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ORIGINAL REPORT

Prognostic Index for Acute- and Lymphoma-Type Adult T-Cell Leukemia/Lymphoma

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ABSTRACT

Purpose
The prognosis of acute- and lymphoma-type adult T-cell leukemia/lymphoma (ATL) is poor, but there is marked diversity in survival outcomes. The aim of this study was to develop a prognostic index (PI) for acute- and lymphoma-type ATL (ATL-PI).

Patients and Methods
In a retrospective review, data from 807 patients newly diagnosed with acute- and lymphoma-type ATL between January 2000 and May 2009 were evaluated. We randomly divided subjects into training (n = 404) and validation (n = 403) samples, and developed a PI using a multivariable fractional polynomial model.

Results
Median overall survival time (MST) for the 807 patients was 7.7 months. The Ann Arbor stage (I and II *v* III and IV), performance status (0 to 1 *v* 2 to 4), and three continuous variables (age, serum albumin, and soluble interleukin-2 receptor [sIL-2R]) were identified as independent prognostic factors in the training sample. Using these variables, a prognostic model was devised to identify different levels of risk. In the validation sample, MSTs were 3.6, 7.3, and 16.2 months for patients at high, intermediate, and low risk, respectively ($P < .001$; $\chi^2 = 89.7$, 2 *df*; log-rank test). We also simplified the original ATL-PI according to dichotomizing age at 70 years, serum albumin at 3.5 g/dL, and sIL-2R at 20,000 U/mL and developed an easily calculable PI with prognostic discrimination power ($P < .001$; $\chi^2 = 74.2$, 2 *df*; log-rank test).

Conclusion
The ATL-PI is a promising new tool for identifying patients with acute- and lymphoma-type ATL at different risks.

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INTRODUCTION

Adult T-cell leukemia/lymphoma (ATL) is a peripheral T-cell malignancy caused by human T-cell lymphotropic virus type I (HTLV-1).[1,2] HTLV-1 is endemic to the southwestern region of Japan, Caribbean basin, Central and South America, and western Africa. The cumulative incidence of ATL is estimated to be approximately 2.5% to 5% among HTLV-1 carriers.[3,4] Patients with ATL present with characteristic clinical features such as increased abnormal lymphocytes with cerebriform or flower-like nuclei (flower cells) in the peripheral blood, hypercalcemia, skin lesions, generalized lymphadenopathy, and hepatosplenomegaly accompanied by opportunistic infections.[1,5] A previous report by the Japan Clinical Oncology Group-Lymphoma Study Group (JCOG-LSG) identified five prognostic factors for ATL, including advanced performance status (PS), high lactic dehydrogenase (LDH), age of 40 years or older, total involved lesions, and hypercalcemia, on the basis of an analysis of 854 patients with newly diagnosed ATL registered between 1983 and 1987.[6] The JCOG-LSG then proposed four clinical subtypes: acute, lymphoma, chronic, and smoldering types. This system is known as Shimoyama classification and is based on prognostic factors and clinical features of the disease.[7] This classification is now widely used for determining therapeutic strategy. Generally, the prognosis of acute- and lymphoma-type ATL is poor, whereas that of the chronic and smoldering types is better. More than two decades have passed since the pivotal reports by JCOG-LSG, and ATL management has improved over this period. Recently, an International Consensus Meeting recommended treatment using chemotherapies

such as a vincristine, cyclophosphamide, doxorubicin, and prednisolone (VCAP) plus doxorubicin, ranimustine, and prednisolone (AMP) plus vindesine, etoposide, carboplatin, and prednisolone (VECP), which is a sequential combination chemotherapy consisting of VCAP, AMP, and VECP[8,9] with or without subsequent allogeneic hematopoietic cell transplantation (HCT) for acute- and lymphoma-type ATL, and a combination of interferon alfa and zidovudine (IFN/AZT) for acute-type ATL outside of clinical trials.[10]

However, there are diverse clinical courses and survival outcomes among patients with acute- and lymphoma-type ATL. Therefore, it is necessary to establish a prognostic index (PI) for a risk-adapted approach and to improve the quality of clinical trials. To determine prognosis in patients with acute- and lymphoma-type ATL, we elucidated prognostic factors by performing a nationwide survey of patients diagnosed during the past decade and developed a PI.

PATIENTS AND METHODS

Patients

We conducted a retrospective survey of patients with ATL diagnosed between January 1, 2000, and May 31, 2009, in Japan. The inclusion criterion for this investigation was a diagnosis of acute- and lymphoma-type ATL based on Shimoyama classification. Patients who had undergone allogeneic HCT were excluded from this analysis because there is an undetermined impact on survival using this novel intervention. All clinical data as well as the validity of diagnosis of ATL were centrally reviewed by two expert hematologists.

Clinical Data

We collected information regarding sex, age, institutional based–clinical subtype, WBC counts, neutrophil counts, lymphoid cell counts, abnormal lymphoid cell counts, hemoglobin, platelet counts, serum total protein, serum albumin, blood urea nitrogen (BUN), LDH, soluble interleukin-2 receptor (sIL-2R), presence of hypercalcemia, C-reactive protein, maximum tumor size, "B" symptoms, PS by Eastern Cooperative Oncology Group (ECOG), Ann Arbor stage, and number of lesions of involved lymph nodes, as well as the sites and number of involved extranodal lesions. We defined leukemic stage IV disease as the presence of more than 1% of abnormal lymphocytes in peripheral blood according to the definition for diagnosing acute- and lymphoma-type ATL in Shimoyama classification.[7] Overall survival (OS) was calculated from the time of diagnosis to the date of death by any cause or to the last follow-up date.

Approval of the study procedure was obtained from the ethics committee and institutional review board of the coordinating center (Fukuoka University) and at each participating center on the basis of their institutional policies.

Statistical Analysis

The data set was randomly split into either a training sample for developing a PI or a validation sample for evaluating the obtained PI. Continuous variables were not categorized a priori because categorizing a predictor would result in an inevitable loss of information.[11] We applied parametric models based on two-degree fractional polynomial (FP) functions to retain relevant variables continuous.[12] For each continuous variable X, one or two terms of the form X^p were fitted with powers, p, which were chosen from (-2, -1, -0.5, 0, 0.5, 1, 2, and 3). The association of each variable with OS was evaluated using a univariable FP model, and variables showing a P value of less than .05 were considered candidate predictors. Then, the multivariable FP (MFP) procedure using backward elimination was performed. The backward elimination was based on closed testing,[12] and a P value of less than .05 was used for variable selection. A continuous PI from the final MFP model was categorized into three risk groups, with two optimal cutoff points in the continuous PI found by maximizing the log-rank statistics according to the minimal P value approach.

An explorative simplification of our continuous PI was developed, dichotomizing all the predictors a priori according to their standard cutoff points. Concordance between three risk groups from the simplified PI and those from the original PI was measured using weighted κ.

Survival curves were estimated using the Kaplan-Meier method and compared using the log-rank test. All statistical analyses were performed with SAS version 9.2 (SAS Institute, Cary, NC) with %mfp8 macro[13] and MATLAB (Mathworks, Natick, MA). All P values were reported as two-sided.

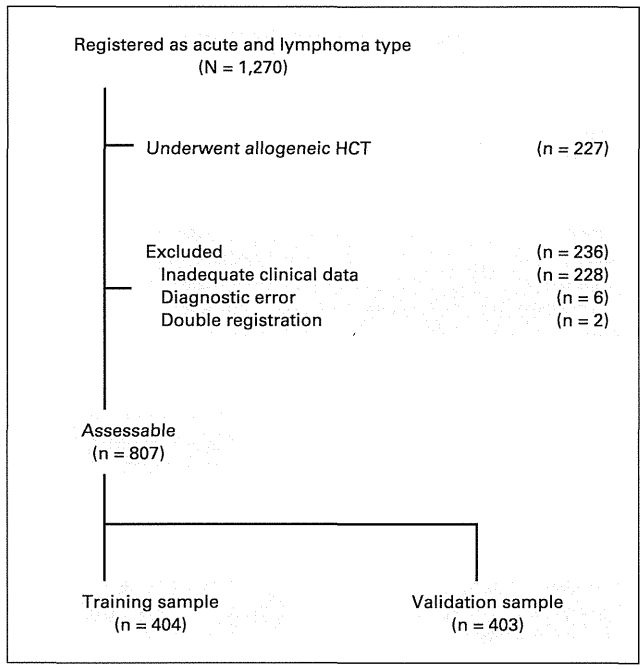

Fig 1. CONSORT flowchart of patients: 1,270 patients diagnosed with acute- and lymphoma-type adult T-cell leukemia/lymphoma were registered. Of these patients, 227 patients were excluded because they had undergone allogeneic hematopoietic cell transplantation (HCT). Two hundred thirty-six patients were excluded for the following reasons: 228 for inadequate clinical data at diagnosis because they had at least one missing value of covariates in Table 1, six for diagnostic error, and two for double registration. The remaining 807 patients were analyzed and randomly divided into training (n = 404) and validation (n = 403) samples.

RESULTS

Patient Characteristics

Data from 1,270 patients with acute- and lymphoma-type ATL were submitted from 81 institutions across Japan (Fig 1). A total of 227 patients had undergone allogeneic HCT and were excluded. Of the remaining 1,043 patients, 236 patients were excluded for the following reasons: 228 for inadequate clinical data at diagnosis because they had at least one missing value of covariates in Table 1, six for diagnostic error, and two for double registration. Thus 807 patients were analyzed for the development of the PI. Baseline characteristics are shown in Table 1. Deaths were observed in 641 patients (79%), and the median overall survival time (MST) was 7.7 months (95% CI, 7.0 to 8.7 months). The most common cause of death was progressive disease (81.3%). Death from infection without disease progression was 13.4%.

The number of patients who received initial treatment was 765 (95%), whereas 37 (4.6%) did not receive any treatment, and five were uncertain. Of the 765 patients who had received initial treatment, 755

Table 1. Baseline Characteristics of All Patients (n = 807)

Characteristic	No.	%
Age, years		
Median	67	
Range	35-91	
Sex		
Female	383	47
Male	424	53
Subtype		
Acute type	564	70
Lymphoma type	243	30
Neutrophil count, × 10^9/L		
Median	5.2	
Range	0.16-37	
Hemoglobin level, g/dL		
Median	13	
Range	7.4-18.0	
Platelet count, × 10^9/L		
Median	206	
Range	8-885	
Serum total protein, g/dL		
Median	6.6	
Range	3.2-8.9	
Serum albumin, g/dL		
Median	3.6	
Range	1.8-5.8	
BUN, mg/dL		
Median	16	
Range	3.6-118.3	
LDH, IU/L		
Median	621	
Range	127-13,813	
LDH > 2 × ULN	457	57
Soluble IL-2R, U/mL		
Median	22,800	
Range	303-683,000	
Hypercalcemia present	279	35
Increased CRP present	576	65
Ann Arbor stage		
I-II	77	10
III-IV	730	90
ECOG PS		
0-1	396	49
2-4	411	51
B symptoms present	252	31
No. of lymph node lesions		
Median	3	
Range	0-8	
No. of extranodal sites		
Median	1	
Range	0-7	
No. of total involved lesions		
Median	4	
Range	0-13	
Bone marrow involvement present	252	31
Liver involvement present	96	12
Spleen involvement present	138	17
Pleural effusion present	97	12
Ascites present	63	8

NOTE. The soluble IL-2R level by pg/mL can be converted to U/mL using the formula: value (pg/mL) × 0.113.

Abbreviations: BUN, blood urea nitrogen; CRP, C-reactive protein; ECOG PS, Eastern Cooperative Oncology Group performance status; IL-2R, interleukin-2 receptor; LDH, lactate dehydrogenase; ULN, upper limit of normal.

Table 2. Results of Variable Selection by the MFP Model in the Training Sample (n = 404)

Variable	HR	95% CI	*P*
Stage			
I-II	1.00		
III-IV	1.91	1.25 to 2.92	.003
ECOG PS			
0-1	1.00		
2-4	1.42	1.13 to 1.80	.003
Age, years (continuous)	1.02	1.01 to 1.03	.007
Serum albumin, g/dL (continuous)	0.70	0.57 to 0.87	.001
Log_{10}(sIL-2R), U/mL (continuous)	1.45	1.19 to 1.76	< .001

Abbreviations: ECOG PS; Eastern Cooperative Oncology Group performance status; HR, hazard ratio; MFP, multivariable fractional polynomial; sIL-2R, soluble interleukin-2 receptor.

had chemotherapy and 10 patients had undergone lesion-directed treatment (Appendix Fig A1, online only). No patient received IFN/AZT, which is considered a standard treatment for acute-type ATL in the world,[10,14] because this combination of agents has not been approved for ATL in Japan.

Development of the PI

We randomly selected 404 patients (50% of the 807 patients) as a training sample and developed a PI based on this set. First, in univariate analysis with the two-degree univariable FP model, all variables except sex showed *P* values less than .05 (likelihood ratio test). We then performed backward elimination using the MFP model. Variables that remained independently significant included Ann Arbor stage (I or II *v* III or IV), ECOG PS (0 to 1 *v* 2 to 4), and the three continuous variables of age, serum albumin, and sIL-2R. The MFP model yielded a significant nonlinear function for sIL-2R (log transformation), whereas the other four variables fitted linearly, thus allowing an expression of a final multivariate model in terms of the usual Cox regression model. The estimated hazard ratios and their 95% CIs in the final multivariate model in the training sample are shown in Table 2. A linear risk function based on Cox regression coefficients (ie, the log of hazard ratios), which hereafter we call ATL-PI, was as follows: ATL-PI = 0.65 (if stage = III or IV) + 0.35 (if ECOG PS > 1) + 0.016 × age (years) − 0.36 × albumin (g/dL) + 0.37 × $\log_{10}$ (sIL-2R [U/mL]).

The median of the ATL-PI in the training sample was 2.13 (range, 0.30 to 3.48), 10% of values were less than 1.31, and 90% of values were less than 2.86. Potential cutoff points between 1.30 and 2.90 were evaluated, and the value of 2.6 showed the best discrimination on the basis of the log-rank test (1 *df*) and was defined as the high-risk group for 91 patients (23%, ATL-PI ≥ 2.6). To define the low-risk group, the value of 1.6 was chosen as the best discriminator using the log-rank test (2 *df*), and 76 patients were classified as low risk (19%, ATL-PI < 1.6). The distribution of ATL-PI was similar in the validation sample (n = 403) with high-, intermediate-, and low-risk groups of 99 (25%), 232 (56%), and 72 (18%) patients, respectively, using the designated cutoff points. The three risk groups according to the ATL-PI were effectively prognostic in the validation sample, as shown in Figure 2 ($P < .001$; $\chi^2 = 89.7$, 2 *df*; log-rank test). MSTs were 3.6 (95% CI, 2.4 to 4.6), 7.3 (95% CI, 6.4 to 8.5), and 16.2 (95% CI, 14.5 to 24.7) months for patients at high, intermediate, and low risk, respectively, and OS rates

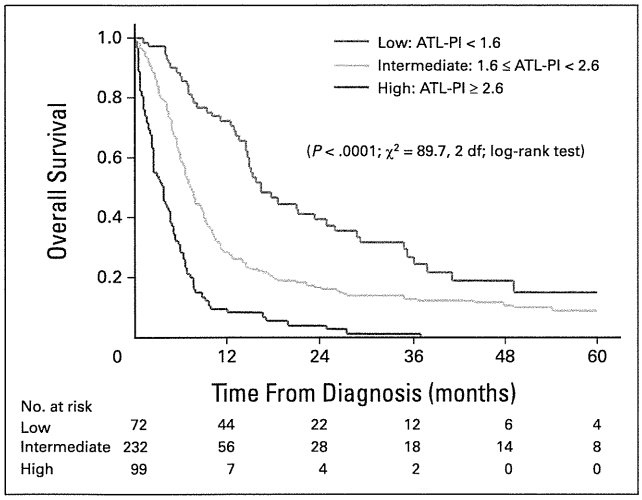

Fig 2. Overall survival curves for the validation sample (n = 403) according to the adult T-cell leukemia/lymphoma prognostic index (ATL-PI): An ATL-PI score was calculated as 0.65 (if stage = III or IV) + 0.35 (if Eastern Cooperative Oncology Group performance status > 1) + 0.016 × age (years) − 0.36 × albumin (g/dL) + 0.37 × $\log_{10}$[soluble interleukin-2 receptor (U/mL)].

Table 3. Results of Cox Regression Model With Dichotomized Covariates in the Training Sample (n = 404)

Variable	HR	95% CI	*P*	Score
Stage				
I-II	1.00			
III-IV	2.17	1.43 to 3.30	< .001	2
ECOG PS				
0-1	1.00			
2-4	1.51	1.20 to 1.90	.001	1
Age, years				
≤ 70	1.00			
> 70	1.45	1.15 to 1.83	.002	1
Serum albumin, g/dL				
≥ 3.5	1.00			
< 3.5	1.42	1.12 to 1.79	.003	1
sIL-2R, U/mL				
≤ 20,000	1.00			
> 20,000	1.37	1.09 to 1.73	.008	1

NOTE. The five variables are those selected by the multivariable fractional polynomial model. In fitting the Cox model, age, serum albumin, and sIL-2R were dichotomized. The last column shows an assigned score for each variable in the calculation of the simplified adult T-cell leukemia/lymphoma prognostic index.

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; HR, hazard ratio; sIL-2R, soluble interleukin-2 receptor.

at 2 years were 4% (95% CI, 1% to 10%), 17% (95% CI, 12% to 22%), and 39% (95% CI, 27% to 51%), respectively.

Simplified ATL-PI

In the previous section, we described how a continuous PI was established from a model in which all relevant covariates were kept continuous. This PI was then used to categorize the three risk groups. Although this procedure is statistically valid for deriving the categorized risk groups,[11] to make the scoring system easier and clinically practicable, we simplified the system by initially dichotomizing individual continuous covariates. Median values of the identified continuous prognostic factors for age, serum albumin, and sIL-2R were 67 years, 3.6 g/dL, and 21,500 U/mL in the training sample, respectively. Therefore, we set the clinically appropriate cutoff points at 70 years for age, 3.5 g/dL for serum albumin, and 20,000 U/mL for sIL-2R and subsequently fitted a multivariate Cox model based on these dichotomizations in the training sample (Table 3). The estimated Cox regression coefficients were 0.77, 0.41, 0.37, 0.35, and 0.31 for the Ann Arbor stage, ECOG PS, age, albumin, and sIL-2R, respectively. From the weights of these variables, we defined a simplified ATL-PI as follows: simplified ATL-PI = 2 (if stage = III or IV) + 1 (if ECOG PS > 1) + 1 (if age > 70 years) + 1 (if albumin < 3.5 g/dL) + 1 (if sIL-2R > 20,000 U/mL).

On the basis of the best discriminations according to the log-rank test in the training sample, scores from 0 to 2 were categorized into the low-risk group, 3 and 4 into the intermediate-risk group, and from 5 to 6 into the high-risk group. The simplified ATL-PI was then applied to the validation sample, which showed a distribution from 0 through 6 (0, n = 13; 1, n = 10; 2, n = 54; 3, n = 112; 4, n = 96; 5, n = 78; 6, n = 40). Frequencies of the three risk groups were 118 patients (29%), 208 patients (52%), and 77 patients (19%), for high-, intermediate-, and low-risk groups, respectively. This classification yielded a high concordance with the original ATL-PI (weighted κ, 0.82) in the validation sample and resulted in a good separation of OS curves ($P < .001$; $\chi^2 = 74.2$, 2 *df*; log-rank test). Survival curves of the three groups according to the simplified ATL-PI are shown in Figure 3. MSTs were 4.6 (95% CI, 2.6 to 5.4), 7.0 (95% CI, 6.3 to 8.6), and 16.2 (95% CI, 13.4 to 23.2) months, and the 2-year OS rates were 6% (95% CI, 2% to 12%), 17% (95% CI, 12% to 23%), 37% (95% CI, 25% to 49%) for patients at high, intermediate, and low risk, respectively. These results indicated that the simplified ATL-PI also had good prognostic power in the validation sample.

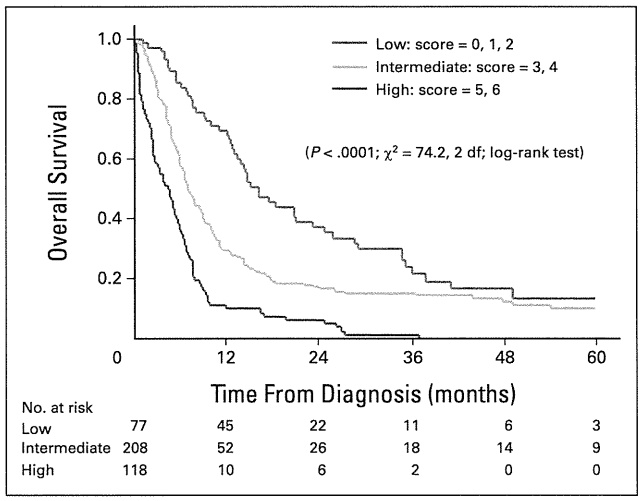

Fig 3. Overall survival curves for the validation sample (n = 403) according to the simplified adult T-cell leukemia/lymphoma prognostic index (ATL-PI): The score for the simplified ATL-PI was calculated as 2 (if stage = III or IV) + 1 (if Eastern Cooperative Oncology Group performance status > 1) + 1 (if age > 70 years) + 1 (if albumin < 3.5 g/dL) + 1 (if soluble interleukin-2 receptor > 20,000 U/mL).

Age-Adjusted ATL-PI

The simplified ATL-PI was applied to a subgroup of patients who were 60 years of age or younger (n = 109) or 70 years of age or younger (n = 255). The predictive capability of the previously determined risk factors other than age was evaluated within each age subgroup in the validation sample. Scores from 0 to 2 were categorized into the low-risk group, 3 and 4 into the intermediate-risk group, and 5 into the high-risk group. The three risk groups according to this age-adjusted ATL-PI were effectively prognostic in patient subgroups younger than 60 or 70 years of age (Appendix Fig A2, online only). MSTs were 2.8 (95% CI, 0.4 to 5.4), 6.5 (95% CI, 5.8 to 9.1), and 16.2 (95% CI, 13.4 to 35.1) months for patients at high, intermediate, and low risk among those younger than 60 years and 3.1 (95% CI, 2.1 to 5.3), 6.7 (95% CI, 5.6 to 8.4), and 16.2 (95% CI, 12.8 to 21.0) months among those younger than 70 years, respectively.

Application of ATL-PI to Patients With Allogeneic HCT

We applied the simplified ATL-PI to 192 patients with allogeneic HCT in whom data was available for five variates. The numbers of high-risk patients were as few as 12 patients (6%), whereas 97 (51%) and 83 (43%) patients showed intermediate and low risk, respectively. MSTs were 9.2 (95% CI, 4.2 to 12.7), 14.0 (95% CI, 11.0 to 17.9), and 14.3 (95% CI, 11.3 to 26.0) months at high, intermediate, and low risk, respectively (Appendix Fig A3). No statistical difference was observed among the three groups ($P = .08$; $\chi^2 = 5.04$, 2 *df*; log-rank test).

DISCUSSION

PIs for specified subentities of malignant lymphoma have involved the International Prognostic Index (IPI) for diffuse large B-cell lymphoma (DLBCL),[15] follicular lymphoma IPI for follicular lymphoma,[16] and PI for advanced Hodgkin's lymphoma.[17] PI for T-cell lymphoma, including peripheral T-cell lymphoma unspecified and extranodal natural killer T-cell lymphoma, nasal type, were also reported.[18,19] However, there have been no studies regarding PI for acute- or lymphoma-type ATL. The aim of this study was to develop a system for risk stratification in patients with acute- and lymphoma-type ATL. Importantly, this is the largest study to analyze prognosis among patients with acute- and lymphoma-type ATL, and the ATL-PI is the first PI for this cohort enabling differentiation among three subgroups with significantly different prognoses. The simplified version of the ATL-PI demonstrated a similar power of prognostic discrimination.

The ATL-PI consists of five factors: Ann Arbor stage, ECOG PS, age, serum albumin, and sIL-2R. In our multivariate analysis, the most significant factor concerning prognostic relevance to survival was the Ann Arbor stage (I or II *v* III or IV). Ann Arbor stage has been included in prognostic indices for other types of lymphoma but not emphasized in ATL because many patients with acute type fall into stage IV as a result of the leukemic phase of the disease. The prognostic significance of the Ann Arbor stage can be translated into better survival in patients with acute- and lymphoma-type ATL with limited disease. Serum sIL-2R level[20,21] was a significant novel indicator in our analyses. Notably, the survival impact of the serum sIL-2R levels was stronger than LDH levels, which are commonly included in PIs for many types of malignant lymphoma. It is thus conceivable that serum sIL-2R can be a new marker of tumor load in ATL.

Recent analysis of 126 patients from the International Peripheral T-Cell Lymphoma Project suggested that the IPI, which is commonly used in the management of patients with DLBCL,[15] is also a useful tool for predicting clinical outcome of patients with ATL.[22] However, in contrast to our study, most patients registered in the previous project had lymphoma type. We applied the IPI to 403 patients in the validation sample and confirmed that most patients were allocated into the intermediate- or high-risk groups, whereas patients in the low-risk group accounted for only 5.7%; the median age of 67 years in our analysis was higher than that in patients involved in the IPI study (56 years),[15] and many more patients with ATL than with DLBCL were in stage IV as a result of frequent leukemic manifestation in the peripheral blood. Moreover, 89% of patients surpassed the normal upper limit of LDH in our study. A similar tendency was observed in applying the PI for peripheral T-cell lymphoma unspecified to the validation sample.[18]

We additionally investigated the simplified ATL-PI according to chemotherapeutic regimens. The MSTs were 4.8, 7.3, and 14.7 months for patients with a cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP)/CHOP-like regimen at high, intermediate, and low risk, respectively, and 5.3, 8.7, and 14.9 months for patients with VCAP-AMP-VECP, respectively. Thus the simplified ATL-PI was not affected by chemotherapeutic regimens.

We excluded patients treated with allogeneic HCT in our analysis because allogeneic HCT has an undetermined impact on survival. In fact, allogeneic HCT may have the potential to put some patients into cure, thus significantly prolonging their survival, whereas allogeneic HCT causes an observed treatment-related mortality of up to 43%,[23-25] implying that prognoses of a specific fraction of patients are perturbed by this intervention. We applied the simplified ATL-PI to patients who received allogeneic HCT, but it was not possible to distinguish patient subgroups between low and intermediate risks. This may be because transplantation was applied to a particular population who could complete induction treatment and survived until transplantation (6 months median since diagnosis), regardless of their risk classification. The predominant difference appears in the intermediate-risk group, where the MSTs were 14.0 and 6.5 months for patients with allogeneic HCT and standard therapy, respectively, suggesting that allogeneic HCT might have improved the prognosis for the group, although this should be interpreted with caution because of the potential bias in patient selection for transplant. There is a need for a larger study to address this issue.

In conclusion, we proposed an original ATL-PI and its simplified version including five prognostic factors for acute- and lymphoma-type ATL. The ATL-PI, the first PI for acute- and lymphoma-type ATL, is a promising platform that can be used to determine optimal treatment based on risk stratification and for well-controlled clinical trials. Further international studies including patients treated with IFN/AZT, which is a common treatment for acute-type ATL outside Japan, is warranted to assess the power of the ATL-PI.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

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REFERENCES

1. Uchiyama T, Yodoi J, Sagawa K, et al: Adult T-cell leukemia: Clinical and hematologic features of 16 cases. Blood 50:481-492, 1977
2. Poiesz BJ, Ruscetti FW, Gazdar AF, et al: Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc Natl Acad Sci U S A 77:7415-7419, 1980
3. Murphy EL, Hanchard B, Figueroa JP, et al: Modelling the risk of adult T-cell leukemia/lymphoma in persons infected with human T-lymphotropic virus type I. Int J Cancer 43:250-253, 1989
4. Yamaguchi K, Watanabe T: Human T lymphotropic virus type-I and adult T-cell leukemia in Japan. Int J Hematol 76:240-245, 2002 (suppl 2)
5. Ishitsuka K, Tamura K: Treatment of adult T-cell leukemia/lymphoma: Past, present, and future. Eur J Haematol 80:185-196, 2008
6. Major prognostic factors of patients with adult T-cell leukemia-lymphoma: A cooperative study—Lymphoma Study Group (1984-1987). Leuk Res 15:81-90, 1991
7. Shimoyama M: Diagnostic criteria and classification of clinical subtypes of adult T-cell leukaemia-lymphoma: A report from the Lymphoma Study Group (1984-87). Br J Haematol 79:428-437, 1991
8. Yamada Y, Tomonaga M, Fukuda H, et al: A new G-CSF-supported combination chemotherapy, LSG15, for adult T-cell leukaemia-lymphoma: Japan Clinical Oncology Group Study 9303. Br J Haematol 113:375-382, 2001
9. Tsukasaki K, Utsunomiya A, Fukuda H, et al: VCAP-AMP-VECP compared with biweekly CHOP for adult T-cell leukemia-lymphoma: Japan Clinical Oncology Group Study JCOG9801. J Clin Oncol 25:5458-5464, 2007
10. Tsukasaki K, Hermine O, Bazarbachi A, et al: Definition, prognostic factors, treatment, and response criteria of adult T-cell leukemia-lymphoma: A proposal from an international consensus meeting. J Clin Oncol 27:453-459, 2009
11. Royston P, Altman DG, Sauerbrei W: Dichotomizing continuous predictors in multiple regression: A bad idea. Stat Med 25:127-141, 2006
12. Royston P, Sauerbrei W: Multivariable Model-building: A Pragmatic Approach to Regression Analysis Based on Fractional Polynomials for Modelling Continuous Variables. Hoboken, NJ, Wiley, 2008
13. Sauerbrei W, Meier-Hirmer C, Benner A, et al: Multivariable regression model building by using fractional polynomials: Description of SAS, STATA and R programs. Comput Stat Data Anal 50:3464-3485, 2006
14. Bazarbachi A, Plumelle Y, Carlos Ramos J, et al: Meta-analysis on the use of zidovudine and interferon-alfa in adult T-cell leukemia/lymphoma showing improved survival in the leukemic subtypes. J Clin Oncol 28:4177-4183, 2010
15. A predictive model for aggressive non-Hodgkin's lymphoma: The International Non-Hodgkin's Lymphoma Prognostic Factors Project. N Engl J Med 329:987-994, 1993
16. Solal-Céligny P, Roy P, Colombat P, et al: Follicular lymphoma international prognostic index. Blood 104:1258-1265, 2004
17. Hasenclever D, Diehl V: A prognostic score for advanced Hodgkin's disease: International Prognostic Factors Project on Advanced Hodgkin's Disease. N Engl J Med 339:1506-1514, 1998
18. Gallamini A, Stelitano C, Calvi R, et al: Peripheral T-cell lymphoma unspecified (PTCL-U): A new prognostic model from a retrospective multicentric clinical study. Blood 103:2474-2479, 2004
19. Lee J, Suh C, Park YH, et al: Extranodal natural killer T-cell lymphoma, nasal-type: A prognostic model from a retrospective multicenter study. J Clin Oncol 24:612-618, 2006
20. Kamihira S, Atogami S, Sohda H, et al: Significance of soluble interleukin-2 receptor levels for evaluation of the progression of adult T-cell leukemia. Cancer 73:2753-2758, 1994
21. Araki K, Harada K, Nakamoto K, et al: Clinical significance of serum soluble IL-2R levels in patients with adult T cell leukaemia (ATL) and HTLV-1 carriers. Clin Exp Immunol 119:259-263, 2000
22. Suzumiya J, Ohshima K, Tamura K, et al: The International Prognostic Index predicts outcome in aggressive adult T-cell leukemia/lymphoma: Analysis of 126 patients from the International Peripheral T-Cell Lymphoma Project. Ann Oncol 20:715-721, 2009
23. Kami M, Hamaki T, Miyakoshi S, et al: Allogeneic haematopoietic stem cell transplantation for the treatment of adult T-cell leukaemia/lymphoma. Br J Haematol 120:304-309, 2003
24. Fukushima T, Miyazaki Y, Honda S, et al: Allogeneic hematopoietic stem cell transplantation provides sustained long-term survival for patients with adult T-cell leukemia/lymphoma. Leukemia 19: 829-834, 2005
25. Hishizawa M, Kanda J, Utsunomiya A, et al: Transplantation of allogeneic hematopoietic stem cells for adult T-cell leukemia: A nationwide retrospective study. Blood 116:1369-1376, 2010

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Case Report

Stevens–Johnson Syndrome associated with mogamulizumab treatment of adult T-cell leukemia/lymphoma

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We report an adult T-cell leukemia/lymphoma patient suffering from Stevens–Johnson Syndrome (SJS) during mogamulizumab (humanized anti-CCR4 monoclonal antibody) treatment. There was a durable significant reduction of the $CD4^+CD25^{high}FOXP3^+$ regulatory T (Treg) cell subset in the patient's PBMC, and the affected inflamed skin almost completely lacked FOXP3-positive cells. This implies an association between reduction of the Treg subset by mogamulizimab and occurrence of SJS. The present case should contribute not only to our understanding of human pathology resulting from therapeutic depletion of Treg cells, but also alert us to the possibility of immune-related severe adverse events such as SJS when using mogamulizumab. We are currently conducting a clinical trial of mogamulizumab for CCR4-negative solid cancers (UMIN000010050), specifically aiming to deplete Treg cells. (*Cancer Sci* 2013; 104: 647–650)

Adult T-cell leukemia/lymphoma (ATL) is an aggressive peripheral T-cell neoplasm caused by HTLV-1. The disease is resistant to conventional chemotherapeutic agents, and has a very poor prognosis.[1] Mogamulizumab (KW-0761) is a defucosylated humanized monoclonal antibody targeting CC chemokine receptor 4 (CCR4).[2] A phase I clinical trial for relapsed CCR4-positive peripheral T-cell neoplasms, including ATL, and a phase II study for relapsed ATL have been conducted with mogamulizumab.[3,4] This agent was subsequently approved for the treatment of relapsed or refractory ATL in Japan, the first country in the world to do so, in March 2012. Mogamulizimab went on sale on 29 May 2012. The interim report for the post-marketing surveillance from 29 May to 28 September 2012 revealed skin-related severe adverse events (SAE), as defined by the Medical Dictionary for Regulatory Activities Terminology/Japan, in nine patients. Thus, during only the first 4 months of use, 9 skin-related SAE, including 4 cases of Stevens–Johnson Syndrome (SJS)/toxic epidermal necrolysis (TEN) were reported, with 1 SJS/TEN fatality. These skin-related, potentially fatal SAE are certainly a challenge to the free use of this agent and clearly require investigation. Therefore, here we report an informative ATL patient suffering from SJS on mogamulizumab treatment, focusing on the reduction of the regulatory T (Treg) cell subset ($CD4^+CD25^{high}FOXP3^+$) caused by the antibody.

Case Report

A 71-year old woman was admitted due to elevation of her lymphocyte count. She had been diagnosed as suffering from acute-type ATL nearly 5 months prior to admission. She had received VCAP-AMP-VECP chemotherapy[5] followed by oral sobuzoxane in another hospital, and achieved a transient partial remission. We started mogamulizumab to treat the flare-up of ATL disease (Fig. 1). Grade 1 skin eruptions appeared around her neck after three antibody infusions. Because we were also giving her antibacterial (ciprofloxacin hydrochloride), fungal (itraconazole), pneumocystic (sulfamethoxazole-trimethoprim) and viral (aciclovir) prophylaxes in addition to stomach medicine (lansoprazole), we judged the skin event to be due to drug eruption caused by one of these concomitant drugs. Therefore, we stopped all five, but continued with mogamulizumab. Despite their discontinuation and treatment with topical steroids, the skin rashes continued to worsen. We started the patient on 30 mg oral prednisolone, which improved the skin symptoms. The patient was then able to complete the eight planned infusions, and oral prednisolone was tapered off. She was discharged from hospital 8 days after her eighth infusion (day 65), and thereafter seen as an outpatient. However, she had to be readmitted as an emergency patient at day 75 because of fulminant skin rashes. These included erythemas, scale-like plaques, vesicles, blisters and erosions over many areas of the body. Her lips were swollen and oral mucosa was erosive (Fig. 2a). Skin biopsy revealed marked liquefaction, degeneration and perivascular inflammation with dominant CD8-positive cells but almost complete lack of FOXP3-positive cells (Fig. 2b). We diagnosed her as a SJS, and immediately started steroid pulse therapy (methylprednisolone 500 mg/day ×3 days), followed by oral prednisolone. Her skin and mucosal lesions improved gradually, and became inactive. At the same time, her general condition improved. Thus, we again tapered the steroid dose, and she was discharged at day 144. However, she had to come back yet again as an emergency patient on day 151 for the same reason as before, with fulminant skin rashes. We prescribed her mini-steroid pulse therapy (methylprednisolone 125 mg/day ×1 day), followed by oral prednisolone. Once more, her skin lesions improved gradually. Over this whole period, complete ATL remission was maintained by mogamulizumab. The HTLV-1 provirus load in PBMC pre-treatment, and at days 121 and 162 was 750.1, 0.0 (under the limit of detection) and 0.8 copies/1000 cells, respectively. These post-treatment values are strikingly low, considering that median HTLV-1

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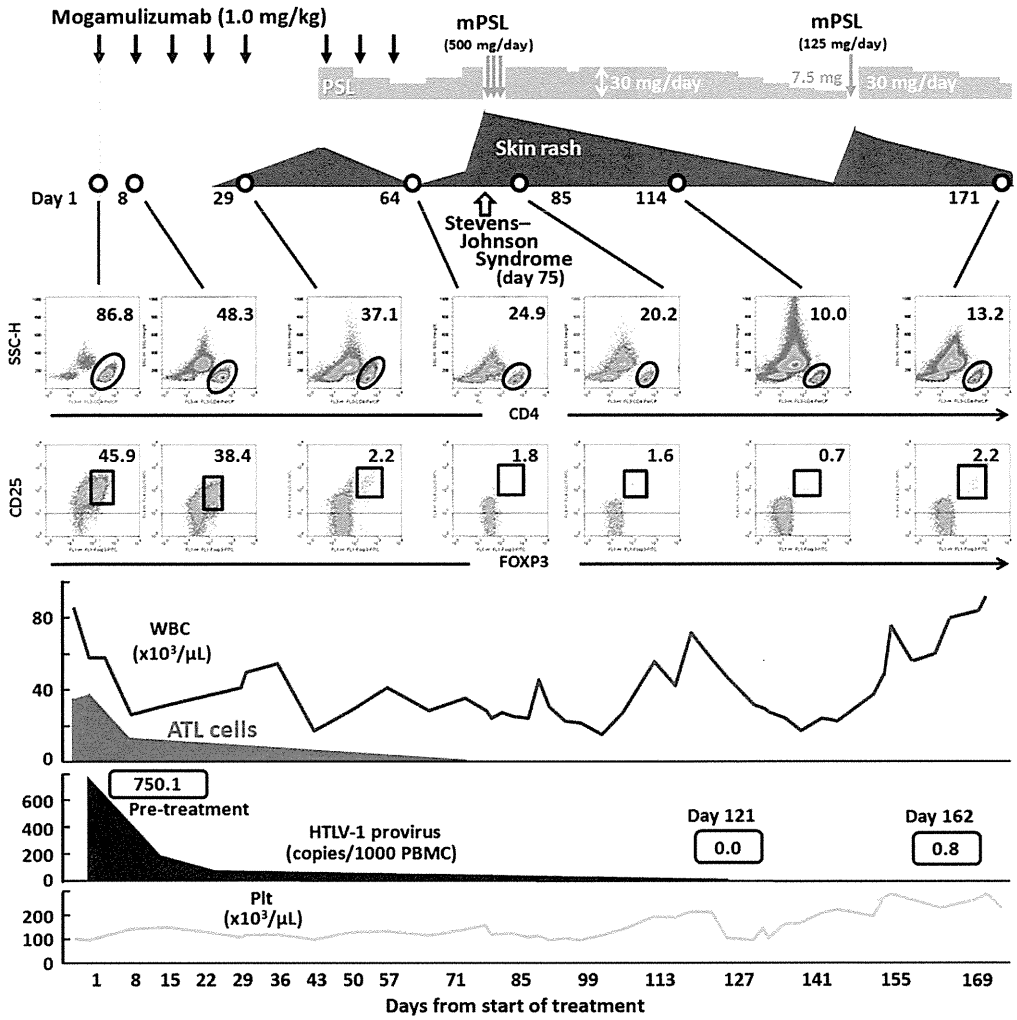

Fig. 1. Clinical course of an ATL patient receiving mogamulizumab monotherapy. ATL; adult T-cell leukemia/lymphoma; mPSL, methylprednisolone; Plt, platelet PSL; prednisolone; WBC, white blood cell.

load in asymptomatic carriers reported by other investigators is 18.0 copies/1000 cells.[6]

We also analyzed CD4, CD25 and FOXP3 expression by PBMC during and after antibody treatment (Fig. 1, middle panels). Before treatment, the majority of the patient's PBMC consisted of CD4-positive and CD25-positive ATL cells. Just before the 5th antibody infusion (day 29), around the time when her skin rash first appeared, the proportion of $CD25^{high}FOXP3^{+}/CD4^{+}$ cells was markedly reduced, to 2.2%. This is low even compared to healthy individuals ($CD25^{high}FOXP3^{+}/CD4^{+}$ cells, mean 3.3%, median 3.3%, range 2.6–4.4%) (Fig. 3). Around the time of SJS onset, the proportion of cells in the Treg subset was further reduced. The proportion of $CD25^{high}FOXP3^{+}/CD4^{+}$ cells at days 64, 85 and 114 was 1.8%, 1.6% and 0.7%, respectively. The striking reduction of the Treg subset persisted until 4 months after the last of the eight antibody infusions (day 171).

Discussion

Drugs often induce adverse cutaneous reactions of varying severity, ranging from simple uncomplicated eruptions to potentially fatal eruptions, such as SJS and TEN, within the spectrum of severe adverse reactions affecting skin and mucosa. Although many factors that might cause variability in the clinical course of such adverse reactions have been suggested, it remains unknown which factors are predominantly involved in these processes. The most prevalent severe drug eruptions are thought to be mediated by drug-reactive T-cells,[7] although we also need to be aware of the alternative view that severe drug eruptions are due to a dysregulated immune system. In this regard, an effect mediated by Treg cells is a likely candidate in severe drug eruptions. Indeed, it is reported that Treg cells can prevent experimentally-induced epidermal injury mimicking TEN in an animal model.[8] Furthermore, Takahashi *et al.* (2009) report that Treg cell function is profoundly impaired in patients with TEN.[9] Consistent with these reports, a marked reduction of the Treg subset was observed in the present case.

Mogamulizumab is the first therapeutic agent targeting CCR4, which is expressed on Treg cells,[10,11] to receive marketing approval anywhere in the world. The reduction of the Treg subset seen here was not specific to the present case, but is commonly observed in ATL patients receiving mogamulizumab. In fact, skin rashes were observed as a frequent non-hematologic adverse event (AE) (63%), mostly occurring

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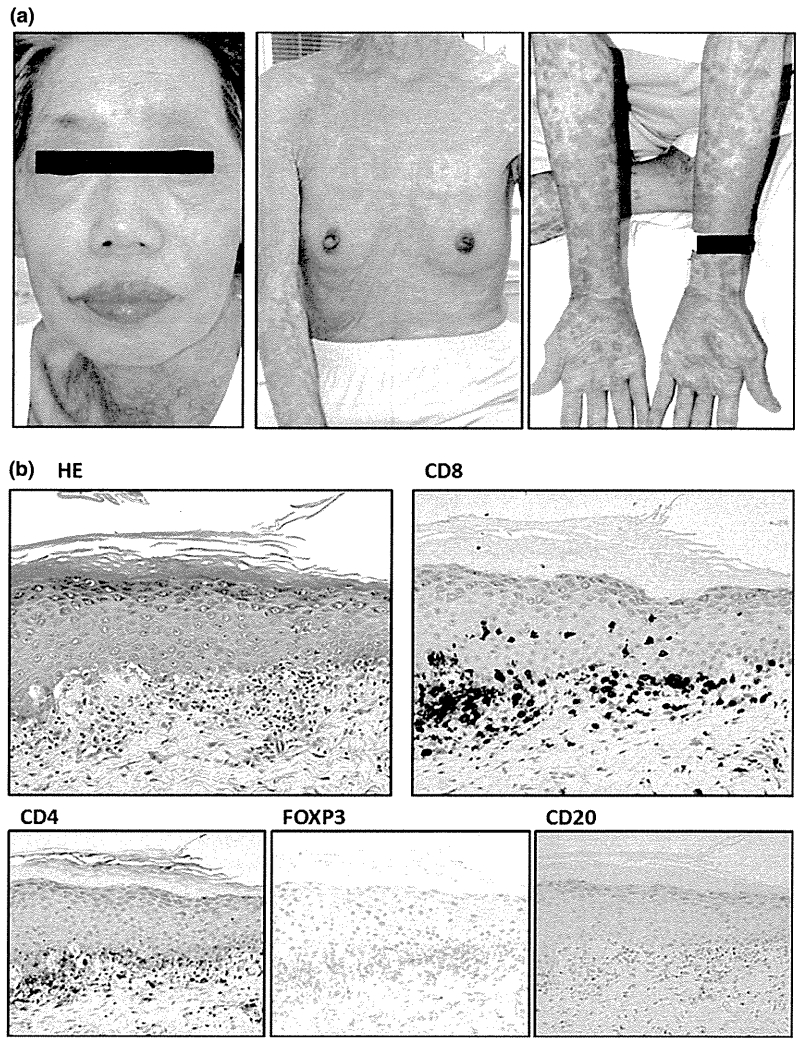
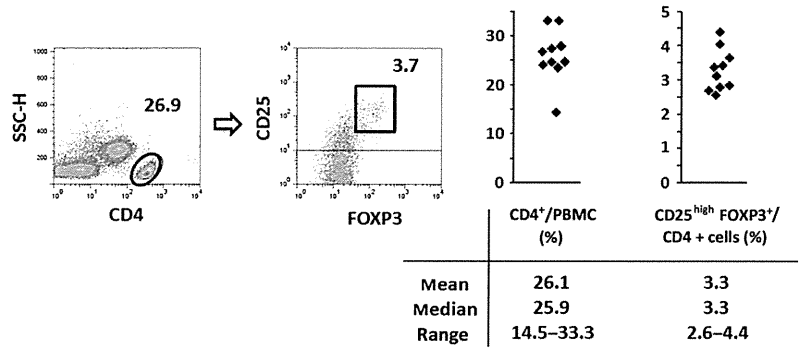


Fig. 2. (a) Macroscopic observations of the patient's skin on the day she was diagnosed with Stevens–Johnson Syndrome. (b) Corresponding skin biopsy showing liquefaction, degeneration and perivascular inflammation with dominant CD8-positive cells but almost no FOXP3-positive cells.



	$CD4^+$/PBMC (%)	$CD25^{high}$ $FOXP3^+$/ CD4 + cells (%)
Mean	26.1	3.3
Median	25.9	3.3
Range	14.5–33.3	2.6–4.4

Fig. 3. $CD4^+CD25^{high}FOXP3^+$ regulatory T cells in PBMC from healthy volunteers (n = 10).

after the fourth or subsequent infusions in the phase II study.[4] The present case was one of these patients. It has been reported that alterations in $CD4^+CD25^+FOXP3^+$ Treg cell frequencies and/or function may contribute to various types of autoimmune diseases.[12] Because the CCR4 molecule aids lymphocyte skin-specific homing,[13] it is not unexpected that skin rashes, which could be an immune-related AE, will be frequently observed in ATL patients receiving mogamulizumab. Because it is an urgent issue to identify which factors determine the severity of immune-related skin disorders associated with mogamulizumab treatment, further investigation on this matter are clearly warranted.

However, reduction of Treg cells is a promising strategy for boosting antitumor immunity in cancer patients, because these cells are increased in the tumor microenvironment and may play an important role in tumor escape from host immunity in several different types of cancer.[14,15] Thus, reduction of Treg cells by mogamulizumab in cancer patients would have both potential benefits leading to enhanced antitumor immunity, but also pose risks of autoimmune disease. The skin-related SAE, including SJS/TEN, are representative of the latter. Currently, several clinical trials of mogamulizumab are being conducted worldwide, not only for ATL, but also other types of lymphoma. In addition, we are currently conducting a clinical trial of mogamulizumab for CCR4-negative solid cancers (UMIN000010050), specifically aiming to deplete Treg cells. Therefore, it is a matter of some urgency to establish the safest and most effective treatment strategies for using mogamulizumab not only in ATL patients but also other types of cancer, to maximize benefit and minimize risk.

In summary, the present case should contribute not only to our understanding of human pathology resulting from therapeutic depletion of Treg cells, but also alert us to the possibility of immune-related SAE, such as SJS/TEN, when using mogamulizumab.

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References

1 Ishida T, Ueda R. Antibody therapy for Adult T-cell leukemia-lymphoma. *Int J Hematol* 2011; **94**: 443–52.
2 Ishii T, Ishida T, Utsunomiya A *et al.* Defucosylated humanized anti-CCR4 monoclonal antibody KW-0761 as a novel immunotherapeutic agent for adult T-cell leukemia/lymphoma. *Clin Cancer Res* 2010; **16**: 1520–31.
3 Yamamoto K, Utsunomiya A, Tobinai K *et al.* 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. *J Clin Oncol* 2010; **28**: 1591–8.
4 Ishida T, Joh T, Uike N *et al.* Defucosylated anti-CCR4 monoclonal antibody (KW-0761) for relapsed adult T-cell leukemia-lymphoma: a multicenter phase ii study. *J Clin Oncol* 2012; **30**: 837–42.
5 Tsukasaki K, Utsunomiya A, Fukuda H *et al.* VCAP-AMP-VECP compared with biweekly CHOP for adult T-cell leukemia-lymphoma: Japan Clinical Oncology Group Study JCOG9801. *J Clin Oncol* 2007; **25**: 5458–64.
6 Sonoda J, Koriyama C, Yamamoto S *et al.* HTLV-1 provirus load in peripheral blood lymphocytes of HTLV-1 carriers is diminished by green tea drinking. *Cancer Sci* 2004; **95**: 596–601.
7 Nassif A, Bensussan A, Boumsell L *et al.* Toxic epidermal necrolysis: effector cells are drug-specific cytotoxic T cells. *J Allergy Clin Immunol* 2004; **114**: 1209–15.
8 Azukizawa H, Sano S, Kosaka H, Sumikawa Y, Itami S. Prevention of toxic epidermal necrolysis by regulatory T cells. *Eur J Immunol* 2005; **35**: 1722–30.
9 Takahashi R, Kano Y, Yamazaki Y, Kimishima M, Mizukawa Y, Shiohara T. Defective regulatory T cells in patients with severe drug eruptions: timing of the dysfunction is associated with the pathological phenotype and outcome. *J Immunol* 2009; **182**: 8071–9.
10 Iellem A, Mariani M, Lang R *et al.* Unique chemotactic response profile and specific expression of chemokine receptors CCR4 and CCR8 by CD4(+) CD25(+) regulatory T cells. *J Exp Med* 2001; **194**: 847–53.
11 Ishida T, Ishii T, Inagaki A *et al.* Specific recruitment of CC chemokine receptor 4-positive regulatory T cells in Hodgkin lymphoma fosters immune privilege. *Cancer Res* 2006; **66**: 5716–22.
12 Michels-van Amelsfort JM, Walter GJ, Taams LS. $CD4^+$ $CD25^+$ regulatory T cells in systemic sclerosis and other rheumatic diseases. *Expert Rev Clin Immunol* 2011; **7**: 499–514.
13 Campbell JJ, Haraldsen G, Pan J *et al.* The chemokine receptor CCR4 in vascular recognition by cutaneous but not intestinal memory T cells. *Nature* 1999; **400**: 776–80.
14 Jacobs JF, Nierkens S, Figdor CG, de Vries IJ, Adema GJ. Regulatory T cells in melanoma: the final hurdle towards effective immunotherapy? *Lancet Oncol* 2012; **13**: e32–42.
15 Ishida T, Ueda R. Immunopathogenesis of lymphoma: focus on CCR4. *Cancer Sci* 2011; **102**: 44–50.

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ORIGINAL REPORT

Multicenter Phase II Study of Mogamulizumab (KW-0761), a Defucosylated Anti-CC Chemokine Receptor 4 Antibody, in Patients With Relapsed Peripheral T-Cell Lymphoma and Cutaneous T-Cell Lymphoma

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ABSTRACT

Purpose
CC chemokine receptor 4 (CCR4) is expressed by peripheral T-cell lymphomas (PTCLs) and is associated with poor outcomes. Mogamulizumab (KW-0761) is a defucosylated humanized anti-CCR4 antibody engineered to exert potent antibody-dependent cellular cytotoxicity. This multicenter phase II study evaluated the efficacy and safety of mogamulizumab in patients with relapsed PTCL and cutaneous T-cell lymphoma (CTCL).

Patients and Methods
Mogamulizumab (1.0 mg/kg) was administered intravenously once per week for 8 weeks to patients with relapsed CCR4-positive PTCL or CTCL. The primary end point was the overall response rate, and the secondary end points included safety, progression-free survival (PFS), and overall survival (OS).

Results
A total of 38 patients were enrolled, and 37 patients received mogamulizumab. Objective responses were noted for 13 of 37 patients (35%; 95% CI, 20% to 53%), including five patients (14%) with complete response. The median PFS was 3.0 months (95% CI, 1.6 to 4.9 months), and the median OS was not calculated. The mean maximum and trough mogamulizumab concentrations (± standard deviation) after the eighth infusion were 45.9 ± 9.3 and 29.0 ± 13.3 μg/mL, respectively. The most common adverse events were hematologic events, pyrexia, and skin disorders, all of which were reversible and manageable.

Conclusion
Mogamulizumab exhibited clinically meaningful antitumor activity in patients with relapsed PTCL and CTCL, with an acceptable toxicity profile. Further investigation of mogamulizumab for treatment of T-cell lymphoma is warranted.

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INTRODUCTION

Mature T/natural killer (NK)-cell neoplasms comprise approximately 20 subclassified heterogeneous groups of non-Hodgkin lymphomas (NHLs) that account for approximately 10% of NHLs in Western countries[1-3] and approximately 25% of NHLs in Japan.[4,5] Mature T/NK-cell neoplasms are largely subdivided into peripheral T-cell lymphoma (PTCL) and cutaneous T-cell lymphoma (CTCL), and different treatment strategies are used for each of these entities.[1,6]

According to the WHO classification, PTCL includes peripheral T-cell lymphoma not otherwise specified (PTCL-NOS), angioimmunoblastic T-cell lymphoma (AITL), and anaplastic large-cell lymphoma (ALCL).[1-3] Cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) and CHOP-like regimens have been widely used as the standard first-line treatment for patients with PTCL.[7,8] With the exception of those patients with anaplastic lymphoma kinase-positive ALCL, the efficacy of these combination therapies is unsatisfactory because those who achieve remission eventually experience relapse and poor outcomes.[3,9] Several agents have been approved by the US Food and Drug Administration for the treatment of relapsed or refractory (Rel/Ref) PTCL: pralatrexate, romidepsin for Rel/Ref PTCL, and brentuximab vedotin for Rel/Ref ALCL. The overall response rates

(ORRs) were reported to be 29% and 25% for PTCL and 86% for ALCL, respectively.[10-12]

CTCL can be classified as mycosis fungoides (MF), Sézary syndrome, or cutaneous ALCL. The majority of cases of CTCL in Japan consist of MF.[13] The therapeutic approaches and outcomes for these conditions are primarily dependent on disease stage.[6,7,14] Patients with advanced stage CTCL who relapse after systemic chemotherapies and those with transformed MF have particularly poor outcomes.[15,16] Recently, the US Food and Drug Administration approved agents for Rel/Ref CTCL treatment, including vorinostat, denileukin diftitox, and romidepsin, with ORRs of 30%, 30%, and 34%, respectively.[17-19] However, there are few treatment options or approved agents for CTCL in Japan, partly because of its low prevalence here.[5,12,13]

CC chemokine receptor 4 (CCR4) is a marker for type 2 helper T cells or regulatory T (Treg) cells and is expressed on tumor cells in approximately 30% to 65% of patients with PTCL.[20,21] CCR4-positive patients (eg, in the PTCL-NOS subgroup) have a shorter survival time when compared with CCR4-negative patients.[21-23] Further, CCR4 expression increases with advancing disease stage in patients with MF/Sézary syndrome.[24]

Mogamulizumab (KW-0761) is a humanized anti-CCR4 monoclonal antibody with a defucosylated Fc region that enhances antibody-dependent cellular cytotoxicity.[25,26] In vitro antibody-dependent cellular cytotoxicity assay and in vivo studies in a humanized mouse model revealed that mogamulizumab exhibited potent antitumor activity against T-cell lymphoma cell lines and against primary CTCL cells from patients.[26-28]

In a phase I study of patients with relapsed adult T-cell leukemia-lymphoma (ATL) and PTCL/CTCL, mogamulizumab was well tolerated up to a dose of 1.0 mg/kg. An ORR of 31% (five of 16) was obtained, including one partial response (PR) among three patients with PTCL/CTCL.[29] Mogamulizumab yielded an ORR of 50% (13 of 26) for relapsed CCR4-positive ATL in a subsequent phase II study.[30] In the United States, a phase I/II study for patients with Rel/Ref CTCL revealed that mogamulizumab was well tolerated with an ORR of 37% (14 of 38, 8% complete response [CR], 29% PR) and a median PFS of 341 days.[31]

The present report describes the results of a multicenter phase II study in Japan that was designed to assess the efficacy and safety of mogamulizumab in patients with relapsed CCR4-positive PTCL or CTCL.

PATIENTS AND METHODS

Study Design and Treatment

This was a multicenter, single-arm phase II study conducted at 15 Japanese centers. At least 35 patients were required to detect a lower limit of the 95% CI that exceeded the 5% threshold, and the expected ORR for mogamulizumab was 25% with a statistical power of 90%.[10,29]

All patients gave written informed consent before enrollment. Patients received intravenous infusions of 1.0 mg/kg mogamulizumab once per week for 8 weeks. Dose modification of mogamulizumab was not allowed. Oral antihistamine and acetaminophen were given before each dose of mogamulizumab as premedication.[29,30] A systemic corticosteroid (hydrocortisone 100 mg intravenously) was also administered before the first dose of mogamulizumab to prevent an infusion reaction. The same dose of hydrocortisone was administered before the second and subsequent administrations at the investigators' discretion. The plasma concentrations of mogamulizumab and antimogamulizumab antibodies in plasma were determined by using enzyme-linked immunosorbent assays.[29,30] Blood samples were collected from all patients who received at least one dose of mogamulizumab at times determined by the protocol for pharmacokinetic analyses. Maximum plasma mogamulizumab concentration and trough concentration parameters were calculated from 0 to 7 days after the eight doses. T-cell subsets and NK cell distribution were also investigated by flow cytometry during and after mogamulizumab treatment. This study was conducted in accordance with the Declaration of Helsinki and in compliance with Good Clinical Practices. The protocol was approved by the institutional review board at each participating institution.

Patients

Patients who were ≥ 20 years of age and who had CCR4-positive PTCL or CTCL with relapse after their last systemic chemotherapy were eligible for participation. Patients who were refractory to their most recent therapy were not eligible for this study. Histopathological subtypes were assessed and reclassified by the Independent Pathology Review Committee according to the 2008 WHO classification.[1] CCR4 expression was determined by immunohistochemistry by using an anti-CCR4 monoclonal antibody (KM2160) and was confirmed by central review, as described previously.[29] In brief, CCR4 expression was classified according to the proportion of stained tumor cells (negative, < 10%; 1+, 10% to <25%; 2+, 25% to < 50%; 3+, ≥ 50%). Staging of nodal/extranodal and/or cutaneous lesions was performed if the lesions met the following requirements: nodal and extranodal lesions were > 1.5 cm in measurable length on cross-sectional computed tomography images, cutaneous lesions were identifiable on visual inspection, and peripheral blood abnormal lymphocyte count was ≥ 1,000/μL and comprised ≥ 5% of total leukocytes. All patients were required to have an Eastern Cooperative Oncology Group performance status of 0 to 2. Other notable eligibility criteria regarding laboratory values were as follows: neutrophil count ≥ 1,500/μL, platelet count ≥ 50,000/μL, hemoglobin level ≥ 8.0 g/dL, AST level ≤ 2.5× the upper limit of normal (ULN), ALT level ≤ 2.5× the ULN, total bilirubin level ≤ 1.5× the ULN, and serum creatinine level ≤ 1.5× the ULN. Patients were excluded if they had any severe complications, such as CNS involvement or a bulky lymphoma mass requiring emergent radiotherapy, a history of allogeneic stem-cell transplantation, active concurrent cancers, an active infection, or positivity for hepatitis B virus DNA, hepatitis B surface antigen, hepatitis C virus antibody, or human immunodeficiency virus antibody.

Efficacy and Safety Assessment

The primary objective was to assess the best overall response, and the secondary objectives included assessments of the best response according to disease site, progression-free survival (PFS), and overall survival (OS). Efficacy was evaluated by the Independent Efficacy Assessment Committee according to modified response criteria based on the International Working Group Criteria.[32,33] Cutaneous lesions were evaluated by using the modified Severity Weighted Assessment Tool.[34] In addition, treatment efficacy in patients with CTCL was evaluated by using a Global Response Score.[35] Responses were assessed after the fourth and eighth mogamulizumab infusions and at 2 and 4 months after the end of treatment. Treatment was discontinued if progressive disease (PD) was evident. PD and survival were monitored until at least 4 months after the completion of dosing. For safety evaluations, adverse events (AEs) were graded according to the National Cancer Institute Common Terminology Criteria for AEs, version 4.0.

Statistical Analysis

PFS and OS were analyzed by using the Kaplan-Meier method. PFS was defined as the time from the first dose of mogamulizumab to progression, relapse, or death by any cause (whichever occurred first). OS was measured from the day of the first dose to death by any cause.

RESULTS

Patient Characteristics

Sixty-five patients were screened, and 64 biopsy specimens were histologically confirmed as PTCL or CTCL by the Independent Pathology Review Committee. In total, 50 (78%) of the 64 screened

patients were CCR4-positive. Of these, 38 eligible patients were enrolled in the study and 37 received at least one infusion of mogamulizumab. One patient withdrew because of an infectious complication before dosing. Patient characteristics, histopathology subtypes, and previous systemic therapies are shown in Table 1.

Table 1. Baseline Patient Demographic and Clinical Characteristics

Characteristic*	Patients (N = 37) No.	%	Patients With PTCL (n = 29) No.	%	Patients With CTCL (n = 8) No.	%
Age, years						
Median	64		67		50	
Range	33-80		33-80		36-70	
≥ 65	18	49	17	59	1	13
Sex						
Male	23	62	20	69	3	38
Female	14	38	9	31	5	63
ECOG performance status						
0	24	65	19	66	5	63
1	12	32	10	34	2	25
2	1	3	0	0	1	13
Elevated LDH level†	21	57	18	62	3	38
Bone marrow involvement	7	19	7	24	0	0
No. of previous systemic regimens						
Median	2		2		3	
Range	1-6		1-5		1-6	
1	14	38	13	45	1	13
2	15	41	12	41	3	38
≥ 3	8	22	4	14	4	50
Types of systemic therapy						
Chemotherapy	37	100	29	100	8	100
CHOP/CHOP-like regimen	36	97	29	100	7	88
DeVIC	6	16	4	14	2	25
CHASE	5	14	5	17	0	0
Single-agent therapy	5	14	0	0	5	63
Other	10	27	10	34	0	0
Auto-PBSCT	3	8	3	10	0	0
Radiotherapy	9	24	5	17	4	50
Intensity of CCR4 expression‡						
1+	6	16	4	14	2	25
2+	6	16	4	14	2	25
3+	25	68	21	72	4	50
Histopathology by central review						
PTCL-NOS	16	43	16	55		
AITL	12	32	12	41		
ALCL, ALK negative	1	3	1	4		
MF	7	19			7	88
c-ALCL	1	3			1	13

Abbreviations: AITL, angioimmunoblastic T-cell lymphoma; ALCL, anaplastic large-cell lymphoma; ALK, anaplastic lymphoma kinase; c-ALCL, cutaneous anaplastic large-cell lymphoma; CHASE, cyclophosphamide, cytosine arabinoside, etoposide, and dexamethasone; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; CTCL, cutaneous T-cell lymphoma; DeVIC, dexamethasone, etoposide, ifosfamide, and carboplatin; ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; MF, mycosis fungoides; NOS, not otherwise specified; PBSCT, peripheral-blood stem-cell transplantation; PTCL, peripheral T-cell lymphoma.

*Of the 38 patients enrolled, 37 received at least one infusion of mogamulizumab.

†Elevated LDH level: higher LDH level than upper limit of the normal range.

‡The denominator used for the intensity of CC chemokine receptor 4 (CCR4) expression is based on subjects who were positive for CCR4 by immunohistochemistry.

Of the 37 patients who received mogamulizumab, 25 (68%) completed the planned course of eight infusions. Nine patients (24%) discontinued treatment because of PD, and three patients (8%) due to serious AEs.

Efficacy

The ORR for the 37 treated patients was 35% (13 of 37; 95% CI, 20% to 53%), and 14% of patients (five of 37) achieved a CR, of which one was unconfirmed (Table 2). Reponses (CR/PR) were observed in at least one patient with each subtype of disease, but the ORR differed between subtypes. The ORR was 34% (10 of 29; 95% CI, 18% to 54%) in patients with PTCL (three of 16 for PTCL-NOS, six of 12 for AITL, and one of one for ALCL, anaplastic lymphoma kinase-negative) and 38% (three of eight; 95% CI, 9% to 76%) in those with CTCL (two of seven for MF and one of one for cutaneous ALCL). In addition, ORR in patients with CTCL was 50% (four of eight; 95% CI, 16% to 84%) according to the Global Response Score.

Total ORR did not significantly correlate with CCR4 expression level, patient age, or the number of previous chemotherapy regimens. The response rates for lymph node and cutaneous lesions were 33% (11 of 33) and 58% (seven of 12), respectively.

The median PFS was 3.0 months (95% CI, 1.6 to 4.9 months) for the entire population and 2.0 months for patients with PTCL. Although the median OS was not reached for the entire population at the

Table 2. Best Response (N = 37)

Parameter	No. of Patients	No. of Patients With Best Response CR/CRu	PR	SD	PD	Response Rate (%)*
Overall response	37	5	8	13	11	35
Histopathology by central review						
PTCL	29	5†	5	9	10	34
PTCL-NOS	16	1	2	6	7	19
AITL	12	3	3	3	3	50
ALCL, ALK negative	1	1†	0	0	0	100
CTCL	8	0	3	4	1	38
MF	7	0	2	4	1	29
c-ALCL	1	0	1	0	0	100
Age, years						
< 65	19	1†	6	7	5	37
≥ 65	18	4	2	6	6	33
Intensity of CCR4 expression						
1+	6	1	1	3	1	33
2+	6	1	2	2	1	50
3+	25	3†	5	8	9	32
No. of previous systemic regimens						
1	14	3	3	6	2	43
2	15	1	1	6	7	13
≥ 3	8	1†	4	1	2	63

Abbreviations: AITL, angioimmunoblastic T-cell lymphoma; ALCL, anaplastic large-cell lymphoma; ALK, anaplastic lymphoma kinase; c-ALCL, cutaneous anaplastic large-cell lymphoma; CCR4, CC chemokine receptor 4; CR, complete response/complete remission; CRu, uncertain complete response/uncertain complete remission; CTCL, cutaneous T-cell lymphoma; MF, mycosis fungoides; NOS, not otherwise specified; PD, progressive disease; PR, partial response/partial remission; PTCL, peripheral T-cell lymphoma; SD, stable disease.

*Response rate (%): 100 × number of responders/number of subjects in each category included in the efficacy analysis set.

†Among the patients who showed CR/CRu, one showed CRu.

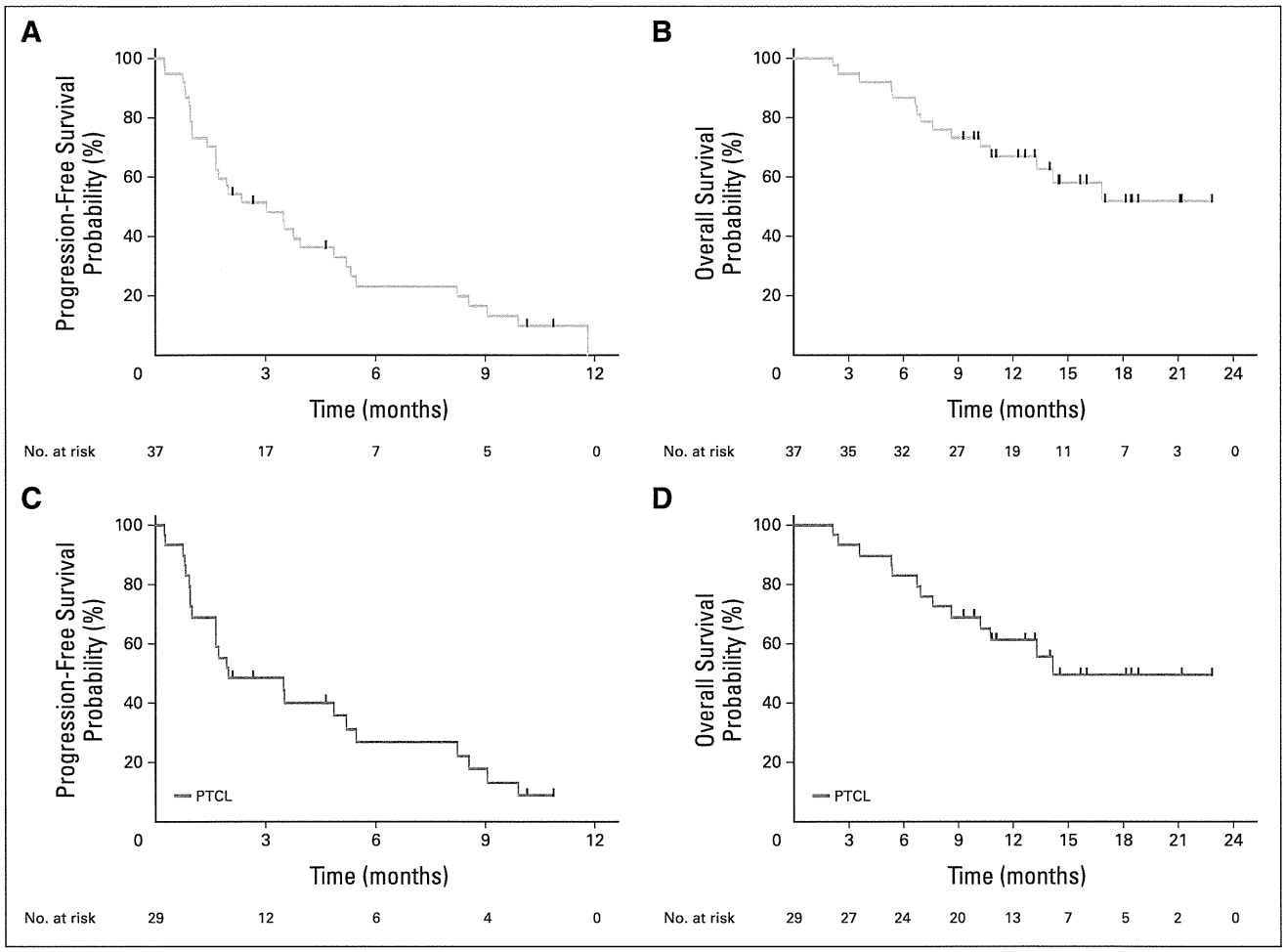

Fig 1. Kaplan-Meier curves of (A) estimated progression-free survival (median, 3.0 months), (B) overall survival (median not reached), (C) progression-free survival in patients with peripheral T-cell lymphoma (PTCL; median, 2.0 months), and (D) overall survival in patients with PTCL (median, 14.2 months).

time of this report, it was 14.2 months for patients with PTCL (Fig 1). Moreover, the median PFS of all 13 responders was 5.5 months, and for PTCL responders (n = 10), it was 8.2 months.

Safety

The most common treatment-related AEs of all grades and treatment-related AEs of grade 3/4 were lymphocytopenia (81%, 73%), neutropenia (38%, 19%), and leukocytopenia (43%, 14%), whereas the most common nonhematologic AE was pyrexia (30%; grade 2 or lower) (Table 3). Lymphocytopenia occurred in 30 patients (81%) and was noted after the first dose in 26 of these patients. For 19 of the patients, lymphocyte counts were $< 800/\mu L$ (grades 2 to 4) before the first dosing. The lymphocyte count ultimately recovered to normal or baseline levels in all patients.

Infusion reaction (24%; grade 2 or lower) occurred primarily at the first infusion, after which it became less frequent, and all patients recovered. No infusion prolongation/interruption was caused by the infusion reaction.

In addition, treatment-related skin disorders were commonly reported (all grades, 51%; grade 3/4, 11%) when grouped according to system organ class. Of the 19 patients who suffered from skin disorder complications, 15 patients experienced improvement, whereas the remaining patients discontinued treatment because of PD or switched to other post treatments. One patient who had a history of psoriasis before the study treatment developed two serious skin disorders (toxicoderma and psoriasis vulgaris) during the study period.

Fifteen serious treatment-related AEs were observed among eight patients (22%); these AEs included grade 3 polymyositis in one patient, grade 2 cytomegalovirus retinitis in two patients, and grade 4 second primary malignancy in one patient with AITL. All patients improved over time, and there were no deaths related to AEs.

Pharmacokinetics and Pharmacodynamics

The mean maximum mogamulizumab concentration and trough mogamulizumab concentration ($\pm$ standard deviation) in plasma after the eighth infusion were 45.9 $\pm$ 9.3 and 29.0 $\pm$ 13.3 μg/mL, respectively. Antimogamulizumab antibodies were not detected after dosing in any patients. These results were consistent with the findings of a previous study of patients with ATL.[30] As an exploratory study, we assessed the effect of mogamulizumab on the number of CD4+/CD25+/Foxp3+ cells (the Treg cell subset) and CD45+/CD16+/CD56+ cells (the NK cell subset). Patients given

Table 3. Treatment-Related Adverse Events (N = 37)

Adverse Event*	All Grades		Grade ≥ 3	
	No.	%	No.	%
Hematologic				
Lymphocytopenia	30	81	27	73
Leukocytopenia	16	43	5	14
Thrombocytopenia	14	38	1	3
Neutropenia	14	38	7	19
Anemia	5	14	2	5
Febrile neutropenia	1	3	1	3
Nonhematologic				
Pyrexia	11	30	0	0
Infusion reaction	9	24	0	0
ALT increased	8	22	1	3
ALP increased	8	22	1	3
Hypophosphatemia	6	16	1	3
Hypokalemia	2	5	1	3
Infection	1	3	1	3
Oral candidiasis	1	3	1	3
Pneumonia	1	3	1	3
Herpes esophagitis	1	3	1	3
Polymyositis	1	3	1	3
Second primary malignancy†	1	3	1	3
Skin and subcutaneous tissue disorders (SOC)	19	51	4	11
Rash papular	6	16	1	3
Rash erythematous	5	14	1	3
Psoriasis	2	5	1	3
Rash maculopapular	2	5	1	3
Toxic skin eruption	2	5	1	3

Abbreviations: ALP, alkaline phosphatase; SOC, System Organ Class (according to the Medical Dictionary for Regulatory Activities).
*Treatment-related adverse events that were reported in at least 15% of patients or that were of grade 3-4 severity.
†Diffuse large B-cell lymphoma was reported in one patient with angioimmunoblastic T-cell lymphoma.

mogamulizumab exhibited a profound depletion of the Treg cell subset during treatment, and cell levels had not returned to baseline 4 months after the last dose (Fig A1). Mogamulizumab also caused a modest decrease in the NK cell subset during treatment (data not shown).

DISCUSSION

This report described results from a single-arm, open-label multicenter phase II study of mogamulizumab in patients with relapsed CCR4-positive PTCL and CTCL.

Mogamulizumab showed promising antitumor activity, with an ORR of 35% (95% CI, 20% to 53%) and a CR/unconfirmed CR of 14%. These data were consistent with those reported with relapsed ATL.[30] It is notable that all three patients who relapsed after autoperipheral blood stem-cell transplantation responded to mogamulizumab. The total ORR is comparable to that of other US Food and Drug Administration-approved drugs, such as pralatrexate and romidepsin.[10,11] However, the present study differed from previous studies in several important respects. Firstly, the patient population was smaller than in the pralatrexate or romidepsin studies. Secondly, since it has been reported that CCR4 expression correlated with advanced disease,[24] it is important to note that although these two studies enrolled relapsed and refractory patients irrespective of their CCR4 expression status, the present study only recruited relapsed patients who were CCR4-positive. However, almost all patients in the present study had good PS compared with those patients in the previous studies. Thirdly, all patients with MF (n = 7) in the present study had relapsed after systemic chemotherapies and were presumed to have advanced stage disease, because all of these patients exhibited clinical skin tumors. Further, four of these seven patients exhibited clinically abnormal lymph node swelling, which does not usually occur at stages lower than IIB.[14,15]

In future study, PFS may also be improved by a longer continuous dosing schedule, such as a phase I/II study for CTCL.[31]

Although the number of patients was relatively small in the present study, the ORR for the AITL group (50%; six of 12) seemed noteworthy, while appearing relatively low in patients with PTCL-NOS (19%; three of 16). However, the three patients with PTCL-NOS who responded to mogamulizumab achieved durable PFS (9.0, 10.1+, and 10.8+ months; +, censored). Further studies are needed to identify which CCR4-positive T-cell lymphoma patients are most likely to benefit from mogamulizumab therapy.

There was no definite correlation between ORR and patient characteristics, such as age, CCR4 expression level, or number of previous systemic regimens. Although our study only included CCR4-positive patients with PTCL and CTCL, a recent US phase I/II study of mogamulizumab included both CCR4-positive and CCR4-negative patients with CTCL.[31] In that study, mogamulizumab exhibited efficacy irrespective of CCR4 expression (positive or negative) or CCR4 expression level, with a continuous dosing schedule.[31] Further studies are needed to define if CCR4 positivity represents a useful predictive biomarker in either PTCL or CTCL.

CCR4-positivity was confirmed in 78% of the 64 screened patients, a higher rate than previously reported.[20,21] However, it is possible that this variation in CCR4 positivity was due to differences in immunohistochemistry assay sensitivity. In our ongoing CTCL phase III study, our protocol permitted recruitment of both CCR4 positive and negative CTCL patients (NCT01728805). This is because the detection limit of CCR4 positivity may not be yet fully established, and mogamulizumab might have antitumor activity against CCR4-negative tumors through the depletion of CCR4-positive regulatory T cells,[36] thus enhancing pre-existing CD8+ cytolytic T-lymphocytes. Based on the latter new concept, an investigator-initiated trial of mogamulizumab against CCR4-negative solid tumors has been initiated (UMIN000010050).

Most of the AEs associated with mogamulizumab were mild and reversible. One patient suffered from polymyositis, an immune-related serious AE, after seven doses of mogamulizumab. The patient improved after steroid pulse therapy, treatment with tacrolimus hydrate, and continuous rehabilitation. Although drug-induced myositis was a possible cause, the relationship between mogamulizumab and myositis was not determined, even after detailed investigation. In our study, skin rash could also represent an immune-related AE, as other immunotherapies, including ipilimumab and zanolimumab, cause similar skin toxicity.[18,36-38] In addition, this may relate to the antitumor mechanism of mogamulizumab, because CCR4 contributes to skin-specific lymphocyte homing.[39] Indeed, a previous study revealed that patients who developed skin disorders ultimately had better therapeutic responses to treatment.[30] In the present study, of the

13 patients who developed grade 2 to 3 skin disorders, five patients achieved CR/PR. Of the 24 patients who developed grade 1 or no skin disorders, eight patients achieved CR/PR. Hence, no clear correlation between skin disorders and response rate was observed in the present study.

As shown in Figure A1, mogamulizumab caused a significant and persistent reduction in the number of Treg cells. This may be responsible for the increased incidence of skin disorders seen in patients with ATL.[30,40] Skin disorders were observed in 19 patients (51%), with grade 3/4 in four cases (11%). This was lower than the proportion of patients who developed skin disorders (67%, 22% in grade 3/4) in a previous study.[30] One patient (4%) with ATL developed Stevens-Johnson syndrome (SJS)[30] and four patients with ATL developed SJS/toxic epidermal necrolysis in postmarketing surveillance of mogamulizumab[40]; however, no cases of SJS/toxic epidermal necrolysis were observed in the present study. Similarly, four of 21 patients with ATL (19%) developed symptoms consistent with SJS[41] after treatment with pralatrexate, whereas no SJS was observed in patients with PTCL[10] after pralatrexate treatment. The risk of severe skin disorders may therefore be lower in patients with PTCL, compared with patients with ATL.

In conclusion, this phase II study revealed that mogamulizumab had promising efficacy and tolerability in patients with relapsed CCR4-positive PTCL and CTCL. Given its novel mechanism of action and favorable toxicity profile compared with multiagent cytotoxic chemotherapy, we might expect the use of mogamulizumab in combination with other agents. Further preclinical and clinical studies of combination therapy will be needed.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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REFERENCES

1. WHO: WHO classification of tumours of haematopoietic and lymphoid tissues (ed 4). Lyon, France, International Agency for Research on Cancer (IARC), 2008
2. O'Leary HM, Savage KJ: Update on the World Health Organization classification of peripheral T-cell lymphomas. Curr Hematol Malig Rep 4:227-235, 2009
3. Vose J, Armitage J, Weisenburger D, et al: International peripheral T-cell and natural killer/T-cell lymphoma study: Pathology findings and clinical outcomes. J Clin Oncol 26:4124-4130, 2008
4. Lymphoma Study Group of Japanese Pathologists: The World Health Organization classification of malignant lymphomas in Japan: Incidence of recently recognized entities. Pathol Int 50:696-702, 2000
5. Aoki R, Karube K, Sugita Y, et al: Distribution of malignant lymphoma in Japan: Analysis of 2260 cases, 2001-2006. Pathol Int 58:174-182, 2008
6. Wollina U: Cutaneous T cell lymphoma: Update on treatment. Int J Dermatol 51:1019-1036, 2012
7. NCCN Clinical Practice Guidelines in Oncology. Non-Hodgkin's lymphomas. Version 1.2013. Fort Washington, PA, NCCN Clinical Practice Guidelines in Oncology, 2013
8. Savage KJ, Chhanabhai M, Gascoyne RD, et al: Characterization of peripheral T-cell lymphomas in a single North American institution by the WHO classification. Ann Oncol 15:1467-1475, 2004
9. Savage KJ: Therapies for peripheral T-cell lymphomas. Hematology Am Soc Hematol Educ Program 2011:515-524, 2011
10. O'Connor OA, Pro B, Pinter-Brown L, et al: Pralatrexate in patients with relapsed or refractory peripheral T-cell lymphoma: Results from the pivotal PROPEL study. J Clin Oncol 29:1182-1189, 2011
11. Coiffier B, Pro B, Prince HM, et al: Results from a pivotal, open-label, phase II study of romidepsin in relapsed or refractory peripheral T-cell lymphoma after prior systemic therapy. J Clin Oncol 30:631-636, 2012
12. Pro B, Advani R, Brice P, et al: Brentuximab vedotin (SGN-35) in patients with relapsed or refractory systemic anaplastic large-cell lymphoma: Results of a phase II study. J Clin Oncol 30:2190-2196, 2012
13. Sugaya M, Hamada T, Kawai K, et al: Guidelines for the management of cutaneous lymphomas (2011): A consensus statement by the Japanese Skin Cancer Society-Lymphoma Study Group. J Dermatol 40:2-14, 2013
14. Olsen E, Vonderheid E, Pimpinelli N, et al: Revisions to the staging and classification of mycosis fungoides and Sézary syndrome: A proposal of the International Society for Cutaneous Lymphomas (ISCL) and the cutaneous lymphoma task force of the European Organization of Research and Treatment of Cancer (EORTC). Blood 110:1713-1722, 2007
15. Kim YH, Liu HL, Mraz-Gernhard S, et al: Long-term outcome of 525 patients with mycosis fungoides and Sézary syndrome. Clinical prognostic factors and risk for disease progression. Arch Dermatol 139:857-866, 2003
16. Diamandidou E, Colome-Grimmer M, Fayad L, et al: Transformation of mycosis fungoides/Sezary syndrome: Clinical characteristics and prognosis. Blood 92:1150-1159, 1998
17. Olsen EA, Kim YH, Kuzel TM, et al: Phase IIB multicenter trial of vorinostat in patients with persistent, progressive, or treatment refractory cutaneous T-cell lymphoma. J Clin Oncol 25:3109-3115, 2007

18. Olsen E, Duvic M, Frankel A, et al: Pivotal phase III trial of two dose levels of denileukin diftitox for the treatment of cutaneous T-cell lymphoma. J Clin Oncol 19:376-388, 2001

19. Whittaker SJ, Demierre MF, Kim EJ, et al: Final results from a multicenter, international, pivotal study of romidepsin in refractory cutaneous T-cell lymphoma. J Clin Oncol 28:4485-4491, 2010

20. Jones D, O'Hara C, Kraus MD, et al: Expression pattern of T-cell-associated chemokine receptors and their chemokines correlates with specific subtypes of T-cell non-Hodgkin lymphoma. Blood 96:685-690, 2000

21. Ishida T, Inagaki H, Utsunomiya A, et al: CXC chemokine receptor 3 and CC chemokine receptor 4 expression in T-cell and NK-cell lymphomas with special reference to clinicopathological significance for peripheral T-cell lymphoma, unspecified. Clin Cancer Res 10:5494-5500, 2004

22. Ohshima K, Karube K, Kawano R, et al: Classification of distinct subtypes of peripheral T-cell lymphoma unspecified, identified by chemokine and chemokine receptor expression: Analysis of prognosis. Int J Oncol 25:605-613, 2004

23. Nakagawa M, Nakagawa-Oshiro A, Karnan S, et al: Array comparative genomic hybridization analysis of PTCL-U reveals a distinct subgroup with genetic alterations similar to lymphoma-type adult T-cell leukemia/lymphoma. Clin Cancer Res 15:30-38, 2009

24. Yagi H, Seo N, Ohshima A, et al: Chemokine receptor expression in cutaneous T cell and NK/T-cell lymphomas: Immunohistochemical staining and in vitro chemotactic assay. Am J Surg Pathol 30: 1111-1119, 2006

25. Shinkawa T, Nakamura K, Yamane N, et al: The absence of fucose but not the presence of galactose or bisecting N-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity. J Biol Chem 278:3466-3473, 2003

26. Niwa R, Sakurada M, Kobayashi Y, et al: Enhanced natural killer cell binding and activation by low-fucose IgG1 antibody results in potent antibody-dependent cellular cytotoxicity induction at lower antigen density. Clin Cancer Res 11:2327-2336, 2005

27. Niwa R, Shoji-Hosaka E, Sakurada M, et al: Defucosylated chimeric anti-CC chemokine receptor 4 IgG1 with enhanced antibody-dependent cellular cytotoxicity shows potent therapeutic activity to T-cell leukemia and lymphoma. Cancer Res 64:2127-2133, 2004

28. Yano H, Ishida T, Inagaki A, et al: Defucosylated anti CC chemokine receptor 4 monoclonal antibody combined with immunomodulatory cytokines: A novel immunotherapy for aggressive/refractory Mycosis fungoides and Sezary syndrome. Clin Cancer Res 13:6494-6500, 2007

29. Yamamoto K, Utsunomiya A, Tobinai K, et al: 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. J Clin Oncol 28:1591-1598, 2010

30. Ishida T, Joh T, Uike N, et al: Defucosylated anti-CCR4 monoclonal antibody (KW-0761) for relapsed adult T-cell leukemia-lymphoma: A multicenter phase II study. J Clin Oncol 10:837-842, 2012

31. Duvic M, Pinter-Brown L, Foss F, et al: Results of a phase 1/2 study for KW-0761, a monoclonal antibody directed against CC chemokine receptor type 4 (CCR4), in CTCL patients. Presented at the 53rd Annual Meeting of the American Society of Hematology, San Diego, CA, December 10-13, 2011 (abstr 962)

32. Cheson BD, Horning SJ, Coiffier B, et al: Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. J Clin Oncol 17:1244-1253, 1999

33. Tsukasaki K, Hermine O, Bazarbachi A, et al: Definition, prognostic factors, treatment, and response criteria of adult T-cell leukemia-lymphoma: A proposal from an international consensus meeting. J Clin Oncol 27:453-459, 2009

34. Stevens SR, Ke MS, Parry EJ, et al: Quantifying skin disease burden in mycosis fungoides-type cutaneous T-cell lymphomas: The severity-weighted assessment tool (SWAT). Arch Dermatol 138:42-48, 2002

35. Olsen EA, Whittaker S, Kim YH, et al: Clinical end points and response criteria in mycosis fungoides and Sézary syndrome: A consensus statement of the International Society for Cutaneous Lymphomas, the United States Cutaneous Lymphoma Consortium, and the Cutaneous Lymphoma Task Force of the European Organisation for Research and Treatment of Cancer. J Clin Oncol 29:2598-2607, 2011

36. Ishida T, Ueda R: CCR4 as a novel molecular target for immunotherapy of cancer. Cancer Sci 97:1139-1146, 2006

37. d'Amore F, Radford J, Relander T, et al: Phase II trial of zanolimumab (HuMax-CD4) in relapsed or refractory non-cutaneous peripheral T cell lymphoma. Br J Haematol 150:565-573, 2010

38. Hodi FS, O'Day JS, McDermott FD, et al: Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 363:711-723, 2010

39. Campbell JJ, Haraldsen G, Pan J, et al: The chemokine receptor CCR4 in vascular recognition by cutaneous but not intestinal memory T cells. Nature 400:776-780, 1999

40. Ishida T, Ito A, Sato F, et al: Stevens-Johnson syndrome associated with mogamulizumab treatment of adult T-cell leukemia/lymphoma. Cancer Sci 104:647-650, 2013

41. Lunning MA, Gonsky J, Ruan J, et al: Pralatrexate in relapsed/refractory HTLV-1 associated adult T-cell lymphoma/leukemia: A New York City multi-institutional experience. Blood 120 (ASH Annual Meeting). 2012 (abstr 2735)

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Appendix

The following review committees and medical experts participated in this trial. Takashi Terauchi, Research Center for Cancer Prevention and Screening National Cancer Center; Ukihide Tateishi, Yokohama City University Graduate School of Medicine; Junichi Tsukada, University of Occupational and Environmental Health; Koichi Nakata, University of Occupational and Environmental Health; Shigeo Nakamura, Nagoya University Graduate School of Medicine; Koichi Ohshima, Kurume University School of Medicine; Tetsuo Nagatani, Hachioji Medical Center of Tokyo Medical University; Akimichi Morita, Nagoya City University Graduate School of Medical Sciences; Kuniaki Ito, National Cancer Center Hospital East; Noriko Usui, Jikei University School of Medicine; Hirokazu Nagai, Clinical Research Center National Hospital Organization Nagoya Medical Center.

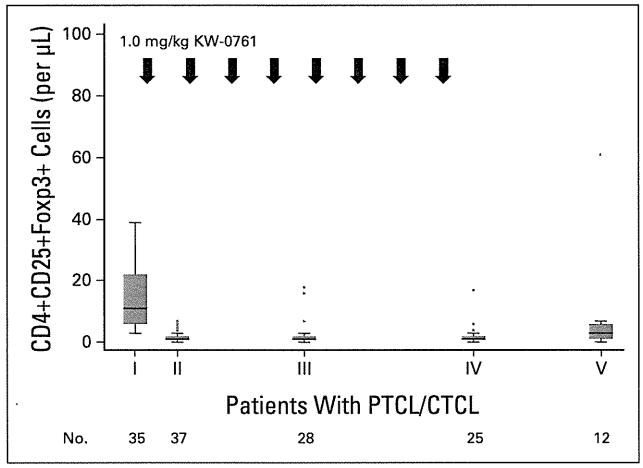

Fig A1. T-cell subset analysis. Numbers of CD4+CD25+Foxp3+ (regulatory T) cells are presented. Blood samples collected at times indicated in the protocol were analyzed. Blood samples were taken (I) just before the first mogamulizumab infusion, (II) just before the second infusion, (III) just before the fifth infusion, (IV) 1 week after the eighth infusion, and (V) 4 months after the eighth infusion. The number of samples used for analysis at each point is indicated below the graph. CTCL, cutaneous T-cell lymphoma; PTCL, peripheral T-cell lymphoma.

HTLV-1 bZIP Factor–Specific CD4 T Cell Responses in Adult T Cell Leukemia/Lymphoma Patients after Allogeneic Hematopoietic Stem Cell Transplantation

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We document human T lymphotropic virus type 1 (HTLV-1) bZIP factor (HBZ)-specific CD4 T cell responses in an adult T cell leukemia/lymphoma (ATL) patient after allogeneic hematopoietic stem cell transplantation (HCT) and identified a novel HLA-DRB1*15:01–restricted HBZ-derived naturally presented minimum epitope sequence, RRRAEKKAADVA (HBZ114–125). This peptide was also presented on HLA-DRB1*15:02, recognized by CD4 T cells. Notably, HBZ-specific CD4 T cell responses were only observed in ATL patients after allogeneic HCT (4 of 9 patients) and not in nontransplanted ATL patients (0 of 10 patients) or in asymptomatic HTLV-1 carriers (0 of 10 carriers). In addition, in one acute-type patient, HBZ-specific CD4 T cell responses were absent in complete remission before HCT, but they became detectable after allogeneic HCT. We surmise that HTLV-1 transmission from mothers to infants through breast milk in early life induces tolerance to HBZ and results in insufficient HBZ-specific T cell responses in HTLV-1 asymptomatic carriers or ATL patients. In contrast, after allogeneic HCT, the reconstituted immune system from donor-derived cells can recognize virus protein HBZ as foreign, and HBZ-specific immune responses are provoked that contribute to the graft-versus-HTLV-1 effect. ***The Journal of Immunology*****, 2014, 192: 940–947.**

Adult T cell leukemia/lymphoma (ATL) is a distinct hematologic malignancy caused by human T lymphotropic virus type 1 (HTLV-1) (1, 2). ATL is resistant to conventional chemotherapeutic agents, and only limited treatment options are available (3). Although early efforts using myeloablative chemoradiotherapy together with autologous hematopoietic stem cell rescue for ATL were associated with a high incidence of relapse and fatal toxicities (4), allogeneic hematopoietic stem cell transplantation (HCT) has been explored as a promising alternative treatment, achieving long-term remission in a proportion of patients with ATL (5, 6). The potential benefit of allogeneic HCT for ATL patients is considered to be due to the high immunogenicity of HTLV-1–infected cells (7–12), which was associated with the existence of posttransplant graft-versus-HTLV-1 and/or graft-versus-ATL effects (13, 14).

HTLV-1 was the first retrovirus to be directly associated with a human malignancy (15, 16), and ~20 million people worldwide are estimated to be infected with this virus (17). Among the HTLV-1 regulatory and accessory genes, *Tax* transforms rodent cells and immortalizes human primary T cells (18–20). In addition, Tax-transgenic mice develop spontaneous tumors (21–24). Another HTLV-1 component gene, *HBZ*, promotes the proliferation of ATL cells (25). Transgenic mice expressing HTLV-1 bZIP factor (HBZ) in their CD4 T cells share many symptoms and immunological features with HTLV-1–infected humans (26). Thus, both *Tax* and *HBZ* are thought to play critical roles in ATL oncogenesis, but there is a marked contrast between them in their expression profiles in primary ATL cells: HBZ expression is constitutive whereas Tax expression is frequently suppressed or minimal in ATL cells (25, 27, 28). Because immune responses against Tax were reported to be strong (7, 8), impaired Tax expression is thought to lead to a survival advantage for HTLV-1–infected cells in the host (2). These observations raise a simple question as to why the expression of Tax, but not HBZ, is impaired, despite both being HTLV-1–derived Ags seen by the human immune system as foreign. In other words, why is it that only HBZ, but not Tax, is constitutively expressed in ATL cells, although it was reported that HBZ is an immunogenic protein recognized by HBZ-specific CTL clones (29, 30). Although several studies (29–31) have been performed to determine the immunogenicity of HBZ, the precise immunological significance of HBZ in HTLV-1–infected individuals has not been fully established. Therefore, the aim of the current study was to clarify the clinical role of HBZ-specific immune responses in HTLV-1–infected individuals.

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Abbreviations used in this article: AC, asymptomatic carrier; ATL, adult T cell leukemia/lymphoma; CR, complete remission; HAM, human T lymphotropic virus type 1–associated myelopathy; HBZ, human T lymphotropic virus type 1 bZIP factor; HCT, hematopoietic stem cell transplantation; HTLV-1, human T lymphotropic virus type 1.

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Materials and Methods

Primary human cells

Blood samples were obtained from healthy volunteers, HTLV-1 asymptomatic carriers (ACs), and ATL patients. Mononuclear cells were isolated with Ficoll-Paque (Pharmacia, Peapack, NJ). Genotyping of HLA-DR, HLA-DQ, and HLA-DP was performed using an WAKFlow HLA-typing kit (WAKUNAGA Pharmacy, Hiroshima, Japan). Diagnosis and classification of clinical subtypes of ATL were according to the criteria proposed by the Japan Lymphoma Study Group (32). All donors provided informed written consent before sampling, according to the Declaration of Helsinki, and the current study was approved by the institutional ethics committees of Nagoya City University Graduate School of Medical Sciences.

Cell lines

ATN-1, MT-1, TL-Om1, and ATL102 are ATL cell lines; MT-2, MT-4, and TL-Su are HTLV-1–immortalized lines; and K562 is a chronic myelogenous leukemia blast crisis cell line (8, 33). Genotyping of HLA-DR, HLA-DQ, and HAL-DP was performed using a WAKFlow HLA-typing kit.

Expansion of HBZ-specific T cells

PBMCs from ATL patients or HTLV-1 ACs were suspended in RPMI 1640 (Cell Science and Technology Institute, Sendai, Japan) supplemented with 10% human serum and 10 μM synthetic HBZ-derived peptides at a cell concentration of 2×10^6/ml. The peptides were purchased from Invitrogen (Carlsbad, CA). The cell suspension (2×10^6 cells) was cultured at 37°C in 5% CO_2 for 2 d, and an equal volume of RPMI 1640 supplemented with 100 IU/ml IL-2 was added. After subsequent culture for 5 d, an equal volume of ALyS505N (Cell Science and Technology Institute) supplemented with 100 IU/ml IL-2 was added, and the cells were cultured with appropriate medium (ALyS505N with 100 IU/ml IL-2) for an additional 7 d.

Abs and flow cytometry

PerCP-conjugated anti-CD8 mAb (SK1; eBioscience, San Diego, CA) and PE-conjugated anti-CD4 mAb [SFCI12T4D11 (T4); Beckman Coulter, Fullerton, CA] were used. For assessing HLA class II expression, PE-conjugated anti–HLA-DR (G46-6; BD Biosciences, San Jose, CA), anti–HLA-DQ (HLA-DQ1; BioLegend, San Diego, CA), or appropriate isotype-control mAbs were used. For intracellular IFN-γ and TNF-α staining, the expanded cells were cocultured with or without target cells or synthetic peptides at 37°C in 5% CO_2 for 3 h, after which brefeldin A (BD Biosciences) was added at 2 μg/ml. The cells were then incubated for an additional 2 h. Subsequently, they were fixed in 10% formaldehyde and stained with FITC-conjugated anti–IFN-γ (45.15; Beckman Coulter) or allophycocyanin-conjugated anti–TNF-α (MAb11; eBioscience) mAbs with 0.25% saponin for 60 min at room temperature. To determine HLA restriction, HLA-blocking experiments were conducted. The expanded cells were preincubated with 20 μg/ml anti–HLA-DR (L243; BioLegend), 20 μg/ml anti–HLA-DQ (1SPVL3; Beckman Coulter), or appropriate isotype control mAbs (20 μg/ml) at 37°C in 5% CO_2 for 1 h, after which they were stimulated with the peptide or the cell lines (ATN-1 and K562). Cells were analyzed on a FACSCalibur (BD Biosciences) with the aid of FlowJo software (Tree Star, Ashland, OR).

Quantitative RT-PCR

Total RNA was isolated with RNeasy Mini Kits (QIAGEN, Tokyo, Japan). Reverse transcription from the RNA to first-strand cDNA was carried out using High Capacity RNA-to-cDNA Kits (Applied Biosystems, Foster City, CA). *HBZ* and *β-actin* mRNA were amplified using TaqMan Gene Expression Assays with the aid of an Applied Biosystems StepOnePlus. The primer set for *HBZ* was as follows: sense, 5′-TCGACCTGAGCTTTAAACTTACCTAGA-3′ and antisense, 5′-GACACAGGCAAGCATCGAAA-3′. All values given are means of triplicate determinations.

Results

T cell responses against synthetic peptides overlapping by 10 aa and covering the entire sequence of the spliced HBZ protein

Because it was reported that HTLV-1 Tax-specific T cells were induced in some ATL patients after allogeneic HCT (10, 11), we initially tried to expand HBZ-specific T cells using PBMCs from an ATL patient who received allogeneic HCT with reduced-intensity conditioning and has been in complete remission (CR) for >3 y (patient #1 after HCT). PBMCs were stimulated with a mixture of 1 16-mer and 19 20-mer synthetic peptides overlapping by 10 aa and covering the entire sequence of the spliced HBZ protein (peptides number 1–20, Fig. 1), at a concentration of 10 μM each. The expanded cells were analyzed by forward scatter height and side scatter height levels, and the lymphocyte population was determined and plotted to show CD4 and CD8 positivity (Fig. 2A, *left panels*). The expanded CD8 T cells responded weakly to stimulation with these 20 overlapping peptides relative to controls without peptide stimulation, as assessed by IFN-γ production (Fig. 2A, *upper middle panels*) but not TNF-α (Fig. 2A, *lower middle panels*). In contrast, the expanded CD4 T cells responded to stimulation by the 20 overlapping peptides by producing both IFN-γ (Fig. 2A, *upper right panels*) and TNF-α (Fig. 2A, *lower right panels*). Because the response of the stimulated and expanded CD4 T cells was stronger than the CD8 response, we focused on the CD4 T cell response against HBZ in patient #1 after HCT.

PBMCs from this patient (#1 after HCT) were stimulated with a mixture of five overlapping peptides consisting of peptides 1–4, 5–8, 9–12, 13–16, and 17–20 (Fig. 1). The expanded CD4 T cells responded to the peptide mixture 9–12 better than to control (no peptides). They produced both IFN-γ (Fig. 2B, *upper panels*) and TNF-α (Fig. 2B, *lower panels*). The expanded CD4 T cells responded very weakly to the peptide mixtures 13–16 and 17–20 by producing TNF-α but not IFN-γ. No responses were observed against the peptide mixtures 1–4 or 5–8 (Fig. 2B). These data indicate that the epitope of HBZ recognized by CD4 T cells from the patient was present in peptides 9–12, within HBZ aa residues 81–130 (Fig. 1).

Next, PBMCs from the same patient were stimulated with four synthetic peptides: 9, 10, 11, and 12. The expanded CD4 T cells responded to peptide 12 by producing both IFN-γ (Fig. 2C, *upper panels*) and TNF-α (Fig. 2C, *lower panels*). The cells did not respond significantly to the other peptides (9, 10, or 11). These results narrow down the specific epitope of HBZ recognized by the CD4 T cells from the patient to a sequence within peptide 12: HBZ aa 111–130 (Fig. 1).

Determination of the minimum epitope sequence of HBZ recognized by CD4 T cells

Seven synthetic peptides (12-1, 12-2, 12-3, 12-4, 12-5, 12-6, 12-7) representing parts of peptide 12 were prepared (Fig. 3A). Responses of the CD4 T cells, which had been stimulated by peptide 12, to these different peptides were tested. The expanded CD4 T cells responded better to peptides 12, 12-1, 12-2, 12-3, and 12-4

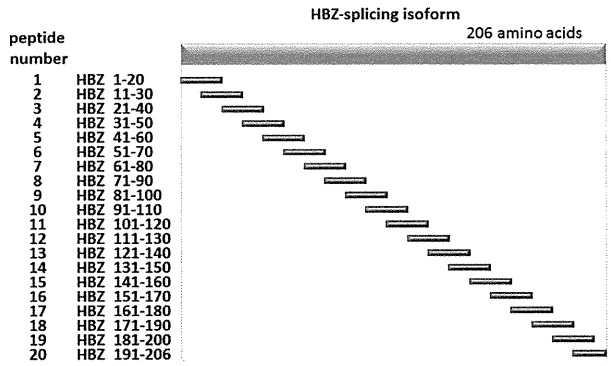

FIGURE 1. Synthetic peptides derived from spliced HBZ. Schematic of 19 20-mer and 1 16-mer synthetic peptides overlapping by 10 aa and covering the entire sequence of the spliced HBZ protein.