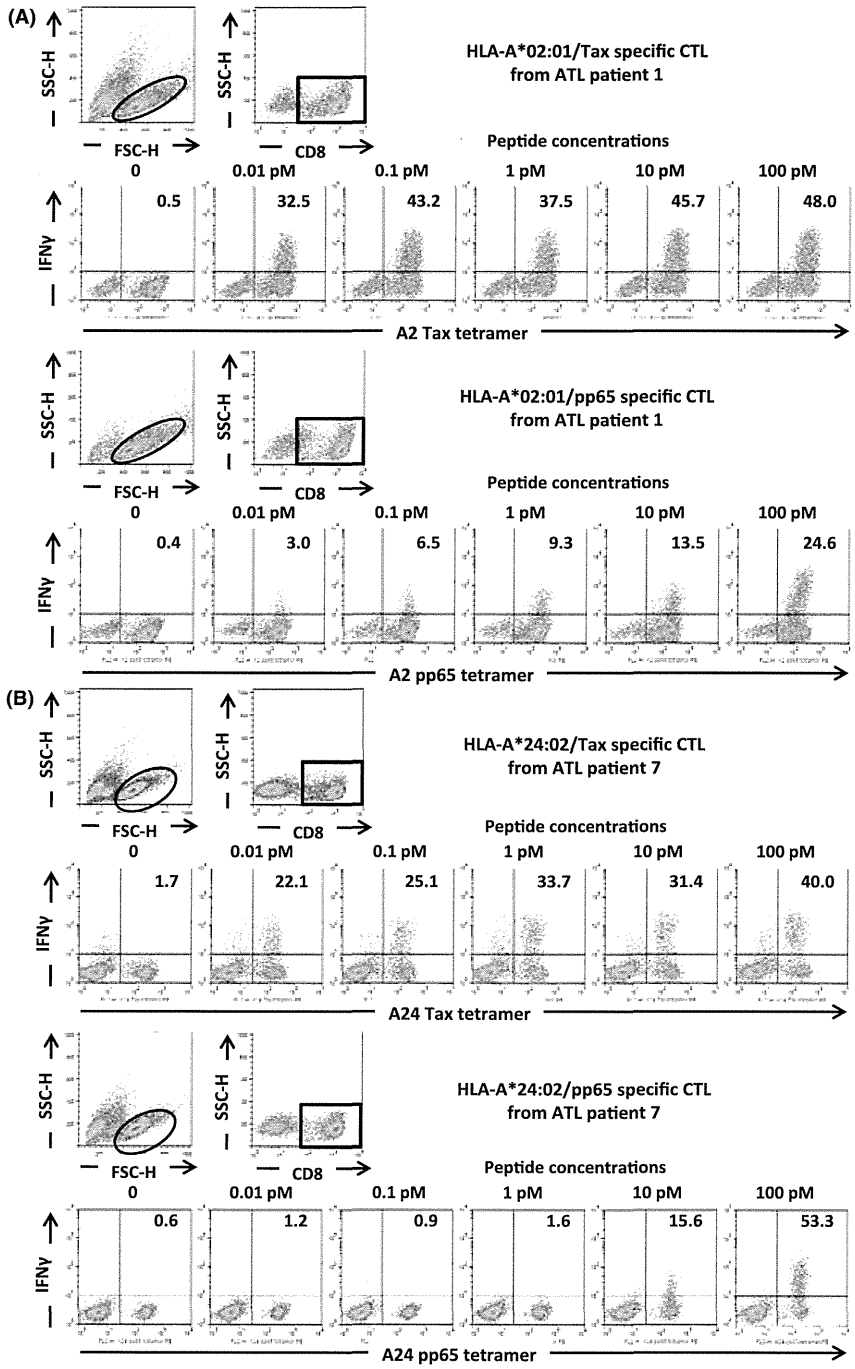

Fig. 2. T-cell receptor avidity of Tax-CTL for Tax epitope peptides. (A) PBMC from adult T-cell leukemia/lymphoma (ATL) patient 1 were stimulated by Tax11–19 peptide, and the expanded cells were then cultured with serial concentration of the cognate peptide. Flow cytometric analyses of those cells are presented. The lymphocyte population was identified by FSC-H and SSC-H levels, and CD8-positive cells gated. These were then plotted according to human leukocyte antigen (HLA)-A*02:01/Tax tetramer-positivity and interferon gamma (IFN-γ) production. The percentages of IFN-γ-producing cells relative to the entire population of HLA-A*02:01/Tax-positive cells are indicated in each panel (upper panels). PBMC from ATL patient 1 were also stimulated by CMV-pp65 495–503 peptide, and then restimulated with the cognate peptide, and flow cytometric analyses of those cells are presented in the same manner as above. The percentages IFN-γ-producing cells relative to the entire population of HLA-A*02:01/CMV-pp65-positive cells are indicated in each panel (lower panels) (B) PBMC from ATL patient 7 were stimulated with Tax301–309 peptide, and then restimulated as above: HLA-A*24:02/Tax301–309 tetramer positivity and IFN-γ production (upper panels). PBMC from ATL patient 7 stimulated with CMV-pp65 495–503 peptide, and treated as above. Each result represents three independent experiments.

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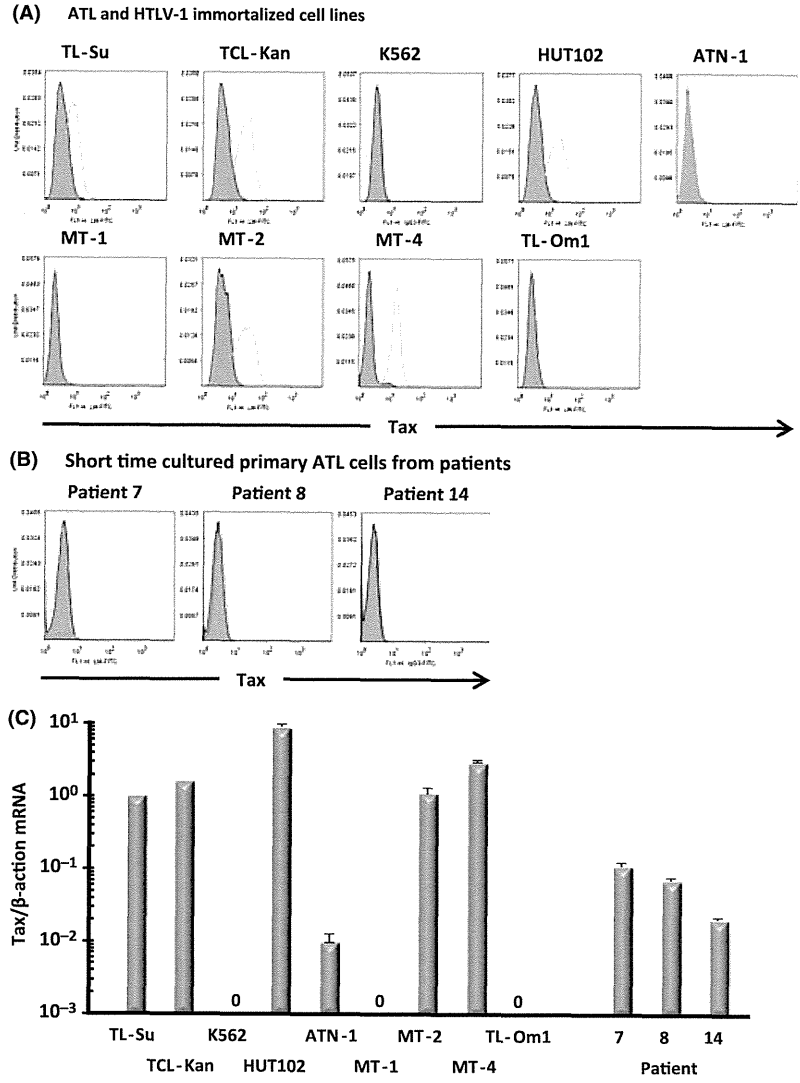

Fig. 3. Expression of human T-lymphotropic virus type 1 (HTLV-1) Tax in adult T-cell leukemia/lymphoma (ATL) cells. (A) Tax expression in ATL cell lines, HTLV-1-immortalized lines and K562 were analyzed by flow cytometry. The cells lines were stained with anti-Tax mAb (blank histograms) or isotype control mAb (filled histograms). (B) Tax expression in short-term cultured ATL cells from patients analyzed by flow cytometry. (C) *Tax* expression in the cell lines and short-term cultured ATL cells from patients analyzed by quantitative RT-PCR by dividing the *Tax* expression level by *β-actin*, resulting in a *Tax/β-actin* mRNA ratio with the expression level in TL-Su set at unity. Columns, mean of triplicate experiments; bars, standard deviation.

(Fig. 7A). *Tax* expression in ATL cells from tumor masses was almost 100-fold higher than in the blood.

ATL mice from patient 12 presented with marked hepatosplenomegaly, but few tumor cells in the blood. *Tax/human CD4* mRNA values of blood cells, liver, and spleen cell suspensions were 0.01337 ± 0.00083, 0.05277 ± 0.00805 and 0.08323 ± 0.00080, respectively. Again, no *Tax/human CD4* mRNA could be detected in bone marrow cells (Fig. 7B).

Adult T-cell leukemia/lymphoma mice from patient 13 also presented with marked hepatosplenomegaly, but also with tumor infiltration into blood and bone marrow. *Tax/human CD4* mRNA values of blood cells, liver, spleen cell suspensions and bone marrow cells were 0.01013 ± 0.00102, 0.12742 ± 0.01524, 0.15411 ± 0.01612 and 0.28881 ± 0.07319, respectively (Fig. 7C).

These observations are consistent with other results from the present study that Tax expression is observed predominantly in actively cycling ATL cells, whereas most primary ATL cells in the peripheral blood are in a quiescent state. Thus, only ATL cells present at the site of active cell proliferation, such as in the tumor masses, liver or spleen, strongly express Tax, but this factor is minimally expressed by the tumor cells in a quiescent state, such as in the blood.

Discussion

The significant findings in the present study are as follows. The efficiency of *in vitro* Tax-CTL expansion was dependent on the stage of disease development following HTLV-1 infection. HTLV-1 Tax-CTL expanded *in vitro* could recognize HLA/Tax-peptide complexes on autologous ATL cells, the Tax expression of which was so low as to be detectable only by RT-PCR and not by flow cytometry. Tax recognition resulted in the production of IFN-γ and killing of the target cells. In an assay of TCR avidity, both HLA-A*02:01-restricted and HLA-A*24:02-restricted Tax-CTL responded to as little as 0.01 pM of the epitope peptide, a concentration much lower than required for recognition of any other viral or tumor antigens. This documents the extremely high TCR avidity of Tax-CTL, which is presumably one of the reasons why these CTL could recognize and kill the autologous ATL cells, despite their very low Tax expression. To the best of our knowledge, this is the first report of Tax-specific CTL from ATL patients specifically recognizing and killing autologous tumor cells that express the Tax antigen. Earlier studies examined the responses of CD8 cells against autologous cells from ATL, HTLV-1-associated myelopathy/tropical spastic paraparesis patients or HTLV-1

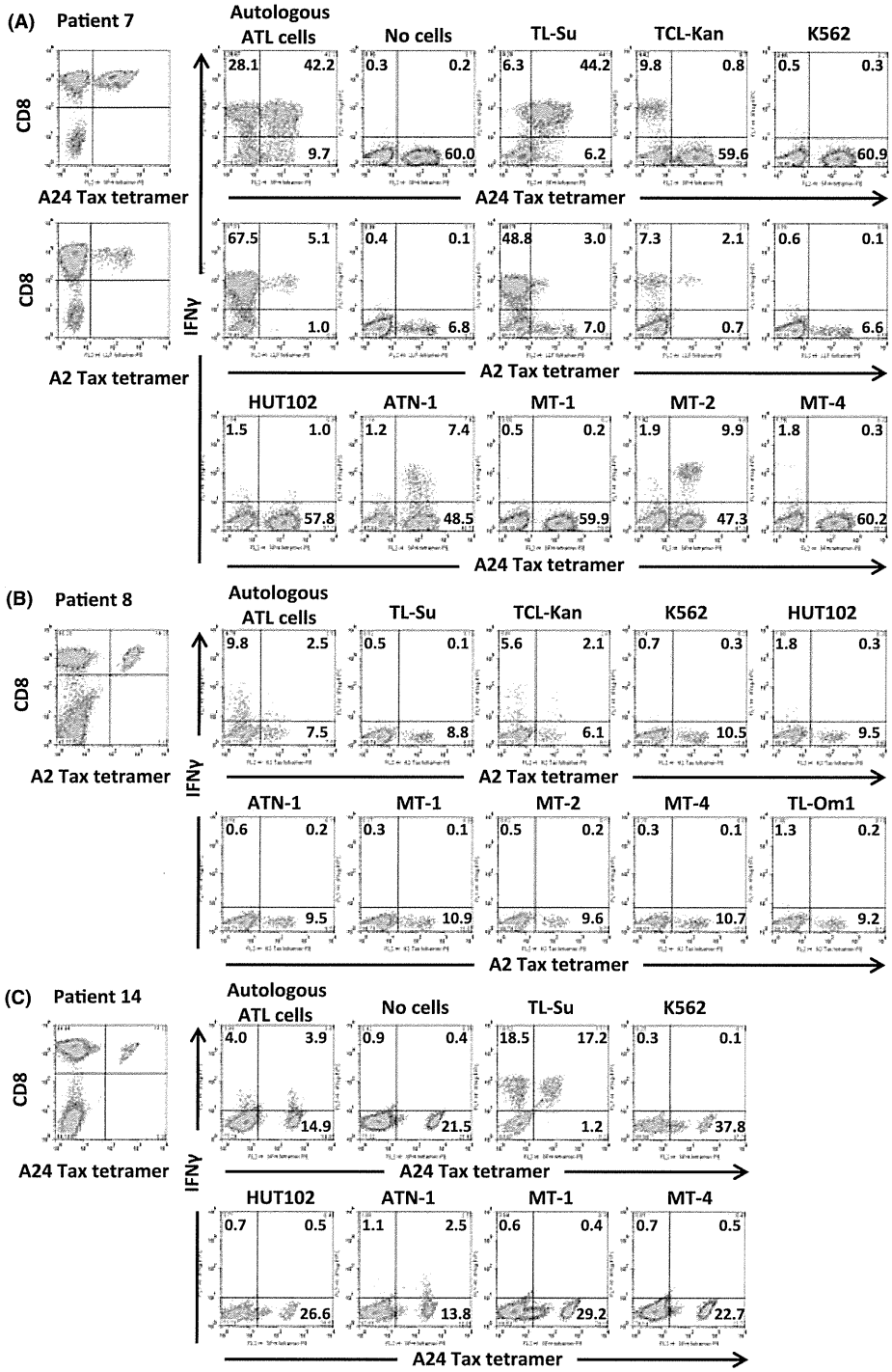

Fig. 4. Tax-specific CTL responses against autologous adult T-cell leukemia/lymphoma (ATL) cells. (A) PBMC from patient 7 were stimulated with human leukocyte antigen (HLA)-A*24:02 restricted Tax301–309 peptide, and the resulting CTL were expanded (upper-left panel). In this culture, HLA-A2-restricted Tax11–19 specific CTL were also expanded (middle-left panel). The expanded cells were co-cultured with autologous ATL cells, ATL cell lines, human T-lymphotropic virus type 1 (HTLV-1)-immortalized lines and K562 (all CD8-negative) for 4 h. CD8-positive cells are plotted according to HLA-A*24:02/Tax301–309 or HLA-A*02:01/zax11–19 tetramer-positivity and interferon gamma (IFN-γ) production, and the percentages in each quadrant are presented in the panels. (B) PBMC from ATL patient 8 at chronic stage were stimulated by Tax11–19 peptide, and the expanded cells co-cultured with the same range of cells as in (A). CD8-positive cells are plotted by HLA-A*0201/Tax11–19 tetramer positivity and IFN-γ production. The HLA-A*02:01/Tax11–19 tetramer recognized HLA-A*02:07-restricted Tax11–19 specific CTL. (C) PBMC from ATL patient 14 were stimulated with Tax301–309 peptide, and treated as in (A, B) above. Each result represents three independent experiments.

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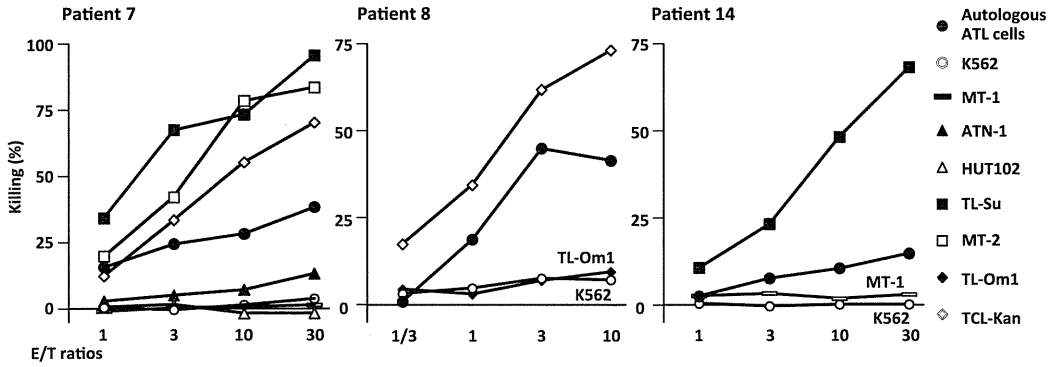

Fig. 5. Lysis of autologous adult T-cell leukemia/lymphoma (ATL) cells by Tax-specific CTL. Tax301–309 peptide-expanded cells from ATL patient 7 (left panel), Tax11–19 peptide-expanded cells from patient 8 (middle panel) and Tax301–309-stimulated patient 14 (right panel) were evaluated for cytotoxicity by a standard 4-h chromium[51] release assay. Lysis was restricted to human leukocyte antigen (HLA)-A*24:02 or HLA-A2 and Tax-positive target cells. Each result represents three independent experiments.

AC. However, in these reports, the nature of the antigens recognized by the CTL is not determined, or the target cells are HTLV-1-infected T-cell lines rather than primary ATL tumor cells. In contrast, the present study clearly demonstrated that Tax antigen expressed by ATL cells was a significant target for CTL from ATL patients in an autologous setting. In addition, Tax expression was observed only in actively cycling ATL cells. This could only be noticed in primary ATL cells from patients, because established ATL cell lines or HTLV-1-immortalized lines, which are commonly used for many types of experiments, are, of course, continuously dividing. These findings collectively demonstrate that the main obstacle to successful immunotherapy targeting Tax, with its very limited expression in ATL cells, could be overcome. Whether primary ATL cells express Tax has been examined in several other studies using tumor cells from patients' blood.[11,12,33] However, the present study demonstrated that most primary ATL cells in the blood are in a quiescent state, in which they express little or no Tax. Proliferating ATL cells are probably mostly to be found in lymph nodes in humans, not in the blood,[35] and these should, therefore, express substantial levels of Tax. Thus, the present findings indicate that Tax is a promising molecular target for immunotherapy in ATL patients, such as adoptive T-cell therapy and/or active vaccination.

As mentioned above, the efficiency of *in vitro* Tax-CTL expansion depended on HTLV-1 disease status. There was a trend towards superior expansion of Tax-CTL in HTLV-1 AC, ATL patients with the indolent variant and ATL patients who were in treatment-induced remission compared with newly diagnosed ATL patients with the aggressive variant. These observations indicate that host immune responses against Tax

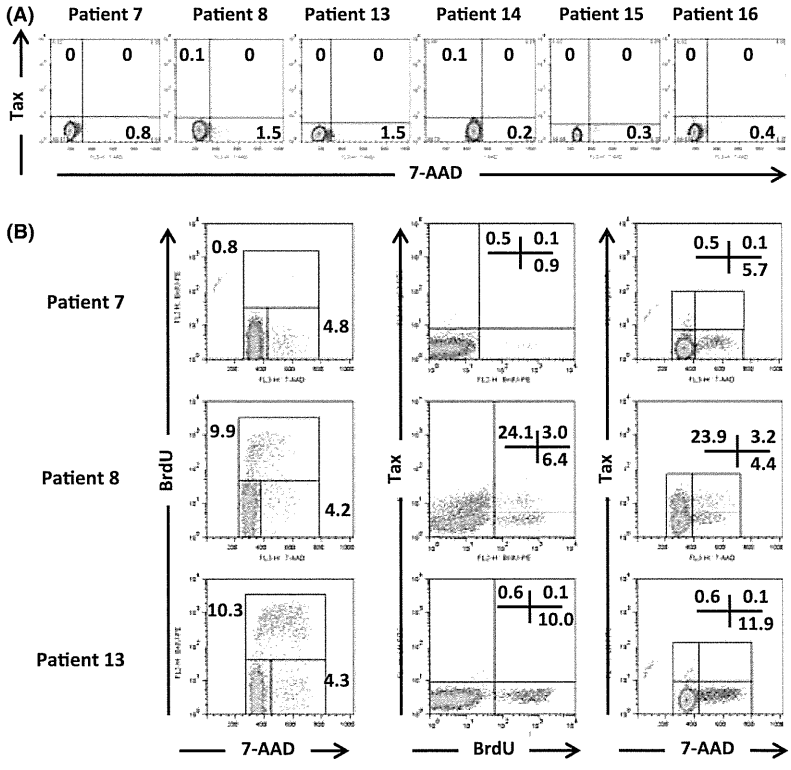

Fig. 6. Tax expression in adult T-cell leukemia/lymphoma (ATL) cells induced by short-term culture. (A) Lack of Tax expression in primary ATL cells from peripheral blood of patients 7, 8, 13, 14, 15 and 16. Cells were in a quiescent state as determined by 7-ADD staining. (B) Cell cycle status and Tax expression of short-term cultured primary ATL cells. Tax expression was induced when cells were actively cycling. Each result represents three independent experiments.

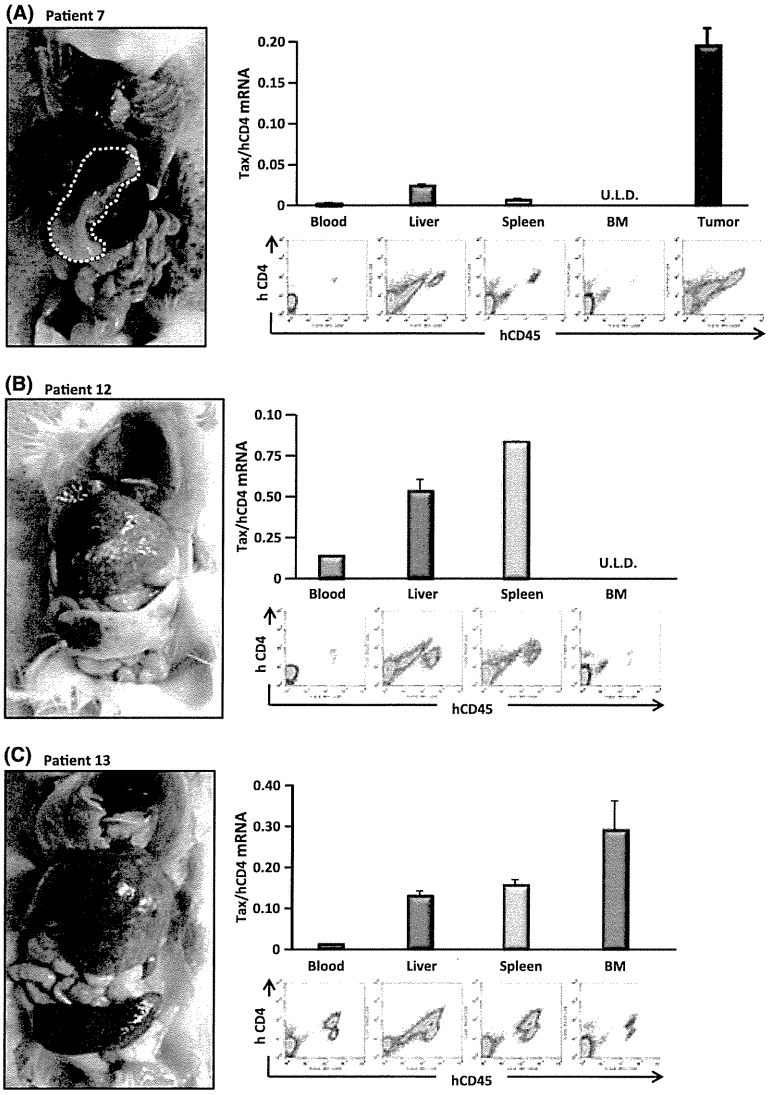

Fig. 7. Tax expression in primary adult T-cell leukemia/lymphoma (ATL) cell-bearing NOG mice. Tax expression of ATL cells in each affected organ of NOG mice bearing primary ATL cells from patient 7 (A), 12 (B) and 13 (C) were evaluated. NOG mice with cells from patient 7 presented with large intraperitoneal tumor masses demarcated by the white dotted lines. *Tax/human CD4* mRNA values of the cells from each organ are presented as bar graphs, where the value for TL-Su was set at unity. Flow cytometric analysis of the cells from each organ determined by human CD45 and CD4 expression is presented. Columns, mean of triplicate experiments; bars, standard deviation. BM, bone marrow; U.L.D., under limit of detection.

play an important role in maintaining the stable status of HTLV-1 AC, indolent ATL patients and ATL in remission. In addition, quantitative and/or functional reduction of Tax-CTL should lead to progression from HTLV-1 AC to ATL, or from indolent to aggressive ATL, or to relapse in ATL patients. Furthermore, the present observations suggest that restoration of substantial anti-Tax responses in some appropriate manner will lead to improvement of ATL disease status.

The efficient expansion of Tax-CTL from PBMC of ATL patients in remission suggests that reducing the number of tumor cells before Tax-targeted immunotherapy could be a crucial factor for successful induction/augmentation of antigen-specific CD8-positive CTL. We have reported that the humanized anti-CCR4 mAb KW-0761 (mogamulizumab) exerted clinically significant antitumor activity in relapsed ATL patients.[36,37] In addition, consistent with the fact that CCR4 is expressed not only on Th2 cells, but also on Treg cells,[21,38–40] KW-0761 treatment resulted in a significant and lasting decrease in CD4+CD25+FOXP3+ cells, including both the tumor cells and endogenous non-ATL Treg cells.[37] Reduction or suppression of Treg cells is expected to be a promising strategy for boosting antitumor immunity in cancer patients, as observed in studies with ipilimumab.[41,42] In fact, Tax-CTL were efficiently expanded from PBMC of patients 2 and 3 who were in CR after KW-0761 treatment. Thus, combining Tax-targeted immunotherapy following reduction of ATL cells and endogenous Treg cell depletion by KW-0761 treatment would be an ideal strategy for ATL immunotherapy.

The efficient expansion of Tax-CTL from PBMC of patients in remission after allogeneic HSCT is consistent with the report that Tax-specific CD8-positive T cells contribute to graft-versus-ATL effects.[13,43] Therefore, Tax-targeted immunotherapy after allogeneic HSCT should be therapeutically effective without increased graft-versus-host disease, which is a frequent and serious complication of this modality.

In conclusion, the present study not only provides a strong rationale for selecting Tax as a possible target for ATL immunotherapy but also contributes to our understanding of the immunopathogenesis driving progression from HTLV-1 AC to ATL, and to devising strategies for preventing this by targeting Tax.

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References

1 Uchiyama T, Yodoi J, Sagawa K, Takatsuki K, Uchino H. Adult T-cell leukemia: clinical and hematologic features of 16 cases. *Blood* 1977; **50**: 481–92.
2 Matsuoka M, Jeang KT. Human T-cell leukaemia virus type 1 (HTLV-1) infectivity and cellular transformation. *Nat Rev Cancer* 2007; **7**: 270–80.
3 Hino S, Sugiyama H, Doi H *et al.* Breaking the cycle of HTLV-I transmission via carrier mothers' milk. *Lancet* 1987; **2**: 158–9.
4 Ishida T, Ueda R. Immunopathogenesis of lymphoma: focus on CCR4. *Cancer Sci* 2011; **102**: 44–50.
5 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* 1991; **79**: 428–37.
6 Akagi T, Ono H, Shimotohno K. Characterization of T cells immortalized by Tax1 of human T-cell leukemia virus type 1. *Blood* 1995; **86**: 4243–9.
7 Kannagi M, Sugamura K, Kinoshita K, Uchino H, Hinuma Y. Specific cytolysis of fresh tumor cells by an autologous killer T cell line derived from an adult T cell leukemia/lymphoma patient. *J Immunol* 1984; **133**: 1037–41.
8 Arnulf B, Thorel M, Poirot Y *et al.* Loss of the ex vivo but not the reinducible CD8+ T-cell response to Tax in human T-cell leukemia virus type 1-infected patients with adult T-cell leukemia/lymphoma. *Leukemia* 2004; **18**: 126–32.
9 Kannagi M, Sugamura K, Sato H, Okochi K, Uchino H, Hinuma Y. Establishment of human cytotoxic T cell lines specific for human adult T cell leukemia virus-bearing cells. *J Immunol* 1983; **130**: 2942–6.
10 Kannagi M, Harada S, Maruyama I *et al.* Predominant recognition of human T cell leukemia virus type I (HTLV-I) pX gene products by human CD8+ cytotoxic T cells directed against HTLV-I-infected cells. *Int Immunol* 1991; **3**: 761–7.
11 Takeda S, Maeda M, Morikawa S *et al.* Genetic and epigenetic inactivation of tax gene in adult T-cell leukemia cells. *Int J Cancer* 2004; **109**: 559–67.
12 Kannagi M, Harashima N, Kurihara K *et al.* Tumor immunity against adult T-cell leukemia. *Cancer Sci* 2005; **96**: 249–55.
13 Harashima N, Kurihara K, Utsunomiya A *et al.* Graft-versus-Tax response in adult T-cell leukemia patients after hematopoietic stem cell transplantation. *Cancer Res* 2004; **64**: 391–9.
14 Ishida T, Utsunomiya A, Iida S *et al.* Clinical significance of CCR4 expression in adult T-cell leukemia/lymphoma: its close association with skin involvement and unfavorable outcome. *Clin Cancer Res* 2003; **9**: 3625–34.
15 Ri M, Iida S, Ishida T *et al.* Bortezomib-induced apoptosis in mature T-cell lymphoma cells partially depends on upregulation of Noxa and functional repression of Mcl-1. *Cancer Sci* 2009; **100**: 341–8.
16 Lozzio CB, Lozzio BB. Human chronic myelogenous leukemia cell line with positive Philadelphia chromosome. *Blood* 1975; **45**: 321–34.
17 Lee B, Tanaka Y, Tozawa H. Monoclonal antibody defining tax protein of human T-cell leukemia virus type-I. *Tohoku J Exp Med* 1989; **157**: 1–11.
18 Ishida T, Iida S, Akatsuka Y *et al.* The CC chemokine receptor 4 as a novel specific molecular target for immunotherapy in adult T-Cell leukemia/lymphoma. *Clin Cancer Res* 2004; **10**: 7529–39.
19 Mori F, Ishida T, Ito A *et al.* Potent antitumor effects of bevacizumab in a microenvironment-dependent human lymphoma mouse model. *Blood Cancer J* 2012; **2**: e67, doi:10.1038/bcj.2012.12 [Epub ahead of print].
20 Ishii T, Ishida T, Utsunomiya A *et al.* Defucosylated humanized anti-CCR4 monoclonal antibody KW-0761 as a novel immunotherapeutic agent for adult T-cell leukemia/lymphoma. *Clin Cancer Res* 2010; **16**: 1520–31.
21 Ishida T, Ueda R. CCR4 as a novel molecular target for immunotherapy of cancer. *Cancer Sci* 2006; **97**: 1139–46.
22 Kozako T, Arima N, Toji S *et al.* Reduced frequency, diversity, and function of human T cell leukemia virus type 1-specific CD8+ T cell in adult T cell leukemia patients. *J Immunol* 2006; **177**: 5718–26.
23 Kannagi M, Hasegawa A, Kinpara S *et al.* Double control systems for human T-cell leukemia virus type 1 by innate and acquired immunity. *Cancer Sci* 2011; **102**: 670–6.
24 Shimizu Y, Takamori A, Utsunomiya A *et al.* Impaired Tax-specific T-cell responses with insufficient control of HTLV-1 in a subgroup of individuals at asymptomatic and smoldering stages. *Cancer Sci* 2009; **100**: 481–9.
25 Kawano N, Shimoda K, Ishikawa F *et al.* Adult T-cell leukemia development from a human T-cell leukemia virus type I carrier after a living-donor liver transplantation. *Transplantation* 2006; **82**: 840–3.
26 Suzuki S, Uozumi K, Maeda M *et al.* Adult T-cell leukemia in a liver transplant recipient that did not progress after onset of graft rejection. *Int J Hematol* 2006; **83**: 429–32.
27 Yano H, Ishida T, Inagaki A *et al.* Regulatory T-cell function of adult T-cell leukemia/lymphoma cells. *Int J Cancer* 2007; **120**: 2052–7.
28 Choi EM, Chen JL, Wooldridge L *et al.* High avidity antigen-specific CTL identified by CD8-independent tetramer staining. *J Immunol* 2003; **171**: 5116–23.
29 Betts MR, Price DA, Brenchley JM *et al.* The functional profile of primary human antiviral CD8+ T cell effector activity is dictated by cognate peptide concentration. *J Immunol* 2004; **172**: 6407–17.
30 Itoh Y, Hemmer B, Martin R, Germain RN. Serial TCR engagement and down-modulation by peptide: MHC molecule ligands: relationship to the quality of individual TCR signaling events. *J Immunol* 1999; **162**: 2073–80.
31 Benlalam H, Linard B, Guilloux Y *et al.* Identification of five new HLA-B*3501-restricted epitopes derived from common melanoma-associated antigens, spontaneously recognized by tumor-infiltrating lymphocytes. *J Immunol* 2003; **171**: 6283–9.
32 Yoshikawa T, Nakatsugawa M, Suzuki S *et al.* HLA-A2-restricted glypican-3 peptide-specific CTL clones induced by peptide vaccine show high avidity and antigen-specific killing activity against tumor cells. *Cancer Sci* 2011; **102**: 918–25.
33 Kurihara K, Harashima N, Hanabuchi S *et al.* Potential immunogenicity of adult T cell leukemia cells in vivo. *Int J Cancer* 2005; **114**: 257–67.
34 Ito A, Ishida T, Utsunomiya A *et al.* Defucosylated anti-CCR4 monoclonal antibody exerts potent ADCC against primary ATLL cells mediated by autologous human immune cells in NOD/Shi-scid, IL-2R gamma(null) mice in vivo. *J Immunol* 2009; **183**: 4782–91.
35 Umino A, Nakagawa M, Utsunomiya A *et al.* Clonal evolution of adult T-cell leukemia/lymphoma takes place in the lymph nodes. *Blood* 2011; **117**: 5473–8.
36 Yamamoto K, Utsunomiya A, Tobinai K *et al.* Phase I study of KW-0761, a defucosylated humanized anti-CCR4 antibody, in relapsed patients with adult T-cell leukemia-lymphoma and peripheral T-cell lymphoma. *J Clin Oncol* 2010; **28**: 1591–8.
37 Ishida T, Joh T, Uike N *et al.* Defucosylated anti-CCR4 monoclonal antibody (KW-0761) for relapsed adult T-cell leukemia-lymphoma: a multicenter phase ii study. *J Clin Oncol* 2012; **30**: 837–42.
38 Imai T, Nagira M, Takagi S *et al.* Selective recruitment of CCR4-bearing Th2 cells toward antigen-presenting cells by the CC chemokines thymus and activation-regulated chemokine and macrophage-derived chemokine. *Int Immunol* 1999; **11**: 81–8.
39 Iellem A, Mariani M, Lang R *et al.* Unique chemotactic response profile and specific expression of chemokine receptors CCR4 and CCR8 by CD4(+) CD25(+) regulatory T cells. *J Exp Med* 2001; **194**: 847–53.
40 Ishida T, Ishii T, Inagaki A *et al.* Specific recruitment of CC chemokine receptor 4-positive regulatory T cells in Hodgkin lymphoma fosters immune privilege. *Cancer Res* 2006; **66**: 5716–22.
41 Hodi FS, O'Day SJ, McDermott DF *et al.* Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 2010; **363**: 711–23.
42 Robert C, Thomas L, Bondarenko I *et al.* Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N Engl J Med* 2011; **364**: 2517–26.
43 Ishida T, Hishizawa M, Kato K *et al.* Allogeneic hematopoietic stem cell transplantation for adult T-cell leukemia-lymphoma with special emphasis on preconditioning regimen: a nationwide retrospective study. *Blood* 2012; doi: 10.1182/blood-2012-03-414490 [Epub ahead of print].

Cancer/testis antigens are novel targets of immunotherapy for adult T-cell leukemia/lymphoma

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Adult T-cell leukemia/lymphoma (ATLL) is an intractable hematologic malignancy caused by human T-lymphotropic virus type 1 (HTLV-1), which infects approximately 20 million people worldwide. Here, we have explored the possible expression of cancer/testis (CT) antigens by ATLL cells, as CT antigens are widely recognized as ideal targets of cancer immunotherapy against solid tumors. A high percentage (87.7%) of ATLL cases (n = 57) expressed CT antigens at the mRNA level: NY-ESO-1 (61.4%), MAGE-A3 (31.6%), and MAGE-A4 (61.4%). CT antigen expression was confirmed by immunohistochemistry. This contrasts with other types of lymphoma or leukemia, which scarcely express these CT antigens. Humoral immune responses, particularly against NY-ESO-1, were detected in 11.6% (5 of 43) and NY-ESO-1–specific CD8$^+$ T-cell responses were observed in 55.6% (5 of 9) of ATLL patients. NY-ESO-1–specific CD8$^+$ T cells recognized autologous ATLL cells and produced effector cytokines. Thus, ATLL cells characteristically express CT antigens and therefore vaccination with CT antigens can be an effective immunotherapy of ATLL. (*Blood*. 2012;119(13): 3097-3104)

Introduction

Adult T-cell leukemia/lymphoma (ATLL) is a distinct hematologic malignancy caused by human T-lymphotropic virus type 1 (HTLV-1).[1,2] HTLV-1 is endemic in southwestern Japan, Africa, South America, and the Caribbean Islands, and approximately 20 million people worldwide are infected.[3] A total of 5% of the infected persons develop ATLL after a long latency period.[2] ATLL cells are CD4-positive; and the majority, if not all, of them express the transcription factor FoxP3 (Forkhead Box P3), CD25, CTLA-4, and CCR4 (CC chemokine receptor 4), and are functionally immunosuppressive, thus phenotypically and functionally resembling naturally occurring regulatory T cells (Tregs).[3-9] Because of its immunosuppressive property and resistance to conventional chemotherapy, aggressive ATLL has a poor prognosis with a mean survival time of less than 1 year.[2,8] A recent phase 3 trial of a dose-intensified multidrug chemotherapy for untreated ATLL patients (acute, lymphoma, and unfavorable chronic types) showed a median progression-free and overall survival of only 7.0 and 12.7 months, respectively.[10] This and other reports indicate that chemotherapy alone is of limited success for ATLL and mostly fails to cure the disease.[10,11] Allogeneic hematopoietic stem cell transplantation has been introduced over the past decade as a potential therapy for ATLL with a long-term remission in only a small fraction of patients who are young, well controlled in disease progression, and have an appropriate stem cell source.[12] More effective strategies to treat ATLL are therefore required.

Several HTLV-1 components have been explored as targets for immunotherapy of ATLL. HTLV-1 Tax, which is crucial for ATLL oncogenesis, has generally been considered to be a main target of the host's cellular immune responses. Yet, the frequency of Tax expression in HTLV-1 infected cells reduces in the course of disease progression, and *Tax* transcripts are detected only in approximately 40% of the established ATLL cases,[13] thus limiting Tax-targeted immunotherapy to a subset of patients. HBZ (HTLV-1 bZIP factor), another HTLV-1 component, which contains an N-terminal transcriptional activation domain and a leucine zipper motif at its C-terminal, also plays an important role in the proliferation of ATLL cells and is detectable in almost all ATLL cases.[2] However, CD8$^+$ T cells specific for HBZ could only recognize peptide-pulsed target cells but not ATLL cells themselves.[14] Furthermore, HTLV-1 is transmitted mainly from mothers to infants through breast milk, and such vertical infection in early life may induce tolerance to the virus and result in insufficient HTLV-1–specific T-cell responses.[15,16] For these reasons, targeting the HTLV-1 components alone may be insufficient for successful immunotherapy of ATLL, necessitating identification of novel tumor-associated target antigens for the immunotherapy.

The expression of cancer/testis (CT) antigens, of which more than 100 have been identified so far, is normally limited to human germ line cells in the testis and in various types of human cancers.[17,18] This restricted expression pattern in normal tissues makes them ideal cancer antigens for tumor immunotherapy.

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*H.N., Y.M., and T.I. contributed equally to this study.

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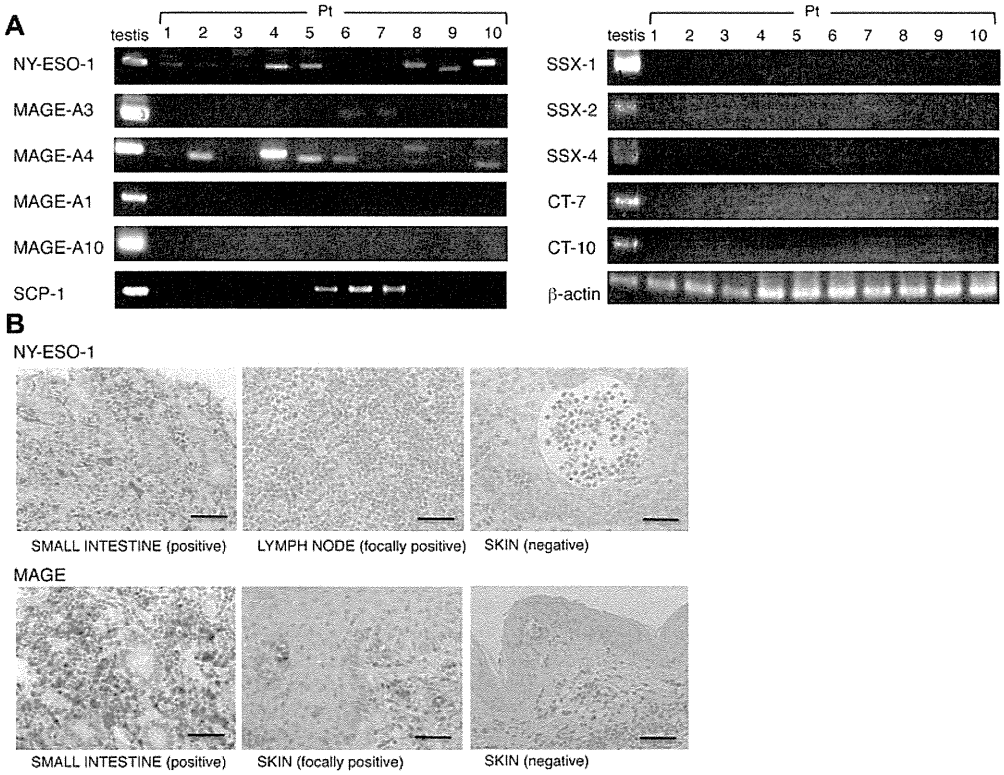

Figure 1. NY-ESO-1, MAGE-A3, and MAGE-A4 are widely expressed by primary ATLL cells. (A) Representative results of RT-PCR analysis for mRNA expression of NY-ESO-1, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A10, CT-7, CT-10, SSX-1, SSX-2, SSX-4, and SCP-1. (B) ATLL samples were subjected to immunohistochemical staining for NY-ESO-1 (E978) mAb and pan-MAGE (57B) mAb. Bar represents 50 μm. These experiments were performed independently at least twice with similar results.

NY-ESO-1 and MAGE family antigens, a subset of CT antigens, are indeed able to elicit spontaneous humoral and cellular immune responses in cancer patients.[17-19] Clinical trials of CT antigen vaccination are currently under way with several types of vaccine formulation, including peptide, protein, and DNA, and some of the treated patients have experienced clinical benefits from vaccination.[19,20] This promising result of CT antigens as a target of tumor immunotherapy has prompted intensive studies of their expression in a wide range of human cancers. However, detailed analysis of CT antigen expression in hematologic disorders has been limited.[21,22] In the present study, we have investigated possible expression of CT antigens by ATLL cells and possible humoral and cellular immune responses against CT antigens in ATLL patients.

Methods

Primary ATLL cells and peripheral blood

Blood or lymph node samples were obtained from ATLL patients, and mononuclear cells were isolated with Ficoll-Paque. Diagnosis and classification of clinical subtypes of ATLL were according to the criteria proposed by the Japan Lymphoma Study Group.[23] All donors provided informed written consent before sampling according to the Declaration of Helsinki, and the present study was approved by the institutional ethics committees of Nagoya City University Graduate School of Medical Sciences, Osaka University, and Imamura Bun-in Hospital.

ATLL cell lines

ATN-1, ATL102, HUT102, MT-2, and MT-1 were previously described.[24,25] TL-Om1 and TL-Su were kindly provided by the Cell Resource Center for Biomedical Research, Tohoku University (Sendai, Japan). MT-4 was purchased from the Health Science Research Resources Bank (Osaka, Japan). MJ was purchased from ATCC. TCL-Kan was kindly provided by Professor M. Kannagi (Tokyo Medical and Dental University, Tokyo, Japan).

Reagents

Phycoerythrin-conjugated anti–IFN-γ (4S.B3) mAb and phycoerythrin-cyanine7–conjugated anti–TNF-α (MAb11) mAb were purchased from eBioscience. Fluorescein isothiocyanate-conjugated anti–IL-2 (MQ1-17H12) mAb and allophycocyanin-cyanine7–conjugated anti-CD8 (SK1) mAb were purchased from BD Biosciences. Anti–NY-ESO-1 mAb (E978, mouse IgG1)[26] and pan-MAGE mAb (57B, mouse IgG1)[27] were purified from hybridoma supernatant by protein G affinity chromatography. Synthetic peptides of NY-ESO-$1_{1\text{-}20}$ (MQAEGRGTGGSTGDADGPGG), NY-ESO-$1_{11\text{-}30}$ (STGDADGPGGPGIPDGPGGN), NY-ESO-$1_{21\text{-}40}$ (PGIPDGPGGNAGGPGEAGAT), NY-ESO-$1_{31\text{-}50}$ (AGGPGEAGATGGRGPRGAGA), NY-ESO-$1_{41\text{-}60}$ (GGRGPRGAGAARASGPGGGA), NY-ESO-$1_{51\text{-}70}$ (ARASGPGGGAPRGPHGGAAS), NY-ESO-$1_{61\text{-}80}$ (PRGPHGGAASGLNGCCRCGA), NY-ESO-$1_{71\text{-}90}$ (GLNGCCRCGARGPESRLLEF), NY-ESO-$1_{81\text{-}100}$ (RGPESRLLEFYLAMPFATPM), NY-ESO-$1_{91\text{-}110}$ (YLAMPFATPMEAELARRSLA), NY-ESO-$1_{101\text{-}120}$ (EAELARRSLAQDAPPLPVPG), NY-ESO-$1_{111\text{-}130}$ (QDAPPLPVPGVLLKEFTVSG), NY-ESO-$1_{119\text{-}143}$ (PGVLLKEFTVSGNILTIRLTAADHR), NY-ESO-$1_{131\text{-}150}$ (NILTIRLTAADHRQLQLSIS), NY-ESO-$1_{139\text{-}160}$ (AADHRQLQLSISSCLQQLSLLM), NY-ESO-$1_{151\text{-}170}$ (SCLQQLSLLMWITQCFLPVF), and NY-ESO-$1_{161\text{-}180}$ (WITQCFLPVFLAQPPSGQRR) were obtained from Invitrogen.

RT-PCR

Total RNA was isolated with RNeasy Mini Kit (QIAGEN). cDNA was synthesized from 0.1 μg of total RNA using SuperScript III reverse

Table 1. Summary of CT antigen expression in ATLL cells from primary ATLL patients

	No. of patients	NY-ESO-1	MAGE	
			A3	A4
Age (mean, 60.6 y)				
≥ 60 y	29	20	12	20
< 60 y	28	15	6	15
Sex				
Male	30	17	8	17
Female	27	18	10	18
Disease type				
Aggressive	40	25 (62.5%)	14 (35.0%)	26 (65.0%)
Acute	37	22	13	24
Lymphoma	3	3	1	2
Indolent	17	9 (52.9%)	4 (23.5%)	9 (52.9%)
Chronic	11	7	2	8
Smoldering	6	3	2	1

mRNA expression of NY-ESO-1, MAGE-A3, and MAGE-A4 in ATLL cells from primary ATLL patients was analyzed with RT-PCR. CT antigen expression was summarized based on several clinical parameters.

transcriptase kit (Invitrogen) and the Oligo (dT) primer in a total volume of 20 μL. cDNA was amplified in a final volume of 20 μL containing 10μM of each CT antigen primer as reported,[21,28] except NY-ESO-1 (sense, 5′-AGT TCT ACC TCG CCA TGC CT-3′; antisense, 5′- TCC TCC TCC AGC GAC AAA CAA-3′) and 0.2 μL of Ex-Taq polymerase (Takara Bio) according to the instructions provided by the manufacturer.

ELISA

Patients' sera were analyzed by ELISA for seroreactivity to bacterially produced recombinant proteins NY-ESO-1/CTAG1B, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A10, CT7/MAGE-C1, CT10/MAGE-C2, SSX1, SSX2, and SSX4.[29] Sera were diluted serially from 1/100 to 1/100 000 and added to low-volume 96-well plates (Corning Life Sciences) coated overnight at 4°C with 1 μg/mL antigen in 25 μL volume and blocked for 2 hours at room temperature with PBS containing 5% nonfat milk. After overnight incubation, plates were extensively washed and rinsed with PBS. Antigen-specific IgG was detected with specific mAb conjugated with alkaline phosphatase (Southern Biotechnology). After addition of ATTOPHOS substrate (Fisher Scientific), absorbance was measured using a fluorescence reader Cytofluor Series 4000 (PerSeptive Biosystems). A reciprocal titer was calculated for each sample as the maximal dilution still significantly reacting to a specific antigen. This value was extrapolated by determining the intersection of a linear trend regression with a cutoff value. The cutoff was defined as 10 times the average of optical density (OD) values from the first 4 dilutions of a negative control pool made of 5 healthy donor sera. In each assay, sera of patients with known presence or absence of specific reactivity were used as controls.

Immunohistochemistry

All tissue specimens were fixed with formalin and embedded in paraffin. Tissue sections of 3 μm thickness on charged glass slides were deparaffinized and rehydrated. Antigen retrieval was performed by autoclave (105°C for 20 minutes) using Tris-EDTA buffer (pH 9.0) as heating solution. Primary mAb for NY-ESO-1 (E978) and pan-MAGE protein (57B) were used at concentrations of 5 μg/mL and 2 μg/mL, respectively. Endogenous peroxidase activity was blocked with 3% hydrogen peroxidase and 1% sodium azide. Simple stain max-PO (Multi; Nichirei) was used for secondary detection; 3,3′-diamino-benzidine was used as chromogen. Human testicular tissue served as positive control. All histological pictures were captured by NIKON Eclipse microscope and NIKON Image software system (Version 3.22).

In vitro sensitization

NY-ESO-1–specific $CD8^+$ and $CD4^+$ T cells were presensitized as described previously.[30,31] Briefly, $CD8^+$ T cells and $CD4^+$ T cells were isolated from peripheral blood mononuclear cells (PBMCs) using a CD8 Microbeads and a $CD4^+$ T cell Isolation Kit, respectively (Miltenyi Biotec). The purity of isolated populations was confirmed to be more than 90%. The non-$CD8^+$/$CD4^+$ cell population was pulsed with 10μM of pooled peptides overnight and was used as antigen-presenting cells (APCs). After irradiation, 5 to 10×10^5 APCs were added to round-bottom 96-well plates (Corning Life Sciences) containing 1 to 5×10^5 $CD8^+$ or $CD4^+$ T cells and were fed with IL-2 (10 U/mL; Roche Diagnostics) and IL-7 (20 ng/mL; R&D Systems). Subsequently, one-half of the medium was replaced by fresh medium containing IL-2 (20 U/mL) and IL-7 (40 ng/mL) twice per week.

Intracellular cytokine staining

$CD8^+$ T cells from PBMCs from ATLL patients were presensitized for 10 to 18 days. These presensitized $CD8^+$ T cells were restimulated for 6 hours with peptide-pulsed T-APCs,[31] and GolgiStop reagent (BD Biosciences) was added 1 hour later. Cells were stained for cell surface markers and for intracellular cytokines, such as IFN-γ and TNF-α after permeabilization. Results were analyzed by flow cytometry (FACSCanto; BD Biosciences) and FlowJo Version 7.6.5 software for Macintosh (TreeStar).

Tetramer assay

Tetramer staining was performed as previously described.[31] Briefly, presensitized $CD8^+$ T cells were stained with phycoerythrin-labeled tetramers (prepared by Drs P. Guillaume and I. Luescher at the Ludwig Institute Core Facility, Lausanne, Switzerland) for 15 minutes at 37°C before additional staining with allophycocyanin-cyanine7–conjugated anti-CD8 mAb for 15 minutes at 4°C. After washing, results were analyzed by FACSCanto and FlowJo Version 7.6.5 software.

Statistical analysis

The significance of the difference in each CT antigen expression between 2 groups was assessed by Fisher exact test. *P* values less than .05 were considered significant.

Results

A subset of CT antigens is highly expressed in ATLL cell lines

To examine possible expression of 11 CT antigens (NY-ESO-1, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A10, CT-7, CT-10, SSX-1, SSX-2, SSX-4, and SCP-1) by ATLL cells, we assessed mRNA expression by RT-PCR in 10 ATLL cell lines (MT-1, MT-2, MT-4, MJ, ATL102, ATN-1, TL-Om-1, TL-Su, TCL-kan, and HUT102). In sharp contrast to a previous report showing that the majority of T-cell lymphomas did not express CT antigens except SCP-1,[21] a high percentage of ATLL cell lines expressed NY-ESO-1, MAGE-A3, and MAGE-A4 (90%, 50% and 70%, respectively) in the present study (supplemental Table 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). SCP-1, another CT antigen, was also detected in 40% of ATLL cell lines, as in other T-cell lymphomas (supplemental Table 1).

NY-ESO-1, MAGE-A3, and MAGE-A4 are widely expressed in primary ATLL cells

Given the high percentage of CT antigen mRNA expression in ATLL cell lines, we next examined the expression of 11 CT antigens in primary tumor cells from 57 individual ATLL patients. As shown in Figure 1A, Table 1, and supplemental Table 2, NY-ESO-1, MAGE-A3, and MAGE-A4 mRNA expression was detected in 61.4% (35 of 57), 31.6% (18 of 57), and 61.4% (35 of 57), respectively, of primary ATLL patients. SCP-1 expression was

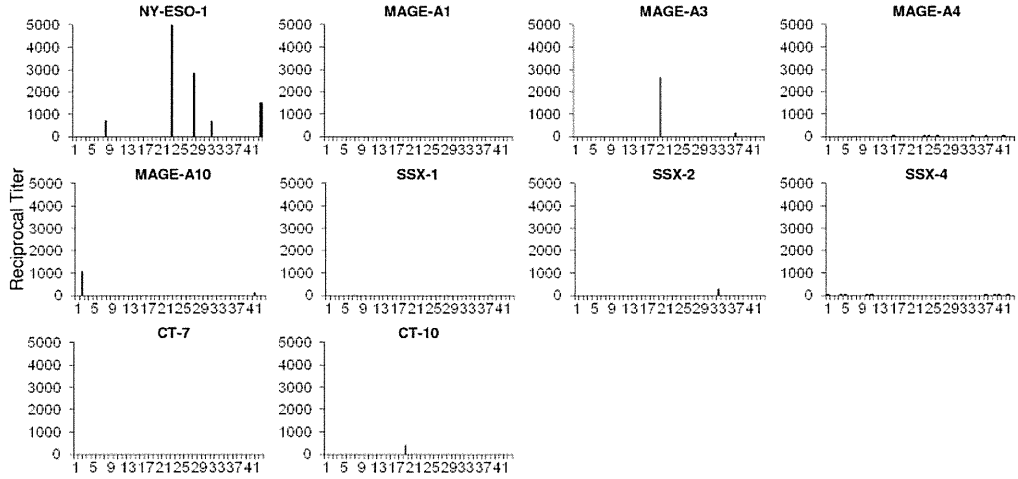

Figure 2. Humoral immune responses against NY-ESO-1 are detected in a subset of patients with ATLL. Sera were collected from 43 primary ATLL patients, and antibody responses against 10 CT antigens (NY-ESO-1, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A10, CT-7, CT-10, SSX-1, SSX-2, and SSX-4) were analyzed by ELISA as described in "ELISA." This experiment was performed at least twice with similar results.

also detected in 23.2% (13 of 56). Among 4 subtypes (acute, lymphoma, chronic, and smoldering) of ATLL, the acute and lymphoma types show aggressive clinical courses (aggressive types), whereas the chronic and smoldering types progress more indolently (indolent types).[2,23] There were no significant differences between the ATLL patients with aggressive and indolent types in the expression of NY-ESO-1, MAGE-A3, and MAGE-A4; yet there was a trend for more frequent expression of CT antigens in aggressive ATLL types (Table 1). Immunohistochemical analysis confirmed the expression of NY-ESO-1 and pan-MAGE at the protein level in samples available for pathologic analysis (Figure 1B). By contrast, expression of other CT antigens was limited: MAGE-A1, 8.8% (3 of 34); MAGE-A10, 0% (0 of 33); CT-7, 0% (0 of 33); CT-10, 0% (0 of 33); SSX-1, 0% (0 of 33); SSX-2, 0% (0 of 33); and SSX-4, 0% (0 of 33) by RT-PCR.

Taken together, CT antigens, such as NY-ESO-1, MAGE-A3 and MAGE-A4, are expressed in a significant fraction of primary ATLL cases, and 87.7% (50 of 57) of ATLL patients expressed at least one of these 3 CT antigens.

Humoral immune responses against NY-ESO-1 are detected in a subset of ATLL patients

We next asked whether ATLL patients spontaneously developed humoral and cellular immune responses specific for CT antigens. Serum samples from 43 primary ATLL patients were assessed by ELISA for the reactivity to 10 CT antigens (NY-ESO-1, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A10, CT-7, CT-10, SSX-1, SSX-2, and SSX-4). Significant antibody titers were detected against NY-ESO-1 in 5 of 43 (11.6%) patients and against MAGE-A1, MAGE-A3, and MAGE-A4 in 0% (0 of 43), 2.3% (1 of 43), and 0% (0 of 43), respectively (Figure 2). Humoral immune responses against CT antigens whose expression was not detected by RT-PCR (MAGE-A10, CT-7, CT-10, SSX-1, SSX-2, and SSX-4) were limited (Figure 2). These data collectively indicate that NY-ESO-1 expressed in primary ATLL cells elicits spontaneous antibody responses in ATLL patients as in patients with NY-ESO-1 expressing solid tumors.[19]

Cellular immune responses against NY-ESO-1 in ATLL patients

We then assessed CT antigen-specific cellular immune responses in ATLL patients. As the majority of ATLL patients are in a severe anemic state as a characteristic of the disease and also because of intensive chemotherapy, we collected PBMCs from patients in partial or complete remission, except patient 8 with an indolent ATLL type. Considering this limitation of available sample sizes and predominant humoral immune responses against NY-ESO-1, we focused on NY-ESO-1 as a parameter of cellular immune responses. With sufficient amounts of PBMCs from 9 ATLL patients (patients 1, 2, 4, 8, 13, 14, 19, 27, and 43), NY-ESO-1 expression by ATLL cells was confirmed by RT-PCR in each patient, except for patient 13 (no detection) and patient 43 (unavailability of sample; supplemental Table 2). $CD8^+$ T cells were presensitized with autologous $CD4^-CD8^-$ PBMCs pulsed with a pool of NY-ESO-1 peptides, and antigen-specific $CD8^+$ T cells were analyzed with NY-ESO-1/HLA tetramers corresponding to the HLA allele of each patient. NY-ESO-1–specific $CD8^+$ T cells were detected in ATLL patients 2, 4, 14, and 43 (Figure 3A). The results indicate that NY-ESO-1–specific $CD8^+$ T-cell responses spontaneously develop in a subset of patients harboring NY-ESO-1 expressing ATLL.

NY-ESO-1–specific $CD8^+$ T cells produce cytokines and recognize an autologous ATLL cell line

With the presence of NY-ESO-1–specific $CD8^+$ T cells in ATLL patients, we further analyzed their cytokine production in response to a pool of NY-ESO-1 peptides or autologous tumor cells. Sufficient amounts of PBMCs for the cytokine analysis were available from 3 ATLL patients (patients 4, 14, and 19), in which NY-ESO-1 expression was confirmed by RT-RCR (supplemental Table 2). NY-ESO-1–specific $CD8^+$ T cells were detected with NY-ESO-1/MHC tetramers in 2 of them (patients 4 and 14), but relevant tetramers were not available for another patient (patient 19). NY-ESO-1–specific $CD8^+$ T cells prepared from these 3 patients by presensitization with NY-ESO-1 peptide-pulsed $CD4^-CD8^-$ PBMCs produced IFN-γ and/or TNF-α by intracellular cytokine staining (Figure 3B). In patient 14, the frequency of NY-ESO-1–specific $CD8^+$ T cells producing IFN-γ was much higher than NY-ESO-1–specific $CD8^+$ T cells detected by NY-ESO-1/HLA-Cw*0304 tetramer, suggesting that this patient may have $CD8^+$ T cells recognizing other epitopes of NY-ESO-1 (Figure 3A-B). Taken together, NY-ESO-1–specific $CD8^+$ T cells were detected in 5 of 9 (55.6%) ATLL patients.

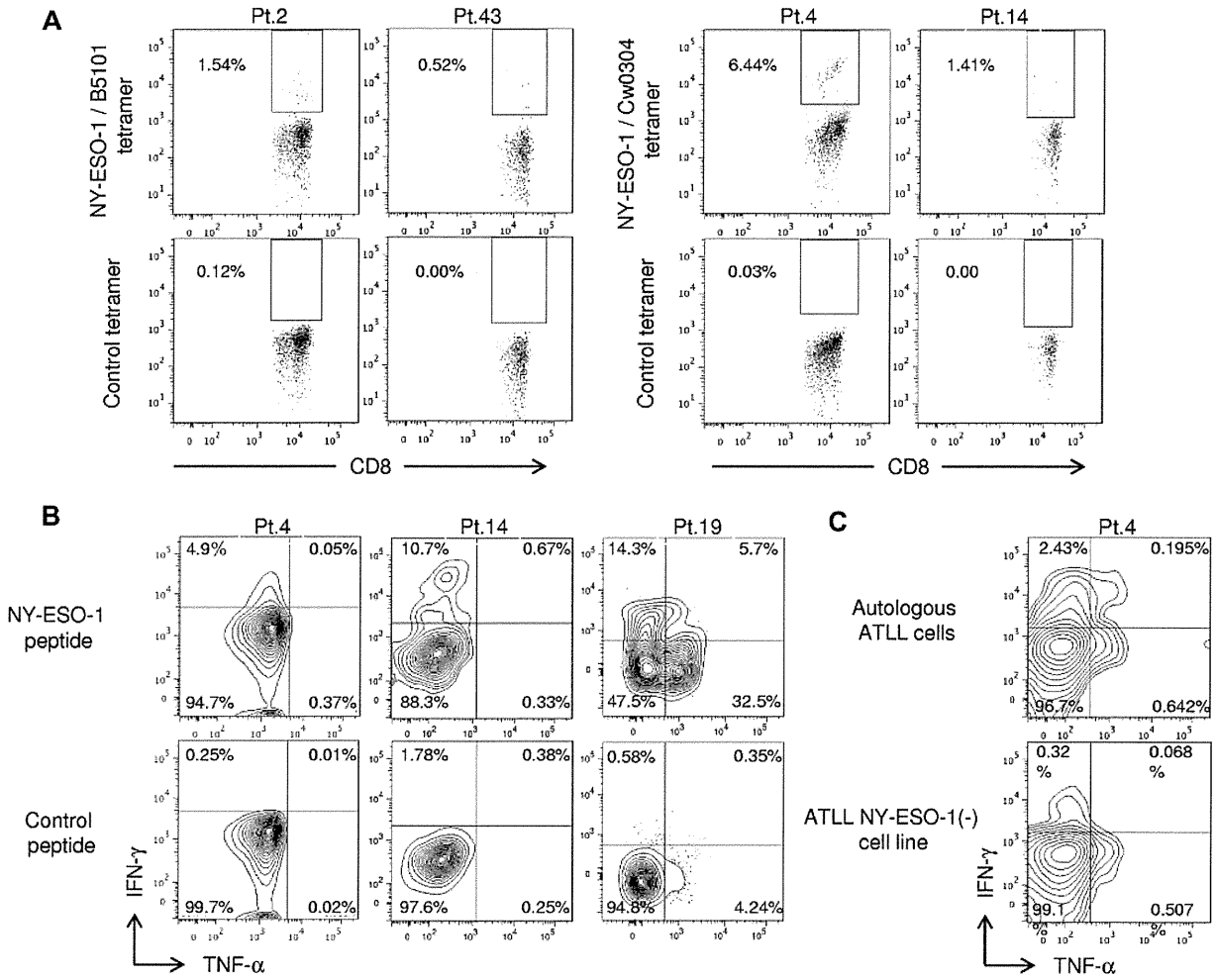

Figure 3. NY-ESO-1–specific CD8$^+$ T cells are detected in ATLL patients. CD8$^+$ T cells derived from PBMCs of patients 1, 2, 4, 8, 13, 14, 19, 27, and 43 were presensitized by CD4$^-$ CD8$^-$ PBMCs pulsed with NY-ESO-1 peptides covering the entire sequence of NY-ESO-1 as described in "In vitro sensitization." (A) Induction of specific CD8$^+$ T cells was analyzed by staining with NY-ESO-1/HLA tetramers indicated. Cytokine (IFN-γ and TNF-α) secreting capacity of NY-ESO-1–specific CD8$^+$ T cells was analyzed by intracellular cytokine staining for recognition of (B) autologous activated T-cell APCs pulsed with NY-ESO-1 peptides or (C) autologous ATLL cells. These experiments were performed independently at least twice with similar results.

We also examined whether NY-ESO-1–specific CD8$^+$ T cells from patient 4 recognized autologous ATLL cells. They produced IFN-γ and TNF-α against autologous ATLL cells expressing NY-ESO-1 but not against a control HLA-matched ATLL cell line (ATL-102) without NY-ESO-1 expression (Figure 3C). Collectively, these data indicate that NY-ESO-1–specific CD8$^+$ T cells are present in ATLL patients and are able to recognize and kill autologous leukemic cells.

CD4$^+$ T-cell responses against NY-ESO-1 in ATLL patients

To determine whether NY-ESO-1–specific CD4$^+$ T cells were present in these ATLL patients, CD4$^+$ T cells derived from PBMCs obtained from ATLL patients (patients 1, 2, 4, 8, 13, 14, 19, 27, and 43) were presensitized by CD4$^-$CD8$^-$ PBMCs pulsed with a pool of NY-ESO-1 peptides, and assessed for cytokine production by intracellular cytokine staining. Of 9 patients, NY-ESO-1–specific CD4$^+$ T cells were detected only in one patient (patient 19), who was in complete remission after receiving allogeneic hematopoietic stem cell transplantation (Figure 4). Thus, NY-ESO-1–specific CD4$^+$ T cells are present in a subset of ATLL patients, but a much lower frequency than CD8$^+$ T-cell responses, partly because the presence of ATLL cells, which are CD4$^+$, may make the detection difficult and possibly because CD25$^+$CD4$^+$ Tregs are present in the CD4$^+$ T-cell fraction.

Discussion

Since the initial description of ATLL as a unique type of T-cell leukemia/lymphoma,[1] various therapeutic attempts have been made. Yet, the prognosis of ATLL is still poor despite advances in our knowledge regarding the oncogenic process of the disease.[10,23] Here, we have examined CT antigen expression and its immunogenicity in ATLL patients to explore the potential for immunotherapy of ATLL by targeting CT antigens. We found that CT antigens, such as NY-ESO-1, MAGE-A3, and MAGE-A4, were highly expressed in ATLL. In particular, the frequencies of NY-ESO-1 and MAGE-A4 expression (61.4% and 61.4%, respectively) at the mRNA level were higher than or comparable to those in other malignancies. For example, the frequency of NY-ESO-1 and MAGE-A4 expression was 32% to 45% and 28%, respectively, in malignant melanoma, 24% to 33% and 63% to 90.2% in esophageal cancer, 30% to 43%

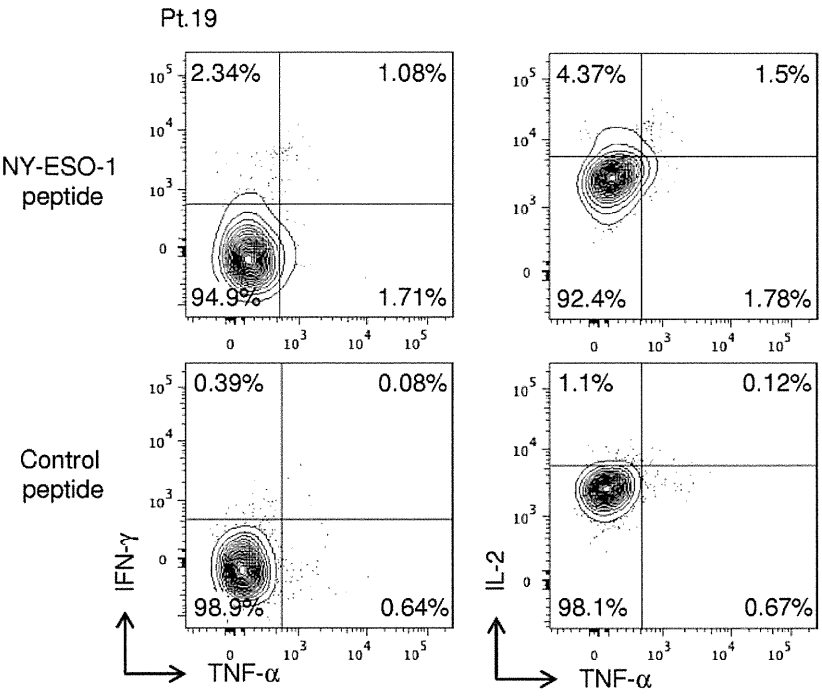

Figure 4. NY-ESO-1–specific CD4+ T cells are present in an ATLL patient (patient 19) receiving an allogeneic hematopoietic stem cell transplantation. $CD4^+$ T cells were presensitized by $CD4^-$ $CD8^-$ PBMCs pulsed with NY-ESO-1 peptide covering the entire sequence of NY-ESO-1 as described in "In vitro sensitization." Induction of NY-ESO-1–specific $CD4^+$ T cells was analyzed by intracellular cytokine staining using autologous activated T-cell APCs pulsed with NY-ESO-1 peptides. These experiments were performed independently twice with similar results.

and 57% in ovarian cancer, and 18% to 35% and 33% in bladder cancer.[19,32,33] In addition, we have revealed that NY-ESO-1 was immunogenic in ATLL patients and elicited specific humoral and cellular immune responses in a subset of patients. These data strongly support CT antigens as novel targets for ATLL immunotherapy.

The frequency of humoral immune responses in ATLL patients was much higher against NY-ESO-1 compared with other CT antigens, such as MAGE-A3 and MAGE-A4. Although NY-ESO-1 expression by ATLL cells is presumably required for antibody induction, the level of NY-ESO-1 mRNA expression did not reflect the induction of humoral immune responses (supplemental Table 2). This lack of correlation appears in part to be the result of immunologic properties of NY-ESO-1 antigen itself.[19,26] It has been shown that polymeric structures of NY-ESO-1 through disulfide bonds and their interaction with calreticulin-TLR4 on immature dendritic cell surface are required to induce phagocytosis of NY-ESO-1 protein and deliver danger signals for making the protein immunogenic.[34] In addition, some non-MAGE-A members of the MAGE family are ubiquitously expressed and therefore possibly in more stable tolerance compared with NY-ESO-1. These properties of NY-ESO-1 and MAGE proteins might make the former more immunogenic than the latter.

Despite high immunogenicity of NY-ESO-1, the frequency (13.6%, 3 of 22) of primary ATLL patients who spontaneously developed NY-ESO-1 antibody responses against NY-ESO-1 expressing leukemic cells was lower compared with the frequencies in patients with malignant melanoma or non-small-cell lung cancers expressing NY-ESO-1 (~ 50%).[19,26] This may reflect the feature of ATLL that tumor cells from the majority of ATLL patients express Foxp3, a key transcription factor for $CD25^+CD4^+$ Tregs.[6,9,35-37] ATLL cells from a subset of patients indeed appear to function as Tregs and contribute to profound immunosuppression that hampers the host's immune responses.[6,36] Alternatively or additionally, the chemokine CCL22 produced by ATLL cells might enhance the migration of CCR4-expressing $CD25^+CD4^+$ Tregs to tumor sites.[38] It remains to be determined whether Tregs in ATLL patients or ATLL cells themselves suppress NY-ESO-1–specific immune responses. Yet, it has been shown that helper T-cell responses against NY-ESO-1 are subject to active suppression by Tregs in patients with solid tumors and healthy persons.[30,37] Further studies are required to understand immunosuppressive property of ATLL to enhance immune responses against CT antigens in ATLL patients.

NY-ESO-1–specific cellular immune responses were detected in a significant number (5 of 9, 55.6%) of ATLL patients in partial or complete remission. This indicates that reducing the number of ATLL cells before CT antigen-targeted immunotherapy may be a crucial component to successfully induce/augment antigen–specific $CD8^+$ T cells. We have recently reported that humanized anti-CCR4 mAb, KW-0761, showed clinically significant antitumor activity as a salvage therapy for patients with relapsed ATLL.[8,39] Patients 2, 4, and 14, from whom we detected NY-ESO-1–specific $CD8^+$ T cells, were in complete or partial remission after anti-CCR4 mAb treatment.[8,39] As CCR4 is expressed on ATLL cells as well as $CD25^+CD4^+FOXP3^+$ Tregs, in addition to T-helper type 2 cells,[8,40,41] anti-CCR4 mAb treatment can reduce not only ATLL cells but also endogenous $CD25^+CD4^+FOXP3^+$ cells,[42] thereby contributing to evoking NY-ESO-1–specific $CD8^+$ T-cell responses. Indeed, the high frequency of NY-ESO-1–specific $CD8^+$ T cells detectable in vitro in ATLL patients could be the result in part of the absence of Tregs. Thus, combining CT antigen-targeted immunotherapy after reduction of endogenous Tregs as well as ATLL cells by anti-CCR4 mAb treatment would be an ideal strategy for ATLL immunotherapy.

NY-ESO-1–specific $CD4^+$ and $CD8^+$ T-cell responses observed in patient 19 who was in a complete remission after allogeneic hematopoietic stem cell transplantation suggest an association of immune responses against CT antigens with a graft-versus-ATLL effect. This indicates that CT antigen-targeted immunotherapy combined with allogeneic stem cell transplantation may augment the efficacy of the current allogeneic hematopoietic stem cell

transplantation for treating ATLL. In addition, as HTLV-1 Tax-specific $CD8^+$ T cells reportedly contribute to graft-versus-ATLL effects,[43] a combination immunotherapy with CT antigen and Tax after stem cell transplantation might also be therapeutically effective. Further, considering the reported high efficacy of NY-ESO-1–targeted adoptive T-cell therapy against malignant melanoma and synovial cell sarcoma,[44,45] similar adoptive T-cell therapy for ATLL could also be effective.

ATLL is, to our knowledge, the first example of high expression of CT antigens in lymphomas or leukemia, as detailed analyses of CT antigen expression in other hematologic disorders have been limited.[21,22] It contrasted with a previous report showing that T-cell lymphoma lacks expression of CT antigens, except SCP-1. As a probable mechanism for transcriptional activation of the CT antigen genes in malignant cells, it has been suggested that the expression of CT antigen is induced by CpG island hypomethylation at the promoter regions.[18] An inflammatory environment created by infection may also trigger NY-ESO-1 expression. It cannot be excluded that viral components may promote this hypomethylation at the promoter regions or elicit inflammatory environment, thereby inducing CT antigen expression. Yet, we failed to observe any correlation between viral gene expression, such as Tax and HBZ and CT antigen expression (supplemental Table 2). In addition, high titers of HTLV-1 Gag/Env antibody responses were not associated with the induction of humoral immune responses against CT antigens (supplemental Table 2). Peripheral T-cell lymphomas, not otherwise specified (PTCL-NOS) are particularly heterogeneous; yet, the CCR4-positive subset of PTCL-NOS might be a distinct disease entity whose clinicopathologic features and genomic profiles were reportedly very similar to the lymphoma type of ATLL.[24,46,47] PTCL-NOS, especially the CCR4-positive subset, have a very poor prognosis like ATLL, and no standard treatment strategies are available.[48] Notably, we have found that CCR4-positive PTCL-NOS also expressed NY-ESO-1 at a high frequency (H.N., T.I., R.U. and S.S., unpublished data, August 2011). These data, when taken together, indicate that the frequent expression of CT antigens in ATLL may not be induced by HTLV-1 infection itself, but rather it may constitute a novel subtype of T-cell leukemia/lymphoma that expresses CCR4 and CT antigens regardless of HTLV-1 infection.

A small population (~ 5%) of HTLV-1–infected persons progresses to ATLL after a long latency period of approximately 50 to 70 years.[2] Although the detailed mechanisms of this leukemogenesis from HTLV-1 infection to ATLL have not yet been well elucidated, host immune responses against HTLV-1–infected cells have been suspected to play an important role.[8,16,49] Interestingly, some of HTLV-1–infected asymptomatic HTLV-1 carriers harbored significant titers of antibody against NY-ESO-1, MAGE-A3, and MAGE-A4, despite that CT antigens, including NY-ESO-1, MAGE-A3, and MAGE-A4, were not detected at the mRNA level in their PBMCs (supplemental Figure 1). This discrepancy raises several possibilities; for example, the number or the frequency of HTLV-1–infected cells expressing CT antigens in circulating PBMCs may be too low to detect the antigen. Alternatively, it is possible that HTLV-1–infected cells expressing CT antigens may be eliminated in HTLV-1–infected asymptomatic carriers by anti-CT antigen-specific immune responses as tumor immunosurveillance,[50] whereas antibody responses may linger on. Future studies with a large cohort of HTLV-1–infected asymptomatic carriers need to address the kinetics of their CT antigen expression during a long latency period and their immune responses against the antigen in the course of ATLL development. The studies will not only provide a rationale for including CT antigens as possible targets of ATLL immunotherapy but also contribute to our understanding of the multistep oncogenesis of ATLL and devising preventive strategies for ATLL by targeting CT antigens.

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Authorship

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References

1. Uchiyama T, Yodoi J, Sagawa K, Takatsuki K, Uchino H. Adult T-cell leukemia: clinical and hematologic features of 16 cases. *Blood.* 1977;50(3):481-492.
2. Matsuoka M, Jeang KT. Human T-cell leukaemia virus type 1 (HTLV-1) infectivity and cellular transformation. *Nat Rev Cancer.* 2007;7(4):270-280.
3. Goncalves DU, Proietti FA, Ribas JG, et al. Epidemiology, treatment, and prevention of human T-cell leukemia virus type 1-associated diseases. *Clin Microbiol Rev.* 2010;23(3):577-589.
4. Uchiyama T, Sagawa K, Takatsuki K, Uchino H. Effect of adult T-cell leukemia cells on pokeweed

mitogen-induced normal B-cell differentiation. *Clin Immunol Immunopathol.* 1978;10(1):24-34.

5. Yagi H, Nomura T, Nakamura K, et al. Crucial role of FOXP3 in the development and function of human CD25+CD4+ regulatory T cells. *Int Immunol.* 2004;16(11):1643-1656.
6. Matsubara Y, Hori T, Morita R, Sakaguchi S, Uchiyama T. Phenotypic and functional relationship between adult T-cell leukemia cells and regulatory T cells. *Leukemia.* 2005;19(3):482-483.
7. Chen S, Ishii N, Ine S, et al. Regulatory T cell-like activity of Foxp3+ adult T cell leukemia cells. *Int Immunol.* 2006;18(2):269-277.
8. Ishida T, Ueda R. Immunopathogenesis of lymphoma: focus on CCR4. *Cancer Sci.* 2011;102(1):44-50.
9. Satou Y, Yasunaga J, Zhao T, et al. HTLV-1 bZIP factor induces T-cell lymphoma and systemic inflammation in vivo. *PLoS Pathog.* 2011;7(2):e1001274.
10. Tsukasaki K, Utsunomiya A, Fukuda H, et al. VCAP-AMP-VECP compared with biweekly CHOP for adult T-cell leukemia-lymphoma: Japan Clinical Oncology Group Study JCOG9801. *J Clin Oncol.* 2007;25(34):5458-5464.
11. Tsukasaki K, Hermine O, Bazarbachi A, et al. Definition, prognostic factors, treatment, and response criteria of adult T-cell leukemia-lymphoma: a proposal from an International Consensus Meeting. *J Clin Oncol.* 2009;27(3):453-459.
12. Hishizawa M, Kanda J, Utsunomiya A, et al. Transplantation of allogeneic hematopoietic stem cells for adult T-cell leukemia: a nationwide retrospective study. *Blood.* 2010;116(8):1369-1376.
13. Takeda S, Maeda M, Morikawa S, et al. Genetic and epigenetic inactivation of tax gene in adult T-cell leukemia cells. *Int J Cancer.* 2004;109(4):559-567.
14. Suemori K, Fujiwara H, Ochi T, et al. HBZ is an immunogenic protein, but not a target antigen, for human T-cell leukemia virus type 1-specific cytotoxic T lymphocytes. *J Gen Virol.* 2009;90(8):1806-1811.
15. Verhasselt V, Milcent V, Cazareth J, et al. Breast milk-mediated transfer of an antigen induces tolerance and protection from allergic asthma. *Nat Med.* 2008;14(2):170-175.
16. Shimizu Y, Takamori A, Utsunomiya A, et al. Impaired Tax-specific T-cell responses with insufficient control of HTLV-1 in a subgroup of individuals at asymptomatic and smoldering stages. *Cancer Sci.* 2009;100(3):481-489.
17. Simpson AJ, Caballero OL, Jungbluth A, Chen Y-T, Old LJ. Cancer/testis antigens, gametogenesis and cancer. *Nat Rev Cancer.* 2005;5(8):615-625.
18. Caballero OL, Chen Y-T. Cancer/testis (CT) antigens: potential targets for immunotherapy. *Cancer Sci.* 2009;100(11):2014-2021.
19. Gnjatic S, Nishikawa H, Jungbluth AA, et al. NY-ESO-1: review of an immunogenic tumor antigen. *Adv Cancer Res.* 2006;95:1-30.
20. Dougan M, Dranoff G. Immune therapy for cancer. *Annu Rev Immunol.* 2009;27:83-117.
21. Xie X, Wacker HH, Huang S, et al. Differential expression of cancer testis genes in histological subtypes of non-Hodgkin's lymphomas. *Clin Cancer Res.* 2003;9(1):167-173.
22. Greiner J, Ringhoffer M, Taniguchi M, et al. mRNA expression of leukemia-associated antigens in patients with acute myeloid leukemia for the development of specific immunotherapies. *Int J Cancer.* 2004;108(5):704-711.
23. 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.* 1991;79(3):428-437.
24. Ishida T, Utsunomiya A, Iida S, et al. Clinical significance of CCR4 expression in adult T-cell leukemia/lymphoma: its close association with skin involvement and unfavorable outcome. *Clin Cancer Res.* 2003;9(10):3625-3634.
25. Ri M, Iida S, Ishida T, et al. Bortezomib-induced apoptosis in mature T-cell lymphoma cells partially depends on upregulation of Noxa and functional repression of Mcl-1. *Cancer Sci.* 2009;100(2):341-348.
26. Stockert E, Jager E, Chen Y-T, et al. A survey of the humoral immune response of cancer patients to a panel of human tumor antigens. *J Exp Med.* 1998;187(8):1349-1354.
27. Rimoldi D, Salvi S, Schultz-Thater E, Spagnoli GC, Cerottini JC. Anti-MAGE-3 antibody 57B and anti-MAGE-1 antibody 6C1 can be used to study different proteins of the MAGE-A family. *Int J Cancer.* 2000;86(5):749-751.
28. Gure AO, Chua R, Williamson B, et al. Cancer-testis genes are coordinately expressed and are markers of poor outcome in non-small cell lung cancer. *Clin Cancer Res.* 2005;11(22):8055-8062.
29. Gnjatic S, Ritter E, Buchler MW, et al. Seromic profiling of ovarian and pancreatic cancer. *Proc Natl Acad of Sci U S A.* 2010;107(11):5088-5093.
30. Nishikawa H, Jager E, Ritter G, Old LJ, Gnjatic S. CD4+CD25+ regulatory T cells control the induction of antigen-specific CD4+ helper T-cell responses in cancer patients. *Blood.* 2005;106(3):1008-1011.
31. Nishikawa H, Sato E, Briones G, et al. In vivo antigen delivery by a Salmonella typhimurium type III secretion system for therapeutic cancer vaccines. *J Clin Invest.* 2006;116(7):1946-1954.
32. Duffour MT, Chaux P, Lurquin C, Cornelis G, Boon T, van der Bruggen P. A MAGE-A4 peptide presented by HLA-A2 is recognized by cytolytic T lymphocytes. *Eur J Immunol.* 1999;29(10):3329-3337.
33. Barrow C, Browning J, MacGregor D, et al. Tumor antigen expression in melanoma varies according to antigen and stage. *Clin Cancer Res.* 2006;12(3):764-771.
34. Liu YN, Tian XL, Leitner WW, et al. Polymeric structure and host Toll-like receptor 4 dictate immunogenicity of NY-ESO-1 antigen in vivo. *J Biol Chem.* 2011;286(43):37077-37084.
35. Karube K, Ohshima K, Tsuchiya T, et al. Expression of FoxP3, a key molecule in CD4CD25 regulatory T cells, in adult T-cell leukaemia/lymphoma cells. *Br J Haematol.* 2004;126(1):81-84.
36. Yano H, Ishida T, Inagaki A, et al. Regulatory T-cell function of adult T-cell leukemia/lymphoma cells. *Int J Cancer.* 2007;120(9):2052-2057.
37. Nishikawa H, Sakaguchi S. Regulatory T cells in tumor immunity. *Int J Cancer.* 2010;127(4):759-767.
38. Toulza F, Nosaka K, Tanaka Y, et al. Human T-lymphotropic virus type 1-induced CC chemokine ligand 22 maintains a high frequency of functional FoxP3+ regulatory T cells. *J Immunol.* 2010;185(1):183-189.
39. Yamamoto K, Utsunomiya A, Tobinai K, et al. Phase I study of KW-0761, a defucosylated humanized anti-CCR4 antibody, in relapsed patients with adult T-cell leukemia-lymphoma and peripheral T-cell lymphoma. *J Clin Oncol.* 2010;28(9):1591-1598.
40. Iellem A, Mariani M, Lang R, et al. Unique chemotactic response profile and specific expression of chemokine receptors CCR4 and CCR8 by CD4+CD25+ regulatory T cells. *J Exp Med.* 2001;194(6):847-853.
41. Ishida T, Iida S, Akatsuka Y, et al. The CC chemokine receptor 4 as a novel specific molecular target for immunotherapy in adult T-cell leukemia/lymphoma. *Clin Cancer Res.* 2004;10(22):7529-7539.
42. Ishida T, Joh T, Uike U, et al. Defucosylated anti-CCR4 monoclonal antibody (KW-0761) for relapsed adult T-cell leukemia-lymphoma: a multicenter phase II study [published online ahead of print February 6, 2012]. *J Clin Oncol.* doi: 10.1200/JCO.2011.37.3472.
43. Harashima N, Kurihara K, Utsunomiya A, et al. Graft-versus-Tax response in adult T-cell leukemia patients after hematopoietic stem cell transplantation. *Cancer Res.* 2004;64(1):391-399.
44. Hunder NN, Wallen H, Cao J, et al. Treatment of metastatic melanoma with autologous CD4+T cells against NY-ESO-1. *N Engl J Med.* 2008;358(25):2698-2703.
45. Robbins PF, Morgan RA, Feldman SA, et al. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. *J Clin Oncol.* 2011;29(7):917-924.
46. Ishida T, Inagaki H, Utsunomiya A, et al. CXC chemokine receptor 3 and CC chemokine receptor 4 expression in T-cell and NK-cell lymphomas with special reference to clinicopathological significance for peripheral T-cell lymphoma, unspecified. *Clin Cancer Res.* 2004;10(16):5494-5500.
47. Nakagawa M, Nakagawa-Oshiro A, Karnan S, et al. Array comparative genomic hybridization analysis of PTCL-U reveals a distinct subgroup with genetic alterations similar to lymphoma-type adult T-cell leukemia/lymphoma. *Clin Cancer Res.* 2009;15(1):30-38.
48. Weisenburger DD, Savage KJ, Harris NL, et al. Peripheral T-cell lymphoma, not otherwise specified: a report of 340 cases from the International Peripheral T-cell Lymphoma Project. *Blood.* 2011;117(12):3402-3408.
49. Kannagi M, Harashima N, Kurihara K, et al. Tumor immunity against adult T-cell leukemia. *Cancer Sci.* 2005;96(5):249-255.
50. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science.* 2011;331(6024):1565-1570.

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ORIGINAL REPORT

Defucosylated Anti-CCR4 Monoclonal Antibody (KW-0761) for Relapsed Adult T-Cell Leukemia-Lymphoma: A Multicenter Phase II Study

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ABSTRACT

Purpose
Adult T-cell leukemia-lymphoma (ATL) is usually resistant to conventional chemotherapies, and there are few other treatment options. Because CC chemokine receptor 4 (CCR4) is expressed on tumor cells from most patients with ATL, KW-0761, a humanized anti-CCR4 monoclonal antibody, which markedly enhances antibody-dependent cellular cytotoxicity, was evaluated in the treatment of patients with relapsed ATL.

Patients and Methods
A multicenter phase II study of KW-0761 for patients with relapsed, aggressive CCR4-positive ATL was conducted to evaluate efficacy, pharmacokinetic profile, and safety. The primary end point was overall response rate, and secondary end points included progression-free and overall survival from the first dose of KW-0761. Patients received intravenous infusions of KW-0761 once per week for 8 weeks at a dose of 1.0 mg/kg.

Results
Of 28 patients enrolled onto the study, 27 received at least one infusion of KW-0761. Objective responses were noted in 13 of 26 evaluable patients, including eight complete responses, with an overall response rate of 50% (95% CI, 30% to 70%). Median progression-free and overall survival were 5.2 and 13.7 months, respectively. The mean half-life period after the eighth infusion was 422 ± 147 hours (± standard deviation). The most common adverse events were infusion reactions (89%) and skin rashes (63%), which were manageable and reversible in all cases.

Conclusion
KW-0761 demonstrated clinically meaningful antitumor activity in patients with relapsed ATL, with an acceptable toxicity profile. Further investigation of KW-0761 for treatment of ATL and other T-cell neoplasms is warranted.

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INTRODUCTION

Adult T-cell leukemia-lymphoma (ATL) is an aggressive peripheral T-cell neoplasm caused by human T-cell lymphotropic virus type I. The disease is resistant to conventional chemotherapeutic agents, and there currently exist limited treatment options; thus, it has a poor prognosis.[1-4] A recent phase III trial for previously untreated patients with aggressive ATL (acute, lymphoma, or unfavorable chronic type) age 33 to 69 years demonstrated that a dose-intensified multidrug regimen, VCAP-AMP-VECP (vincristine, cyclophosphamide, doxorubicin, and prednisone; doxorubicin, ranimustine, and prednisone; and vindesine, etoposide, carboplatin, and prednisone), resulted in median progression-free (PFS) and overall survival (OS) of 7.0 and 12.7 months, respectively.[5] This remains unsatisfactory compared with responses in other hematologic malignancies. Allogeneic hematopoietic stem-cell transplantation has evolved into a potential approach to treating patients with ATL over the last decade. However, only a small fraction of patients with ATL have the opportunity to benefit from transplantation, such as those who are younger, have achieved sufficient disease control, and have an appropriate stem-cell source.[6,7] Therefore, the development of alternative treatment strategies for patients with ATL is an urgent issue.

Because CC chemokine receptor 4 (CCR4) is expressed on tumor cells from most patients with ATL,[8,9] we postulated that it might represent a novel molecular target for immunotherapy. Accordingly, KW-0761, a next-generation humanized anti-CCR4 immunoglobulin G1 (IgG1) monoclonal antibody (mAb) with a defucosylated Fc region, which markedly enhances antibody-dependent cellular cytotoxicity (ADCC), was developed.[10,11] We demonstrated that robust ADCC by the defucosylated anti-CCR4 mAb against primary tumor cells from patients with ATL mediated by autologous effector cells was triggered both in vitro and in a humanized mouse model in vivo.[11-13] These promising preclinical results prompted us to conduct a phase I clinical trial of KW-0761 for patients with relapsed CCR4-positive peripheral T-cell lymphoma (PTCL), including ATL. This study demonstrated good tolerability, predictable pharmacokinetics, and preliminary evidence of potent antitumor activity and resulted in a recommended dose of 1.0 mg/kg for subsequent clinical trials.[14] Herein, we report the results of a multicenter phase II study designed to assess the efficacy, pharmacokinetic profile, and safety of KW-0761 monotherapy in patients with relapsed CCR4-positive aggressive ATL.

PATIENTS AND METHODS

Patients

Patients 20 years of age or older with CCR4-positive aggressive ATL (acute, lymphoma, or unfavorable chronic type)[1,4] who had relapsed after at least one prior chemotherapy regimen were eligible. The unfavorable chronic type of ATL was defined by the presence of at least one of the following three factors: low serum albumin, high lactate dehydrogenase, or high blood urea nitrogen concentration.[5] CCR4 expression was determined by immunohistochemistry or flow cytometry using a mouse anti-CCR4 mAb (KM2160)[8,14] and confirmed by a central review committee. All patients were required to have an Eastern Cooperative Oncology Group performance status of 0 to 2. Eligibility criteria also included the following laboratory values: absolute neutrophil count $\geq$ 1500/μL, platelet count $\geq$ 50,000/μL, hemoglobin $\geq$ 8.0 g/dL, AST $\leq$ 2.5 $\times$ the upper limit of the normal range (UNL), ALT [lteuq] 2.5 $\times$ UNL, total bilirubin $\leq$ 1.5 $\times$ UNL, serum creatinine $\leq$ 1.5 $\times$ UNL, corrected serum calcium $\leq$ 11.0 mg/dL, and arterial partial oxygen pressure $\geq$ 65 mmHg or arterial blood oxygen saturation $\geq$ 93%. Patients were excluded if they had an active infection, a history of organ transplantation, active concurrent cancers, CNS involvement, a bulky mass requiring emergent radiotherapy, or seropositivity for hepatitis B virus antigen, hepatitis C virus antibody, or HIV antibody.

Study Design

This study was a multicenter, single-arm, phase II trial. Objectives of the study were to evaluate the efficacy, pharmacokinetic profile, and safety of KW-0761 monotherapy. Patients received intravenous infusions of KW-0761 once per week for 8 weeks at a dose of 1.0 mg/kg.[14] Oral antihistamine and acetaminophen were administered before each KW-0761 infusion to prevent infusion reactions. The primary end point was overall response rate (ORR), and secondary end points included the best response by disease site, PFS, and OS. Objective responses were assessed after the fourth and eighth infusions of KW-0761 by an independent efficacy assessment committee according to the modified response criteria for ATL.[4] It was estimated that 25 patients would be required to detect a lower limit of the 95% CI exceeding the 5% threshold of ORR based on the assumptions that the minimum required ORR for a new drug for relapsed, aggressive ATL is 5%,[15] with an expected ORR for KW-0761 of 30%[14] with 90% power. Adverse events (AEs) were graded according to the National Cancer Institute Common Terminology Criteria for AEs, version 3.0. The presence of human anti-KW-0761 antibodies in the patients' plasma was examined using enzyme-linked immunosorbent assay. Blood samples collected at times strictly in accordance with the protocol were employed for the pharmacokinetic analysis. Samples were obtained from patients who had received at least one dose of KW-0761 up to all eight doses. When any event resulted in an alteration in the infusion protocol, only those samples taken before the alteration were used for the analysis. The following parameters were calculated for plasma KW-0761: maximum drug concentration and trough drug concentration of each KW-0761 administration, area under the blood concentration time curve from 0 to 7 days after the first and eighth doses, and half-life period ($t_{1/2}$) after the eighth dose. As an additional research parameter, we investigated blood T-cell subset distribution during and after KW-0761 treatment and compared these values with those of 10 healthy donors as controls (five men, five women; median age, 45 years; range, 41 to 57 years).

Statistical Analysis

Survival estimates were calculated using the Kaplan-Meier method. PFS was defined as the time from the first dose of KW-0761 to progression, relapse, or death resulting from any cause, whichever occurred first. OS was measured from the day of the first dose to death resulting from any cause. Regarding T-cell subset analysis, differences between the patients' values before KW-0761 treatment and those of the controls were examined using the Mann-Whitney U-test. Differences between KW-0761 pretreatment values and those at each time point after KW-0761 treatment were examined using the Wilcoxon signed-rank test. All analyses were performed with SPSS Statistics 17.0 (SPSS, Chicago, IL). In this study, $P < .05$ was considered significant.

Study Oversight

The study was sponsored by Kyowa Hakko Kirin Company (Tokyo, Japan). The academic investigators and the sponsor were jointly responsible for the study design. The protocol was approved by the institutional review board at each participating site, and all patients and controls provided written informed consent before enrollment according to the Declaration of Helsinki.

RESULTS

Patients

Of the 28 patients enrolled onto the study, 27 (12 men, 15 women) received at least one infusion of KW-0761. One patient was withdrawn for aggravation of the general condition before the administration of KW-0761. Demographics and clinical characteristics of the 27 patients are summarized in Table 1. Median age was 64 years (range, 49 to 83). The disease subtypes included 14 acute, six lymphoma, and seven unfavorable chronic type ATL. Of these 27 patients, 14 (52%) completed the schedule of eight planned infusions. Of the remaining 13 patients, 11 (41%) discontinued treatment because of disease progression, one (4%) because of skin rash, and another (4%) because of concurrent colon cancer, for which this patient was excluded from the efficacy evaluation.

Efficacy of KW-0761

Of 26 patients evaluable for efficacy, objective responses were noted in 13 patients (ORR, 50%; 95% CI, 30% to 70%), including eight complete responses (CRs). Responses according to disease site were 100% (13 of 13; all CRs) for blood, 63% (five of eight) for skin, and 25% (three of 12) for nodal and extranodal lesions. Responses according to disease subtype were 43% (six of 14) for acute, 33% (two of six) for lymphoma, and 83% (five of six) for unfavorable chronic type ATL. Responses according to number of prior chemotherapy regimens were 48% (10 of 21) in those who had one prior regimen and 60% (three of five) for those who had two or three prior regimens. Median PFS and OS were 5.2 and 13.7 months, respectively (Figs 1A, 1B).

Table 1. Patient Demographics and Clinical Characteristics (n = 27)*

Characteristic	No.	%
Age, years		
Median	64	
Range	49-83	
≥ 65	13	48
Sex		
Male	12	44
Female	15	56
ECOG performance status†		
0	15	56
1	7	26
2	5	19
Disease subtype		
Acute	14	52
Lymphoma	6	22
Chronic	7	26
Prior chemotherapy regimens, No.		
1	22	82
2	3	11
3	2	7

Abbreviation: ECOG, Eastern Cooperative Oncology Group.
*Of 28 patients enrolled, 27 received at least one infusion of KW-0761.
†ECOG performance status scores range from 0 (normal activity) to 5 (death), with higher scores indicating more severe disability.

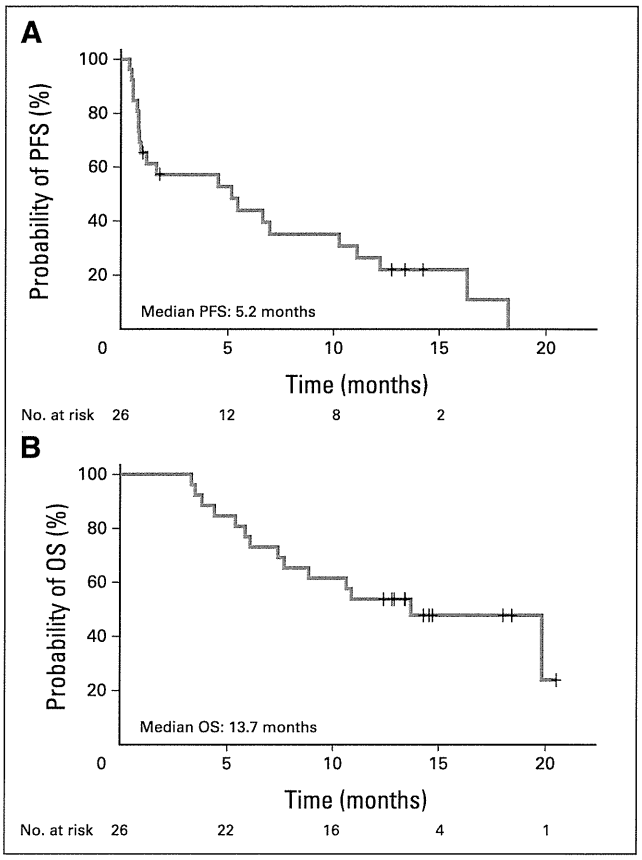

Fig 1. Kaplan-Meier curves of estimated (A) progression-free survival (PFS; median, 5.2 months) and (B) overall survival (OS; median, 13.7 months).

Pharmacokinetics

KW-0761 plasma concentrations over eight infusions, once per week, at 1.0 mg/kg are shown in Figure 2. Mean maximum drug concentration and trough drug concentration (± standard deviation) of the eighth infusion were 42.9 ± 14.2 μg/mL and 33.6 ± 10.6 μg/mL, respectively. Mean area under the blood concentration time curve from 0 to 7 days after the eighth infusion was 6,297 ± 1,812 μg × hours/mL. The mean $t_{1/2}$ after the eighth infusion was 422 ± 147 hours.

AEs

Table 2 lists AEs that occurred in at least 15% of patients or at grades 3 to 4, which were determined as possibly, probably, or definitely KW-0761 related. The most common nonhematologic AE was an infusion reaction (89%). In addition, 80% or more of the following recorded AEs occurred along with an infusion reaction: fever, chills, tachycardia, hypertension, nausea, and hypoxemia (Table 2). These events occurred primarily at the first infusion, becoming less frequent with subsequent treatments. The infusion reactions and component events were transient, and all patients recovered, although some needed systemic steroids. Skin rashes were observed as another frequent nonhematologic AE (63%), mostly occurring after the fourth or subsequent infusions. Of the 14 patients who developed grade 2 or higher skin rashes, objective responses were noted in 13 patients (93%), including eight CRs. On the other hand, of the 12 patients who developed no or grade 1 skin rashes, no objective responses were observed. A typical clinical course of the rash is depicted in Appendix Figures A1A and A1B (online only). The skin rash observed in this patient appeared after the seventh infusion, and the corresponding skin biopsy revealed mild perivascular CD8-positive cells dominating an inflammatory reaction, with an absence of ATL cells. The skin rash recovered on application of topical steroid. Of the 17 patients who developed skin rashes, one developed Stevens-Johnson syndrome, which was determined as possibly KW-0761 related, although that patient also received trimethoprim/sulfamethoxazole, fluconazole, and acyclovir for prevention of infection according to the protocol. This patient stopped those preventive agents and was treated with

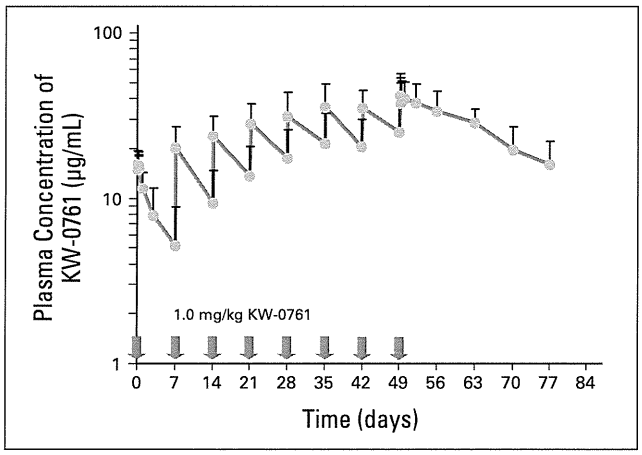

Fig 2. Pharmacokinetics of KW-0761. Mean KW-0761 plasma concentrations during and after 1.0 mg/kg KW-0761 infusions once per week for 8 weeks. Bar indicates upper limit of standard deviation.

Table 2. Adverse Events (n = 27)*

Adverse Event	Grade (No. of patients) 1	2	3	4	All Grades No. of Patients	%	Infusion Reaction Related (No. of patients) All Grades	≥ Grade 2
Nonhematologic								
Infusion reaction	1	22	1	0	24	89		
Fever	20	2	0	0	22	82	18	2
Rash	3	9	5	0	17	63	1	0
Chills	14	2	0	0	16	59	16	2
ALT	5	4	2	0	11	41		
AST	3	5	2	0	10	37		
Tachycardia	9	0	0	0	9	33	9	0
Hypertension	6	2	0	0	8	30	8	1
Albuminemia	7	1	0	0	8	30		
ALP	4	2	0	0	6	22		
Weight gain	5	0	0	0	5	19		
Nausea	4	1	0	0	5	19	5	1
Hyponatremia	5	0	0	0	5	19		
Hypoxemia	0	2	3	0	5	19	4	4
Hypotension	2	2	0	0	4	15	3	1
Pruritus	0	3	1	0	4	15		
γ-GTP	0	1	3	0	4	15		
Hypophosphatemia	0	4	0	0	4	15		
Hyperuricemia	4	0	0	0	4	15		
Hypercalcemia	1	1	0	1	3	11		
Hypokalemia	1	0	2	0	3	11		
Erythema multiforme†	0	0	1	0	1	4		
Hyperglycemia	0	0	1	0	1	4		
Tumor lysis syndrome	0	0	1	0	1	4		
Metabolic/laboratory, other‡	4	7	3	0	14	52		
Hematologic								
Lymphopenia§	0	6	9	11	26	96		
Leukocytopenia	3	7	8	0	18	67		
Thrombocytopenia	7	2	3	2	14	52		
Neutropenia	5	4	5	0	14	52		
Hemoglobin	4	3	1	0	8	30		

Abbreviations: ALP, alkaline phosphatase; BUN, blood urea nitrogen; CRP, C-reactive protein; GTP, glutamyl transpeptidase.

*Of 28 patients enrolled, 27 received at least one infusion of KW-0761. Listed are adverse events determined as possibly, probably, or definitely KW-0761 related that occurred in at least 15% of patients or were of grade 3 to 4 severity.

†One patient diagnosed as having Stevens-Johnson syndrome.

‡Other metabolic and laboratory test abnormalities included hypoproteinemia, BUN elevation, CRP, glycosuria, hypochloremia, and hyperammoniemia.

§Lymphopenia included decrease of abnormal lymphocytes.

systemic steroids, but improvement required the passage of 4 months. Lymphopenia, including a decrease in the number of ATL cells, occurred in 26 (96%) of the 27 patients. Grades 3 to 4 thrombocytopenia was observed in five patients (19%) but was not associated with bleeding, and grade 3 neutropenia also occurred in five patients but did not lead to a febrile episode. The latter two hematologic AEs improved in all patients. None of the patients developed detectable anti–KW-0761 antibody.

T-Cell Subset Analysis

The numbers of circulating blood CD4+ CCR4+, CD4+ CD25+ FOXP3+, CD4+ CCR4−, and CD4− CD8+ cells from KW-0761–treated patients and those from the 10 controls are presented as box and whisker plots in each graph (Appendix Figs A2A to A2D, online only). The numbers of CD4+ CCR4+ and CD4+ CD25+ FOXP3+ cells in patients with ATL before treatment were significantly higher than those in the controls but were significantly reduced after the first KW-0761 infusion. The reduction lasted for at least 4 months after the eighth infusion (Appendix Figs A2A, A2B; online only). The numbers of CD4+ CCR4−, and CD4− CD8+ cells in patients with untreated ATL were significantly lower than those in the controls. KW-0761 treatment led to a transient further reduction of those cells; however, recovery took place by the fifth infusion (Appendix Figs A2C, A2D; online only).

DISCUSSION

In the present multicenter phase II study, KW-0761 monotherapy demonstrated significant responses in patients with relapsed ATL with an acceptable toxicity profile. An ORR of 50% and median PFS and OS values of 5.2 and 13.7 months, respectively, were observed. Because the lower limit for an ORR with a 95% CI was 30%, this study met the primary end point. These results suggest an improvement over what has been achieved with other agents in relapsed ATL.[15] Cladribine was associated with an ORR of 7% (one of 15 patients),[16] and irinotecan hydrochloride treatment had an ORR of 38% (five of 13 patients) with a median duration of response of 31 days.[17] Antiviral therapy consisting of a combination of zidovudine and interferon, which has been proposed as a standard first-line therapy in leukemic subtypes of ATL,[18] was initially reported as having a median OS of 3.0 months in 19 patients with acute or lymphoma type ATL.[19] In addition, White et al[20] reported three objective responses lasting longer than 1 month with zidovudine plus interferon in 18 patients with ATL, of whom 15 had received prior therapy. Those observations collectively suggest that KW-0761 may offer an advantage over or provide an additional therapeutic option to the currently available therapy for relapsed ATL, although there were no direct comparisons.

On examining the results of ATL treatment according to disease site, disease in blood seemed to be more sensitive to KW-0761 than at other disease sites. Currently, we are unable to fully explain this difference; however, factors such as the KW-0761 delivery or the amount of ADCC effector cells such as natural killer (NK) cells and monocytes/macrophages in each disease site may be important.

Pharmacokinetic analyses demonstrated that the $t_{1/2}$ after the eighth administration of KW-0761 was nearly the same as that of circulating endogenous human IgG1, indicating good stability of this antibody in vivo. In addition, no anti-KW-0761 antibody was detected, suggesting that the antigenicity of this novel defucosylated mAb is not likely to be a problem clinically, consistent with findings in our preceding phase I study.[14]

The infusion reactions observed in the present study may also provide novel insights into problems associated with antibody therapy. It is generally recognized that complement plays a major role in infusion reactions,[21] but this mechanism cannot apply to KW-0761, because the agent is unable to mediate complement-dependent cytotoxicity.[11] Therefore, the infusion reactions observed here may have a different mechanism compared with those of other antibody therapies, such as rituximab. KW-0761 has a defucosylated Fc region, which markedly enhances ADCC because of increased binding affinity to the

Fcγ receptor on effector cells. Defucosylated IgG1 is a more potent activator of NK cells than nondefucosylated IgG1 during ADCC.[22] We surmise that the infusion reactions to KW-0761 were mainly induced by cytokines and related cytotoxic molecules released from highly activated NK cells.

The present study demonstrated that compared with the levels in the controls, KW-0761 led to a significant and lasting decrease in the number of CD4+ CCR4+ but not CD4+ CCR4− or CD4− CD8+ cells in patients with ATL. Consistent with the fact that CCR4 is expressed not only on T-helper type 2 cells but also on regulatory T (Treg) cells,[23-26] KW-0761 treatment also resulted in a significant and lasting decrease in CD4+ CD25+ FOXP3+ cells, including both ATL cells and endogenous non-ATL Treg cells.[27-29] Reduction or suppression of Treg cells is expected to be a potentially promising strategy for boosting antitumor immunity in patients with cancer, as observed in studies with ipilimumab,[30-33] although ipilimumab and KW-0761 have different targets; the former suppresses Treg cell function, and the latter decreases their number. Hence, KW-0761 could also lead to activation of antitumor immunity, which might also contribute to its potent anti-ATL response. Because ipilimumab causes immune-related AEs such as diarrhea and colitis, we were especially vigilant in monitoring for this type of AE. Because CCR4 contributes to lymphocyte skin-specific homing,[34] it was not surprising that skin rashes, which could be an immune-related AE, were frequently observed in the present KW-0761 study. Skin rashes, including the most severe case of Stevens-Johnson syndrome, the causal association of which with concomitant medications other than KW-0761 could not be excluded, proved to be manageable, and patients improved in all cases, although some needed systemic or topical steroid treatment. The observed better responses to KW-0761 in patients with grade 2 or higher skin rashes were highly impressive. However, the underlying mechanisms for this finding are not clear; thus, further detailed investigation is warranted. All of the 14 patients who developed grade 2 or higher skin rashes received five or more KW-0761 infusions according to the protocol, whereas only three of the 12 patients who developed no or grade 1 skin rashes received five or more KW-0761 infusions. This suggests the possibility that skin rashes were associated with the number of KW-0761 infusions. The Cochran-Mantel-Haenszel test stratified by the number of KW-0761 infusions ($\leq$ four $v \geq$ five) indicated a significant association between clinical response and skin rashes (no or grade 1 v grades 2 to 4; $P = .009$). However, the sample size is insufficient to draw such a conclusion.

Following on a phase III study (JCOG9801 [Japan Clinical Oncology Group 9801]) for untreated aggressive ATL,[5] the present promising results for KW-0761 monotherapy prompted us to conduct a subsequent randomized trial of VCAP-AMP-VECP chemotherapy with or without KW-0761 for previously untreated ATL (Clinicaltrials.gov: NCT01173887). CCR4 is also expressed on tumor cells from a subgroup of PTCL other than ATL, which also has an unfavorable prognosis.[2,35,36] Thus, we are currently conducting a phase II study of KW-0761 monotherapy for relapsed CCR4-positive PTCL (Clinicaltrials.gov:NCT01192984). In addition, Duvic et al[37] recently reported a phase I/II study of KW-0761 for refractory cutaneous T-cell lymphoma. They found that KW-0761 was well tolerated at doses of 0.1 to 1.0 mg/kg, and a promising ORR of 39% (15 of 38 patients) was achieved, although expression of CCR4 on lymphoma cells was not included as one of the eligibility criteria (Clinicaltrials.gov: NCT00888927). Furthermore, clinical trials of KW-0761 for patients with Hodgkin's lymphoma may be worth trying, because it has been reported that Hodgkin's lymphoma tumor cells produce CCR4 ligand molecules, and migratory CCR4-expressing Treg cells prevent a host immune attack on tumor cells, thereby creating an immunologically favorable environment for the tumor cells.[38]

Although this phase II study offers a novel promising treatment option (KW-0761) for patients with relapsed ATL, some limitations should be discussed. First, the present phase II study was relatively small, with consequent limitations on drawing definitive conclusions about the efficacy and safety profile of KW-0761. Second, patients received different prior systemic chemotherapy regimens, which could affect the results of the present study. Finally, the enrolled patients all had aggressive ATL, but three clinical subtypes (acute, lymphoma, and unfavorable chronic type) were included. Although there may be no significant differences in susceptibility to conventional chemotherapies between these subtypes, the heterogeneity of the enrolled patients might have affected the results.

In conclusion, this multicenter phase II study demonstrated that KW-0761 monotherapy showed clinically meaningful antitumor activity in patients with relapsed ATL, with an acceptable toxicity profile. Further investigation of KW-0761 for ATL and other T-cell neoplasms is warranted on the basis of the present results.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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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. Vose J, Armitage J, Weisenburger D: International peripheral T-cell and natural killer/T-cell lymphoma study: Pathology findings and clinical outcomes. J Clin Oncol 26:4124-4130, 2008
3. Uchiyama T, Yodoi J, Sagawa K, et al: Adult T-cell leukemia: Clinical and hematologic features of 16 cases. Blood 50:481-492, 1977
4. Tsukasaki K, Hermine O, Bazarbachi A, et al: Definition, prognostic factors, treatment, and response criteria of adult T-cell leukemia-lymphoma: A proposal from an international consensus meeting. J Clin Oncol 27:453-459, 2009
5. Tsukasaki K, Utsunomiya A, Fukuda H, et al: VCAP-AMP-VECP compared with biweekly CHOP for adult T-cell leukemia-lymphoma: Japan Clinical Oncology Group Study JCOG9801. J Clin Oncol 25:5458-5464, 2007
6. Utsunomiya A, Miyazaki Y, Takatsuka Y, et al: Improved outcome of adult T cell leukemia/lymphoma with allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant 27:15-20, 2001
7. Hishizawa M, Kanda J, Utsunomiya A, et al: Transplantation of allogeneic hematopoietic stem cells for adult T-cell leukemia: A nationwide retrospective study. Blood 116:1369-1376, 2010
8. Ishida T, Utsunomiya A, Iida S, et al: Clinical significance of CCR4 expression in adult T-cell leukemia/lymphoma: Its close association with skin involvement and unfavorable outcome. Clin Cancer Res 9:3625-3634, 2003
9. Yoshie O, Fujisawa R, Nakayama T, et al: Frequent expression of CCR4 in adult T-cell leukemia and human T-cell leukemia virus type 1-transformed T cells. Blood 99:1505-1511, 2002
10. Shinkawa T, Nakamura K, Yamane N, et al: The absence of fucose but not the presence of galactose or bisecting N-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity. J Biol Chem 278:3466-3473, 2003
11. Ishii T, Ishida T, Utsunomiya A, et al: Defucosylated humanized anti-CCR4 monoclonal antibody KW-0761 as a novel immunotherapeutic agent for adult T-cell leukemia/lymphoma. Clin Cancer Res 16:1520-1531, 2010
12. Ishida T, Iida S, Akatsuka Y, et al: The CC chemokine receptor 4 as a novel specific molecular target for immunotherapy in adult T-cell leukemia/lymphoma. Clin Cancer Res 10:7529-7539, 2004
13. Ito A, Ishida T, Utsunomiya A, et al: Defucosylated anti-CCR4 monoclonal antibody exerts potent ADCC against primary ATLL cells mediated by autologous human immune cells in NOD/Shi-scid, IL-2R gamma(null) mice in vivo. J Immunol 183: 4782-4791, 2009
14. Yamamoto K, Utsunomiya A, Tobinai K, et al: Phase I study of KW-0761, a defucosylated humanized anti-CCR4 antibody, in relapsed patients with adult T-cell leukemia-lymphoma and peripheral T-cell lymphoma. J Clin Oncol 28:1591-1598, 2010
15. Tobinai K: Current management of adult T-cell leukemia/lymphoma. Oncology (Williston Park) 14: 1250-1256, 2009
16. Tobinai K, Uike N, Saburi Y, et al: Phase II study of cladribine (2-chlorodeoxyadenosine) in relapsed or refractory adult T-cell leukemia-lymphoma. Int J Hematol 77:512-517, 2003
17. Tsuda H, Takatsuki K, Ohno R, et al: Treatment of adult T-cell leukaemia-lymphoma with irinotecan hydrochloride (CPT-11): CPT-11 Study Group on Hematological Malignancy. Br J Cancer 70:771-774, 1994
18. Bazarbachi A, Plumelle Y, Carlos Ramos J, et al: Meta-analysis on the use of zidovudine and interferon-alfa in adult T-cell leukemia/lymphoma showing improved survival in the leukemic subtypes. J Clin Oncol 28:4177-4183, 2010
19. Gill PS, Harrington W Jr, Kaplan MH, et al: Treatment of adult T-cell leukemia-lymphoma with a combination of interferon alfa and zidovudine. N Engl J Med 332:1744-1748, 1995
20. White JD, Wharfe G, Stewart DM, et al: The combination of zidovudine and interferon alpha-2B in the treatment of adult T-cell leukemia/lymphoma. Leuk Lymphoma 40:287-294, 2001
21. van der Kolk LE, Grillo-López AJ, Baars JW, et al: Complement activation plays a key role in the side-effects of rituximab treatment. Br J Haematol 115:807-811, 2001
22. Niwa R, Sakurada M, Kobayashi Y, et al: Enhanced natural killer cell binding and activation by low-fucose IgG1 antibody results in potent antibody-dependent cellular cytotoxicity induction at lower antigen density. Clin Cancer Res 11:2327-2336, 2005
23. Imai T, Nagira M, Takagi S, et al: Selective recruitment of CCR4-bearing Th2 cells toward antigen-presenting cells by the CC chemokines thymus and activation-regulated chemokine and macrophage-derived chemokine. Int Immunol 11: 81-88, 1999
24. Iellem A, Mariani M, Lang R, et al: Unique chemotactic response profile and specific expression of chemokine receptors CCR4 and CCR8 by CD4(+)CD25(+) regulatory T cells. J Exp Med 194: 847-853, 2001
25. Yagi H, Nomura T, Nakamura K, et al: Crucial role of FOXP3 in the development and function of human CD25+CD4+ regulatory T cells. Int Immunol 16:1643-1656, 2004
26. Ishida T, Ueda R: CCR4 as a novel molecular target for immunotherapy of cancer. Cancer Sci 97:1139-1146, 2006
27. Ishida T, Ueda R: Immunopathogenesis of lymphoma: Focus on CCR4. Cancer Sci 102:44-50, 2011
28. Karube K, Ohshima K, Tsuchiya T, et al: Expression of FoxP3, a key molecule in CD4CD25 regulatory T cells, in adult T-cell leukaemia/lymphoma cells. Br J Hematol 126:81-84, 2004
29. Yano H, Ishida T, Inagaki A, et al: Regulatory T-cell function of adult T-cell leukemia/lymphoma cells. Int J Cancer 120:2052-2057, 2007
30. Hodi FS, O'Day SJ, McDermott DF, et al: Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 363:711-723, 2010
31. Takahashi T, Tagami T, Yamazaki S, et al: Immunologic self-tolerance maintained by $CD25^{+}CD4^{+}$ regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. J Exp Med 192:303-310, 2000
32. Zou W: Regulatory T cells, tumour immunity and immunotherapy. Nat Rev Immunol 6:295-307, 2006
33. O'Day SJ, Hamid O, Urba WJ: Targeting cytotoxic T-lymphocyte antigen-4 (CTLA-4): A novel strategy for the treatment of melanoma and other malignancies. Cancer 110:2614-2627, 2007
34. Campbell JJ, Haraldsen G, Pan J, et al: The chemokine receptor CCR4 in vascular recognition by cutaneous but not intestinal memory T cells. Nature 400:776-780, 1999
35. Ishida T, Inagaki H, Utsunomiya A, et al: CXCR3 and CCR4 expression in T-cell and NK-cell lymphomas with special reference to clinicopathological significance for peripheral T-cell lymphoma, unspecified. Clin Cancer Res 10:5494-5500, 2004
36. Weisenburger DD, Savage KJ, Harris NL, et al: Peripheral T-cell lymphoma, not otherwise specified: A report of 340 cases from the International Peripheral T-cell Lymphoma Project. Blood 117: 3402-3408, 2011
37. Duvic M, Pinter-Brown L, Foss FM, et al: Results of a phase 1/2 study for KW-0761, a monoclonal antibody directed against CC chemokine receptor type 4 (CCR4), in CTCL patients. Blood 116, 2010 (abstr 962)
38. Ishida T, Ishii T, Inagaki A, et al: Specific recruitment of CC chemokine receptor 4-positive regulatory T cells in Hodgkin lymphoma fosters immune privilege. Cancer Res 66:5716-5722, 2006

