ANTICANCER RESEARCH *34*: 5287-5294 (2014)

Promise of Combining a Bcl-2 Family Inhibitor with Bortezomib or SAHA for Adult T-cell Leukemia/Lymphoma

NAOKO KUNAMI, HIROO KATSUYA, RUMIKO NOGAMI, KENJI ISHITSUKA and KAZUO TAMURA

Department of Internal Medicine, Division of Medical Oncology, Hematology and Infectious Diseases, Fukuoka University, Jonan, Fukuoka, Japan

Abstract. *Background: Adult T-cell leukemia/lymphoma (ATL) is an aggressive malignancy of peripheral T-lymphocytes and its prognosis still remains very poor. Materials and Methods: The potential of combining the Bcl-2 homology 3 mimetic ABT-737, which blocks Bcl-2, Bcl-X_L, and Bcl-w, with either the proteasome inhibitor bortezomib or histone deacetylase (HDAC) inhibitor suberoylanilide hydroxamic acid (SAHA) to inhibit the growth of human T-lymphotropic virus type-I (HTLV-1) infected T-cell lines and its mechanism was further evaluated. Results: ABT-737 synergistically induced apoptosis when combined with either bortezomib or SAHA in HTLV-1 infected T-cell lines and fresh ATL cells. Bortezomib increased the expression of Noxa, which subsequently enhanced the formation of Mcl-1-Noxa complexes, resulting in the functional neutralization of Mcl-1, an inducer of resistance to ABT-737. On the other hand, SAHA reduced the expression of survivin, an anti-apoptotic molecule that confers drug resistance on ATL cells. Conclusion: The combination of ABT-737 with bortezomib or SAHA is promising for the treatment of ATL.*

Adult T-cell leukemia/lymphoma (ATL) is an aggressive malignancy of peripheral T-lymphocytes associated with human T-cell lymphotropic virus type I (HTLV-1) (1, 2). The clinical subtypes of ATL have been divided into acute, lymphoma, chronic and smoldering (1), and a recent retrospective study showed that the median survival times of patients with these subtypes were 8.3, 10.6, 30.2 and 36.7 months and overall survival rates at 4 years were 11.4%, 16.2%, 35.0% and 43.2%, respectively (3). Novel therapeutic options such as allogeneic stem cell transplantation and anti-CCR4 monoclonal antibody have been recently introduced; however, the outcome of patients with ATL is still very poor and novel therapeutic approaches are urgently required.

Members of the Bcl-2 family proteins are critical regulators of apoptosis and interactions between anti-apoptotic and pro-apoptotic members are the major determinants of cell death and survival. ABT-737 is a small molecule Bcl-2 homology 3 (BH3) mimetic that binds to surface hydrophobic grooves of anti-apoptotic Bcl-2 family members and has been shown to strongly and selectively inhibit Bcl-2, Bcl-X_L and Bcl-w, but not Mcl-1 or A1. In preclinical studies, ABT-737 exhibited single-agent activity and also increased the sensitivities of malignant lymphoma and small cell lung carcinoma cell lines to chemotherapeutics (4, 5). ABT-263 (Navitoclax), an analogue of ABT-737, was also reported to be effective on various cancer-derived cell lines *in vitro*, *in vivo* animal model, and in several clinical trials (6-11). We previously demonstrated that Bcl-2 and Bcl-X_L, as well as the Mcl-1 protein, were highly expressed in ATL cells and HTLV-1 infected T-cell lines. Fresh tumor cells derived from ATL patients were less sensitive to ABT-737 than those derived from chronic lymphocytic leukemia (CLL) patients *in vitro*, however, ABT-737 synergistically enhanced apoptosis induced by current key conventional chemotherapeutics in HTLV-1 infected T-cell lines. Furthermore, the inhibition of Mcl-1 expression significantly enhanced the induction of apoptosis by ABT-737 (12). In addition, the nuclear factor-kappa-light-chain-enhancer of activated B (NF-κB) pathway, which induces anti-apoptotic and survival signals, was also shown to be constitutively activated in ATL cells (13).

The clinical activity of proteasome inhibitors against multiple myeloma has already been established. Proteasome inhibition is known to affect various intracellular signaling pathways, including NF-κB, cell-cycle regulation, modulation of Bcl-2 family members and accumulation of p53 (14). Previous studies have demonstrated that the proteasome inhibitor bortezomib induced cell death in HTLV-I infected T-cell lines and ATL cells, and its potential activity has also been reported in a xenograft murine model

Correspondence to: Kenji Ishitsuka, Division of Medical Oncology, Hematology and Infectious Diseases, Fukuoka University, 7-45-1 Nanakuma, Jonan, Fukuoka 814-0180, Japan. Tel: +81 928011011, Fax: +81 928655656, e-mail: kenjiishitsuka@fukuoka-u.ac.jp

Key Words: Adult T-cell leukemia/lymphoma, Bcl-2 family protein, ABT-737, bortezomib, suberoylanilide hydroxamic acid.

(15-18). We are currently conducting a multi-center phase II clinical trial of bortezomib in relapsed and refractory ATL patients (clinical trial registry number; UMIN000004061).

Histone deacetylase inhibitors (HDACi) are novel agents that inhibit the enzymatic activity of HDAC. HDACi can induce the acetylation of histone and non-histone proteins, which have been shown to affect various physiological and pathological processes controlling apoptosis, autophagy, cell cycle, immune response, inflammation and metabolism through its downstream molecular targets (19). HDACi were shown to influence the expression and activity of apoptotic proteins favoring a pro-apoptotic response and lowered the cellular apoptotic threshold (20). HDACi, such as suberoylanilide hydroxamic acid (SAHA), romidepsin and panobinostat, have shown promise in pre-clinical and/or clinical studies against T-cell malignancies including ATL (21, 22). However, the overexpression of anti-apoptotic Bcl-2 or Bcl-X_L has been previously shown to reduce the efficacy of HDACi (23, 24). ABT-737 has been reported to sensitize Bcl-2 overexpressing tumors to HDACi-mediated apoptosis (25). Therefore, we postulated that ABT-737, combined with SAHA, may synergistically induce apoptosis in HTLV-1-infected T-cells.

In the present study, we examined the synergistic effects of the combination of ABT-737 with bortezomib or SAHA and further clarified the molecular sequences of HTLV-1 infected T-cell lines.

Materials and Methods

Cells. Two HTLV-1 infected T-cell lines MT-1 and MT-2 (kindly provided by Dr. Miyoshi I. (Kochi University, Nangoku, Japan)) were used in this study. MT-1 cells were established from the peripheral blood (PB) tumor cells of ATL patients (26), while MT-2 cells were established from cord blood T-cells by the co-cultivation of normal human cord lymphocytes and PB tumor cells of an ATL patient (27). Fresh PB ATL cells were separated by density sedimentation using LymphoPrep (PROGEN Biotechnik, Heidelberg Germany) from heparinized PB obtained from two acute-type ATL patients whose number of ATL cells comprised more than 85% of mononuclear cells, after obtaining informed consent. Cells were cultured at 37˚C in Roswell Park Memorial Institute (RPMI) 1640 containing 15% fetal bovine serum (Sigma, St Louis, MO, USA), 2 μM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco, Grand Island, NY, USA).

Reagents. An inhibitor of Bcl-2 family proteins, ABT-737 was provided by Abbott Laboratories (Abbott Park, IL, USA). The proteasome inhibitor bortezomib, HDACi SAHA and survivin inhibitor YM-155 were obtained from Toronto Research Chemicals Inc. (Ontario, Canada), Cayman Chemical (Michigan, IL, USA) and Selleck Chemicals (Houston, TX, USA), respectively. The pan-caspase inhibitor z-VAD-fmk (Z-VAD) was obtained from Bachem (Bubendorf, Switzerland). The Cell Counting Kit-8 (Dojindo, Kumamoto, Japan) was used to assess cellular proliferation by a colorimetric assay.

Detection of apoptosis. APO 2.7 staining (Immunotech, Marseille, France) was used to determine apoptosis and was evaluated using an EPICS XL flow cytometer (Beckman Coulter, Hialeah, FL, USA).

Western blotting. Western blotting was performed as previously described (28) with the following antibodies: anti-caspase 3, -caspase 9, -PARP, -Bcl-2, -Bcl-X_L, -Bcl-w and -survivin (Cell Signaling, Danvers, MA, USA), and -Noxa and -Mcl-1 (Santa Cruz Biotechnology city, CA, USA). Immunoblotting with anti-α-Tubulin (Cell Signaling) confirmed equivalent protein loading.

Immunoprecipitation. Cells cultured under the indicated conditions were harvested, washed twice with ice-cold PBS, lysed in radioimmunoprecipitation assay (RIPA) buffer (Wako Pure Chemical Industries, Osaka, Japan), incubated with 2 μg anti-Mcl-1 antibody and 15 μl protein G-Sepharose was added. The immunocomplexes were collected and detected by Western blotting.

Analysis of drug synergy. The effects of combining ABT-737 with either bortezomib or SAHA were evaluated using the Chou-Talalay method to determine the combination index using the CalcuSyn software (Biosoft, Ferguson, MO, USA). Each affected fraction (Fa) was calculated by comparing the absorbance values of drug-treated wells measured by a colorimetric assay, to the absorbance of control wells. The drug concentration that induced Fa=0.25 signified a 75% decrease in absorbance and growth (*i.e.*, IC_{25} concentration). Background absorbance was set at Fa=1. Based on this approach, combination index (CI) values of <0.9 were considered synergistic, >1.1 were antagonistic and values of 0.9 to 1.1 were additive (29, 30).

Results

Either bortezomib or SAHA synergistically enhanced the effects of ABT-737. We first examined the single-agent activity of ABT-737, bortezomib or SAHA on HTLV-1 infected T-cell lines by the colorimetric assay and showed that they inhibited the growth of MT-1 and MT-2 cells (Figure 1A). We then investigated the effects of combining ABT-737 with either bortezomib or SAHA. Dose-effect and Fa-CI plots revealing the effects of fixed drug ratio combinations are shown in Figure 1B. The combination of ABT-737 with bortezomib or SAHA displayed a strong synergism (CI<0.9) for inhibiting the growth of MT-1 and MT-2 cells.

To clarify the *in vitro* anti-tumor effects achieved by these combinations, we examined the induction of apoptosis in MT-1, MT-2 cells and fresh ATL cells, treated by ABT-737 with or without bortezomib or SAHA. Apoptosis induced by ABT-737 was significantly enhanced by either bortezomib or SAHA. Of note, it was accompanied by the cleavage of caspase 3, caspase 9 and PARP, and was blocked by the pan-caspase inhibitor Z-VAD in MT-1 and MT-2 cells (Figure 2A and B).

Bortezomib induced the expression of Noxa. To explore the mechanism of the synergistic effects of ABT-737 and

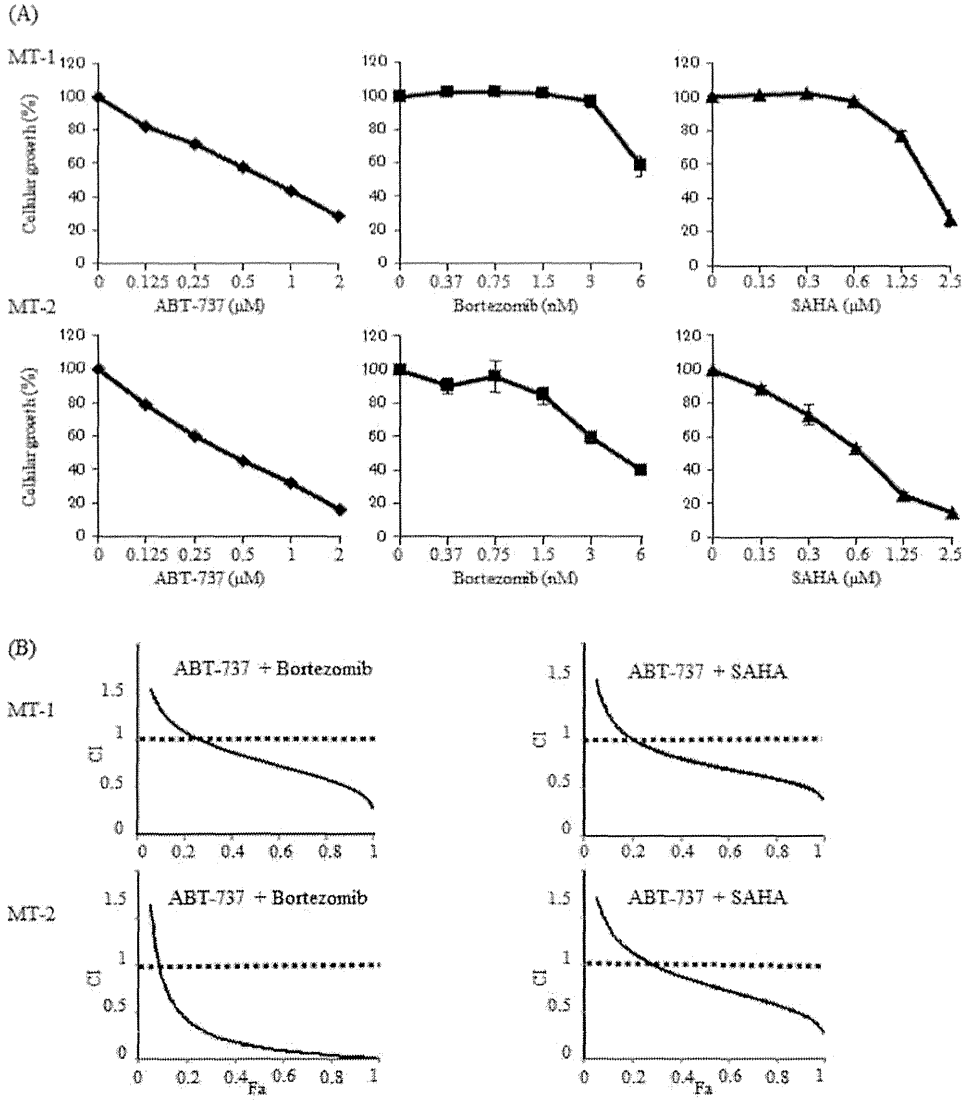

Figure 1. *Combination of ABT-737 with bortezomib or SAHA synergistically inhibited the growth of HTLV-1-infected T-cell lines. (A) The growth inhibition of MT-1 and MT-2 cells by ABT-737, bortezomib, and SAHA assessed by a colorimetric assay after a 72-h culture. Data represent means±SD (standard deviation) of 3 independent experiments. (B) MT-1 and MT-2 cells were treated with ABT-737 in combination with either bortezomib or SAHA for 72 h and the effects of the combined treatments were evaluated using the CalcuSyn software. Dose–effect and Fa-CI plots, illustrating the effects of fixed drug ratio combinations, are depicted. CI values <0.9 were considered synergistic, >1.1 were antagonistic and values of 0.9 to 1.1 were additive.*

bortezomib, we examined the modulation of Bcl-2 family proteins in MT-1 and MT-2 cells. Bortezomib induced the expression of Noxa and Mcl-1 without modulating the expression of Bcl-2, Bcl-w or Bcl-X_L (Figure 3A). Immunoprecipitation using the anti-Mcl-1 antibody revealed the enhanced formation of the Mcl-1-Noxa complex in both cell lines treated with bortezomib-alone and ABT-737 combined with bortezomib (Figure 3B).

SAHA reduced the expression of survivin. We next elucidated the mechanism of synergy between ABT-737 and SAHA. No significant change was observed in the expression of Bcl-2, Bcl-w or Bcl-X_L. SAH- alone and ABT-737 combined with SAHA slightly decreased the expression of Mcl-1. The expression of survivin was reduced by SAHA and was significantly reduced by combining SAHA with ABT-737 (Figure 4). To confirm the

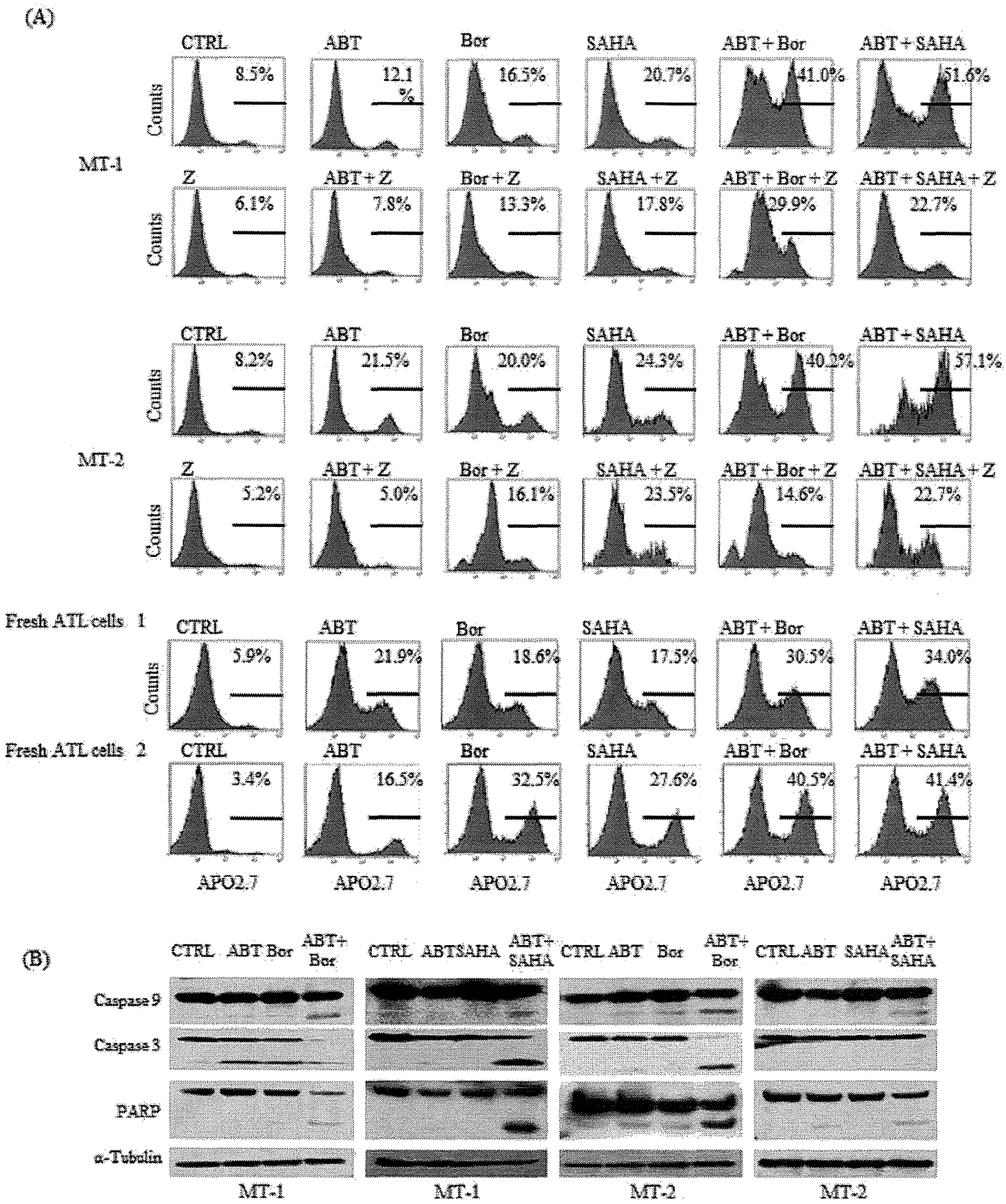

Figure 2. *Combination of ABT-737 and bortezomib or SAHA synergistically induced apoptosis in HTLV-1-infected T-cell lines and fresh ATL cells. (A) MT-1 and MT-2 cells were treated by ABT-737 (1 μM), bortezomib (4 nM), SAHA (2 μM) or by their combination in the absence or presence of Z-VAD (25μM) for 72 h. Fresh ATL cells were treated by ABT-737 (1 μM), bortezomib (4 nM), SAHA (1 μM) or by their combination for 72 h. The induction of apoptosis was assessed by using the APO2.7 assay. The percentage of APO2.7-positive cells is shown. Data are representative of two independent experiments. CTRL, Control; ABT, ABT-737; Bor, bortezomib; Z, Z-VAD. (B) Cells were treated with ABT (1 μM), Bor (4 nM), SAHA (2 μM) or their combination for 24 h. Whole-cell lysates were subjected to western blotting to assess the cleavage of caspase 9, caspase 3 and PARP. CTRL, Control; ABT, ABT-737; Bor, bortezomib.*

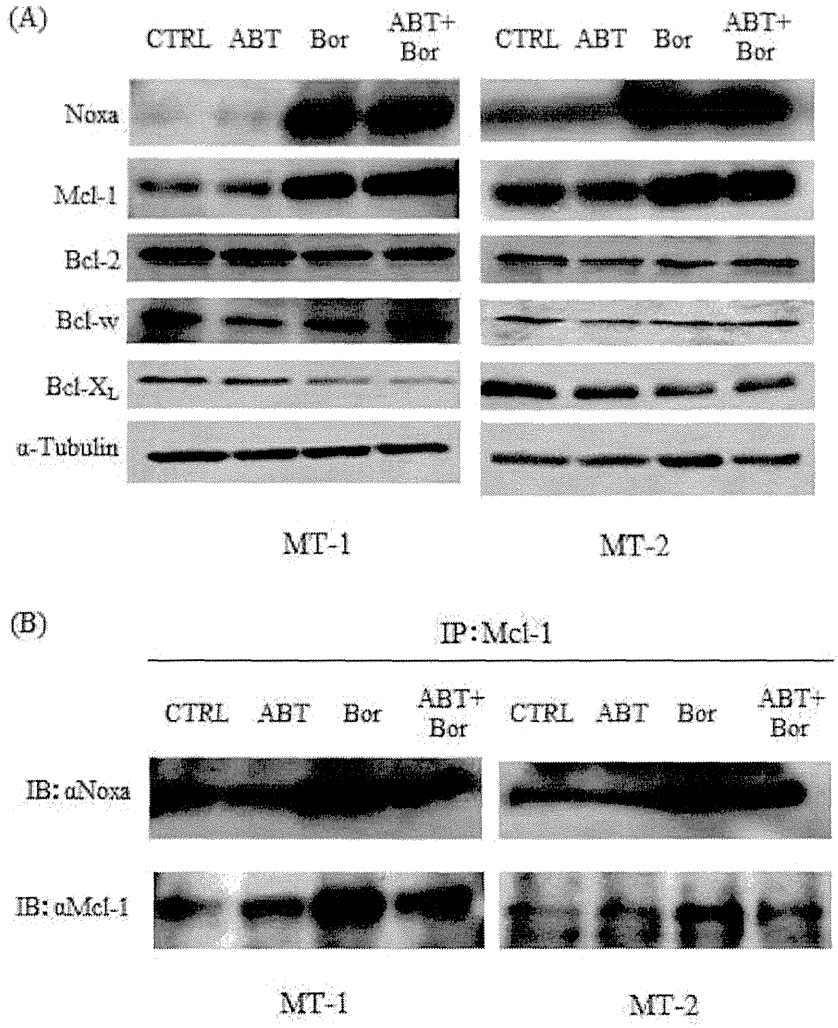

Figure 3. *The effects of the combination treatment with ABT-737 and bortezomib on NOXA and Mcl-1 protein expression. (A) Cells were treated with ABT (1 μM), Bor (4 nM) or combination of both for 24 h. Whole-cell lysates were subjected to western blotting to assess Noxa, Mcl-1, Bcl-2, Bcl-w and Bcl-X_L. CTRL, control; ABT, ABT-737; Bor, bortezomib. (B) Cells were treated with ABT (1 μM), Bor (4 nM) or combination of both for 24 h. Whole-cell lysates were immunoprecipitated with the anti-Mcl-1 antibody. Immunoprecipitates were then detected by western blotting using the anti-Noxa or anti-Mcl-1 antibody. CTRL, control; ABT, ABT-737; Bor, bortezomib.*

biological relevance of survivin in ABT-737-induced apoptosis, we demonstrated that the small- molecule inhibitor of survivin YM-155 significantly enhanced apoptosis induced by ABT-737 (Figure 5).

Discussion

In the present study, we investigated the synergistic anti-ATL effects by combining ABT-737 and a proteasome inhibitor, bortezomib, or an HDACi, SAHA, in HTLV-1 infected T-cell lines and elucidated their mechanism of action.

ABT-737 in combination with bortezomib induced a synergistic apoptotic response in HTLV-1 infected T-cell lines and fresh ATL cells. Mcl-1 cleavage by bortezomib has been reported in multiple myeloma cells (31). The cleavage of Mcl-1 was not observed in our study; however, bortezomib up-regulated the expression of Noxa, resulting in the enhanced formation of Mcl-1-Noxa complexes. Because the BH3 domain of Noxa has been shown to antagonize Mcl-1, the up-regulation of Noxa resulted in the functional repression of Mcl-1 (32). Thus, the mechanism that enhances the activity of ABT-737 by bortezomib is suggested to cancel the anti-apoptotic effect of Mcl-1.

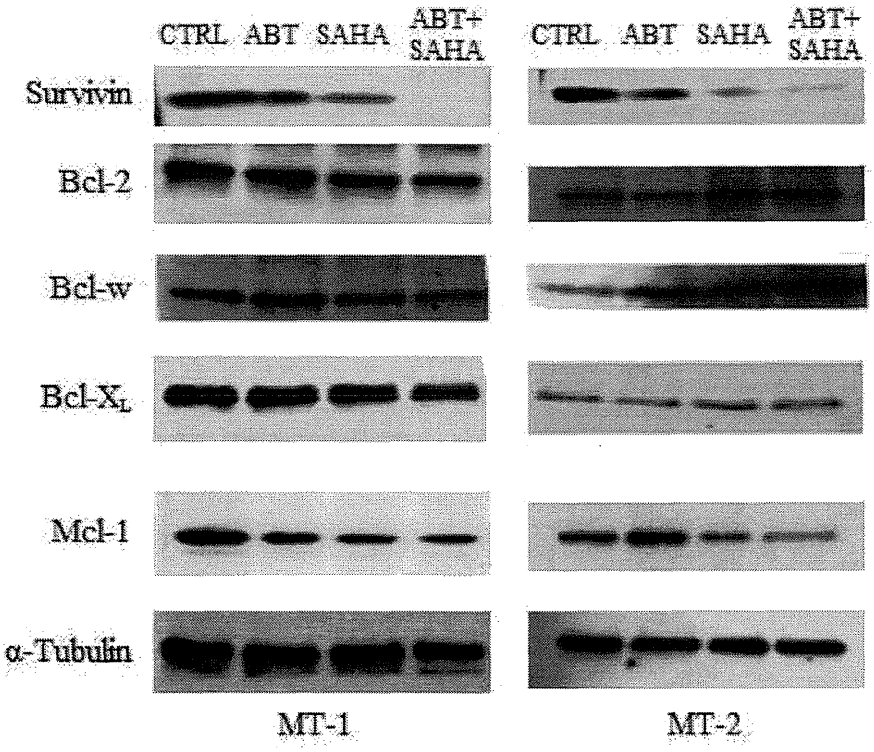

Figure 4. *The effect of combination of ABT-737 and SAHA on survivin levels. Cells were treated with ABT (1 μM), SAHA (2 μM) or their combination for 24 h. Whole-cell lysates were subjected to Western blotting to assess survivin, Bcl-2, Bcl-w, Bcl-X$_L$ and Mcl-1. CTRL, control; ABT, ABT-737= Bor, bortezomib.*

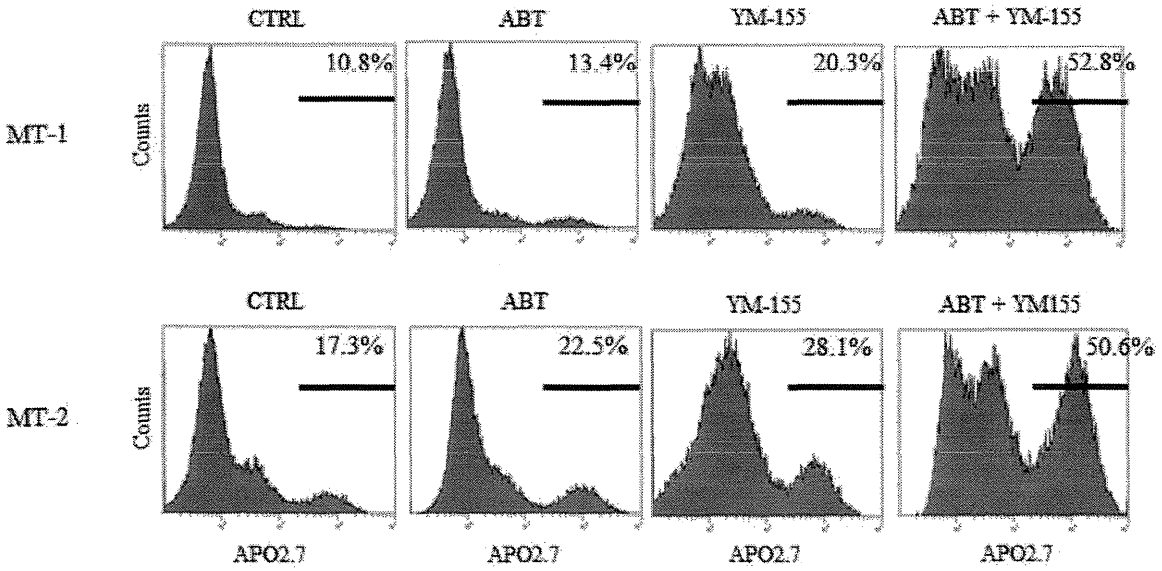

Figure 5. *Inhibition of survivin sensitizes HTLV-1-infected T-cell lines to ABT-737. Cells were treated with ABT (1 μM), YM-155 (10 nM) or their combination for 72 h and induction of apoptosis was assessed using an APO2.7 assay. The percentage of APO2.7-positive cells is shown. CTRL, Control; ABT, ABT-737.*

The combination of ABT-737 and SAHA also synergistically enhanced the induction of apoptosis in HTLV-1-infected T-cells by down-regulating the expression of survivin. Caspases are regulated by several intrinsic inhibitors of apoptosis proteins, in which survivin is a major one. Previous studies have shown that survivin induced resistance to ABT-737 (33, 34). In ATL, survivin has been suggested to be an important anti-apoptotic molecule that confers drug resistance, and high mRNA expression of survivin was shown to be a risk factor for the prognosis (35, 36). We also demonstrated that the inhibition of survivin by YM-155 sensitized MT-1 and MT-2 cells for induction of apoptosis by ABT-737. Our results suggest that SAHA increases sensitivity to ABT-737 by down-regulating the expression of survivin. The down-regulation of Mcl-1 was less significant than that of survivin, but may also contribute to the induction of apoptosis. Consequently, the combination of ABT-737 and SAHA is a promising treatment that targets multiple anti-apoptotic molecules such as Bcl-2, Bcl-X_L, Bcl-w, Mcl-1 and survivin in HTLV-1 infected T-cells.

ABT-737 is a known inhibitor of some anti-apoptotic proteins. Bortezomib targets the proteasome/ubiquitin pathway, which is active on apoptosis pathway regulators, while SAHA targets histone and non-histone deacetylases and indirectly modulates apoptosis. Numerous regulators are known to be involved in apoptotic pathways; therefore, inhibiting a single pathway is not sufficient. ABT-737, bortezomib and SAHA by themselves exhibited limited activity against HTLV-1 infected T-cell lines. However, ABT-737 combined with bortezomib or SAHA synergistically enhanced the induction of apoptosis because they could compensate for the functional deficits of each other. Taken together, these novel strategies targeting multiple apoptotic pathways appear promising for ATL but should be validated by *in vivo* animal model and clinical studies.

Conflicts of Interest

The Authors declare no conflicts of interest.

Acknowledgements

This work was supported by Health and Labor Sciences Research Grants for Clinical Research (H23-rinkensui-ippan-011) from the Ministry of Health, Labour and Welfare of Japan, and funds (No.:127006) from the Central Research Institute of Fukuoka University (K.I.).

References

1 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.

2 Ishitsuka K and Tamura K: Treatment of adult T-cell leukemia/lymphoma: past, present, and future. Eur J Haematol *80*: 185-196, 2008.

3 Tamura K, Katsuya H, Ishitsuka K, Utsunomiya A, Hanada S, Eto T, Moriuchi Y, Saburi Y, Yamanaka T and Suzumiya J: A nationwide survey of adult T-cell leukemia/lymphoma (ATL) newly diagnosed over the last decade in Japan. Ann Oncol *23*: ix348, 2012.

4 Oltersdorf T, Elmore SW, Shoemaker AR, Armstrong RC, Augeri DJ, Belli BA, Bruncko M, Deckwerth TL, Dinges J, Hajduk PJ, Joseph MK, Kitada S, Korsmeyer SJ, Kunzer AR, Letai A, Li C, Mitten MJ, Nettesheim DG, Ng S, Nimmer PM, O'Connor JM, Oleksijew A, Petros AM, Reed JC, Shen W, Tahir SK, Thompson CB, Tomaselli KJ, Wang B, Wendt MD, Zhang H, Fesik SW and Rosenberg SH: An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature *435*: 677-681, 2005.

5 Hann CL, Daniel VC, Sugar EA, Dobromilskaya I, Murphy SC, Cope L, Lin X, Hierman JS, Wilburn DL, Watkins DN and Rudin CM: Therapeutic efficacy of ABT-737, a selective inhibitor of Bcl-2, in small cell lung cancer. Cancer Res *68*: 2321-2328, 2008.

6 Tse C, Shoemaker AR, Adickes J, Anderson MG, Chen J, Jin S, Johnson EF, Marsh KC, Mitten MJ, Nimmer P, Roberts L, Tahir SK, Xiao Y, Yang X, Zhang H, Fesik S, Rosenberg SH and Elmore SW: ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor. Cancer Res *68*: 3421-3428, 2008.

7 Shoemaker AR, Mitten MJ, Adickes J, Ackler S, Refici M, Ferguson D, Oleksijew A, O'Connor JM, Wang B, Frost DJ, Bauch J, Marsh K, Tahir SK, Yang X, Tse C, Fesik SW, Rosenberg SH and Elmore SW: Activity of the Bcl-2 family inhibitor ABT-263 in a panel of small cell lung cancer xenograft models. Clin Cancer Res *14*: 3268-3277, 2008.

8 Wilson WH, O'Connor OA, Czuczman MS, LaCasce AS, Gerecitano JF, Leonard JP, Tulpule A, Dunleavy K, Xiong H, Chiu YL, Cui Y, Busman T, Elmore SW, Rosenberg SH, Krivoshik AP, Enschede SH and Humerickhouse RA: Navitoclax, a targeted high-affinity inhibitor of BCL-2, in lymphoid malignancies: a phase 1 dose-escalation study of safety, pharmacokinetics, pharmacodynamics, and antitumour activity. Lancet Oncol *11*: 1149-1159, 2010.

9 Gandhi L, Camidge DR, Ribeiro de Oliveira M, Bonomi P, Gandara D, Khaira D, Hann CL, McKeegan EM, Litvinovich E, Hemken PM, Dive C, Enschede SH, Nolan C, Chiu YL, Busman T, Xiong H, Krivoshik AP, Humerickhouse R, Shapiro GI and Rudin CM: Phase I study of Navitoclax (ABT-263), a novel Bcl-2 family inhibitor, in patients with small-cell lung cancer and other solid tumors. J Clin Oncol *29*: 909-916, 2011.

10 Roberts AW, Seymour JF, Brown JR, Wierda WG, Kipps TJ, Khaw SL, Carney DA, He SZ, Huang DC, Xiong H, Cui Y, Busman TA, McKeegan EM, Krivoshik AP, Enschede SH and Humerickhouse R: Substantial susceptibility of chronic lymphocytic leukemia to BCL2 inhibition: results of a phase I study of navitoclax in patients with relapsed or refractory disease. J Clin Oncol *30*: 488-496, 2012.

11 Rudin CM, Hann CL, Garon EB, Ribeiro de Oliveira M, Bonomi PD, Camidge DR, Chu Q, Giaccone G, Khaira D, Ramalingam SS, Ranson MR, Dive C, McKeegan EM, Chyla BJ, Dowell BL, Chakravartty A, Nolan CE, Rudersdorf N, Busman TA, Mabry MH, Krivoshik AP, Humerickhouse RA, Shapiro GI and Gandhi L: Phase II study of single-agent Navitoclax (ABT-263) and biomarker correlates in patients with relapsed small cell lung cancer. Clin Cancer Res *18*: 3163-3169, 2012.

12 Ishitsuka K, Kunami N, Katsuya H, Nogami R, Ishikawa C, Yotsumoto F, Tanji H, Mori N, Takeshita M, Miyamoto S and Tamura K: 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. Cancer Lett *317*: 218-225, 2012.

13 Mori N, Fujii M, Ikeda S, Yamada Y, Tomonaga M, Ballard DW and Yamamoto N: Constitutive activation of NF-kappaB in primary adult T-cell leukemia cells. Blood *93*: 2360-2368, 1999.

14 Adams J: The proteasome: a suitable antineoplastic target. Nat Rev Cancer *4*: 349-360, 2004.

15 Tan C and Waldmann TA: Proteasome inhibitor PS-341, a potential therapeutic agent for adult T-cell leukemia. Cancer Res *62*: 1083-1086, 2002.

16 Satou Y, Nosaka K, Koya Y, Yasunaga JI, Toyokuni S and Matsuoka M: Proteasome inhibitor, bortezomib, potently inhibits the growth of adult T-cell leukemia cells both *in vivo* and *in vitro*. Leukemia *18*: 1357-1363, 2004.

17 Nasr R, El-Sabban ME, Karam JA, Dbaibo G, Kfoury Y, Arnulf B, Lepelletier Y, Bex F, de Thé H, Hermine O and Bazarbachi A: Efficacy and mechanism of action of the proteasome inhibitor PS-341 in T-cell lymphomas and HTLV-I associated adult T-cell leukemia/lymphoma. Oncogene *24*: 419-430, 2005.

18 Hamamura RS, Ohyashiki JH, Kurashina R, Kobayashi C, Zhang Y, Takaku T and Ohyashiki K: Induction of heme oxygenase-1 by cobalt protoporphyrin enhances the antitumour effect of bortezomib in adult T-cell leukaemia cells. Br J Cancer *97*: 1099-1105, 2007.

19 Tang J, Yan H and Zhuang S: Histone deacetylases as targets for treatment of multiple diseases. Clin Sci *124*: 651-662, 2013.

20 Bots M and Johnstone RW: Rational Combinations Using HDAC Inhibitors. Clin Cancer Res *15*: 3970-3977, 2009.

21 O'Connor OA, Heaney ML, Schwartz L, Richardson S, Willim R, MacGregor-Cortelli B, Curly T, Moskowitz C, Portlock C, Horwitz S, Zelenetz AD, Frankel S, Richon V, Marks P and Kelly WK: Clinical experience with intravenous and oral formulations of the novel histone deacetylase inhibitor suberoylanilide hydroxamic acid in patients with advanced hematologic malignancies. J Clin Oncol *24*: 166-173, 2006.

22 Hasegawa H, Yamada Y, Tsukasaki K, Mori N, Tsuruda K, Sasaki D, Usui T, Osaka A, Atogami S, Ishikawa C, Machijima Y, Sawada S, Hayashi T, Miyazaki Y and Kamihira S: LBH589, a deacetylase inhibitor, induces apoptosis in adult T-cell leukemia/lymphoma cells *via* activation of a novel RAIDD-caspase-2 pathway. Leukemia *25*: 575-587, 2011.

23 Ellis L, Bots M, Lindemann RK, Bolden JE, Newbold A, Cluse LA, Scott CL, Strasser A, Atadja P, Lowe SW and Johnstone RW: The histone deacetylase inhibitors LAQ824 and LBH589 do not require death receptor signaling or a functional apoptosome to mediate tumor cell death or therapeutic efficacy. Blood *114*: 380-393, 2009.

24 Thompson RC, Vardinogiannis I and Gilmore TD: The sensitivity of diffuse large B-cell lymphoma cell lines to histone deacetylase inhibitor-induced apoptosis is modulated by BCL-2 family protein activity. PLoS One *8*: e62822, 2013.

25 Whitecross KF, Alsop AE, Cluse LA, Wiegmans A, Banks KM, Coomans C, Peart MJ, Newbold A, Lindemann RK and Johnstone RW: Defining the target specificity of ABT-737 and synergistic anti-tumor activities in combination with histone deacetylase inhibitors. Blood *113*: 1982-1891, 2009.

26 Miyoshi I, Kubonishi I, Sumida M, Hiraki S, Tsubota T, Kimura I, Miyamoto K and Sato J: A novel T-cell line derived from adult T-cell leukemia. Gann *71*: 155-156, 1980.

27 Miyoshi I, Kubonishi I, Yoshimoto S, Akagi T, Ohtsuki Y, Shiraishi Y, Nagata K and Hinuma Y: Type C virus particles in a cord T-cell line derived by co-cultivating normal human cord leukocytes and human leukaemic T cells. Nature *294*: 770-771, 1981.

28 Ishitsuka K, Hideshima T, Hamasaki M, Raje N, Kumar S, Podar K, Le Gouill S, Shiraishi N, Yasui H, Roccaro AM, Tai YZ, Chauhan D, Fram R, Tamura K, Jain J and Anderson KC: Novel inosine monophosphate dehydrogenase inhibitor VX-944 induces apoptosis in multiple myeloma cells primarily *via* caspase-independent AIF/Endo G pathway. Oncogene *24*: 5888–5896, 2005.

29 Chou TC and Talalay P: Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv Enzyme Regul *22*: 27-55, 1984.

30 Temmink OH, Hoogeland MF, Fukushima M and Peters GJ: Low folate conditions may enhance the interaction of trifluorothymidine with antifolates in colon cancer cells. Cancer Chemother Pharmacol *57*: 171-179, 2006.

31 Podar K, Gouill SL, Zhang J, Opferman JT, Zorn E, Tai YT, Hideshima T, Amiot M, Chauhan D, Harousseau JL and Anderson KC: A pivotal role for Mcl-1 in Bortezomib-induced apoptosis. Oncogene *27*: 721-731, 2008.

32 Willis SN and Adams JM: Life in the balance: how BH3-only proteins induce apoptosis. Curr Opin Cell Biol *17*: 617-625, 2005.

33 Zhao X, Ogunwobi OO and Liu C: Survivin inhibition is critical for Bcl-2 inhibitor-induced apoptosis in hepatocellular carcinoma cells. PLoS One *6*: e21980, 2011.

34 Jane EP, Premkumar DR, DiDomenico JD, Hu B, Cheng SY and Pollack IF: YM-155 potentiates the effect of ABT-737 in malignant human glioma cells *via* survivin and Mcl-1 down-regulation in an EGFR-dependent context. Mol Cancer Ther *12*: 326-338, 2013.

35 Nakayama K and Kamihira S: Survivin an important determinant for prognosis in adult T-cell leukemia: a novel biomarker in practical hemato-oncology. Leuk Lymphoma *43*: 2249-2255, 2002.

36 Che XF, Zheng CL, Owatari S, Mutoh M, Gotanda T, Jeung HC, Furukawa T, Ikeda R, Yamamoto M, Haraguchi M, Arima N and Akiyama S: Overexpression of survivin in primary ATL cells and sodium arsenite induces apoptosis by down-regulating survivin expression in ATL cell lines. Blood *107*: 4880-4887, 2006.

Received July 2, 2014
Revised July 22, 2014
Accepted July 24, 2014

Instructions to Authors 2014

General Policy. ANTICANCER RESEARCH (AR) will accept original high quality works and reviews on all aspects of experimental and clinical cancer research. The Editorial Policy suggests that priority will be given to papers advancing the understanding of cancer causation, and to papers applying the results of basic research to cancer diagnosis, prognosis, and therapy. AR will also accept the following for publication: (a) Abstracts and Proceedings of scientific meetings on cancer, following consideration and approval by the Editorial Board; (b) Announcements of meetings related to cancer research; (c) Short reviews (of approximately 120 words) and announcements of newly received books and journals related to cancer, and (d) Announcements of awards and prizes.

The principal aim of AR is to provide prompt publication (print and online) for original works of high quality, generally within 1-2 months from final acceptance. Manuscripts will be accepted on the understanding that they report original unpublished works on the cancer problem that are not under consideration for publication by another journal, and that they will not be published again in the same form. All authors should sign a submission letter confirming the approval of their article contents. All material submitted to AR will be subject to review, when appropriate, by two members of the Editorial Board and by one suitable outside referee. The Editors reserve the right to improve manuscripts on grammar and style.

The Editors and Publishers of AR accept no responsibility for the contents and opinions expressed by the contributors. Authors should warrantee due diligence in the creation and issuance of their work.

NIH Open Access Policy. The journal acknowledges that authors of NIH funded research retain the right to provide a copy of the final manuscript to the NIH four months after publication in ANTICANCER RESEARCH, for public archiving in PubMed Central.

Copyright. Once a manuscript has been published in ANTICANCER RESEARCH, which is a copyrighted publication, the legal ownership of all published parts of the paper has been transferred from the Author(s) to the journal. Material published in the journal may not be reproduced or published elsewhere without the written consent of the Managing Editor or Publisher.

Format. Two types of papers may be submitted: (i) Full papers containing completed original work, and (ii) review articles concerning fields of recognisable progress. Papers should contain all essential data in order to make the presentation clear. Reasonable economy should be exercised with respect to the number of tables and illustrations used. Papers should be written in clear, concise English. Spelling should follow that given in the "Shorter Oxford English Dictionary".

Manuscripts. Submitted manuscripts should not exceed fourteen (14) pages (approximately 250 words per double - spaced typed page), including abstract, text, tables, figures, and references (corresponding to 4 printed pages). Papers exceeding four printed pages will be subject to excess page charges. All manuscripts should be divided into the following sections:
(a) *First page* including the title of the presented work [not exceeding fifteen (15) words], full names and full postal addresses of all Authors, name of the Author to whom proofs are to be sent, key words, an abbreviated running title, an indication "review", "clinical", "epidemiological", or "experimental" study, and the date of submission. (Note: The order of the Authors is not necessarily indicative of their contribution to the work. Authors may note their individual contribution(s) in the appropriate section(s) of the presented work); (b) *Abstract* not exceeding 150 words, organized according to the following headings: Background/Aim - Materials and Methods/Patients and Methods - Results - Conclusion; (c) *Introduction;* (d) *Materials and Methods/Patients and Methods;* (e) *Results;* (f) *Discussion;* (g) *Acknowledgements;* (h) *References*. All pages must be numbered consecutively. Footnotes should be avoided. Review articles may follow a different style according to the subject matter and the Author's opinion. Review articles should not exceed 35 pages (approximately 250 words per double-spaced typed page) including all tables, figures, and references.

Figures. All figures (whether photographs or graphs) should be clear, high contrast, at the size they are to appear in the journal: 8.00 cm (3.15 in.) wide for a single column; 17.00 cm (6.70 in.) for a double column; maximum height: 20.00 cm (7.87 in.). Graphs must be submitted as photographs made from drawings and must not require any artwork, typesetting, or size modifications. Symbols, numbering and lettering should be clearly legible. The number and top of each figure must be indicated. Colour plates are charged.

Tables. Tables should be typed double-spaced on a separate page, numbered with Roman numerals and should include a short title.

References. Authors must assume responsibility for the accuracy of the references used. Citations for the reference sections of submitted works should follow the standard form of "Index Medicus" and must be numbered consecutively. In the text, references should be cited by number. Examples: 1 Sumner AT: The nature of chromosome bands and their significance for cancer research. Anticancer Res 1: 205-216, 1981. 2 McGuire WL and Chamnes GC: Studies on the oestrogen receptor in breast cancer. In: Receptors for Reproductive Hormones (O' Malley BW, Chamnes GC (eds.). New York, Plenum Publ Corp., pp 113-136, 1973.

Nomenclature and Abbreviations. Nomenclature should follow that given in "Chemical Abstracts", "Index Medicus", "Merck Index", "IUPAC –IUB", "Bergey's Manual of Determinative Bacteriology", The CBE Manual for Authors, Editors and Publishers (6th edition, 1994), and MIAME Standard for Microarray Data. Human gene symbols may be obtained from the HUGO Gene Nomenclature Committee (HGNC) (http://www.gene.ucl.ac.uk/). Approved mouse nomenclature may be obtained from http://www.informatics.jax.org/. Standard abbreviations are preferable. If a new abbreviation is used, it must be defined on first usage.

Clinical Trials. Authors of manuscripts describing clinical trials should provide the appropriate clinical trial number in the correct format in the text.

For International Standard Randomised Controlled Trials (ISRCTN) Registry (a not-for-profit organization whose registry is administered by Current Controlled Trials Ltd.) the unique number must be provided in this format: ISRCTNXXXXXXXX (where XXXXXXXX represents the unique number, always prefixed by "ISRCTN"). Please note that there is no space between the prefix "ISRCTN" and the number. Example: ISRCTN47956475.

For Clinicaltrials.gov registered trials, the unique number must be provided in this format: NCTXXXXXXXX (where XXXXXXXX represents the unique number, always prefixed by 'NCT'). Please note that there is no space between the prefix 'NCT' and the number. Example: NCT00001789.

Ethical Policies and Standards. ANTICANCER RESEARCH agrees with and follows the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" established by the International Committee of Medical Journal Editors in 1978 and updated in October 2001 (www.icmje.org). Microarray data analysis should comply with the "Minimum Information About Microarray Experiments (MIAME) standard". Specific guidelines are provided at the "Microarray Gene Expression Data Society" (MGED) website. Presentation of genome sequences should follow the guidelines of the NHGRI Policy on Release of Human Genomic Sequence Data. Research involving human beings must adhere to the principles of the Declaration of Helsinki and Title 45, U.S. Code of Federal Regulations, Part 46, Protection of Human Subjects, effective December 13, 2001. Research involving animals must adhere to the Guiding Principles in the Care and Use of Animals approved by the Council of the American Physiological Society. The use of animals in biomedical research should be under the careful supervision of a person adequately trained in this field and the animals must be treated humanely at all times. Research involving the use of human foetuses, foetal tissue, embryos and embryonic cells should adhere to the U.S. Public Law 103-41, effective December 13, 2001.

Submission of Manuscripts. Please follow the Instructions to Authors regarding the format of your manuscript and references. There are 3 ways to submit your article (NOTE: Please use only one of the 3 options. Do not send your article twice.):

1. To submit your article online please visit: IIAR-Submissions (http://www.iiar-anticancer.org/submissions/login.php)
2. You can send your article via e-mail to journals@iiar-anticancer.org. Please remember to always indicate the name of the journal you wish to submit your paper. The text should be sent as a Word document (*doc) attachment. Tables, figures and cover letter can also be sent as e-mail attachments.
3. You can send the manuscript of your article via regular mail in a USB stick, DVD, CD or floppy disk (including text, tables and figures) together with three hard copies to the following address:
   John G. Delinasios
   International Institute of Anticancer Research (IIAR)
   Editorial Office of ANTICANCER RESEARCH,
   IN VIVO, CANCER GENOMICS and PROTEOMICS.
   1st km Kapandritiou-Kalamou Road
   P.O. Box 22, GR-19014 Kapandriti, Attiki
   GREECE

Submitted articles will not be returned to Authors upon rejection.

Galley Proofs. Unless otherwise indicated, galley proofs will be sent to the first-named Author of the submission. Corrections of galley proofs should be limited to typographical errors. Reprints, PDF files, and/or Open Access may be ordered after the acceptance of the paper. Requests should be addressed to the Editorial Office.

bjh research paper

Japan Clinical Oncology Group (JCOG) prognostic index and characterization of long-term survivors of aggressive adult T-cell leukaemia-lymphoma (JCOG0902A)

Takuya Fukushima,[1] Shogo Nomura,[2] Masanori Shimoyama,[3] Taro Shibata,[2] Yoshitaka Imaizumi,[4] Yoshiyuki Moriuchi,[5] Takeaki Tomoyose,[6] Kimiharu Uozumi,[7] Yukio Kobayashi,[8] Noriyasu Fukushima,[9] Atae Utsunomiya,[10] Mitsutoshi Tara,[11] Kisato Nosaka,[12] Michihiro Hidaka,[13] Naokuni Uike,[14] Shinichiro Yoshida,[15] Kazuo Tamura,[16] Kenji Ishitsuka,[16] Mitsutoshi Kurosawa,[17] Masanobu Nakata,[18] Haruhiko Fukuda,[2] Tomomitsu Hotta,[3] Kensei Tobinai[8] and Kunihiro Tsukasaki[19]

[1]*Laboratory of Haematoimmunology, School of Health Sciences, Faculty of Medicine, University of the Ryukyus, Nishihara-cho,* [2]*JCOG Data Centre, Multi-institutional Clinical Trial Support Centre, National Cancer Centre, Tokyo,* [3]*Multicentre-institutional Clinical Trial Support Centre, National Cancer Centre, Tokyo,* [4]*Department of Haematology, Atomic Bomb Disease and Hibakusha Medicine Unit, Atomic Bomb Disease Institute, Nagasaki University, Nagasaki,* [5]*Department of Haematology, Sasebo City General Hospital, Sasebo,* [6]*Division of Endocrinology, Diabetes and Metabolism, Haematology, Rheumatology (Second Department of Internal Medicine), Graduate School of Medicine, University of the Ryukyus, Nishihara-cho,* [7]*Department of Haematology and Immunology, Kagoshima University Hospital, Kagoshima,* [8]*Department of Haematology, National Cancer Centre Hospital, Tokyo,* [9]*Division of Haematology, Respiratory Medicine and Oncology, Department of Internal Medicine, Faculty of Medicine, Saga University, Saga,* [10]*Department of Haematology, Imamura Bun-in Hospital, Kagoshima,* [11]*Department of Haematology, Kagoshima City Hospital, Kagoshima,* [12]*Department of Haematology, Kumamoto University of Medicine, Kumamoto,* [13]*Department of Internal Medicine, National Hospital Organization Kumamoto Medical Centre, Kumamoto,* [14]*Department of*

Summary

This study evaluated the clinical features of 276 patients with aggressive adult T-cell leukaemia-lymphoma (ATL) in 3 Japan Clinical Oncology Group (JCOG) trials. We assessed the long-term survivors who survived >5 years and constructed a prognostic index (PI), named the JCOG-PI, based on covariates obtained by Cox regression analysis. The median survival time (MST) of the entire cohort was 11 months. In 37 patients who survived >5 years, no disease-related deaths in 10 patients with lymphoma-type were observed in contrast to the 10 ATL-related deaths in other types. In multivariate analysis of 193 patients, the JCOG-PI based on corrected calcium levels and performance status identified moderate and high risk groups with an MST of 14 and 8 months respectively (hazard ratio, 1·926). The JCOG-PI was reproducible in an external validation. Patients with lymphoma-type who survived >5 years might have been cured. The JCOG-PI is valuable for identifying patients with extremely poor prognosis and will be useful for the design of future trials combining new drugs or investigational treatment strategies.

Keywords: adult T-cell leukaemia-lymphoma, Japan Clinical Oncology Group trials, long-term survivors, prognostic index.

First published online 14 June 2014
doi:10.1111/bjh.12962

Haematology, National Hospital Organization Kyushu Cancer Centre, Fukuoka, [15]*Department of Haematology, National Hospital Organization Nagasaki Medical Centre, Omura,* [16]*Department of Medicine, Division of Medical Oncology, Haematology and Infectious Diseases, Fukuoka University, Fukuoka,* [17]*Department of Haematology, National Hospital Organization Hokkaido Cancer Centre, Sapporo,* [18]*Department of Haematology, Sapporo Hokuyu Hospital, Sapporo, and* [19]*Department of Haematology National Cancer Centre Hospital East, Kashiwa, Japan*

Received 14 January 2014; accepted for publication 10 April 2014
Correspondence: Takuya Fukushima, MD, Laboratory of Haematoimmunology, School of Health Sciences, Faculty of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-0215, Japan.
E-mail: fukutaku@med.u-ryukyu.ac.jp

Adult T-cell leukaemia-lymphoma (ATL) is a distinct peripheral T-lymphocytic malignancy associated with human T-cell lymphotropic virus type I (HTLV-1) (Uchiyama *et al*, 1977; Poiesz *et al*, 1980; Hinuma *et al*, 1981; Miyoshi *et al*, 1981; Yoshida *et al*, 1982). Classification of clinical subtypes into acute, lymphoma, chronic and smouldering was proposed based on prognostic factors, clinical features and the natural history of the disease (Shimoyama, 1991). Patients with aggressive ATL (i.e., acute, lymphoma and unfavourable chronic types) have frequently been treated as a subtype of aggressive non-Hodgkin lymphoma (NHL), whereas those with indolent ATL (i.e., favourable chronic and smouldering types) have been managed as a subtype of chronic lymphoid leukaemia (Shimoyama, 1994; Tobinai & Watanabe, 2004). Aggressive ATL typically has a very poor prognosis compared with aggressive B-cell lymphomas, such as diffuse large B-cell lymphoma and peripheral T-cell lymphoma excluding ATL (The International Non-Hodgkin's Lymphoma Prognostic Factor Project's, 1993; Shimoyama, 1994; Gallamini *et al*, 2004; Watanabe *et al*, 2010). In the 1980's, patients with aggressive ATL were reported to have a median survival time (MST) of approximately 8 months, with a 2-year survival rate of <5% because of the multidrug-resistant phenotype of their malignant tumour cells, rapid proliferation of the tumour cells, a large tumour burden with multi-organ failure, hypercalcaemia, and/or frequent opportunistic infections (Lymphoma Study Group, 1991; Shimoyama, 1991, 1994; Tobinai & Watanabe, 2004).

The Japan Clinical Oncology Group (JCOG)-Lymphoma Study Group (LSG) has conducted consecutive clinical trials to improve the survival of patients with ATL. Earlier trials (JCOG7801, JCOG8101, and JCOG8701) revealed poor prognosis of ATL compared with other aggressive NHLs (Shimoyama *et al*, 1988; Tobinai *et al*, 1994). Furthermore, the disappointing results with conventional chemotherapies in the 1980s and the proposal for a subtype classification of ATL led us to conduct clinical trials with new agents that exclusively targeted aggressive ATL. The first phase II trial, JCOG9109 (1991–1993), evaluated combination chemotherapy with deoxycoformycin, an inhibitor of adenosine deaminase, which had been effective as a single agent against relapsed or refractory ATL (Tobinai *et al*, 1992). However, the results were disappointing with an MST of 7 months, similar to the findings of previous JCOG-LSG trials (Tsukasaki *et al*, 2003). The next phase II trial, JCOG9303 (1994–1996), evaluated the chemotherapy regimen VCAP-AMP-VECP (LSG15) against aggressive ATL. This dose-intensified multi-agent chemotherapy consisted of vincristine, cyclophosphamide, doxorubicin (DXR) and prednisone (PSL) for VCAP, DXR, ranimustine and PSL for AMP, and vindesine, etopside, carboplatin and PSL for VECP, supported by granulocyte colony-stimulating factor and intrathecal (IT) prophylaxis with methotrexate (MTX) and PSL. This phase II trial showed promising results, with complete remission (CR) and overall response rates of 36% and 81%, respectively, and an MST of 13 months at the expense of haematological and other toxicities (Yamada *et al*, 2001). Based on these results, we proceeded to the phase III trial JCOG9801 (1998–2003), which compared a modified VCAP-AMP-VECP regimen (shortened from 7 to 6 courses), to which cytarabine was added to the IT prophylaxis, *versus* CHOP (cyclophosphamide, DXR, vincristine and PSL)-14 supported by granulocyte

colony-stimulating factor and IT prophylaxis identical to the former regimen. The CR and 3-year overall survival (OS) were higher in the modified VCAP-AMP-VECP arm than in the CHOP-14 arm (40% vs. 25% and 24% vs. 13% respectively), suggesting that the former is a more effective regimen at the expense of greater toxicity for patients with newly diagnosed aggressive ATL (Tsukasaki *et al*, 2007).

Through these 3 JCOG trials for patients with aggressive ATL, the 5-year OS was improved, from 5% in the 1980's to 15% in the 1990s. To characterize the long-term survivors of aggressive ATL and to develop a new prognostic index (PI) for the disease, we performed a combined analysis (JCOG0902A) of all the patients enrolled in the 3 JCOG trials.

Methods

Study population

A total of 276 patients who were registered in the 3 JCOG trials described above were enrolled in this study (Yamada *et al*, 2001; Tsukasaki *et al*, 2003, 2007). Some patients did not receive anti-viral therapy using interferon-alpha and zidovudine because these drugs for ATL was not covered by the National Health Insurance in Japan. The eligibility criteria for the 3 JCOG trials were detailed in previous reports (Yamada *et al*, 2001; Tsukasaki *et al*, 2003, 2007). Briefly, patients were eligible to participate if they had aggressive ATL (i.e., acute, lymphoma, or unfavourable chronic type) with no prior chemotherapy, were aged 15–69 years and had preserved organ functions, no proven central nervous system (CNS) involvement and a performance status (PS) of 0–3 or 4 due to hypercalcaemia caused by ATL. The diagnosis of ATL was made based on seropositivity for HTLV-1 antibody and histologically and/or cytologically proven peripheral T-cell malignancy. Monoclonal integration of HTLV-1 provirus was analysed in 104 of 276 patients studied. Among these 104 patients, integration was detected in 100 patients and not detected in four patients.

The PI for the JCOG trials, which we refer to as the JCOG-PI, was constructed from the data of patients who participated in these trials (training set) and was then applied to an external validation set. The external validation set consisted of 136 patients who had not participated in prior JCOG studies but had received anthracycline-containing regimens as initial chemotherapy at three sites (Nagasaki University Hospital, Nagasaki Medical Centre, and Sasebo City General Hospital) under the remit of the JCOG-LSG. These patients were a subset of those from a previous retrospective study (Katsuya *et al*, 2012) and their OS and corrected calcium levels were reviewed.

Data and analysis sets

The endpoint of this study was OS, defined as the duration between registration to each JCOG trial and death from any cause or censored at the last follow up in living patients. For the validation data set, we substituted the date of treatment initiation for the date of registration.

Candidate covariates were sex, age, Eastern Cooperative Oncology Group (ECOG) PS, B symptoms, clinical stage, liver involvement, lactate dehydrogenase, blood urea nitrogen (BUN), corrected calcium levels, serum total protein, serum albumin, white blood cell count, total (normal and abnormal) lymphocyte count, neutrophil count and platelet count. We excluded the treatment regimen from the covariates because our aim was to create an index that could stratify the patients' prognosis and be applicable to future clinical trials evaluating various promising regimens. Cut-off values were determined clinically by dividing the continuous biological and laboratory test variables into no more than three categories. The data of 193 patients with a complete set of candidate covariates were used for the training set (Fig 1).

The protocol of this study was reviewed and approved by the JCOG Protocol Review Committee.

Statistical analysis

Patients who survived >5 years were categorized according to ATL subtype (acute, lymphoma or unfavourable chronic types). In addition, to evaluate the ATL-related death events for each subtype, a disease-specific mortality curve was estimated, for only those patients who survived >2 years, by means of a competing risks framework (Kalbfleisch & Prentice, 2002). The proportion of patients who survived >5 and >10 years was calculated to evaluate the association between long-term survival and CR (including CR unconfirmed) for initial treatment. The proportion of cases with

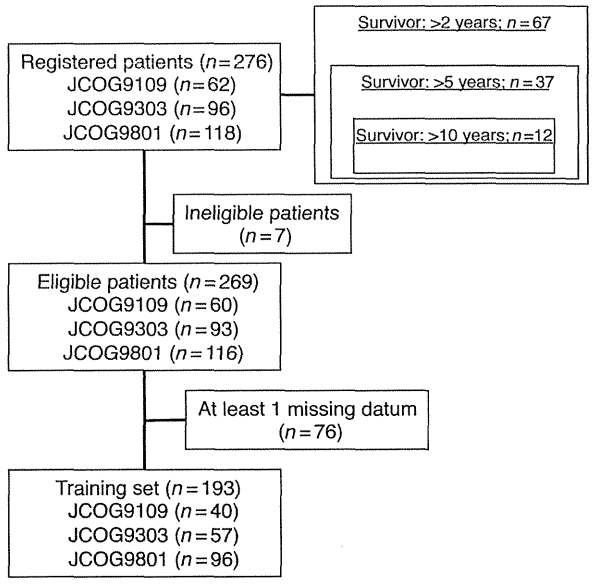

Fig 1. Patient disposition of the training set.

CNS involvement was compared among the JCOG trial regimens in an exploratory evaluation of the efficacy of prophylactic IT treatment. The prophylactic IT treatments against CNS involvement were: none in JCOG9109, MTX and PSL in JCOG9303, and MTX, cytarabine and PSL in both regimens in JCOG9801. Confidence intervals (CIs) for all the above proportions were computed using the Clopper–Pearson method (Clopper & Pearson, 1934).

Analyses for the development and validation of the JCOG-PI were performed according to a pre-specified analysis plan. The JCOG-PI consisted of risk groups that were developed using Cox's proportional hazards model. Before constructing the JCOG-PI, covariates with several definitions were selected for those with the smallest Akaike's Information Criteria (Akaike, 1973) on univariate analysis. Next, we verified the correlations between covariates to avoid multi-colinearity. Stepwise Cox regression analysis was then performed to identify unfavourable prognostic factors for constructing the JCOG-PI. The entry criterion was $P < 0{\cdot}20$ and the removal criterion was $P > 0{\cdot}15$.

The maximum number of risk group strata was set at three, based on the opinions of JCOG-LSG members who commented that too many strata were impractical for evaluating risk. The risk group was divided with patients equally distributed. The log-rank test was used to assess the discrepancy between the risk groups and the Kaplan–Meier method was applied to estimate OS.

All statistical analysis was performed using SAS Release 9·1 (SAS Institute, Inc, Cary, NC, USA). All reported P values are two-sided and $P < 0{\cdot}05$ was considered statistically significant.

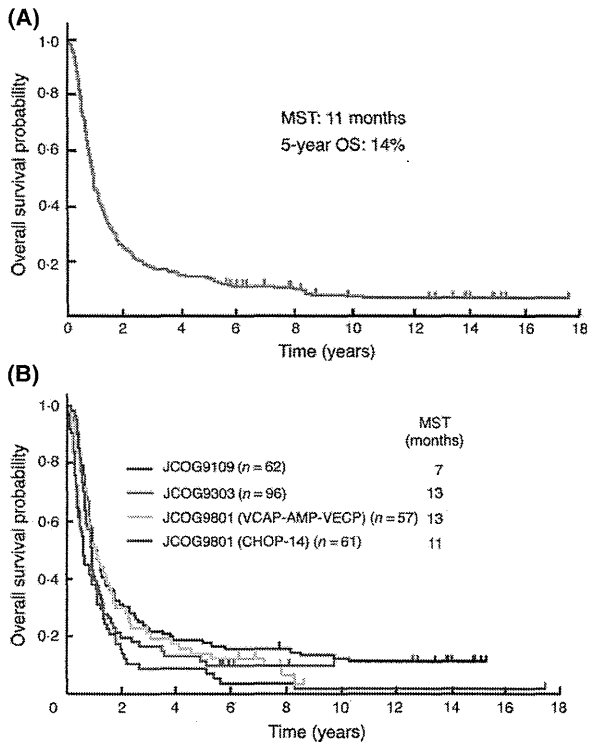

Fig 2. Overall survival (OS) of all registered patients in 3 Japan Clinical Oncology Group (JCOG) trials and according to treatment regimens. (A) OS of all 276 registered patients. Median survival time (MST) and the 5-year OS were 11 months and 14%, respectively.(B) OS according to different treatment regimens. MST was 7 months in JCOG9109, 13 months in JCOG9303, 13 months in VCAP-AMP-VECP of JCOG9801 and 11 months in CHOP-14 of JCOG9801.

Results

Patient characteristics

A total of 276 patients were registered in the 3 trials (JCOG9109, $n = 62$; JCOG9303, $n = 96$; and JCOG9801, $n = 118$) from 58 institutions in Japan. The MST and the 5-year OS of all patients were 11 months and 14% respectively (Fig 2A). The OS of each treatment regimen during the long follow up reconfirmed the findings of each original report (Fig 2B) (Yamada *et al*, 2001; Tsukasaki *et al*, 2003, 2007). Clinical characteristics are shown in Table I.

Long-term survivors according to subtype and initial response

The disease-specific mortality curve of patients who survived >2 years according to subtype is presented in Fig 3. Among the 37 patients (acute, $n = 22$; lymphoma, $n = 8$; unfavourable chronic, $n = 7$) who survived >5 years, there were no ATL-related deaths in lymphoma type, which was in contrast to the 10 ATL-related deaths in the acute and unfavourable chronic types after 5 years.

Of the 276 patients, 88 (32%) achieved CR with initial treatment. Of these 88 patients, 24 (27%) patients had survived >5 years and 11 (13%) patients had survived >10 years. Of the remaining 188 patients who did not achieve CR, 13 (17%) patients who survived >5 years and only 1 (0·5%) patient survived >10 years.

CNS involvement by treatment regimen

CNS involvement was 1·6% (95% CI, 0·04–8·7) in JCOG9109, 6·3% (95% CI, 2·3–13·1) in JCOG9303, and 3·5% (95% CI, 0·4–12·1) in the VCAP-AMP-VECP arm and 8·2% (95% CI, 2·7–18·1) in the CHOP-14 arm of JCOG9801. No significant differences in the proportion of CNS involvement were observed among the regimens.

Development of the PI

In univariate analyses, three covariates showed significant associations with OS, namely PS, corrected calcium level and serum total protein (all $P < 0{\cdot}05$; Table II). Stepwise Cox regression analysis returned three unfavourable prognostic

Table I. Clinical characteristics of 15 covariates in all 276 registered patients.

		JCOG9109 (n = 62)	JCOG9303 (n = 96)	JCOG9801 (n = 118)	Total (n = 276)
Initial date of registration		November 1991	January 1994	July 1998	
Final date of registration		July 1993	December 1996	October 2003	
Number of sites		30	20	27	49
Sex	Male/female	38/24	54/42	61/57	153/123
Age, years	≥20, <30	0	1	0	1
	≥30, <40	2	7	6	15
	≥40, <50	14	29	20	63
	≥50,<60	27	24	44	95
	≥60, <70	19	35	48	102
PS	0/1	23/22	19/25	49/46	91/93
	2/3/4/NE	7/9/1/0	17/9/8/18	18/4/1/0	42/22/10/18
B symptoms	+/−/NE	22/36/4	39/57/0	45/73/0	106/166/4
Stage	I/II/III/IV	1/4/8/49	2/6/14/74	0/4/8/106	3/14/30/229
Liver invasion	+/−	10/52	20/76	25/93	55/221
LDH, iu/l	<−1 × ULN/>	9/53	10/86	20/98	39/237
BUN, mmol/l	<−1 × ULN/>/NE	47/14/1	80/15/1	107/11/0	234/40/2
Corrected Ca, mmol/l	<2·75/≥/NE	49/9/4	75/16/5	93/25/0	217/50/9
Serum protein, g/l	<60/≥/NE	18/44/0	27/69/0	30/87/1	75/200/1
Albumin g/l	<35/35–40/≥40/NE	18/26/15/3	35/39/18/4	28/64/26/0	81/129/59/1
WBC ($\times 10^9$/l)	<3/≥	48/14	77/19	104/14	229/47
Lymphocytes ($\times 10^9$/l)*	<4/4–15/≥15/NE	28/16/14/4	54/19/23/0	64/33/20/1	146/68/57/5
Neutrophils ($\times 10^9$/l)	<8/≥/NE	49/12/1	75/21/0	94/24/0	218/57/1
Platelets ($\times 10^9$/l)	<150/≥	16/46	19/77	19/99	54/222

B symptoms: fever, night sweats, and weight loss.
JCOG, Japan Clinical Oncology Group; ECOG PS, Eastern Cooperative Oncology Group performance status; Ca, calcium level; WBC, white blood cell count; ULN, upper limit of normal; NE, not evaluated.
*total (normal + abnormal) lymphocyte count.

factors associated with OS, namely a high, corrected calcium level, high PS (2–4), and the existence of B symptoms, although the third factor was not statistically significant (Table II). Table II also presents the results of the model when the two significant factors of corrected calcium and ECOG PS were included. The hazard ratios (HRs) estimated by this model were 1·574 (95% CI, 1·088–2·277; P = 0·016) for corrected calcium and 1·554 (95% CI, 1·120–2·157; P = 0·008) for ECOG PS.

The four categories consisting of the two prognostic factors (corrected calcium level and PS) were combined into a dichotomous PI, named the JCOG-PI, by considering its potential for clinical use. Similarly, we constructed a dichotomous PI including B symptoms with two prognostic factors. We excluded B symptoms from further assessment because the Akaike Information Criteria of JCOG-PI (1537·8) was smaller than that of PI (1545·6).

According to the JCOG-PI, the MST and 5-year OS were 14 months and 18% in patients with both corrected calcium <2·75 mmol/l and a PS of 0 or 1 (moderate-risk group) and were 8 months and 4% in patients with corrected calcium ≥2·75 mmol/l and/or a PS of 2–4 (high-risk group) respectively (Fig 4A). The HR and 95% CI were 1·926 and 1·423–2·606 respectively (P < 0·0001).

External validation

Nine patients in the validation set of 136 patients had missing corrected calcium or PS data, resulting in 127 evaluable patients (Fig 5). The median and longest follow-up periods were 9 months and 97 months, respectively. The HR was 2·138 (95% CI, 1·414–3·233, P = 0·0003) with an MST of 18 months and 6 months in the moderate- and high-risk groups respectively and JCOG-PI showed good reproducibility (Fig 4B).

Discussion

In this first prospective analysis of a large cohort of aggressive ATL patients from prospective clinical trials conducted after the clinical subtype classification of ATL was introduced, we constructed the JCOG-PI based on corrected calcium level and PS and validated it with external data. The ascertained discrepancy was stronger among the external validation set. In addition, OS of high-risk patients was worse in the external validation set than in the training set, probably reflecting poor organ functions and other unfavourable prognostic factors in patients not participating in clinical trials. The OS of the moderate-risk patients was better in the

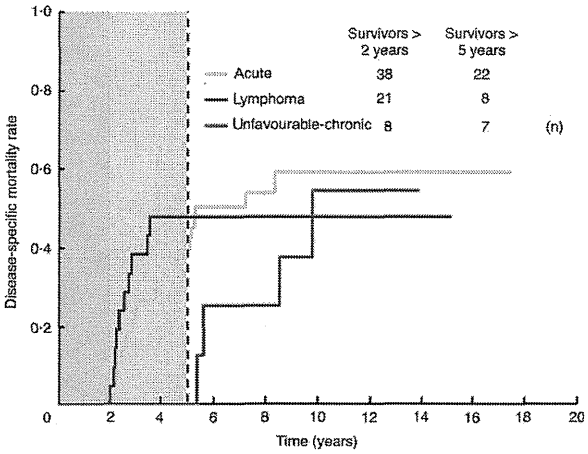

Fig 3. ATL-related deaths of patients who survived >2 years according to subtype. Among the 37 patients who survived >5 years, there were no ATL-related deaths in lymphoma type in contrast to the 10 ATL-related deaths in other types after 5 years.

external validation set than in the training set, possibly reflecting recent advances in treatment, including chemotherapy and allogeneic haematopoietic stem cell transplantation (allo-HSCT).

In our analysis of patients who survived >5 years, no ATL-related deaths occurred in those with lymphoma type, which is in contrast to the ATL-related deaths seen among patients with acute or unfavourable chronic type (Fig 3). This suggests that about 10% of patients with lymphoma type survived >5 years, most of whom might have been cured. Although abnormalities of comparative genomic hybridization might differ between acute and lymphoma types (Oshiro *et al*, 2006), the difference in clinical course between lymphoma type and acute or unfavourable chronic type remains unclear, and further analyses on the molecular and biological features of these types are needed.

Of the 276 patients studied, 20 received an allo-HSCT. The 5-year OS rate of these patients was 40%, compared with 12% in patients who did not undergo transplantation

Table II. Results of univariate and multivariate analyses in the training set (n = 193).

Factor		Univariate analysis HR (95%CI)	*P* value	Pre-planned multivariate analysis (AIC = 1545·6) HR (95%CI)	*P* value	Model used for constructing JCOG-PI (AIC = 1537·8) HR (95%CI)	*P* value
Ca, mmol/l	<2·75	Ref		Ref		Ref	
	≥2·75	1·742 (1·214–2·498)	0·002	1·688 (1·156–2·466)	0·007	1·574 (1·088–2·277)	0·016
ECOG PS	0–1	Ref		Ref		Ref	
	2–4	1·680 (1·219–2·314)	0·001	1·493 (1·073–2·078)	0·018	1·554 (1·120–2·157)	0·008
B symptoms	−	Ref		Ref			
	+	1·249 (0·926–1·685)	0·145	1·288 (0·945–1·755)	0·109		
Sex	Male	Ref					
	Female	0·999 (0·743–1·342)	0·994				
Age, years	<60	Ref					
	≥60	1·108 (0·818–1·502)	0·504				
Stage	I–II	Ref					
	III–IV	1·293 (0·682–2·451)	0·429				
Liver invasion	−	Ref					
	+	1·238 (0·867–1·768)	0·241				
LDH, iu/l	≤ULN	Ref					
	>1 × ULN	1·325 (0·840–2·091)	0·226				
BUN, mmol/l	≤ULN	Ref					
	>1 × ULN	1·332 (0·871–2·036)	0·184				
Serum protein, g/l	<60	Ref					
	≥60	0·642 (0·457–0·901)	0·010				
Lymphocytes, $\times 10^9$/l	<4	Ref					
	4–14·9 (vs. <4)	1·110 (0·785–1·570)	0·553				
	≥15 (vs. <4)	1·102 (0·747–1·626)	0·626				
Neutrophils, $\times 10^9$/l	<8	Ref					
	≥8	1·271 (0·888–1·817)	0·189				
Platelets, $\times 10^9$/l	<150	Ref					
	≥150	0·900 (0·626–1·294)	0·569				

AIC, Akaike's Information Criteria; JCOG, Japan Clinical Oncology Group; PI, Prognostic index; HR, hazard ratio; CI, confidence interval; Ref, reference; ECOG PS, Eastern Cooperative Oncology Group performance status; LDH, lactate dehydrogenase; BUN, blood urea nitrogen.

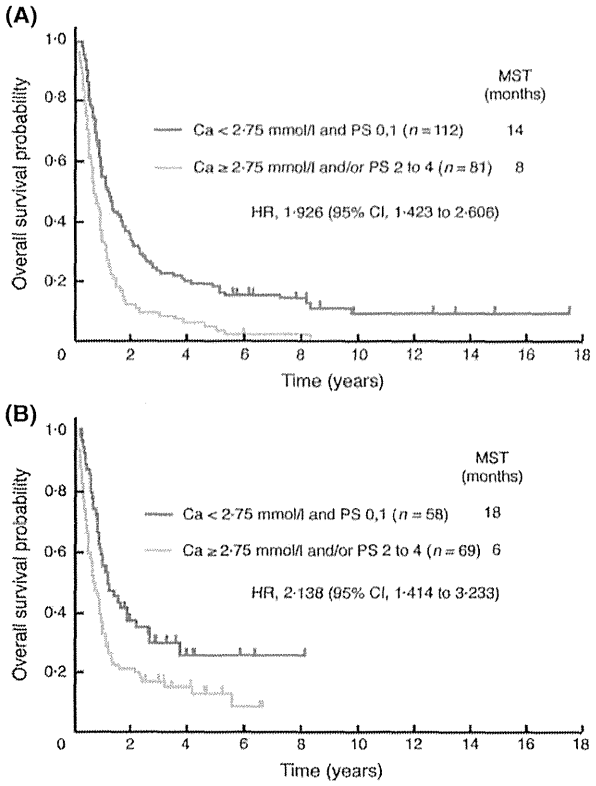

Fig 4. Overall survival of the patients in the training set and in the external validation set according to the JCOG-PI. (A) OS in the training set. The median survival time (MST) and 5-year OS were 14 months and 18% in moderate-risk group (blue line) and were 8 months and 4% in high-risk group (yellow line), respectively (B) OS in the validation set. The MST of 18 months and 6 months in the moderate- (blue line) and high-risk (yellow line) groups, respectively, and JCOG-PI showed good reproducibility.

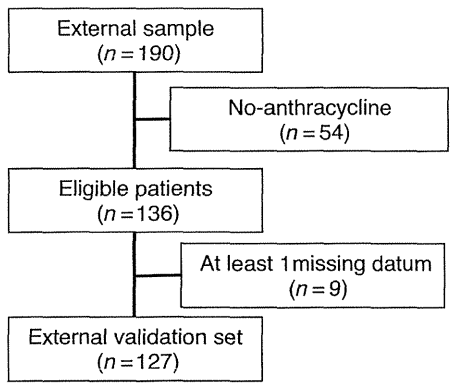

Fig 5. Patient disposition of the external validation set.

(data not shown). However, it was too difficult to evaluate the efficacy of allo-HSCT in our cohort because the disease status at transplantation and the duration from registration to transplantation were rather heterogeneous and the transition to allo-HSCT was time-dependent. To adjust this time-dependent causality, periodical data collection of, for example, indicators of treatment and time-dependent confounders, is necessary. The causal relationship between allo-HSCT and OS should be evaluated in a future prospective trial.

Several reports have revealed risk factors for ATL. In a prospective randomized trial against NHL parsimonious conducted between 1981 and 1983, Shimoyama *et al* (1988) demonstrated that poor PS and high lactate dehydrogenase levels were poor prognostic factors in patients with advanced T-cell lymphoma/leukaemia, including ATL. In a Japanese nationwide survey of 854 patients, a multivariate analysis identified major prognostic indicators of ATL as poor PS, high lactate dehydrogenase levels, age ≥40 years, >3 involved lesions and hypercalcaemia (Lymphoma Study Group, 1991). These factors were then used to construct a risk model. Additional factors reportedly associated with poor prognosis, as determined by multivariate analyses, include thrombocytopenia (Yamada *et al*, 1997), eosinophilia (Utsunomiya *et al*, 2007), bone marrow involvement (Takasaki *et al*, 2007), high interleukin (IL)5 and IL10 serum levels (Inagaki *et al*, 2006), C-C chemokine receptor 4 (CCR4) expression (Ishida *et al*, 2003), lung resistance-related protein (Ohno *et al*, 2001), *TP53* mutation (Tawara *et al*, 2006) and *CDKN2A* deletion (Yamada *et al*, 1997). Specific to chronic-type ATL, multivariate analysis has identified high lactate dehydrogenase levels, high blood urea nitrogen levels and low albumin levels as poor prognostic factors in several retrospective analyses (Shimoyama, 1994).

Recently, an ATL-PI consisting of Ann Arbour clinical stage, PS, age, serum albumin level and soluble IL2 receptor level was used to identify three risk groups for patients with acute and lymphoma types of ATL (Katsuya *et al*, 2012). However, in that study, both the ATL-PI and the risk grouping in the 1980's were constructed based on the results of questionnaires collected retrospectively; hence the treatments used were diverse and the prognostic factors might not have been evaluated homogeneously, in contrast to present study based on the three prospective trials (Lymphoma Study Group, 1991; Katsuya *et al*, 2012).

In the present study, monoclonal integration of HTLV-1 was not detected in four of 104 patients analysed. It was previously demonstrated that about 20% of patients with lymphoma-type ATL did not have monoclonal integration of HTLV-1, by Southern blot analysis, when investigating lymph node specimens (Ohshima *et al*, 1998). From this aspect, the possibility that a fraction of patients with the lymphoma type in the present study had non-ATL-peripheral T-cell lymphoma cannot be completely excluded. Further studies are required to differentiate lymphoma-type ATL from non-ATL-peripheral T-cell lymphoma by analysing monoclonal integration of the HTLV-1 provirus by Southern blot analysis or integration site-specific polymerase chain reaction.

In this study, the median age of 56 years in the training set was notably younger than that in other recent reports and that of the average population of patients with ATL. The

population investigated in the present study represents a selection of fairly young and physically fit patients with preserved organ functions. Although we expected to define a favourable prognosis group in the international PI for aggressive NHL, which consists mostly of diffuse large B-cell lymphoma, the difference in the OS between the two risk groups was small. This finding was similar to a recent retrospective nationwide survey in Japan of all patients with acute or lymphoma type at each institute (Katsuya *et al*, 2012). Therefore, the JCOG-PI could not be used to identify patients with aggressive ATL who could be treated with intensive chemotherapy alone and spared from more intensive therapy, such as allo-HSCT, as is the case with the ATL-PI (Katsuya *et al*, 2012). However, we did manage to identify patients with extremely poor prognosis despite undergoing intensive chemotherapy in clinical trials. These patients might be candidates for future trials that combine new agents or investigational strategies.

Recently, the results of several phase I and II trials using a defucosylated anti-CCR4 antibody for relapsed patients with aggressive ATL have demonstrated clinically meaningful antitumour activity and an acceptable toxicity profile (Yamamoto *et al*, 2010; Ishida *et al*, 2012a). Moreover, allo-HSCT with myeloablative and reduced intensity conditioning for patients with aggressive ATL has been reported to cure diseases associated with the graft-*versus*-ATL effect, despite the high transplant-related mortality (Hishizawa *et al*, 2010; Ishida *et al*, 2012b; Kanda *et al*, 2012). To further improve patient outcomes, two trials are ongoing in Japan: a phase II trial of VCAP-AMP-VECP followed by allo-HSCT with myeloablative conditioning for patients aged <55 years with aggressive ATL (JCOG 0907), and a randomized phase II trial of VCAP-AMP-VECP with or without anti-CCR4 antibody (Jo *et al*, 2013).

In conclusion, patients with lymphoma-type ATL who survived >5 years might have been cured, which is in contrast to long-term survivors with acute or unfavourable chronic type. The JCOG-PI, based on corrected calcium levels and PS, is a simple and valuable tool for identifying patients with aggressive ATL having extremely poor prognosis in clinical trials, and it will be useful for the design of future studies combining new drugs or investigational strategies.

Acknowledgements

The authors are grateful to all the physicians and data managers at the institutions of Japan Clinical Oncology Group (JCOG) – Lymphoma Study Group (LSG). The authors thank all the members of the JCOG Data Centre. This work was supported by the National Cancer Centre Research and Development Fund Nos. 23-A-16 and 23-A-17, Grants-in-Aid Nos. 2S-1, 5S-1, 8S-1, 11S-1, 14S-1, 17S-1, 20S-1, 20S-6, 1-1, 4-5, 7-29, 9-10, 15-11, 16-12, 19-8 and 21-6-3 and a grant (H23-gan rinsho-ippan-022) for the Cancer Research from the Ministry of Health, Labour and Welfare of Japan (1990 to present), for the Second-Term Ten-Year Strategy for Cancer Control from the Ministry of Health and Welfare (1994 to 2004) and for Basic Research from the Science and Technology Agency (1991 to 1993).

Authorship

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

Disclosure

The authors report no potential conflict of interest.

References

Akaike, H. (1973) Information theory and an extension of the maximum likelihood principle. In: *Proceedings of the 2nd International Symposium on Information theory* (ed. by B.N. Petrov & F. Caski), pp. 267–281. Akademinai, Budapest.

Clopper, C.J. & Pearson, E.S. (1934) The use of confidence or fiducial limits illustrated in the case of the binomial. *Biometrika*, **26**, 404–413.

Gallamini, A., Stelitano, C., Calvi, R., Bellei, M., Mattei, D., Vitolo, U., Martelli, M., Brusamolino, E., Iannitto, E., Zaja, F., Cortelozzo, S., Rigacci, L., Devizzi, L., Todeschini, G., Santini, G., Brugiatelli, M., Federico, M. & Linfomi, I.I. (2004) Peripheral T-cell lymphoma unspecified (PTCL-U): a new prognostic model from a retrospective multicentric clinical study. *Blood*, **103**, 2474–2479.

Hinuma, Y., Nagata, K., Hanaoka, M., Nakai, M., Matsumoto, T., Kinoshita, K.I., Shirakawa, S. & Miyoshi, I. (1981) Adult T-cell leukemia: antigen in an ATL cell line and detection of antibodies to the antigen in human sera. *Proceedings of the National Academy of Sciences of the United States of America*, **78**, 6476–6480.

Hishizawa, M., Kanda, J., Utsunomiya, A., Taniguchi, S., Eto, T., Moriuchi, Y., Tanosaki, R., Kawano, F., Miyazaki, Y., Masuda, M., Nagafuji, K., Hara, M., Takanoshi, M., Kai, S., Suzuki, R., Kawase, T., Matsuo, K., Nagamura-Inoue, T., Kato, S., Sakamaki, H., Morishima, Y., Okamura, J., Ichinohe, T. & Uchiyama, T. (2010) Transplantation of allogeneic hematopoietic stem cells for adult T-cell leukemia: a nationwide retrospective study. *Blood*, **116**, 1369–1376.

Inagaki, A., Ishida, T., Ishii, T., Komatsu, H., Iida, S., Ding, J., Yonekura, K., Takeuchi, S., Takatsuka, Y., Utsunomiya, A. & Ueda, R. (2006) Clinical significance of serum Th1-, Th2- and regulatory T cells-associated cytokines in adult T-cell leukemia/lymphoma: high interleukin-5 and -10 levels are significant unfavorable prognostic factors. *International Journal of Cancer*, **118**, 3054–3061.

Ishida, T., Utsunomiya, A., Iida, S., Inagaki, H., Takatsuka, Y., Kusumoto, S., Takeuchi, G., Shimizu, S., Ito, M., Komatsu, H., Wakita, A., Eimoto, T., Matsushima, K. & Ueda, R. (2003) Clinical significance of CCR4 expression in adult T-cell leukemia/lymphoma: its close association with skin involvement and unfavorable outcome. *Clinical Cancer Research*, **9**, 3625–3634.

Ishida, T., Joh, T., Uike, N., Yamamoto, K., Utsunomiya, A., Yoshida, S., Saburi, Y., Miyamoto,

T., Takemoto, S., Suzushima, H., Tsukasaki, K., Nosaka, K., Fujiwara, H., Ishitsuka, K., Inagaki, H., Ogura, M., Akinaga, S., Tomonaga, M., Tobinai, K. & Ueda, R. (2012a) Defucosylated anti-CCR4 monoclonal antibody (KW-0761) for relapsed adult T-cell leukemia-lymphoma: a multicenter phase II study. *Journal of Clinical Oncology*, **30**, 837–842.

Ishida, T., Hishizawa, M., Kato, K., Tanosaki, R., Fukuda, T., Taniguchi, S., Eto, T., Takatsuka, Y., Miyazaki, Y., Moriuchi, Y., Hidaka, M., Akashi, K., Uike, N., Sakamaki, H., Morishima, Y., Kato, K., Suzuki, R., Nishiyama, T. & Utsunomiya, A. (2012b) Allogeneic hematopoietic stem cell transplantation for adult T-cell leukemia-lymphoma with special emphasis on preconditioning regimen: a nationwide retrospective study. *Blood*, **120**, 1734–1741.

Jo, T., Ishida, T., Takemoto, S., Suzushima, H., Uozumi, K., Yamamoto, K., Uike, N., Saburi, Y., Nosaka, K., Utsunomiya, A., Tobinai, K., Fujiwara, H., Ishitsuka, K., Yoshida, S., Taira, N., Moriuchi, Y., Imada, K., Miyamoto, T., Tomonaga, M. & Ueda, R. (2013) Randomized phase II study of mogamulizumab (KW-0761) plus VCAP-AMP-VECP (mLSG15) versus mLSG15 alone for newly diagnosed aggressive adult T-cell leukemia-lymphoma (ATL). *Journal of Clinical Oncology*, **31**, 519s, abstract 8506.

Kalbfleisch, J.D. & Prentice, R.L. (2002) *The Statistical Analysis of Failure Time Data*, 2nd edn. John Wiley & Sons Inc, New York, NY.

Kanda, J., Hishizawa, M., Utsunomiya, A., Taniguchi, S., Eto, T., Moriuchi, Y., Tanosaki, R., Kawano, F., Miyazaki, Y., Masuda, M., Nagafuji, K., Hara, M., Takanashi, M., Kai, S., Atsuta, Y., Suzuki, R., Kawase, T., Matsuo, K., Nagamura-Inoue, T., Kato, S., Sakamaki, H., Morishima, Y., Okamura, J., Ichinohe, T. & Uchiyama, T. (2012) Impact of graft-versus-host disease on outcome after allogeneic hematopoietic cell transplantation for adult T-cell leukemia: a retrospective cohort study. *Blood*, **119**, 2141–2148.

Katsuya, H., Yamanaka, T., Ishitsuka, K., Utsunomiya, A., Sasaki, H., Hanada, S., Eto, T., Moriuchi, Y., Saburi, Y., Miyahara, M., Sueoka, E., Uike, N., Yoshida, S., Yamashita, K., Tsukasaki, K., Suzushima, H., Ohno, Y., Matsuoka, H., Jo, T., Suzumiya, J. & Tamura, K. (2012) Prognostic index for acute- and lymphoma-type adult T-cell leukemia/lymphoma. *Journal of Clinical Oncology*, **30**, 1635–1640.

Lymphoma Study Group (1991) Major prognostic factors of patients with adult T-cell leukemia-lymphoma: a cooperative study-Lymphoma Study Group (1984-1987). *Leukemia Research*, **15**, 81–90.

Miyoshi, I., Kubonishi, I., Yoshimoto, S., Akagi, T., Ohtsuki, Y., Shiraishi, Y., Nagata, K. & Hinuma, Y. (1981) Type C virus particles in a cord T-cell line derived by co-cultivating normal cord leukocytes and human leukaemic T cells. *Nature*, **294**, 770–771.

Ohno, N., Tani, A., Uozumi, K., Hanada, S., Furukawa, T., Akiba, S., Sumizawa, T., Utsunomiya, A., Arima, T. & Akiyama, S. (2001) Expression of functional lung resistance-related protein predicts poor outcome in adult T-cell leukemia. *Blood*, **98**, 1160–1165.

Ohshima, K., Suzumiya, J., Sato, K., Kanda, M., Sugihara, M., Haraoka, S., Takeshita, M. & Kikuchi, M. (1998) Nodal T-cell lymphoma in an HTLV-1-endemic area: proviral HTLV-1 DNA, histological classification and clinical evaluation. *British Journal of Haematology*, **101**, 703–711.

Oshiro, A., Tagawa, H., Ohshima, K., Karube, K., Uike, N., Tashiro, Y., Utsunomiya, A., Masuda, M., Takasu, N., Nakamura, S., Morishima, Y. & Seto, M. (2006) Identification of subtype-specific genomic alterations in aggressive adult T-cell leukemia/lymphoma. *Blood*, **107**, 4500–4507.

Poiesz, B.J., Ruscetti, F.W., Gazdar, A.F., Bunn, P.A., Minna, J.D. & Gallo, R.C. (1980) Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proceedings of the National Academy of Sciences of the United States of America*, **77**, 7415–7419.

Shimoyama, M. (1991) Diagnostic criteria and classification of clinical subtypes of adult T-cell leukaemia-lymphoma: a report from the Lymphoma Study Group (1984-87). *British Journal of Haematology*, **79**, 428–437.

Shimoyama, M. (1994) Chemotherpy of ATL. In: *Adult T-Cell Leukemia* (ed. by Takatsuki, K.), pp. 221–237. Oxford University Press, Oxford, United Kingdom.

Shimoyama, M., Ota, K., Kikuchi, M., Yunoki, K., Konda, S., Takatsuki, K., Ichimaru, M., Ogawa, M., Kimura, I. & Tominaga, S. (1988) Chemotherapeutic results and prognostic factors of patients with advanced non-Hodgkin's lymphoma treated with VEPA or VEPA-M. *Journal of Clinical Oncology*, **6**, 128–141.

Takasaki, Y., Iwanaga, M., Tsukasaki, K., Kusano, M., Sugahara, K., Yamada, Y., Kamihira, S., Ikeda, S. & Tomonaga, M. (2007) Impact of visceral involvement and blood cell count abnormalities on survival in adult T-cell leukemia/lymphoma (ATLL). *Leukemia Research*, **31**, 751–757.

Tawara, M., Hogerzeil, S.J., Yamada, Y., Yakasaki, Y., Soda, H., Hasegawa, H., Murata, K., Ikeda, S., Imaizumi, Y., Sugahara, K., Tsuruda, K., Tsukasaki, K., Tomonaga, M., Hirakata, Y. & Kamihira, S. (2006) Impact of p53 aberration on the progression of adult T-cell leukemia/lymphoma. *Cancer Letters*, **234**, 249–255.

The International Non-Hodgkin's Lymphoma Prognostic Factor Project (1993) A predictive model for aggressive non-Hodgkin's lymphoma: the International Non-Hodgkin's Lymphoma Prognostic Factors Project. *New England Journal of Medicine*, **329**, 987–994.

Tobinai, K. & Watanabe, T. (2004) Adult T-cell leukemia-lymphoma. In: *Clinical Oncology* (ed. by M.D. Abeloff, J.O. Armitage, J.E. Niederhuber & M.C.B. Kastan), 3rd edn, pp. 3109–3130. Elsevier Churchill Livingstone, Philadelphia, PA

Tobinai, K., Shimoyama, M., Inoue, S., Takayasu, S., Kikuni, C., Kozuru, M., Oda, S. & Nakajima, H. (1992) Phase I study of YK-176 (2′-deoxycoformycin) in patients with adult T-cell leukemia-lymphoma. The DCF Study Group. *Japanese Journal of Clinical Oncology*, **22**, 164–171.

Tobinai, K., Shimoyama, M., Minato, K., Shirakawa, S., Kobayashi, T. & Hotta, T. (1994) Japan Clinical Oncology Group phase II trial of second-generation "LSG4 protocol" in aggressive T- and B-lymphoma: a new predictive model for T- and B-lymphoma. *Proceedings, American Society of Clinical Oncology*, **13**, 378a.

Tsukasaki, K., Tobinai, K., Shimoyama, M., Kozuru, M., Uike, N., Yamada, Y., Tomonaga, M., Araki, K., Kasai, M., Takatsuki, K., Tara, M., Mikuni, C. & Hotta, T.; Lymphoma Study Group of the Japan Clinical Oncology Group (2003) Deoxycoformycin-containing combination chemotherapy for adult T-cell leukemia-lymphoma: Japan Clinical Oncology Group Study (JCOG9109). *International Journal of Hematology*, **77**, 164–170.

Tsukasaki, K., Utsunomiya, A., Fukuda, H., Shibata, T., Fukushima, T., Takatsuka, Y., Ikeda, S., Masuda, M., Nagoshi, H., Ueda, R., Tamura, K., Sano, M., Momita, S., Yamaguchi, K., Kawano, F., Hanada, S., Tobinai, K., Shimoyama, M., Hotta, T. & Tomonaga, M.; Japan Clinical Oncology Group Study JCOG9801 (2007) VCAP-AMP-VECP compared with biweekly CHOP for adult T-cell leukemia-lymphoma: Japan Clinical Oncology Group Study JCOG9801. *Journal of Clinical Oncology*, **25**, 5458–5464.

Uchiyama, T., Yodoi, J., Sagawa, K., Takatsuki, K. & Uchino, H. (1977) Adult T-cell leukemia: clinical and hematologic features of 16 cases. *Blood*, **50**, 481–492.

Utsunomiya, A., Ishida, T., Inagaki, A., Ishii, T., Yano, H., Komatsu, H., Iida, S., Yonekura, K., Takeuchi, S., Takatsuka, Y. & Ueda, R. (2007) Clinical significance of a blood eosinophilia in adult T-cell leukemia/lymphoma: a blood eosinophilia is a significant unfavorable prognostic factor. *Leukemia Research*, **31**, 915–920.

Watanabe, T., Kinoshita, T., Itoh, K., Yoshimura, K., Ogura, M., Kagami, Y., Yamaguchi, M., Kurosawa, M., Tsukasaki, K., Kasai, M., Tobinai, K., Kaba, H., Mukai, K., Nakamura, S., Ohshima, K., Hotta, T. & Shimoyama, M. (2010) Pretreatment total serum protein is a significant prognostic factor for the outcome of patients with peripheral T/natural killer-cell lymphomas. *Leukaemia & Lymphoma*, **51**, 813–821.

Yamada, Y., Hatta, Y., Murata, K., Sugawara, K., Ikeda, S., Mine, M., Maeda, T., Hirakata, Y., Kamihira, S., Tsukasaki, K., Ogawa, S., Hirai, H., Koeffler, H.P. & Tomonaga, M. (1997) Deletions of p15 and/or p16 genes as a poor-prognosis factor in adult T-cell leukemia. *Journal of Clinical Oncology*, **15**, 1778–1785.

Yamada, Y., Tomonaga, M., Fukuda, H., Hanada, S., Utsunomiya, A., Tara, M., Sano, M., Ikeda, S., Takatsuki, K., Kozuru, M., Araki, K., Kawano, F., Niimi, M., Tobinai, K., Hotta, T. & Shimoyama, M. (2001) A new G-CSF supported combination chemotherapy, LSG15, for adult T-

cell leukaemia-lymphoma: Japan Clinical Oncology Group Study 9303. *British Journal of Haematology*, **113**, 375–382.

Yamamoto, K., Utsunomiya, A., Tobinai, K., Tsukasaki, K., Uike, N., Uozumi, K., Yamaguchi, K., Yamada, Y., Hanada, S., Tamura, K., Nakamura, S., Inagaki, H., Ohshima, K., Kiyoi, H., Ishida, T., Matsushima, K., Akinaga, S., Ogura, M., Tomonaga, M. & Ueda, R. (2010) 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. *Journal of Clinical Oncology*, **28**, 1591–1598.

Yoshida, M., Miyoshi, I. & Hinuma, Y. (1982) Isolation and characterization of retrovirus from cell lines of human adult T cell leukemia and its implication in the disease. *Proceedings of the National Academy of Sciences of the United States of America*, **79**, 2031–2035.