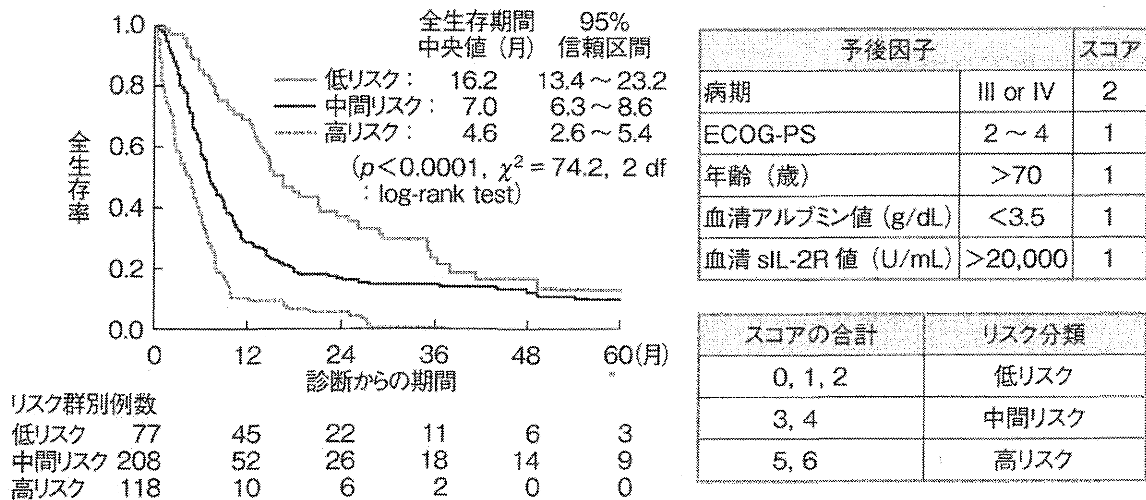

予後因子		スコア
病期	III or IV	2
ECOG-PS	2～4	1
年齢（歳）	>70	1
血清アルブミン値（g/dL）	<3.5	1
血清 sIL-2R 値（U/mL）	>20,000	1

スコアの合計	リスク分類
0, 1, 2	低リスク
3, 4	中間リスク
5, 6	高リスク

図 1　ATL-PI によるリスク別全生存期間

病期，ECOG-PS，年齢，血清アルブミン値，血清 sIL-2R 値の 5 個の予後因子をスコア化することによって，リスク分類を可能にした．リスク群ごとの全生存期間を示す．

（Katsuya H, *et al*：*J Clin Oncol* 2012；30：1635-1640 より引用）

スチン，シクロホスファミド，ドキソルビシン，プレドニゾロン－ドキソルビシン，ラニムスチン，プレドニゾロン－ビンデシン，エトポシド，カルボプラチン，プレドニゾロン）療法や CHOP（シクロホスファミド，ドキソルビシン，ビンクリスチン，プレドニゾロン）療法がしばしば実施されている．

10～25% の症例で中枢神経浸潤がみられることから，初回化学療法時の中枢神経浸潤予防（シタラビン，メトトレキサート，プレドニゾロンの予防的髄腔内投与）は標準治療と考えられる．

(2) 単剤化学療法

エトポシドやソブゾキサンによる単剤治療が，高齢者など多剤併用化学療法不耐例や多剤併用化学療法後の維持投与，再発後の緩和的化学療法として実施される．

(3) 抗 CCR4 抗体療法

CCR4 は正常細胞ではヘルパー 2 型 T 細胞や制御性 T 細胞に発現しているが，ATL 細胞ではほとんどすべてに発現している．新規治療薬モガムリズマブは CCR4 を標的とし，抗体依存性細胞傷害によって抗腫瘍効果を示すヒト化モノクローナル抗体である．

再発・難治性 ATL に対する単剤での第 I，II 相試験では，約半数の患者に有効で[4,5]，すでに再発・難治性の CCR4 陽性 ATL に対する単剤投与が承認されている．また，初発 aggressive ATL に対する VCAP-AMP-VECP 療法とモガムリズマブ併用 VCAP-AMP-VECP 療法の比較第 II 相試験の結果が間もなく公表される．なお，投与に当たっては重篤な皮膚障害など特有の有害事象への注意が必要である．

(4) 同種造血幹細胞移植

同種移植により 25～40% の患者に長期生存が得られ，患者背景の違いはあるが同種移植を受けなかった患者の長期生存の約 10% に比べると非常に良好である．患者の高齢化に伴い骨髄破壊的造血幹細胞移植の適応から外れる患者が多いが，骨髄非破壊的造血幹細胞移植でも遜色のない治療成績が得られるようになってきた[6]．

2. Indolent ATL

Aggressive ATL になるまで無治療経過観察を行うことが標準治療である．しかし最近の後ろ向き研究では，急性型への移行など増悪するまで無治療経過観察が行われた indolent ATL の 5 年，10 年，15 年の生存割合はそれぞれ

47.2%，25.4%，14.1% で，これまで考えられてきたよりも予後不良である[7]．

皮膚が病変の首座である場合は，外用薬，光化学療法や局所放射線照射など皮膚指向性治療が行われる．これらの治療は皮膚局所の症状緩和の手段としては有効であるが，生存期間の改善に貢献するエビデンスはない．

III 海外での ATL 治療

海外ではインターフェロン α/ジドブジン(IFN/AZT)併用療法が，急性型，慢性型，くすぶり型に対する標準治療とされている[8]．特筆すべきことは，単純に比較すると海外で IFN/AZT 併用療法を受けた慢性型，くすぶり型患者の予後は，本邦で無治療経過観察をされた患者より明らかに良好な可能性があることである[9]．そこで JCOG-LSG では indolent ATL を対象に本療法の有用性を科学的に検証する第 III 相試験を先進医療として実施することを計画しており，2013 年夏には開始見込みである．

おわりに

Aggressive ATL と indolent ATL ではそれぞれ異なった治療戦略がとられ，前者では多剤併用化学療法と可能な症例では同種造血幹細胞移植，後者では aggressive ATL になるまで無治療経過観察を行うことが本邦の標準治療である．Aggressive ATL は近年同種造血幹細胞移植や新規治療薬の導入などによりその治療に大きな進歩がみられている．今後は indolent ATL に対する早期治療介入の有用性の検討に関心がもたれる．

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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

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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.

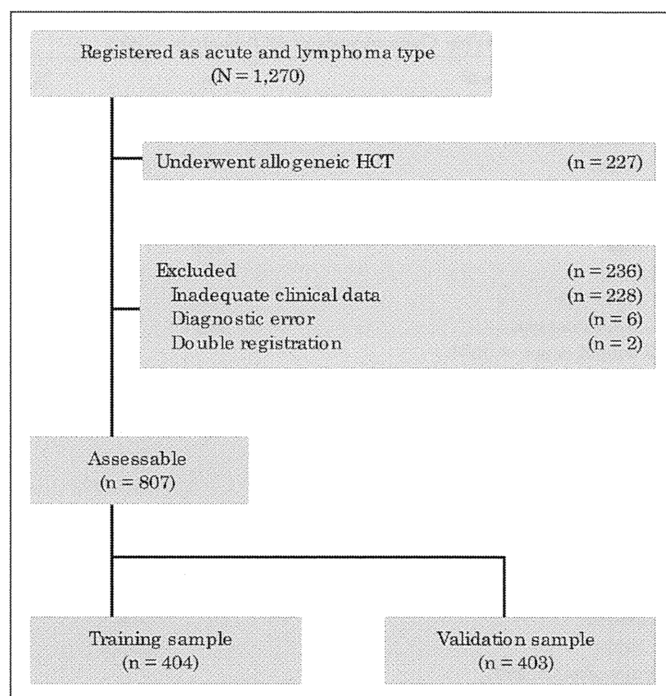

Fig 1. CONSORT flowchart of patients: 1,270 patients diagnosed with acute- and lymphoma-type adult T-cell leukemia/lymphoma were registered. Of these patients, 227 patients were excluded because they had undergone allogeneic hematopoietic cell transplantation (HCT). Two hundred thirty-six patients were 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

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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; χ^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

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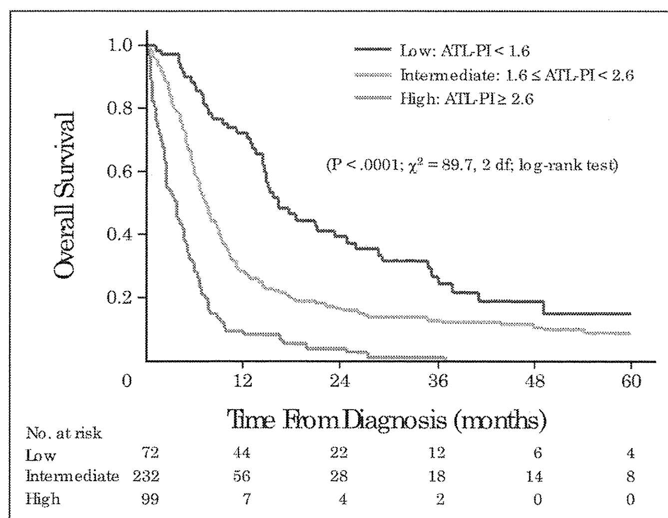

Fig 2. Overall survival curves for the validation sample (n = 403) according to the adult T-cell leukemia/lymphoma prognostic index (ATL-PI): An ATL-PI score was calculated as 0.65 (if stage = III or IV) + 0.35 (if Eastern Cooperative Oncology Group performance status > 1) + 0.016 × age (years) − 0.36 × 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.

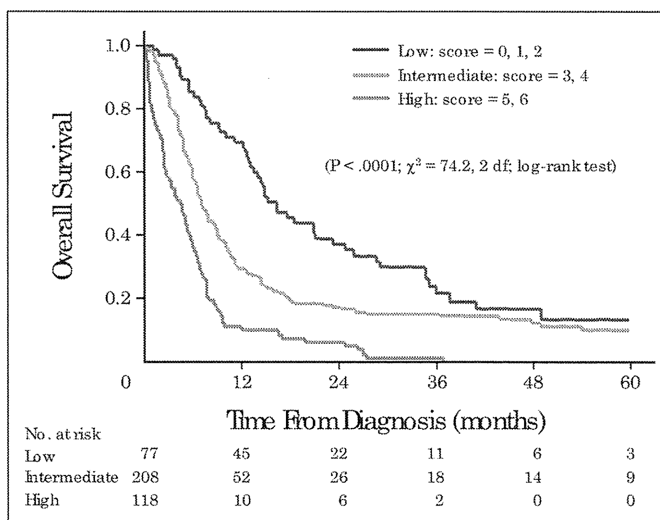

Fig 3. Overall survival curves for the validation sample (n = 403) according to the simplified adult T-cell leukemia/lymphoma prognostic index (ATL-PI): The score for the simplified ATL-PI was calculated as 2 (if stage = III or IV) + 1 (if Eastern Cooperative Oncology Group performance status > 1) +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

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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.

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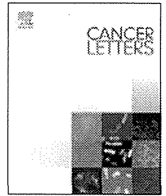

Targeting Bcl-2 family proteins in adult T-cell leukemia/lymphoma: In vitro and in vivo effects of the novel Bcl-2 family inhibitor ABT-737

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abstract

Adult T-cell leukemia/lymphoma (ATLL) is a peripheral T-cell malignancy caused by human T-lymphotropic virus type I (HTLV-1). ABT-737, a small molecule inhibitor of Bcl-2, Bcl-X_L, and Bcl-w, significantly induced apoptosis in HTLV-1 infected T-cell lines as well as in fresh ATLL cells, and synergistically enhanced the cytotoxicity and apoptosis induced by conventional cytotoxic drugs. Moreover, ABT-737 significantly inhibited the in vivo tumor growth of an ATLL mouse model. These results suggest that the use of an agent targeting anti-apoptotic bcl-2 family proteins, either alone or in combination with other conventional drugs, represents a novel promising approach for ATLL.
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1. Introduction

Adult T-cell leukemia/lymphoma (ATLL) is a malignancy of peripheral T-lymphocytes caused by human T-lymphotropic virus type I (HTLV-1). Clinical subtypes of ATLL include smouldering, chronic, lymphoma, and acute types [1]. At present, conventional chemotherapeutic regimens used against other malignant lymphomas are usually administered to aggressive (i.e., acute and lymphoma types) ATLL patients. Treatment by interferon-a in combination with zidovudine has also been shown to be effective according to small-scale studies; however, its efficacy remains to be assessed in larger trials [2,3]. Recent reports of Japanese clinical trials of induction chemotherapy, as well as reports of retrospective analysis of allogeneic hematopoietic stem cell transplantation for aggressive ATLL, showed improvement of the therapeutic outcome, although it still remained extremely poor [4–7].

ABT-737 (Abbott Laboratories, Abbott Park, IL) is a small molecule that occupies the pro-apoptotic Bcl-2 homology domain (BH3) binding groove of anti-apoptotic Bcl-2 family members, and, thereby, strongly and selectively inhibits Bcl-2, Bcl-X_L, and Bcl-w. ABT-737 has been reported to induce apoptosis in a variety of tumor cell lines including chronic lymphocytic leukemia, malignant lymphoma, multiple myeloma, acute myelocytic leukemia, and acute lymphoblastic leukemia as well as in solid tumors [8–15]. Phase I/II clinical trials of ABT-263, an orally available analog of ABT-737, are currently in progress. Promising preliminary results have been reported when ABT-263 is used as a single agent for relapsed or refractory lymphoid malignancies and advanced small cell lung cancer [16,17].

The constitutive activation of NF-j B has been reported to be a characteristic feature of ATLL cells [18], and activated NF-j B induces the expression of anti-apoptotic Bcl-2 family proteins [19]. In fact, Bcl-2 and Bcl-X_L have been reported to be highly expressed and confer resistance to chemotherapy in ATLL cells [20–22]. Therefore, the targeting of Bcl-2 and Bcl-X_L is a promising novel approach for the treatment of ATLL.

In this study, we demonstrate that ABT-737 inhibits the growth of ATLL cells both in vitro and in an in vivo mouse model by inducing apoptosis. Furthermore, ABT-737 enhances the cytotoxicity of conventional drugs towards ATLL cells.

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Table 1
Expression of Bcl-2, Bcl-X_L, and Mcl-1 in lymph node ATLL cells.

Case	Bcl-2	Bcl-X_L	Mcl-1
1	4+	4+	–
2	4+	2+	3+
3	4+	1+	–
4	3+	2+	–
5	3+	1+	3+
6	3+	1+	3+
7	3+	1+	–
8	2+	4+	4+
9	1+	1+	2+
10	4+	–	2+
11	4+	–	–
12	4+	–	–
13	4+	–	–
14	4+	–	–
15	4+	–	–
16	–	4+	4+
17	–	4+	–
18	–	2+	3+
19	–	1+	1+
20	–	1+	–
21	–	–	4+
22	–	–	2+
23	–	–	–
24	–	–	–
25	–	–	–

Protein expression was determined immunohistochemically and the percentage of positively stained cells was quantified using the following scale: –: <10%, 1+: 10–25%, 2+: 25–50%, 3+: 50–75% and 4+:>75%.

2. Materials and methods

2.1. Cells

Three HTLV-1 infected T-cell lines: MT-1, MT-2, and HUT 102, were used in this study. MT-1 and MT-2 cells were kindly provided by Dr. I. Miyoshi (Kochi University, Nangoku, Japan), and HUT 102 cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD). MT-1 and HUT 102 cells were established from peripheral blood (PB) tumor cells of ATLL patients [23,24], while MT-2 cells were established from cord blood T cells by the co-cultivation of normal human cord lymphocytes and PB tumor cells from an ATLL patient [25]. An acute T-cell leukemia cell line free from HTLV-1 Jurkat, and Burkitt lymphoma cell lines Raji and Ramos cells were obtained from the ATCC. Fresh PB tumor cells (PB-ATLL cells) obtained from acute-type ATLL patients whose number of ATLL cells comprised more than 90% of white blood cells, after obtaining informed consent, were separated from heparinized PB by Ficoll-Hipaque density sedimentation. Cells were cultured at 37 °C in RPMI 1640 containing 15% fetal bovine serum (FBS; Sigma, St. Louis, MO), 2 l M L-glutamine, 100 U/mL penicillin, and 100 l g/mL streptomycin (Gibco, Grand Island, NY).

2.2. Reagents

A Bcl-2 family inhibitor, ABT-737, and its less active enantiomer, A-793844, were provided by Abbott Laboratories (Abbott Park, IL). Doxorubicin, vincristine, etoposide, and flavopiridol were obtained from Sigma (St. Louis, MO). Cell Counting Kit-8 (Dojindo, Kumamoto, Japan) was used to assess cellular proliferation by employing a colorimetric assay. The pan-caspase inhibitor z-VAD-fmk was obtained from Bachem (Bubendorf, Switzerland).

2.3. Immunostaining

Twenty-five lymph node specimens that had been biopsied for the purpose of diagnosis and shown to involve ATLL were selected from files in the Department of Pathology at Fukuoka University. Paraffin sections from each of the samples were immunostained with monoclonal antibodies against Bcl-2 (Dako, Glostrup, Denmark), Bcl-X_L (Cell Signaling, Beverly, MA), and Mcl-1 (Millipore, Billerica, MA) using heat-mediated antigen retrieval. Staining results were evaluated semi-quantitatively by two independent observers. Immunostaining was considered negative if less than 10% of the tumor cells were stained. In specimens considered positive,

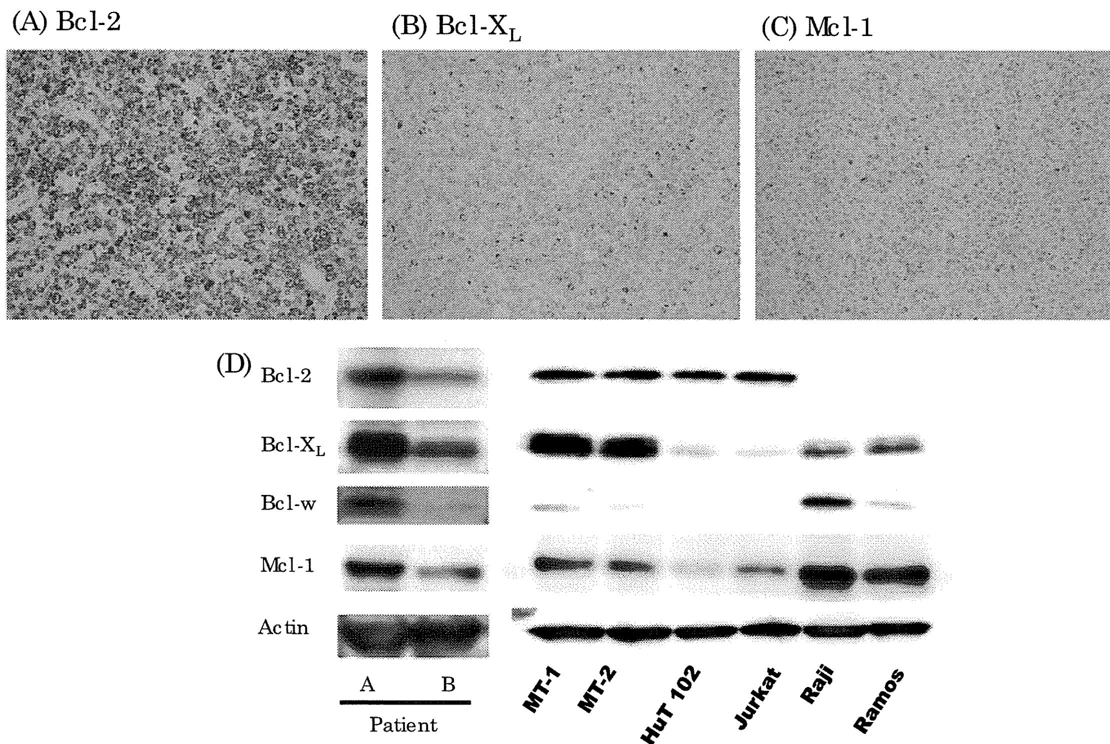

Fig. 1. Expression of Bcl-2 family proteins in ATLL cells and cell lines. The expression of Bcl-2(A), Bcl-X_L(B), and Mcl-1(C) proteins in ATLL cells of lymph node specimens obtained from ATLL patients was determined by immunohistochemistry. Representative results of immunostaining are shown. An Olympus BX41 microscope equipped with a 20×/0.75 objective lens (Olympus, Tokyo, Japan) was used, along with a DP70 digital camera (Olympus). Original magnification 40×. (D) Whole cell lysates of freshly isolated peripheral blood ATLL cells in patients A and B; MT-1, MT-2, HUT 102, Jurkat, Raji, and Ramos cell lines were subjected to Western blotting to assess the expression of Bcl-2, Bcl-X_L, Bcl-w, and Mcl-1 proteins. Actin expression was used as a loading control.

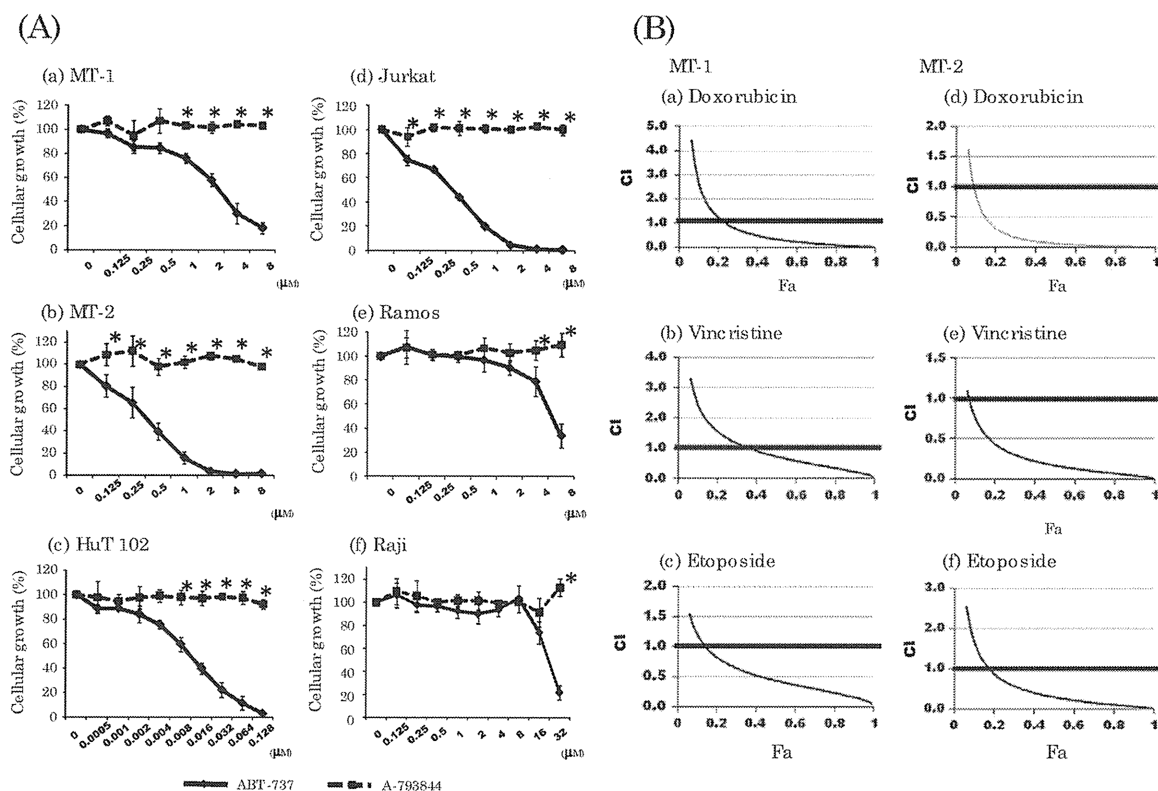

Fig. 2. ABT-737 inhibits the growth of cell lines, including HTLV-1 infected T-cell lines, and augments the cytotoxicity of conventional chemotherapeutic agents towards MT-1 and MT-2 cells. (A) Growth inhibition of cell lines by either ABT-737 or its less active enantiomer A-793844, was assessed by colorimetric assay after 72-h culture (a: MT-1, b: MT-2, c: HUT 102, d: Jurkat, e: Ramos, f: Raji cells). Data represent means ± SD (standard deviation) of 3 independent experiments (*$p < 0.05$ by the Student's t-test). (B) MT-1 (a, b, and c) and MT-2 (d, e, and f) cells were treated with doxorubicin, vincristine, or etoposide in combination with ABT-737 at the fixed ratio for 72 h, and the effects of the combined treatment were evaluated using CalcuSyn software. Fraction affected (Fa)-combination index (CI) plots illustrating the effects of fixed drug ratio combinations are shown. CI values <0.9 are considered synergistic, >1.1 are antagonistic, and values of 0.9–1.1 are additive. Representative data from triplicate experiments are shown.

Table 2
Induction of apoptosis by ABT-737 in MT-1, MT-2, and fresh ATLL cells.

ABT-737 (l M)	MT-1	MT-2	HuT 102	Patient A		Patient B
	72 h	72 h	72 h	48 h	72 h	72 h
0	2.46	3.72	4.28	9.61	25.6	29.5
0.125	n.t.	10.5	74.2	n.t.	n.t.	n.t.
0.25	n.t.	15.4	90.7	n.t.	n.t.	n.t.
0.5	n.t.	24.7	n.t.	n.t.	n.t.	n.t.
1	7.86	45.1	n.t.	30.5	53.2	61.8
2	11.9	n.t.	n.t.	32.8	58.8	53.1
4	21.4	n.t.	n.t.	35.8	61.9	64.5

Cells were treated as indicated, and the percentage of apoptotic cells was determined by flow cytometric analysis using APO2.7. n.t.: not tested.

staining of the tumor was quantified on a scale from 1 to 4 based on the percentage of positively-stained tumor cells, as described previously: –:<10%, 1+: 10–25%, 2+: 25–50%, 3+: 50–75% and 4+:>75% [26].

2.4. Detection of apoptosis

APO 2.7 staining (Immunotech, Marseille, France) and the TdT-mediated d-UTP nick end labeling (TUNEL) assay (MBL, Nagoya, Japan) were used to determine apoptosis, and were evaluated using an EPICS XL flow cytometer (Beckman Coulter, Hialeah, FL).

2.5. Western blotting

Western Blotting was performed as previously described [27] with the following antibodies: anti-caspase 3, -caspase 8, -caspase 9, PARP, Bcl-2, $Bcl\text{-}X_L$, Bcl-w, and Mcl-1 (Cell Signaling). Immunoblotting with anti-b-actin (Cell Signaling) confirmed equivalent protein loading.

2.6. Xenograft severe combined immunodeficient (SCID) mouse model and in vivo treatment

Five-week-old female C.B-17/Icr-SCID mice, obtained from Ryukyu Biotec Co. (Urasoe, Japan), were maintained in containment level 2 cabinets, and provided with autoclaved food and water ad libitum. Mice were engrafted with 1×10^7 HUT 102 cells by subcutaneous injection in the post-auricular region and were randomly placed into 2 cohorts of 5 mice each that received either ABT-737 or vehicle. Treatment was initiated on the day following cell injection. ABT-737, dissolved in 5% dextrose in water containing 30% propylene glycol and 5% Tween 80, was given intraperitoneally every day for 21 days at a dose of 100 mg/kg/day. Control mice received the same volume of vehicle only. The tumor size was monitored once a week. Following treatment, all mice were sacrificed on day 21, blood samples were collected, and sera were analyzed to determine the level of soluble IL-2 receptor a (sIL-2Ra) using a commercially available ELISA kit (BioSource, Camarillo, CA). Tumors were excised at the time of sacrifice, and their weight was measured. Tumors were fixed for paraffin embedding and tissue sectioning, and were subjected to TUNEL staining using a commercially available kit (Takara Bio, Otsu, Japan) to assess the induction of apoptosis. This experiment was performed according to the Guidelines for the Animal Experimentation of the University of the Ryukyus and was approved by the Animal Care and Use Committee of the University of the Ryukyus.

2.7. Analysis of drug synergy

The nature of the interaction between ABT-737 and conventional chemotherapeutic agents was evaluated using the Chou-Talalay method by determing the combination index using CalcuSyn software (Biosoft, Ferguson, MO). MT-1 cells were treated by ABT-737 at the concentration of 0.125–4.0 l M with either of doxorubicin, vincristine or etoposide at the fixed ratio of 100:1, 250:1 or 15:1, respectively. While, MT-2 cells were treated by ABT-737 at the concentration of 0.03–1.0 l M with either of doxorubicin, vincristine or etoposide at the fixed ratio of 25:1, 125:1 or 15:1, respectively. Each fraction affected (Fa) was calculated by comparing the absorbance values of drug-treated wells, measured by the colorimetric assay, to the absorbance of control wells. A drug concentration that induces Fa = 0.25 signifies a 25% decrease in absorbance and growth (i.e., IC_{25} concentration). Background

absorbance was set at Fa = 1. Based on this approach, combination index values <0.9 are considered synergistic, >1.1 are antagonistic, and values 0.9–1.1 are additive [28,29].

3. Results

3.1. ATLL cells express Bcl-2 and Bcl-X_L proteins

Prior to analysis of the effect of targeting anti-apoptotic Bcl-2 family proteins on ATLL cells, we first immunohistochemically analyzed the expression of Bcl-2, Bcl-X_L, and Mcl-1 proteins in 25 lymph node specimens obtained from ATLL patients. Bcl-2 and Bcl-X_L proteins were expressed in 60% (15/25) and 56% (14/25) of the ATLL samples, respectively. In total, 80% (20/25) of the specimens expressed Bcl-2 and/or Bcl-X_L proteins. The anti-apoptotic Mcl-1 protein was expressed in 44%(11/25) of the specimens (Table 1). Representative results of immunostaining for Bcl-2, Bcl-X_L and Mcl-1 are shown in Fig. 1A, B and C.

We subsequently examined the expression of Bcl-2 family proteins by Western blotting in fresh PB-ATLL cells and the cell lines used for analysis of the cytotoxicity of ABT-737 in this study (Fig. 1D). In PB-ATLL cells from 2 patients, expressions of Bcl-2, Bcl-X_L and Mcl-1 protein were observed, while that of Bcl-w varied in cases. In cell lines, Bcl-2 protein was expressed in MT-1, MT-2, HUT 102, and Jurkat cells. Bcl-X_L protein was expressed in MT-1 and MT-2, as well as in Raji and Ramos cells. Bcl-w protein was strongly expressed in Raji cells. While Mcl-1 protein was expressed highly in Raji and Ramos cells, moderately in MT-1, MT-2, and Jurkat cells, and modestly in HUT 102 cells.

3.2. ABT-737. inhibits the growth of HTLV-1 infected T-cell lines

To examine the cytotoxicity of ABT-737, cell lines were cultured with either ABT-737, or its less active enantiomer A-793844, for 72 h, and cell growth was determined using a colorimetric assay (Fig. 2A). ABT-737 inhibited the growth of MT-1, MT-2, HUT 102, Jurkat, and Ramos cells, with 50% inhibition of growth (IC_{50}) at 72 h observed at a concentration of 2.4, 0.23, 0.008, 0.23, and 6.79 l M, respectively. Raji cells were resistant to ABT-737, showing an IC_{50} value of 23.7 l M at 72 h.

3.3. ABT-737. induces apoptosis in HTLV-1 infected T-cell lines and in patients' ATLL cells

In order to further characterize the cytotoxicity of ABT-737 towards ATLL cells, we next analyzed the induction of apoptosis by the flow cytometric detection of APO2.7-positive cells. ABT-737 significantly induced apoptosis in MT-1, MT-2, and HUT 102 cells (Table 2). The induction of apoptosis was also confirmed by the TUNEL assay (data not shown). ABT-737 also induced apoptosis in fresh PB-ATLL cells (Table 2).

3.4. Augmentation of the cytotoxicity of conventional chemotherapeutic agents by ABT-737

To determine if ABT-737 enhances cytotoxicity induced by conventional anti-tumor agents, cells were treated with ABT-737 in combination with either doxorubicin, vincristine, or etoposide for 72 h, and the effects of combined treatment were evaluated using CalcuSyn software. FA-CI plots, which reveal the effects of fixed drug ratio combinations, are depicted in Fig. 2B. All drug combinations exhibited synergistic effects (CI < 0.9) for both cell lines. Cells that were treated first with ABT-737 for 24 h, followed by a exposure to doxorubicin, vincristine, or etoposide, showed a similar synergy (data not shown). Furthermore, the combination of any of these conventional drugs with a lower dose of ABT-737 markedly enhanced drug-induced apoptosis, which is accompanied by caspase activation and PARP cleavage (Fig. 3) and blocked by pretreatment with z-VAD-fmk at the concentration of 25 l M (Fig. 4A and B). While expression of Mcl-1 was not modulated by any of the conventional anti-tumor agents with or without ABT-737 (data not shown).

3.5. ABT-737. suppresses ATLL tumor growth in SCID mice

Finally, we examined the effect of ABT-737 on ATLL in vivo. SCID mice (n = 10) were inoculated with HUT 102 cells. Treatment

Fig. 3. Cleavage of caspases and PARP by treating conventional agents with or without ABT-737. MT-1 (A) and MT-2 (B) cells were treated with doxorubicin (Dox), vincristine (VCR), etoposide (VP-16) or vehicle control, in the absence or presence of lower dose of ABT-737 for 48 h, and whole cell lysates were subjected to Western blotting to determine cleavage of caspases and PARP.

with either ABT-737 (n = 5) or vehicle (n = 5) was initiated on the day following inoculation. On day 21 post-treatment, the mean tumor volume, weight, and serum level of sIL-2Ra were significantly lower than those of vehicle-treated mice ($p < 0.05$ by the Mann–Whitney U-test) (Fig. 5A–D). Moreover, the massive induction of apoptosis in the tumors of mice treated with ABT-737 was observed by immunofluorescent TUNEL assay (Fig. 5E and F). These results suggest that ABT-737 has a strong in vivo anti-ATLL effect through the induction of tumor cell apoptosis.

4. Discussion

Several reasons have been suggested to explain why the therapeutic outcome of aggressive ATLL is very poor. One involves the intrinsic resistance of ATLL cells to conventional chemotherapeutic agents. This resistance is due to their high-level expression of anti-apoptotic proteins [21,22], up-regulation of efflux pumps such as P-glycoprotein and lung resistance related protein [30,31], and their activation of proliferation and survival signals [32,33]. A second reason is the inherent highly-immunocompromised state of ATLL patients that leads to the development of lethal opportunistic infections both before and after the onset of ATLL [34]. Therefore, novel therapeutic strategies, which can overcome the intrinsic resistance of ATLL cells to conventional cytotoxic agents that are commonly used in the clinic, and that induce less collateral damage to normal tissues, would be a promising breakthrough for the treatment of ATLL

Anti-apoptotic Bcl-2 family proteins permit the survival and maintenance of cancer cells by blocking apoptosis. Furthermore, the role of anti-apoptotic Bcl-2 family proteins in resistance to anti-cancer treatment has been widely demonstrated. This critical role of anti-apoptotic Bcl-2 family proteins is due to the fact that most conventional chemotherapeutic anti-cancer agents appear to induce apoptosis via the intrinsic pathway [35–37]. ABT-737, a BH3 mimetic which binds to and inhibits anti-apoptotic Bcl-2 family proteins, induces cell death exclusively through the mitochondrial, intrinsic apoptotic pathway [38]. ABT-737 is thought to function predominantly by blocking Bcl-2, Bcl-X_L, and Bcl-w, while Mcl-1 is a known inducer of resistance to ABT-737 [39,40]. Therefore, sensitivity of ABT-737 is suggested to be correlated with high-level expression of either Bcl-2, Bcl-X_L, or Bcl-w, and with low-level expression of Mcl-1.

In this study, we demonstrated that ABT-737 shows promise in the treatment of ATLL We first validated the concept of targeting Bcl-2 or Bcl-X_L proteins for the treatment of ATLL by showing that 80% of lymph node specimens derived from ATLL patients expressed Bcl-2 and/or Bcl-X_L proteins. These features are in agreement with previous small scale studies of patient specimens and cell lines [20–22]. In addition, Bcl-2 and Bcl-X_L proteins were strongly expressed in PB-ATLL cells in two patients we evaluated. These results clearly confirmed the therapeutic rationale of targeting Bcl-2 or Bcl-X_L for the treatment of ATLL

ABT-737 significantly inhibited the growth of the HTLV-1-infected HUT 102 and MT-2 as well as non-infected Jurkat cells at a dose of lower than 1 l M, while MT-1 and non-infected Ramos cells were less sensitive, and Raji cells were resistant to ABT-737. The expression of Bcl-2, Bcl-X_L, Bcl-w and Mcl-1 proteins was determined to evaluate the relation to the sensitivity of ABT-737. It is possible that many factors other than the proteins examined in this study may affect ABT-737-induced apoptosis; however,

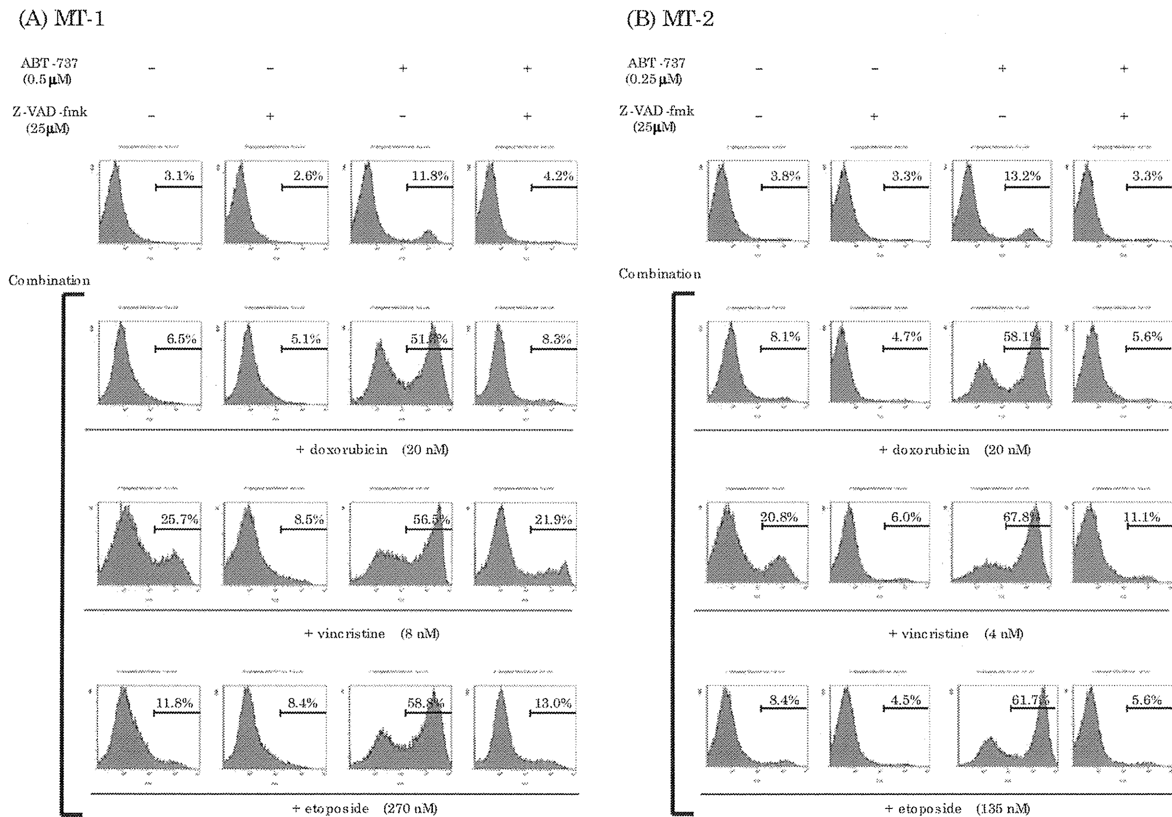

Fig. 4. Induction of apoptosis in MT-1 and MT-2 cells by conventional agents with or without ABT-737. MT-1 (A) and MT-2 (B) cells were treated with doxorubicin, vincristine, etoposide, or the vehicle control, in the presence or absence of ABT-737 for 48 h, and the induction of apoptosis was assessed using an APO2.7 assay. Pretreatment by z-VAD-fmk at the concentration of 25 l M for 1 h prior to exposure to the conventional agents with or without ABT-737 was conducted as indicated. The percentage of APO2.7-positive cells is shown. Representative data from triplicate experiments are shown.

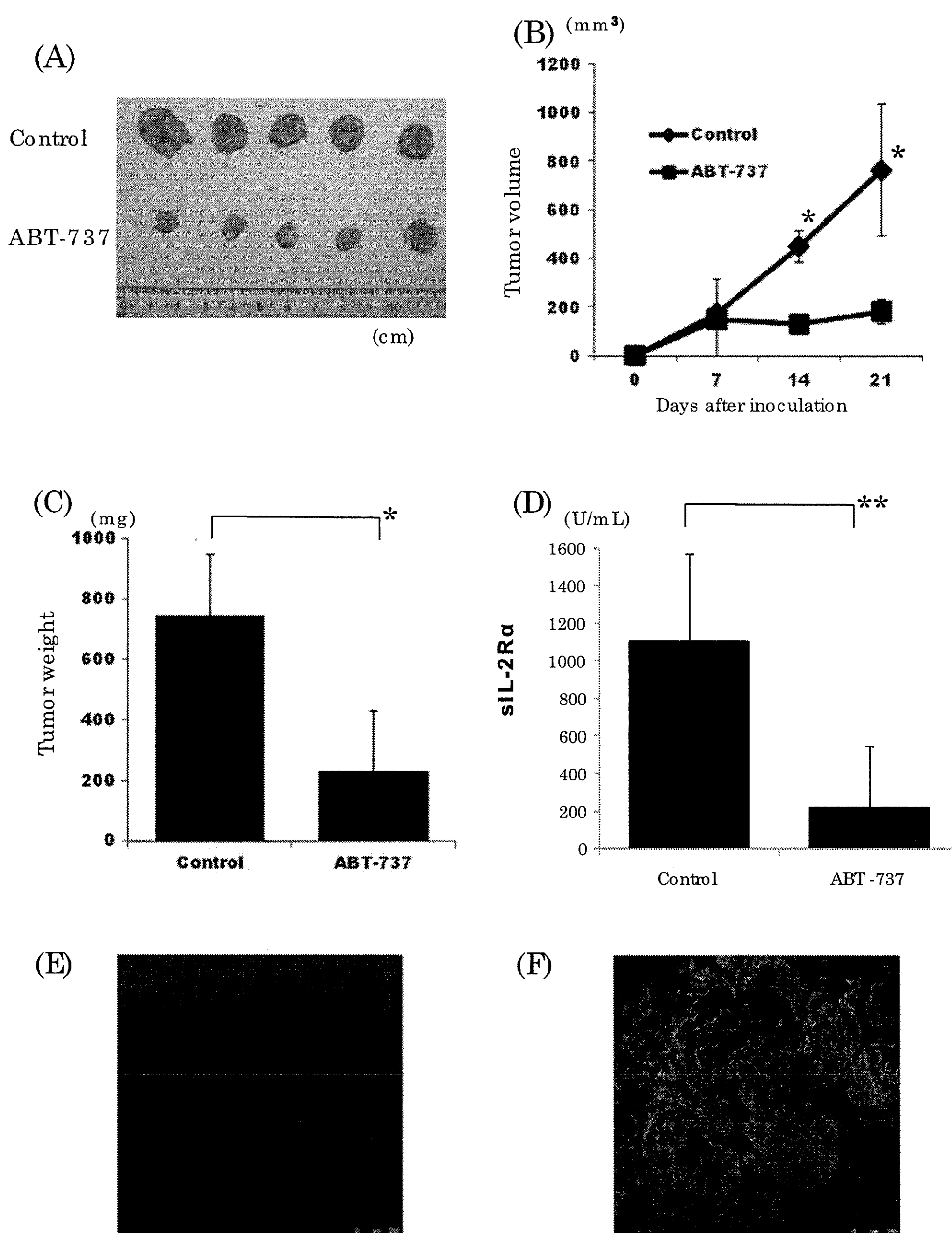

Fig. 5. ABT-737 inhibits the growth of HUT102 cells in SCID mice. HUT102 cells (1×10^7 per mouse) were inoculated subcutaneously into SCID mice. The mice (n = 5/group) were treated with either vehicle or ABT-737 (100 mg/kg/day given intraperitoneally) for 21 days. (A) Tumors excised from mice treated with or without ABT-737 for 21 days are shown. (B) Tumor volumes at 7, 14, and 21 days after cell inoculation. Data represent the mean ± SD of 5 mice in each group (*$p < 0.01$). (C) Tumor weight at the time of sacrifice on day 21. Data represent the mean ± SD of 5 mice in each group (*$p < 0.01$). (D) Serum levels of sIL-2Rα at the time of sacrifice on day 21. Data represent the mean ± SD of 5 mice in each group (*$p < 0.05$). Tumors were excised from mice, treated with (F) or without (E) ABT-737, at the time of sacrifice on day 21, and were subjected to TUNEL assays to assess apoptotic cells (green). Images were captured through an ultraviolet filter using an IX70 microscope (Olympus, Tokyo, Japan). Original magnification 100×.

the low-level expression of Bcl-2 protein and/or high-level expression of Mcl-1 protein tend to be correlated with reduced sensitivity to ABT-737 in these cell lines. Moreover, inhibition of Mcl-1 expression by flavopiridol [41,42] significantly enhanced the induction of apoptosis by ABT-737 in MT-1 and MT-2 cells (data not shown). These results, together with the profile of ATLL cells shown in Table 1, strongly suggest that ABT-737 is a promising agent for the treatment of ATLL, especially patients whose tumor cells are highly positive for Bcl-2 while negative for Mcl-1 are probably the best candidates for this treatments. Most importantly, ABT-737 significantly inhibited tumor cell growth, and induced apoptosis, in a mouse ATLL model.

However, in comparison with CLL cells, whose IC_{50} to induce apoptosis in fresh tumor cells derived from 60 patients reported to be less than 100 nM [43], ATLL cells seems to be less sensitive. One of the possible reasons for distinct sensitivity between ATLL and CLL cells is the constitutive activation of NF-j B pathway which inducing anti-apoptotic and survival signals in ATLL cells [19,43,44]. We next evaluated the synergistic effects of ABT-737 by combining with conventional cytotoxic drugs using MT-1 and MT-2 cells, which are less sensitive to ABT-737 than HUT102 cells. Anti-apoptotic Bcl-2 family proteins are localized in mitochondria, and modulating intrinsic apoptotic signaling, by which most conventional chemotherapeutics induce apoptosis [45,46]. Inhibition

of Bcl-2, Bcl-X_L, and Bcl-w by ABT-737 thereby supposed to enhance the cytotoxicity of conventional chemotherapeutics by overcoming intrinsic resistance to apoptosis. In fact, any of the key chemotherapeutics used for the current treatment of aggressive ATLL, i.e., doxorubicin, vincristine, or etoposide in combination with ABT-737, showed strong synergism in inducing cytotoxicity and caspase-dependent apoptosis.

In conclusion, the results indicate that Bcl-2 anti-apoptotic family proteins are potential targets for the treatment of ATLL, and that ABT-263, the clinically relevant analog of ABT-737 and being investigated in ongoing clinical trials, used either alone, or in combination with conventional drugs, represents a promising novel targeted approach to overcome drug resistance and improve the patient outcome in ATLL.

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