.0)
1994-2003	36	16 (44.4)	20 (55.6)
Total for all years	90	25 (27.8)	65 (72.2)

was granted an exemption from the institutional review board and waived the requirement for written informed consent.

Clinical factors and definitions

Age was dichotomized into 2 groups: 60 years or older and younger than 60 years. Performance status (PS) was based on the 5-grade scale of the World Health Organization. Complications at diagnosis were dichotomized into present and absent. Leukocytosis was defined as white blood cell count of 12×10^9/L or greater with the median value as cutoff level. Lymphocytosis was defined as a total lymphocyte count of 6.5×10^9/L or greater with the median value as cutoff level. Neutrophilia was defined as a neutrophil count of 7.5×10^9/L or greater.[10] Eosinophilia was defined as an eosinophil count of 0.4×10^9/L greater.[11] Lactate dehydrogenase (LDH) and blood urea nitrogen (BUN) were dichotomized into normal and elevated concentrations.[12] Albumin was dichotomized into concentrations of 40.0 g/L (4.0 g/dL) or greater and less than 40.0 g/L (4.0 g/dL).[2] Potential prognostic factors (PPFs) for chronic ATL were defined as those with at least one of the following 3 factors: low serum albumin, high LDH, or high BUN according to previous reports.[13,14] Tumor lesions were evaluated as the number of lymph node lesions, number of extranodal lesions, and number of total involved lesions. Extranodal lesions were defined as follows: bone marrow (BM) involvement as the presence of more than 5% typical ATL cells on a BM smear or detection of their infiltration in a BM biopsy specimen; skin involvement as the presence of ATL infiltration in a skin biopsy specimen or as the clinically presence of typical types of skin lesions such as tumors, nodules, erythema, and papules, if biopsy was impossible; lung involvement as lesions with ATL cell infiltration in a transbronchial lung biopsy specimen or in bronchoalveolar lavage fluid; liver involvement as hepatomegaly determined by any imaging tests or liver biopsy if done; spleen involvement as splenomegaly on any imaging test. All patients had peripheral blood involvement. Both lymph node and extranodal tumor lesions were determined according to Ann Arbor classification.[2] The number of total involved lesions was defined as the sum of lymph node lesions and extranodal lesions.[2] Factors used in analyses were listed in Table 2.

Statistical analysis

OS was defined as the time from the date of first diagnosis to the date of death or the latest contact with the patient. Survival curves were estimated using the Kaplan-Meier method and were compared using the generalized Wilcoxon test. MST was estimated as the time point at which the Kaplan-Meier survival curves crossed 50%. Time to transformation was calculated as the time from the date of the first diagnosis to the date of transformation into the aggressive type (acute or lymphoma type). Univariate and multivariate Cox regression analyses were applied to evaluate prognostic factors for survival. The effects of clinical parameters were evaluated as hazard ratios (HRs) and their 95% confidence intervals (95% CIs). All statistical analyses were performed using SAS software (Version 9.1; SAS Japan Institute). All tests were 2-tailed, and the statistical significance level was set at .05.

Results

Baseline characteristics

The median value of white blood cell count, lymphocyte count, neutrophil count, and eosinophil count was 11.5×10^9/L (range, 3.9-94.4 $\times 10^9$/L), 6.5×10^9/L (range, 0.9-80.2 $\times 10^9$/L), 4.9×10^9/L (range, 1.5-25.5 $\times 10^9$/L), and 0.06×10^9/L (range, 0-3.0 $\times 10^9$/L), respectively. Frequencies of the patients at baseline are summarized in Table 2. Fifty-eight percent of the patients were male, 52% were 60 years or older, and 22% had an advanced PS (2 or more grade). Regarding complications, 35 patients (39%) had some complications at the time of diagnosis, including 13 with chronic pulmonary diseases (10 chronic bronchitis, 2 diffuse panbronchiolitis, and 1 bronchial asthma with chronic bronchitis), 9 with opportunistic infections (3 pneumocystis pneumonia, 2 cryptococcal meningitis, 2 aspergillus pneumonia, 1 cytomegalovirus infection, and 1 pulmonary tuberculosis), 7 with malignancies other than ATL (2 lung cancer, 1 larynx cancer, 1 pharynx cancer, 1 colon cancer, 1 hepatic cell carcinoma, and 1 lip cancer), and 6 with autoimmune diseases (2 infective dermatitis, 1 primary biliary cirrhosis, 1 autoimmune hemolytic anemia, 1 dermatomyositis, and 1 ulcerative colitis). The 6 patients with autoimmune diseases had received a variety of medications as follows: antibiotics for infective dermatitis, ursodeoxycholic acid for primary biliary cirrhosis, prednisolone for autoimmune hemolytic anemia and dermatomyositis, and sulfasalazine for ulcerative colitis. Concerning the hematologic factors, 43 patients (48%) had leukocytosis, 45 (50%) had lymphocytosis, 17 (19%) had neutrophilia, and 17 (19%) had eosinophilia. Regarding the laboratory factors, 28 patients (31%) had a high LDH level (greater than the normal limit). Only 5 of 87 patients (6%) had an abnormal BUN level; 34 of 88 patients (39%) had a low albumin level. Forty-seven patients (55%) had more than 1 of the 3 unfavorable prognostic factors.

Twenty-four patients (27%) had more than 2 involved lymph node lesions. Regarding the extranodal lesions, skin involvement was observed in 46 patients (51%), liver involvement in 15 (17%), spleen involvement in 6 (7%), and pulmonary involvement in 1 (1%). Of the 64 patients who had BM examined, the involvement was observed in 16 patients (25%; data not shown). Twenty percent of the patients (n = 18) had more than 3 extranodal lesions. Regarding the number of total involved lesions (extranodal lesions plus lymph node lesions), more than 4 involved lesions were observed in 24 patients (27%), 2 or 3 involved lesions in 42 patients (46%), and only 1 involved lesion in 24 patients (27%).

Prognosis

Among 90 patients with indolent ATL, 63 (70%) died, with a median duration of follow-up of 4.1 years (range, 8 days to 17.6 years). The estimated 5-, 10-, and 15-year survival rates were 47.2% (95% CI, 36.1%-57.5%), 25.4% (95% CI, 15.3%-36.8%), and 14.1% (95% CI, 6.2%-25.3%), respectively, with an MST of 4.1 years (95% CI, 2.9-6.3 years; Figure 1A). No plateaus were observed in the survival curves for OS. Of the 27 survivors, 12 were alive for more than 10 years. Of the 63 patients who died, 41 (65.1%) died of acute ATL after transformation, 5 (7.9%) died of severe chronic ATL, 11 (17.5%) died of other diseases (3 malignancies other than ATL, 2 chronic pulmonary diseases, 2 opportunistic infections, 2 autoimmune diseases, 1 cardiac failure, and 1 myocardial infarction), 2 died of transplantation-related complications, and 4 died of unknown cause. No significant difference in OS was observed between patients who died of ATL and patients who died of other causes (data not shown). Among 90 patients, 44 (49%) progressed to aggressive ATL (all were acute types), among those, 41 (93%) died. The median time to transformation was 18.8 months (range, 0.3 months to 17.6 years).

Table 2. Survival by baseline clinical factors

Factors	No. of evaluated (% of total)	No. of deaths (%)*	MST, y	Cumulative probability of survival†		
				5-y survival, % (95% CI)	10-y survival, % (95% CI)	P†
Total	90	63 (70)	4.1	47.2 (36.1-57.5)	25.4 (15.3-36.8)	
Clinical subtype						
Smoldering	25 (28)	17 (68)	2.9	39.4 (19.8-58.6)	25.3 (8.2-47.0)	.36
Chronic	65 (72)	46 (71)	5.3	50.2 (37.0-62.0)	26.3 (14.6-39.5)	
Patient-related factors (n = 90)						
Sex						
Male	52 (58)	34 (65)	4.3	48.1 (33.4-61.3)	24.9 (11.8-40.5)	.99
Female	38 (42)	29 (76)	4.1	46.4 (29.5-61.6)	26.5 (12.0-43.4)	
Age						
60 y or older	46 (52)	32 (70)	3.7	45.5 (30.4-59.4)	29.5 (14.8-45.8)	.18
Younger than 60 y	44 (48)	31 (70)	4.5	49.2 (32.9-63.6)	24.0 (11.2-39.3)	
PS						
0	22 (24)	15 (68)	8.4	75.9 (51.4-89.1)	38.9 (16.8-60.7)	.006
1	49 (54)	33 (67)	3.4	41.5 (26.9-55.5)	22.5 (9.7-38.5)	
2 or 3	19 (22)	15 (79)	1.3	27.9 (10.2-49.0)	13.9 (1.3-41.1)	
Complications at diagnosis (n = 90)						
Absent	55 (61)	37 (67)	5.7	54.1 (39.4-66.7)	25.4 (12.9-40.1)	
Present	35 (39)	26 (74)	3.4	36.6 (20.7-52.8)	28.3 (13.5-45.1)	.06
Malignancies other than ATL	7 (8)	6 (86)	0.8	28.6 (4.1-61.2)	28.6 (4.1-61.2)	
Opportunistic infection	9 (10)	7 (78)	1.2	0	0	
Chronic pulmonary disease	13 (14)	10 (77)	4.1	38.5 (14.1-62.8)	25.6 (5.2-53.4)	
Autoimmune disease	6 (7)	3 (50)	11.4	62.5 (14.2-89.3)	62.5 (14.2-89.3)	
Hematologic factors						
WBC count (n = 90)						
At least 12.0 × 10^9/L	43 (48)	32 (74)	3.4	43.0 (27.6-57.5)	22.3 (9.9-37.8)	.24
Less than 12.0 × 10^9/L	47 (52)	31 (66)	5.3	51.0 (35.1-64.8)	28.5 (13.6-45.2)	
Total lymphocyte count (n = 90)						
At least 6.5 × 10^9/L	45 (50)	35 (78)	3.7	43.3 (28.2-57.5)	17.4 (6.8-32.0)	.34
Less than 6.5 × 10^9/L	45 (50)	28 (62)	5.3	51.4 (35.2-65.4)	36.8 (20.9-52.9)	
Neutrophil counts (n = 89)						
At least 7.5 × 10^9/L	17 (19)	14 (82)	2.3	29.4 (10.7-51.1)	14.7 (1.3-42.9)	.05
Less than 7.5 × 10^9/L	72 (81)	48 (67)	5.3	51.0 (38.3-62.4)	28.4 (16.6-41.3)	
Eosinophil count (n = 89)						
At least 0.4 × 10^9/L	17 (19)	11 (65)	4.0	34.9 (13.0-58.0)	23.2 (4.9-49.4)	.47
Less than 0.4 × 10^9/L	72 (81)	51 (71)	4.5	49.2 (36.8-60.5)	27.4 (16.0-40.1)	
Laboratory factors						
LDH (n = 90)						
Greater than NI	28 (31)	23 (82)	1.5	34.8 (17.3-53.0)	14.9 (3.9-32.7)	.004
Less than or equal to NI	62 (69)	40 (65)	5.4	52.9 (39.2-64.8)	31.8 (18.5-45.9)	
BUN (n = 87)						
Greater than NI	5 (6)	5 (100)	2.0	20.0 (0.8-58.2)	0	.18
Less than or equal to NI	82 (94)	56 (68)	4.5	48.9 (37.2-59.6)	28.4 (17.3-40.6)	
Albumin (n = 88)						
Less than 40.0 g/L	34 (39)	22 (65)	3.4	39.9 (22.4-56.8)	25.6 (8.9-46.4)	.22
At least 40.0 g/L	54 (61)	40 (74)	5.3	52.2 (37.9-64.7)	26.6 (14.3-40.6)	
Potential prognostic factors (n = 87)‡						
At least 1	47 (55)	34 (72)	2.9	38.7 (24.1-53.1)	18.1 (6.5-34.3)	.05
None	40 (45)	27 (68)	5.4	56.1 (39.2-70.0)	35.2 (19.3-51.6)	
Tumor lesions (n = 90)						
No. of lymph node lesions						
2 or more	24 (27)	16 (67)	2.1	37.5 (19.0-56.0)	30.0 (12.1-50.4)	.09
0 or 1	66 (73)	47 (71)	5.3	50.9 (37.5-62.8)	23.6 (12.2-37.2)	
No. of extranodal lesions						
3 or more	18 (20)	14 (78)	1.1	29.4 (10.7-51.1)	19.6 (4.2-43.3)	.005
1 or 2	72 (80)	49 (68)	5.3	51.6 (38.9-62.9)	26.8 (15.2-39.7)	
No. of total involved lesions						
4 or more	24 (27)	16 (67)	1.3	34.8 (16.6-53.7)	26.1 (8.8-47.6)	.03
2 or 3	42 (46)	30 (71)	4.5	49.5 (32.7-64.3)	13.1 (3.5-29.1)	
1	24 (27)	17 (71)	5.4	54.5 (32.1-72.4)	44.1 (22.8-63.5)	
Chemotherapy						
Received	12 (13)	12 (100)	1.4	25.0 (6.0-50.5)	0	.01
Not received	78 (87)	51 (65)	5.3	50.8 (38.6-61.8)	31.3 (19.3-44.0)	

WBC indocates white blood cell count; MST, median survival time (years); and NI, normal index.

*Rate of death in evaluated cases.

†Cumulative probability of survival rate was estimated with the Kaplan-Meier method, and the *P* value was calculated with the generalized Wilcoxon test.

‡PPFs indicate at least 1 of the following 3 factors: low serum albumin, high LDH, or high BUN.[13,14]

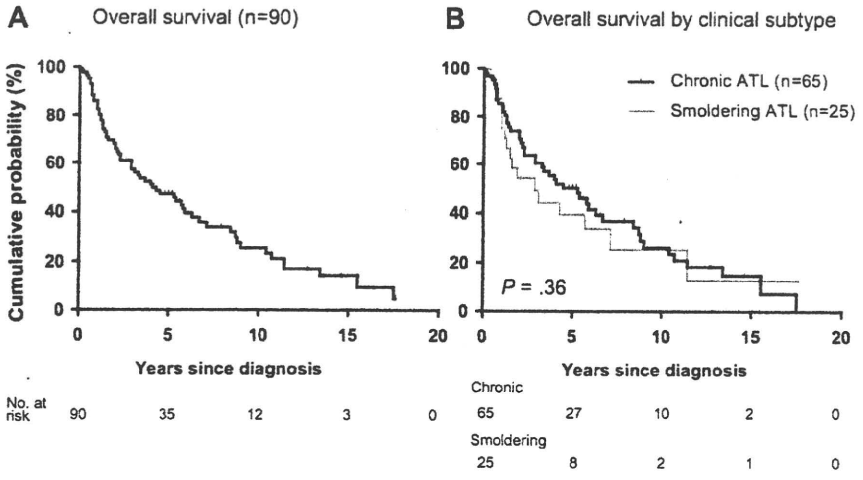

Figure 1. Survival of patients with indolent ATL. (A) For OS (n = 90), the median survival time was 4.1 years (95% CI, 2.9-6.3 years). No plateau was observed in the survival curves for OS. The estimated 5-, 10-, and 15-year survival rates were 47.2% (95% CI, 36.1%-57.5%), 25.4% (95% CI, 15.3%-36.8%), and 14.1% (95% CI, 6.2%-25.3%), respectively. (B) OS by clinical subtype (smoldering type vs chronic type). The estimated 15-year survival rate was 12.7% (95% CI, 1.1%-38.8%) with an MST of 2.9 years for smoldering type and 14.7% (95% CI, 5.7%-27.8%) with an MST of 5.3 years. There was no statistically significant difference ($P = .36$).

Among 25 patients with smoldering ATL, 17 patients (68%) died, and the estimated 15-year survival rate was 12.7% (95% CI, 1.1%-38.8%) with an MST of 2.9 years (95% CI, 1.3-7.1 years). Of the 17 patients who died, 15 died of acute ATL after transformation. Among 65 patients with chronic ATL, 46 (71%) died, and the estimated 15-year survival rate was 14.7% (95% CI, 5.7%-27.8%) with an MST of 5.3 years (95% CI, 2.9-6.7 years). Of the 46 patients who died, 29 died of acute ATL after transformation and 5 died of the disease severity. No statistically significant difference was observed in OS between subtypes ($P = .36$; Figure 1B). The overall estimated 5- and 10-year survival rates of both subtypes are shown in Table 2.

Effects of clinical factors on prognosis

Effects of clinical factors on prognosis were analyzed with the use of all the 90 patients together. Results of prognostic analyses (estimated 5- and 10-year OS rates and MST) with the use of Kaplan-Meier methods are summarized in Table 2. The survival rate was poor for patients with advanced PS ($P = .006$; Figure 2A), neutrophilia ($P = .05$; Figure 2B), and a higher LDH level ($P = .004$; Figure 2C). Patients with at least 1 of 3 PPFs for chronic ATL (a high level of LDH and BUN and a low level of albumin)[13,14] showed a poor survival rate compared with patients without ($P = .05$; Figure 2D). The difference in survival rates between patients with any complications and patients without was marginally significant ($P = .06$). Among patients with any complications, those with malignancies other than ATL or opportunistic infections at diagnosis showed a tendency of poor prognosis, although the number of patients in each category was too small (supplemental Figure 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Although no difference was observed in survival rates between patients with involvement of more than 2 lymph node lesions and patients with less involvement ($P = .09$; Table 2), the survival rate of patients

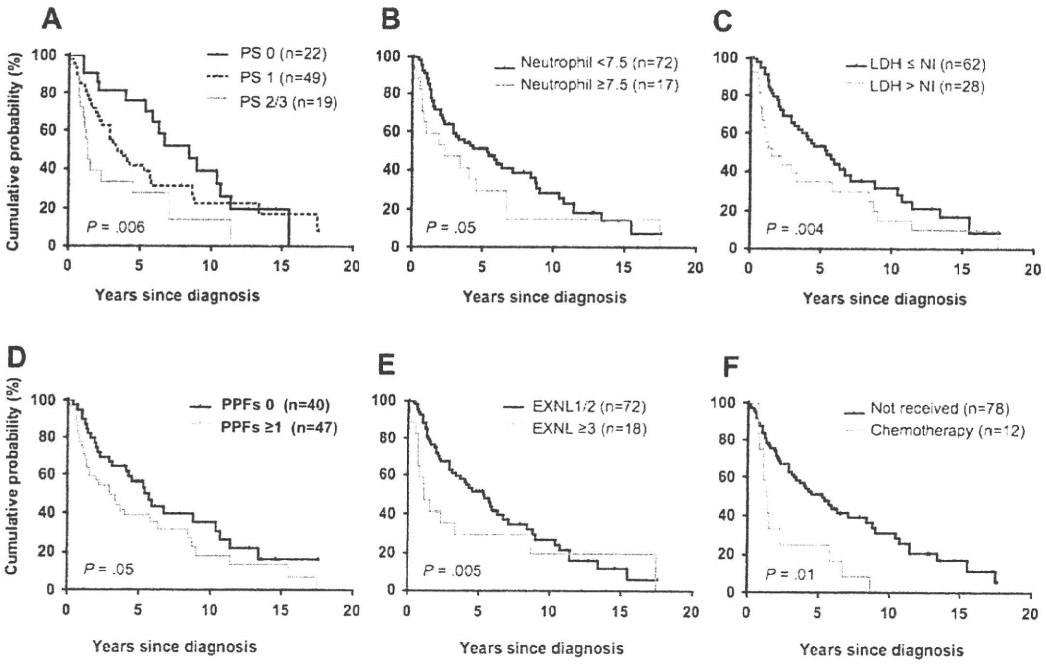

Figure 2. OS by clinical parameters. (A) OS by PS ($P = .006$). (B) OS by neutrophil count ($P = .05$). The unit is $\times 10^9$/L. (C) OS by LDH level ($P = .004$). NI indicates normal index. (D) OS by PPFs for chronic ATL that were defined based on low serum albumin, high LDH, or high BUN according to previous reports[13,14] ($P = .05$). (E) OS by the number of extranodal lesions (EXNL; $P = .005$). (F) OS by treatment states ($P = .01$).

Table 3. Effects of clinical factors on OS in Cox analyses

	All patients (n = 90)						Patients had not received chemotherapy (n = 78)					
	Univariate analysis		Multivariate model A		Multivariate model B		Univariate analysis		Multivariate model C		Multivariate model D	
Clinical factor	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
PS												
0	1		1		1		1		1		1	
1	1.5 (0.8-2.7)	.22	1.4 (0.8-2.8)	.27	1.3 (0.7-2.6)	.37	1.4 (0.7-2.7)	.28	1.6 (0.8-3.1)	.21	1.4 (0.7-2.9)	.30
2 or more	2.5 (1.2-5.2)	.01	2.1 (1.0-4.6)	.06	2.1 (1.0-4.6)	.06	1.7 (0.7-4.0)	.26	1.5 (0.6-3.8)	.39	1.6 (0.6-4.2)	.30
Neutrophil counts												
Less than 7.5 × 10^9/L	1		1		1		1		1		1	
7.5 × 10^9/L or greater	1.6 (0.9-2.9)	.15	1.3 (0.6-2.7)	.45	1.2 (0.6-2.3)	.58	1.3 (0.6-2.7)	.47	1.5 (0.6-3.8)	.43	1.0 (0.5-2.3)	.94
LDH												
Less than or equal to NI	1		1		1		1		1		1	
Greater than NI	1.7 (1.0-2.9)	.04	1.5 (0.8-2.7)	.16	1.5 (0.8-2.6)	.21	1.5 (0.8-2.8)	.19	1.7 (0.9-3.3)	.12	1.6 (0.8-3.1)	.20
No. of extranodal lesions												
0-2	1		1				1		1			
3 or more	1.5 (0.8-2.8)	.16	0.7 (0.3-1.6)	.41			0.9 (0.4-2.2)	.82	0.5 (0.1-1.6)	.22		
No. of total involved lesions												
1	1				1		1				1	
2 or 3	1.2 (0.7-2.2)	.52			0.8 (0.4-1.6)	.52	1.1 (0.6-2.1)	.67			0.9 (0.4-1.7)	.65
4 or more	1.5 (0.7-3.0)	.26			0.9 (0.4-2.1)	.83	1.0 (0.5-2.3)	.96			0.8 (0.3-2.0)	.67
Chemotherapy												
Not received	1		1		1							
Received	2.6 (1.4-5.1)	.003	2.3 (1.1-4.7)	.03	2.0 (1.0-4.2)	.06						

HR indicates hazard ratio; 95% CI, 95% confidence interval; and NI, normal index.

with more than 3 extranodal lesions was significantly poor than the others ($P = .005$; Figure 2E). The survival rate was worse in patients with more than 4 total involvement lesions than in the others (Table 2). Of the extranodal lesions, we additionally examined the effect of skin lesion and BM involvement on survival rates. The survival rate of patients with BM involvement was significantly poor than of patients without ($P = .04$; data not shown), but that of patients with skin involvement was not different from those without ($P = .66$; supplemental Figure 2).

Although most patients in this study had not been treated until their disease progression was similar to B-cell chronic lymphoid leukemia, 12 patients with chronic ATL were treated with chemotherapy immediately after diagnosis because of elevated LDH levels in 8 patients, severe BM involvement in 2 patients, and severe skin involvements in 2 patients. Among them, 2 patients were treated with VCAP (vincristine, cyclophosphamide, doxorubicin, and prednisone)–AMP (doxorubicin, ranimustine, and prednisone)–VECP (vindesine, etoposide, carboplatin, and prednisone),[3] 2 with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone), 4 with CHOP-like, 3 with VEPA (vincristine, etoposide, prednisone, and doxorubicin),[15] and 1 with low-dose etoposide. All of these patients died (MST, 1.4 years; 95% CI, 1.1-2.3 years), and their prognosis was very poor compared with patients not treated ($P = .01$; Figure 2F).

On the basis of results from Kaplan-Meier curves and univariate analysis for each factor, we decided to include PS category, dichotomized neutrophil counts, dichotomized LDH category, dichotomized number of extranodal lesions, the number of total involved lesions, and chemotherapy states into multivariate Cox analysis. Model A included PS category, dichotomized neutrophil counts, dichotomized LDH category, dichotomized number of extranodal lesions, and chemotherapy states. Model B included the same factors as model A except for the number of total involved lesions instead of the number of extranodal lesions. This was because, by definition, a factor of the number of total involved lesions included a factor of the number of extranodal lesions. Results were summarized in Table 3. In model A, advanced PS (≥ 2; HR, 2.1; 95% CI, 1.0-4.6; $P = .06$, borderline significance) and chemotherapy states (HR, 2.3; 95% CI, 1.1-4.7; $P = .03$, significance) were correlated with OS, but the remaining factors were not independent prognostic factors after adjustment for covariate factors. To evaluate effects of clinical factors beyond the effect of chemotherapy states on OS, we also performed additional multivariate analyses for patients who were not received chemotherapy (n = 78; model C and model D in Table 3). We found that there was no clinical parameter that associated with OS.

Discussion

In the present study, we investigated for the first time the long-term clinical course of patients with indolent ATL with a maximum duration of follow-up of 17.6 years. We found that the prognosis of indolent ATL was poor with the MST of 4.1 years, and the estimated 15-year OS rates were 14.1% with no plateau in the survival curve. The prognosis observed in the present study was poorer than expected. Our results confirmed a recent long-term Brazilian study,[6] that showed a poor OS of less than 20% for indolent ATL. In the present study, we showed that 65.1% of patients died of acute ATL with a median time to transformation of 18.8 months. This finding suggests that most patients with indolent ATL will eventually die of aggressive ATL during their long-term course of illness. These findings suggest that even patients with indolent ATL should be carefully observed by frequent clinical visits.

The cause of death in patients with indolent ATL has not been well reported so far. In the present study, patients with indolent ATL died of various causes such as malignancies other than ATL,

chronic pulmonary diseases, opportunistic infections, and autoimmune diseases, in addition to death from acute ATL after transformation. A previous long-term study, which followed-up 50 HTLV-1 carriers with monoclonal proliferation of T lymphocytes (pre-ATL) for 20 years, also reported that 10 patients died of opportunistic infections such as *Pneumocystis* pneumonia or malignancies other than ATL (skin carcinoma, lung cancer, etc).[16] Patients with indolent ATL were also comorbid with a variety of diseases at diagnosis such as chronic pulmonary disease, opportunistic infections, multiple cancers, and autoimmune diseases in the present study. The pathogens responsible for the opportunistic infections were similar to those observed in patients with AIDS associated with HIV. Opportunistic infection was previously reported as a frequent complication in patients with aggressive or with indolent ATL.[2] These findings suggest that helper T-cell function in indolent ATL might be impaired similar to that in AIDS.[17]

We also presented that chronic pulmonary disease, multiple cancers, and autoimmune diseases were frequent as complications at diagnosis in indolent ATL. The reason why indolent ATL had such immune dysregulation remains unknown. It was recently noted that the origin of the ATL cells in a fraction of the patients was from regulatory T cells expressing FoxP3 and CCR4.[18,19] In the present study, 6 patients also had autoimmune diseases. Among them, 3 patients were treated with immunosuppressive drugs, and of those only one patient with smoldering ATL transformed to acute ATL. Therefore, we were not able to evaluate the effect of comorbid autoimmune diseases and immunosuppressive drug therapy on the risk of transformation or poor prognosis so far. Further studies are warranted to elucidate the mechanisms responsible for the development of hyperimmunity or hypoimmunity in patients with indolent ATL.

Although comparison on OS by subtype is not a primary purpose of this study, it was unexpected that survival rates of smoldering ATL (15-year OS, 12.7%) tended to be lower than chronic ATL (15-year OS, 14.7%), and the MST of smoldering ATL (2.9 years) tended to be shorter than chronic ATL (5.3 years; Table 2; Figure 1B). Transformation rates of smoldering ATL and chronic ATL were 60% (n = 15) and 44% (n = 29), respectively (data not shown), which was also unexpected. Although there was no statistically difference in OS, MST, and transformation rate between the 2 groups, our results were different from a previous short-time follow-up study reported by Shimoyama et al[2] (the 4-year survival rates for smoldering type was 62.8%). It was unknown why the rate of smoldering type was poorer than chronic type in the present study. Some previous studies suggested that skin involvements might be a risk factor for poor prognosis of smoldering ATL.[6,20-22] In the present study, the frequency of patients with skin lesion was a little higher in smoldering ATL (n = 14; 56%) than in chronic ATL (n = 32; 49%). The OS of smoldering ATL with skin lesion was worse than that of chronic ATL without skin lesion (supplemental Figure 2), although there was no statistical difference ($P = .5$). Therefore, a possible explanation might be that smoldering ATL with poor conditions (eg, skin involvement) might be disproportionately included in the present study because data were collected at a university hospital, where more advanced cases were referred from city clinics. Another possible explanation might be that the percentage of patients with smoldering-type ATL has increased recently, as shown in Table 1. In recent decades, more patients have been diagnosed with the smoldering type of ATL on the basis of a health examination, including a blood cell count. Some of these patients may have been in the early phase of acute ATL.

Shimoyama et al[2] reported that involved lymph node lesions, extranodal lesions, and total involvement lesions were significantly poor prognostic factors for ATL all together, and low serum albumin, high LDH, or high BUN levels were PPFs for chronic ATL.[13,14] As we expected, patients with at least 1 of 3 known PPFs for chronic ATL (a high level of LDH and BUN and a low level of albumin)[13,14] showed a poor survival rate than patients without (Table 2; Figure 2D). We also confirmed the difference was seen when analyses were performed for chronic ATL only ($P = .03$) but was not seen for smoldering ATL only ($P = .62$; supplemental Figure 3). This suggests that there may be different prognostic factors for smoldering ATL and chronic ATL, respectively. Further detailed studies regarding prognostic factors are needed for individual subtype.

Other than the known 3 potential prognostic factors, an advanced PS, neutrophilia, more than 3 extranodal lesions, more than 4 total involved lesions, and having received chemotherapy were shown to be possible unfavorable prognostic factors for indolent ATL in our Kaplan-Meier analyses (Table 2; Figures 1B, 2A-F). However, in multivariate Cox analyses, only advanced PS and chemotherapy state were associated with OS after adjustment for other covariates (models A and B in Table 3). The poor prognosis in patients with indolent ATL who were treated by chemotherapy was similar to that of the patients with unfavorable chronic ATL who were treated with intensive combination chemotherapy in several clinical trials in Japan.[3,5,23] Although advanced PS was a borderline significant independent poor factor on survival for indolent ATL in the model that used all patients, the factor was not a prognostic factor anymore when data were limited for only untreated patients (models C and D in Table 3). Among 12 patients who received chemotherapy, 7 (58%) had advanced PS at diagnosis. This suggests that patients with advanced PS at diagnosis might have a condition that required treatments, which introduced the disappearance of the effect of advanced PS on survival, even though advanced PS was an independent poor factor.

Regarding the effect of the presence of extranodal lesions on poor survival, we previously reported that BM involvement was a prognostic factor for aggressive ATL.[24] Although we did not present the effect of each extranodal lesion on survival in detail, we also confirmed that the survival rate of patients with BM involvement was significantly poor compared with patients those without BM involvement ($P = .04$; data not shown), but the survival rate of patients with skin involvement was not different compared with those without ($P = .66$; supplemental Figure 2). However, some studies reported that the presence of skin lesions was a possible poor prognostic factor in indolent ATL,[6,20-22] as described earlier. Setoyama et al[21] reported that smoldering cases with a deeper infiltration pattern had a more aggressive course than cases with a superficial infiltration pattern. Degree of skin involvement might be associated with prognosis in indolent ATL.

Previously, our study group noted that some patients showed alterations in tumor suppressor genes (p16 INKA[25,26] or p53[27]) or aneuploidy greater than 1 chromosomal locus by comparative genomic hybridization in ATL cells[28] and that such abnormalities were associated with a poor prognosis. Although we could not perform molecular analyses for all patients in the present study, 7 were examined molecularly, and at least one abnormality was found in each patient (data not shown). They had a poor prognosis and died within 2.5 years. Patients with a poor prognosis who died during the first steep slope in the survival curve (Figure 1A) might have had such genetic alterations.

The primary purpose of this study was to analyze prognosis of smoldering and chronic types together as an indolent type of ATL. Therefore, we were not able to present in detail the difference in

prognostic factors between subtypes, which is one of the limitations in this study. The number of cases evaluated in this study was too small to perform detail analyses for prognostic factors in indolent ATL. Further large-scaled studies are warranted.

In conclusion, the long-term prognosis of patients with indolent ATL was not good without a plateau phase in the survival curve. Further studies are warranted to elucidate patients with indolent ATL who require intensive chemotherapy, allogenic hematopoietic stem cell transplantation (in cases of aggressive ATL), or combination therapy with zidovudine and interferon alfa.[29,30] In addition, new molecular targeting treatments, such as histone deacetylase inhibitors,[31] which have shown promise in the treatment of $CD4^+$ cutaneous T-cell lymphoma, should be taken into consideration for treatment of indolent ATL.

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Authorship

Contribution: Y.T. collected and analyzed the data and wrote the manuscript; M.I. analyzed the data and wrote the manuscript; Y.I., M.T., T.J., T.K., Y.Y., S.K., S.I., Y.M., and M.T. made the diagnoses and treated the patients with ATL; and K.T. organized the study.

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ORIGINAL REPORT

Phase I Study of KW-0761, a Defucosylated Humanized Anti-CCR4 Antibody, in Relapsed Patients With Adult T-Cell Leukemia-Lymphoma and Peripheral T-Cell Lymphoma

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A B S T R A C T

Purpose
KW-0761, a defucosylated humanized anti-CC chemokine receptor 4 (CCR4) antibody, exerts a strong antibody-dependent cellular cytotoxic effect. This phase I study assessed the safety, pharmacokinetics, recommended phase II dose and efficacy of KW-0761 in patients with relapsed CCR4-positive adult T-cell leukemia-lymphoma (ATL) or peripheral T-cell lymphoma (PTCL).

Patients and Methods
Sixteen patients received KW-0761 once a week for 4 weeks by intravenous infusion. Doses were escalated, starting at 0.01, 0.1, 0.5, and finally 1.0 mg/kg by a 3 + 3 design.

Results
Fifteen patients completed the protocol treatment. Only one patient, at the 1.0 mg/kg dose, developed grade 3 dose-limiting toxicities, skin rash, and febrile neutropenia, and grade 4 neutropenia. Other treatment-related grade 3 to 4 toxicities were lymphopenia (n = 10), neutropenia (n = 3), leukopenia (n = 2), herpes zoster (n = 1), and acute infusion reaction/cytokine release syndrome (n = 1). Neither the frequency nor severity of toxicities increased with dose escalation. The maximum tolerated dose was not reached. Therefore, the recommended phase II dose was determined to be 1.0 mg/kg. No patients had detectable levels of anti-KW-0761 antibody. The plasma maximum and trough, and the area under the curve of 0 to 7 days of KW-0761, tended to increase dose and frequency dependently. Five patients (31%; 95% CI, 11% to 59%) achieved objective responses: two complete (0.1; 1.0 mg/kg) and three partial (0.01; 2 at 1.0 mg/kg) responses.

Conclusion
KW-0761 was tolerated at all the dose levels tested, demonstrating potential efficacy against relapsed CCR4-positive ATL or PTCL. Subsequent phase II studies at the 1.0 mg/kg dose are thus warranted.

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The successful use of monoclonal antibodies (mAb) has evolved into a promising approach to treating cancer over the last decade. In the field of hematologic malignancies, development of the therapeutic mAb rituximab has changed the standard of therapy for patients with B-cell lymphomas and has markedly improved prognosis.[1-3] In contrast, the prognosis of patients with T-cell neoplasms remains very poor.[4] The 5-year overall survival (OS) for common subtype of peripheral T-cell lymphoma (PTCL), such as PTCL not otherwise specified (NOS) and angioimmunoblastic T-cell lymphoma, is 32% compared with only 14% for adult T-cell leukemia lymphoma (ATL).[4] A recent phase III trial for newly diagnosed aggressive ATL demonstrated that a dose-intensified multidrug chemotherapy with vincristine, cyclophosphamide, doxorubicin, and prednisone (VCAP), doxorubicin, ranimustine, and prednisone (AMP), and vindesine, etoposide, carboplatin, and prednisone (VECP) was more effective than biweekly cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP).[5] However, the median survival time and OS at 3 years were still unsatisfactory, at approximately 13 months and 24%, respectively.[5,6]

CC chemokine receptor 4 (CCR4) is a chemokine receptor expressed on T-helper type 2[7] and regulatory T cells (Treg).[8-10] Because numerous studies, including our own, have demonstrated CCR4 to be expressed on certain types of T-cell neoplasms,[11-17] we hypothesized that this molecule might represent a novel molecular target for immunotherapy against relapsed or refractory T-cell lymphomas.[16-21] Accordingly, we developed KW-0761, a next generation humanized anti-CCR4 mAb, with a defucosylated Fc region, which markedly enhanced antibody-dependent cellular cytotoxicity (ADCC) due to increased binding affinity to the Fcγ receptor on effector cells.[21,22]

Herein, we report the results of a phase I study designed to assess the safety, pharmacokinetics, recommended phase II dose, and efficacy of KW-0761 in patients with relapsed CCR4-positive ATL and other peripheral T-cell lymphomas (PTCL).

PATIENTS AND METHODS

Investigational Drug and Eligibility

KW-0761 is a defucosylated humanized immunoglobulin G1 (IgG1) 1 mAb generated from a mouse anti-CCR4 mAb[7] by Kyowa Hakko Kirin Co Ltd.[23,24]

Patients between 20 and 69 years of age with CCR4-positive aggressive ATL (acute type, lymphoma type, or unfavorable chronic type)[25,26] or PTCL with CCR4 expression were eligible. CCR4 expression was confirmed by immunohistochemistry or flow cytometry using an anti-CCR4 mAb (KM2160, Kyowa Hakko Kirin Co Ltd),[12,14,15] and confirmed by the review committee with a central evaluation. Patients with relapse after at least one prior course of chemotherapy were eligible. All patients were required to have an Eastern Cooperative Oncology Group performance status of 0 or 1. Eligibility criteria also included the following laboratory values: an absolute neutrophil count $\geq$ 1,500/uL, platelet count $\geq$ 75,000/uL, hemoglobin $\geq$ 8.0 g/dL, AST $\leq$ 2.5 $\times$ the upper limit of the normal range (UNL), ALT $\leq$ 2.5 $\times$ UNL, total bilirubin $\leq$ 1.5 $\times$ UNL, serum creatinine $\leq$ 1.5 $\times$ UNL, corrected serum calcium $\leq$ 11.0 mg/dL, negative for hepatitis B surface antigen and for hepatitis B virus DNA, and arterial partial oxygen pressure $\geq$ 65 mmHg or arterial blood oxygen saturation $\geq$ 90%. All subjects underwent electrocardiography to confirm the absence of abnormalities requiring treatment and that the left ventricular ejection fraction was at least 50%.

Patients were excluded if they had any severe complication, an infectious complication or active tuberculosis, a history of organ transplantation, active concurrent cancers, CNS involvement, a bulky mass requiring emergent radiotherapy, or tested positive for hepatitis C virus antibody and/or HIV antibody.

The institutional review boards of the participating institutions approved this study, and all patients gave written informed consent according to the Declaration of Helsinki.

Study Design

This was a multicenter dose-escalation study with three to six patients at each dose level to determine the maximum-tolerated dose (MTD) and estimate the recommended phase II dose. Cohorts of patients received KW-0761 at 0.01, 0.1, 0.5, and 1.0 mg/kg, weekly for 4 weeks by intravenous infusion. Premedications (antihistamine and antipyretic) were administered before each KW-0761 treatment.

If no dose-limiting toxicity (DLT) was observed in a cohort of three patients at a given dose level, the next cohort of three new patients would be treated with the next higher dose. If DLT was experienced by one or two of the three patients at any dose, three additional patients would be treated at the same dose level. If three or more patients at a given dose level exhibited DLT, this dose would be considered to exceed the MTD and the dose escalation would thus be halted. The recommended phase II dose was defined as one dose level below the MTD or the maximum dose level judged to be tolerable. An expanded cohort of three additional newly enrolled patients was also treated at the recommended phase II dose. Patients who relapsed after achieving responses to KW-0761 were allowed to be re-treated with this antibody.

Toxicity Evaluation and Definition of DLT

Patients treated at each dose level were evaluated weekly during therapy and until 4 weeks after the last infusion to assess toxicity. Toxicity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3. Human anti-KW-0761 antibodies in the plasma of patients were detected by an enzyme-linked immunosorbent assay. The plates were coated with KW-0761 to capture any anti-KW-0761 antibodies, followed by addition of biotinylated KW-0761, and then horseradish peroxidase–labeled avidin. Detection sensitivity of this assay was 5 ng/mL as standard antibody equivalent in plasma.

DLT was defined as an adverse event or a laboratory abnormality that occurred within 28 days after the first infusion, judged to be related to KW-0761 and meeting any of the following criteria: $\geq$ grade 4 hematologic toxicity except lymphopenia, $\geq$ grade 4 symptoms judged to be consistent with an acute infusion reaction/cytokine release syndrome or with tumor lysis

Table 1. Patient Demographic and Clinical Characteristics by Cohort

Characteristic	Cohort and Dosage					Total
	1: 0.01 mg/kg	2: 0.1 mg/kg	3: 0.5 mg/kg	4: 1.0 mg/kg	Expanded: 1.0 mg/kg	
No. of patients	3	4*	3	3	3	16
Median age, years						62
Range	46-68	55-66	60-69	62-64	55-62	46-69
Sex						
Male	2	2	2	0	2	8
Female	1	2	1	3	1	8
Diagnosis						
ATL	2	4	3	2	2	13
PTCL	1 (MF)	0	0	1 (PTCL-NOS)	1 (PTCL-NOS)	3
No. of prior chemotherapy regimens						
1	2	2	2	1	2	9
2	0	0	0	2	0	2
$\geq$ 3	1	2	1	0	1	5

Abbreviations: ATL, adult T-cell leukemia-lymphoma; PTCL, peripheral T-cell lymphoma; NOS, not otherwise specified; MF, mycosis fungoides.
*One patient enrolled at 0.1 mg/kg was withdrawn due to early progressive disease.

