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Clinical outcomes of a novel therapeutic vaccine with Tax peptide-pulsed dendritic cells for adult T cell leukaemia/lymphoma in a pilot study

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Summary

Adult T cell leukaemia/lymphoma (ATL) is a human T cell leukaemia virus type-I (HTLV-I)-infected T cell malignancy with poor prognosis. We herein developed a novel therapeutic vaccine designed to augment an HTLV-I Tax-specific cytotoxic T lymphocyte (CTL) response that has been implicated in anti-ATL effects, and conducted a pilot study to investigate its safety and efficacy. Three previously treated ATL patients, classified as intermediate- to high-risk, were subcutaneously administered with the vaccine, consisting of autologous dendritic cells (DCs) pulsed with Tax peptides corresponding to the CTL epitopes. In all patients, the performance status improved after vaccination without severe adverse events, and Tax-specific CTL responses were observed with peaks at 16–20 weeks. Two patients achieved partial remission in the first 8 weeks, one of whom later achieved complete remission, maintaining their remission status without any additional chemotherapy 24 and 19 months after vaccination, respectively. The third patient, whose tumour cells lacked the ability to express Tax at biopsy, obtained stable disease in the first 8 weeks and later developed slowly progressive disease although additional therapy was not required for 14 months. The clinical outcomes of this pilot study indicate that the Tax peptide-pulsed DC vaccine is a safe and promising immunotherapy for ATL.

Keywords: adult T cell leukaemia/lymphoma, tumour vaccine, dendritic cell, human T cell leukaemia virus type-I, cytotoxic T lymphocyte.

Adult T cell leukaemia/lymphoma (ATL) is an aggressive lymphoproliferative disease caused by human T cell leukaemia virus type-I (HTLV-I) infection (Uchiyama *et al*, 1977; Poiesz *et al*, 1980; Hinuma *et al*, 1981). In particular, the acute and lymphoma types of ATL are characterized by a poor prognosis. Although the chronic and smouldering types

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of ATL exhibit milder disease progression, these diseases also result in poor clinical outcome once they have converted to the acute or lymphoma types.

One reason for the poor clinical outcome associated with ATL is rapid progression of the disease at onset, which requires a prompt diagnosis and effective first-line therapy. Currently available first-line therapies for ATL include intensive multi-agent chemotherapy (Tsukasaki *et al*, 2012), interferon-α combined with zidovudine (Gill *et al*, 1995; Hermine *et al*, 1995) and an anti-CCR4 antibody (mogamulizumab) (Ishida *et al*, 2012).

Frequent relapse is another reason for the poor prognosis of ATL, requiring subsequent administration of second-line therapy that can produce a long-lasting anti-ATL effect. Haematopoietic stem cell transplantation (HSCT) has been reported to achieve a long-lasting remission in 30–40% of ATL patients, although it occasionally induces treatment-related mortality in a similar percentage of recipients (Utsunomiya *et al*, 2001; Okamura *et al*, 2005; Hishizawa *et al*, 2010; Ishida *et al*, 2013). In addition to the graft-*versus*-host response (Tanosaki *et al*, 2008), the actions of Tax-specific cytotoxic T lymphocytes (CTLs) have been implicated in the graft-*versus*-ATL effects of HSCT. This is based on our previous finding that ATL patients who obtained complete remission following HSCT often exhibit activation of $CD8^+$ CTLs specific for HTLV-I Tax (Harashima *et al*, 2004).

In untreated ATL patients, Tax-specific CTLs are either undetectable or dysfunctional, if present (Takamori *et al*, 2011). Although ATL patients are in a severe immune suppressive state, the impaired CTL response is not merely a result of general immune suppression in the advanced disease, but also observed in the patients with earlier stages of the disease in a selective manner for HTLV-I-specific responses (Takamori *et al*, 2011). The anti-tumour effects of Tax-specific T cells have been well characterized in animal models, where Tax-coding DNA and Tax-peptide vaccines have been shown to induce T cell immunity, thus eradicating HTLV-I-infected lymphomas in rats (Ohashi *et al*, 2000; Hanabuchi *et al*, 2001).

The efficacy of the vaccine targeting Tax in human ATL patients remains unclear, and no such treatment has ever been attempted as an actual therapy. This is partly because the HTLV-I gene expression levels are believed to be very low *in vivo* (Kurihara *et al*, 2005; Rende *et al*, 2011), and ATL cells occasionally lack the ability to express Tax (Takeda *et al*, 2004). However, our previous finding of the Tax-specific CTL activation in ATL patients following HSCT from uninfected donors indicated the presence of a sufficient level of Tax expression for the CTL response *in vivo* (Harashima *et al*, 2004).

These findings prompted us to attempt to develop a therapeutic anti-ATL vaccine designed to augment a Tax-specific CTL response that may partly reproduce the long-lasting anti-tumour effects of HSCT as second-line therapy for ATL. For the vaccine antigen, we used synthetic oligopeptides corresponding to the major epitopes recognized by Tax-specific CTL identified in our previous studies of post-HSCT ATL patients (Harashima *et al*, 2004, 2005). These epitopes are restricted to HLA-A2, A24 or A11, all of which are common in the Japanese population. For the vaccine adjuvant, we used autologous dendritic cells (DCs) induced from the peripheral monocytes. Although previous reports suggested dysfunctions of DCs in ATL patients (Makino *et al*, 2000; Hishizawa *et al*, 2004), the monocyte-derived DCs obtained from ATL patients retained the ability of antigen presentation in our preliminary experiments. The use of autologous DCs loaded with tumour antigens have been reported in various tumour vaccine trials of different tumours (Nagayama *et al*, 2003; Ueda *et al*, 2004; Linette *et al*, 2005; Fuessel *et al*, 2006; Thomas-Kaskel *et al*, 2006; Wierecky *et al*, 2006).

The present pilot study investigated the safety and efficacy of the Tax peptide-pulsed dendritic cell (Tax-DC) vaccine when administered to augment Tax-specific CTL responses in ATL patients.

Materials and methods

Study design

This clinical study was approved by the institutional ethics committee and registered as UMIN000011423. Three ATL patients possessing HLA-A*02:01, A*24:02 and/or A*11:01, in stable condition at least 4 weeks after the administration of previous therapy, provided their written informed consent and were enrolled in this study, which investigated the safety and efficacy of the Tax peptide-pulsed DC (Tax-DC) vaccine between September 2012 and February 2013.

HTLV-I proviruses in the peripheral blood mononuclear cells (PBMCs) were examined for the potential Tax expression and conservation of targeted CTL epitopes by analysing their nucleotide sequences beforehand. All patients were subcutaneously administered with Tax peptide-pulsed autologous DCs (5×10^6) three times at 2-week intervals (Fig 1A) at Kyushu University Hospital.

Patients

Patient 1 was a 69-year-old male who was diagnosed with acute ATL in August 2011. After receiving four courses of multi-agent chemotherapy, he achieved stable disease (SD). Although additional treatment with lenalidomide was administered for a few weeks, it was discontinued due to the development of thrombocytopenia. The patient was registered to the study in September 2012.

Patient 2 was a 67-year-old female who was diagnosed with acute ATL in December 2011. She presented with remarkable systemic lymphadenopathy and splenomegaly, in addition to an extremely high level of soluble interleukin-2 receptor (sIL2R; 57 815 u/ml). She received four courses of

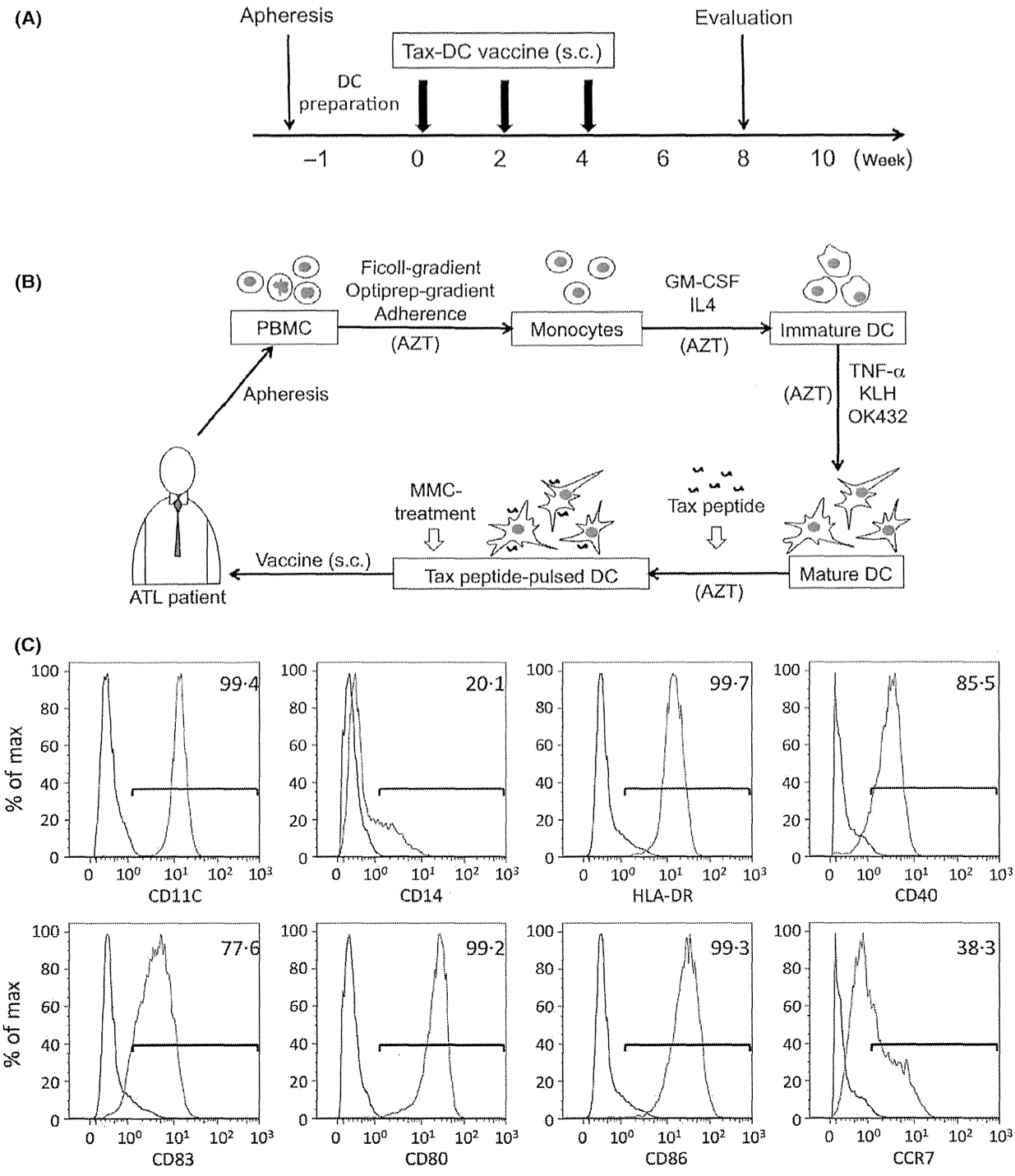


Fig 1. Outline of the Tax-DC vaccine therapy. (A) Schedule for the Tax-DC vaccine therapy. (B) Preparation of the monocyte-derived dendritic cells (DCs). Monocytes were enriched via serial density gradient centrifugation, and the adherent cells were cultured in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin 4 (IL4) for 5 d, followed by 48 h of culture with TNF-α, keyhole limpet haemocyanin (KLH), and OK432. A total of 10 μmol/l of zidovudine (AZT) was added whole throughout the culture. The matured DCs were pulsed with synthetic Tax peptides, treated with Mitomycin C (MMC), and then cryopreserved prior to subcutaneous injection. (C) Representative phenotype of mature dendritic cells prepared from Patient 1 prior to administration, as evaluated using flow cytometry. The red histograms indicate the results of staining with monoclonal antibodies for the indicated molecules, while the black histograms indicate the results of staining with control antibodies. ATL, Adult T cell leukaemia/lymphoma; PBMC, peripheral blood mononuclear cells.

multi-agent chemotherapy and achieved a partial remission (PR). Due to the development of disease recurrence with rapid progression after 2 months, treatment with mogamulizumab and low-dose chemotherapy (sobuzoxane + etoposide) was added. After obtaining a second PR, the patient was registered to the study in November 2012.

Patient 3 was a 56-year-old female diagnosed with acute ATL who presented with severe pneumocystis pneumonia in August 2012. After receiving two courses of multi-agent chemotherapy followed by two courses of mogamulizumab combined with chemotherapy, she achieved a PR. Further intensive treatment was not planned due to the development of severe respiratory dysfunction. The patient was registered to the study in February 2013.

The clinical information of the patients at enrollment is summarized in Table I.

Preparation of Tax peptide-pulsed DCs

Monocyte-derived DCs were generated from apheresis samples collected from the peripheral blood (6 l) of ATL patients at institutional cell processing facilities according to the good manufacturing practice (GMP) standard using a previously reported method, with some modifications (Nagayama *et al*, 2003) (Fig 1B). Briefly, monocytes enriched via serial density gradient centrifugation on Ficoll-Paque Plus (GE Healthcare, Uppsala, Sweden) and density-adjusted Optiprep (1·073 g/ml; Axis-Shield PoC, Oslo, Norway) were cultured at 37°C for 2 h, after which the adherent cells were cultured in CellGro DC medium (CellGenix GmbH, Freiburg, Germany) with 1000 iu/ml of granulocyte-macrophage colony-stimulating factor (Leukine; Bayer HealthCare Pharmaceuticals, Seattle, WA, USA) and 100 iu/ml of IL4 (Miltenyi Biotec, Bergisch Gladbach, Germany) for 5 d. The resulting monocyte-derived DCs were matured in the presence of 10 ng/ml of TNF-α (Miltenyi Biotec) and 12·5 μg/ml of keyhole limpet haemocyanin (KLH; Calbiochem, La Jolla, CA, USA) for 48 h, with 0·1 Clinical unit (Klinische Einheit; KE)/ml of OK432 (Picibanil; Chugai Pharmaceutical Co. Ltd., Tokyo, Japan) for the last 24 h. The matured DCs were pulsed with 2 μg/ml of synthetic peptides (NeoMPS; PolyPeptide Laboratories Group, San Diego, CA, USA), including Tax11-19 (LLFGYPVYV) (Kannagi *et al*, 1992) or Tax301-309 (SFHSLHLLY) (Harashima *et al*, 2004) restricted to HLA-A*02:01 or -A*24:02 respectively, and treated with Mitomycin C (MMC; Kyowa Hakko Kirin Co. Ltd., Tokyo, Japan) (50 μg/ml) in order to inactivate the ATL cells potentially contained in the preparation. As DCs are reported to be susceptible for HTLV-I infection (Jones *et al*, 2008), 10 μmol/l of zidovudine (Retrovir, AZT; GlaxoSmithKline, Research Triangle Park, NC, USA) was added whole throughout the culture to avoid *de novo* infection. The peptide-pulsed DCs were then washed and examined for safety by checking for contamination with bacteria, fungi, mycoplasma and/or endotoxins, then cryopreserved until use. The cells (5×10^6) were subsequently thawed and washed prior to administration.

Evaluation of adverse events and the clinical response

Toxic effects were graded according to the Common Terminology Criteria for Adverse Events version 3.0 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf). The clinical response was evaluated according to the criteria proposed by the international consensus meetings that led to the modification of the Japan Clinical Oncology Group criteria (Tsukasaki *et al*, 2009). Briefly, complete remission (CR) was defined as the disappearance of all clinical, microscopic and radiographic evidence of disease. PR was defined as a ≥50% reduction in the level of measurable disease without the appearance of new lesions. In addition, the diagnosis of a PR was required to satisfy a 50% or greater reduction in the absolute abnormal lymphocyte count in the peripheral blood. Progressive disease (PD) or relapsed disease was defined as a ≥50% increase from the nadir in the sum of the products of measurable disease or the appearance of new lesions, excluding the skin. Stable disease (SD) was defined as the failure to attain CR/PR nor PD.

The soluble IL2 receptor (sIL2R) level, HTLV-I proviral load and Tax-specific CTL response were monitored in addition to the results of general laboratory tests. Adverse effects

Table I. Patient characteristics at enrollment.

	Patient 1	Patient 2	Patient 3
Age (years)/gender	70/ male	68/ female	57/ female
HLA-A allele	24:02, 31:01	24:02, 26:03	02:01, 11:01
Subtype of ATL	Acute	Acute	Acute
Previous therapy	mEPOCH, lenalidomide	mEPOCH, mogamulizumab + PVP	mEPOCH, mogamulizumab + PVP
Disease status	SD	PR	PR
Interval from previous therapy	2·5 months	1·5 months	2 months
Duration since diagnosis	14 months	11 months	6 months
Complication	Allergic dermatitis	Breast cancer, DM, NASH	Interstitial pneumonia

mEPOCH, modified combination chemotherapy with etoposide + prednisone + vincristine + doxorubicin + carboplatin; PVP, combination chemotherapy with sobuzoxane + etoposide; SD, stable disease; PR, partial remission; DM, diabetes mellitus; NASH, nonalcoholic steatohepatitis.

and the clinical response were monitored and evaluated at 8 weeks after the initiation of the Tax-DC vaccine therapy.

Tax-specific CTL analysis

Phycoerythrin (PE)-conjugated HLA-A*0201/Tax11-19, HLA-A*1101/Tax88-96 and HLA-A*2402/Tax301-309 tetramers were purchased from Medical & Biological Laboratories, Co., Ltd. (Nagoya, Japan). Whole blood samples or PBMCs were stained with PE-conjugated Tax/HLA tetramers, together with fluorescein isothiocyanate (FITC)-conjugated anti-human CD3 and PE/cyanin 5 (Cy5)-conjugated anti-human CD8 monoclonal antibodies (mAbs) (BioLegend, San Diego, CA, USA), then fixed in Becton Dickinson (BD) FACS lysing solution (BD Biosciences, San Jose, CA, USA), followed by analysis on the FACS Calibur system using the CELLQUEST software program (BD Biosciences). For staining intracellular IFN-γ production, PBMCs pre-stained with PE-conjugated Tax/HLA tetramers and anti-human CD8-PE/Cy5 mAb were incubated at 37°C for 6 h in the presence of cognate Tax peptides (10 μmol/l), with brefeldin A (10 μg/ml; Sigma Aldrich, St. Louis, MO, USA) for the last 5 h. The cells were then permeablized using BD Cytofix/Cytoperm Fixation/Permeabilization Kit (BD Biosciences) and stained with FITC-conjugated anti-human IFN-γ mAb (4S.B3; BioLegend).

Detection of HTLV-I gene expression

To detect intracellular HTLV-I antigens, cells were serially treated with 4% paraformaldehyde for 10 min and 100% methanol for 10 min on ice, and then stained with Alexa Fluor 488-labelled anti-Tax Lt-4 (Lee *et al*, 1989) or isotype control mAbs followed by flow cytometry.

To quantify HTLV-I *pX* mRNA, total RNA extracted by using Isogen (Nippon Gene, Tokyo, Japan) were treated with DNase (Ambion, Austin, TX, USA), and subjected to quantitative reverse transcription polymerase chain reaction (RT-PCR) with the primer sets specific for HTLV-I *pX* (forward, 5′-CGG ATA CCC AGT CTA CGT GTT TGG AGA CT-3′; reverse, 5′-GAG CCG ATA ACG CGT CCA TCG ATG GGG TCC-3′) and *GAPDH* (forward, 5′-TGA TTT TGG AGG GAT CTC GCT CCT GGA AGA-3′; reverse, 5′-GTG AAG GTC GGA GTC AAC GGA TTT GGT CGT-3′) by using LightCycler Fast Start DNA Master SYBR Green I (Roche Diagnostics, Mannheim, Germany) after reverse transcription with oligo(dT)20 primers. The *pX* mRNA levels were standardized against *GAPDH* mRNA copy numbers.

Results

Feasibility of the DC preparation in ATL patients

We obtained 4·3–10·6 × 10^7 DCs with 72·2–91·3% purity. The cells exhibited the phenotype of mature DCs ($CD11c^+$, $CD80^+$, $CD86^+$, $CD83^+$, $CD40^+$, $HLA\text{-}DR^+$). The representative results obtained in Patient 1 are shown in Fig 1C. The HTLV-I proviral load of the PBMCs in the input apheresis samples were 114·8, 36·7 and 25·5 copies/1000 cells in the three patients respectively, with final loads in the DCs of 5·9, 5·0 and 10·3 copies/1000 cells, respectively.

Clinical courses after the Tax-DC vaccine therapy in the ATL patients

The clinical outcomes of the Tax-DC vaccine therapy in the three patients are summarized in Table II.

Table II. Clinical responses after the Tax-DC vaccine therapy in the three ATL patients.

Clinical response in 8 weeks after initiation of the vaccine therapy	Patient 1*		Patient 2†		Patient 3‡	
Time at evaluation	Pre-therapy	8 weeks	Pre-therapy	8 weeks	Pre-therapy	8 weeks
KPS (%)	70	90	70	80	70	90
LDH (iu/l)	473	245	250	326	329	268
sIL2R (u/ml)	19 056	1866	806	1462	1739	871
HTLV-I PVL (copies/1000 PBMCs)	114·8	12·4	36·7	14·9	17·7	29·6
Clinical response	–	PR	–	SD	–	PR
Long-term outcomes						
TTNT (months from registration)	25+		15		20+	
Survival (months from diagnosis)	39+		34		26+	

KPS, Karnofsky performance status; LDH, lactate dehydrogenase; HTLV-I PVL, human T cell leukaemia virus type-I proviral load, PBMCs, peripheral blood mononuclear cells; SD, stable disease, PR, partial remission; TTNT, time to next anti-tumour therapy.

*The size of the lymph nodes in Patient 1 repeatedly increased and decreased, especially at time points later than 6 months after initiation of vaccine therapy.

†Patient 2 was considered to have developed a progressive disease at 6 months after the initiation of the vaccine therapy.

‡Patient 3 achieved complete remission at 6 months after the initiation of the vaccine therapy.

Patient 1 was positive for HLA-A*24:02 and vaccinated with Tax 301-309 peptide-pulsed DCs. Following the first administration of the Tax-DC vaccine, he developed a fever (grade 2), dermatitis (grade 2) and diarrhoea (grade 1). The white blood cell count, level of ATL cells in the peripheral blood and LDH level in the serum showed remarkable fluctuation during the vaccination, and then stabilized after the third administration of the vaccine (Fig 2A). In Patient 1, the level of sIL2R, which is a sensitive tumour marker for ATL, decreased from 19 056 to 1866 u/ml (normal range: <570 u/ml) by 8 weeks of therapy (Fig 2B). In addition, his surface lymph nodes decreased in size (Fig 2C), and he achieved a partial remission (PR) that persisted for at least 24 weeks. He returned to his normal life, and his Karnofsky performance status (KPS) improved from 70% to 100%. Although the size of the patient's lymph nodes and the level of sIL2R fluctuated at later time points, he has remained in remission for more than 24 months after the completion of the Tax-DC vaccine therapy, without any additional anti-tumour treatment.

Patient 2 had HLA-A*24:02 and was vaccinated with Tax 301–309 peptide-pulsed DCs. She developed a low-grade fever and dermatitis (grade 2) after each vaccine administration. However, no severe adverse events were observed during her clinical course. At 8 weeks of therapy, she was considered to have achieved SD. Although there was no objective response, an improvement in the KPS was noted. She was subsequently considered to have developed PD 6 months after the initiation of the Tax-DC vaccine therapy. Nevertheless, due to slow progression of the disease and her stable general condition, she was followed without any additional anti-tumour therapy until 14 months after the completion of vaccination. The patient died of infection 23 months after the initiation of the vaccine therapy.

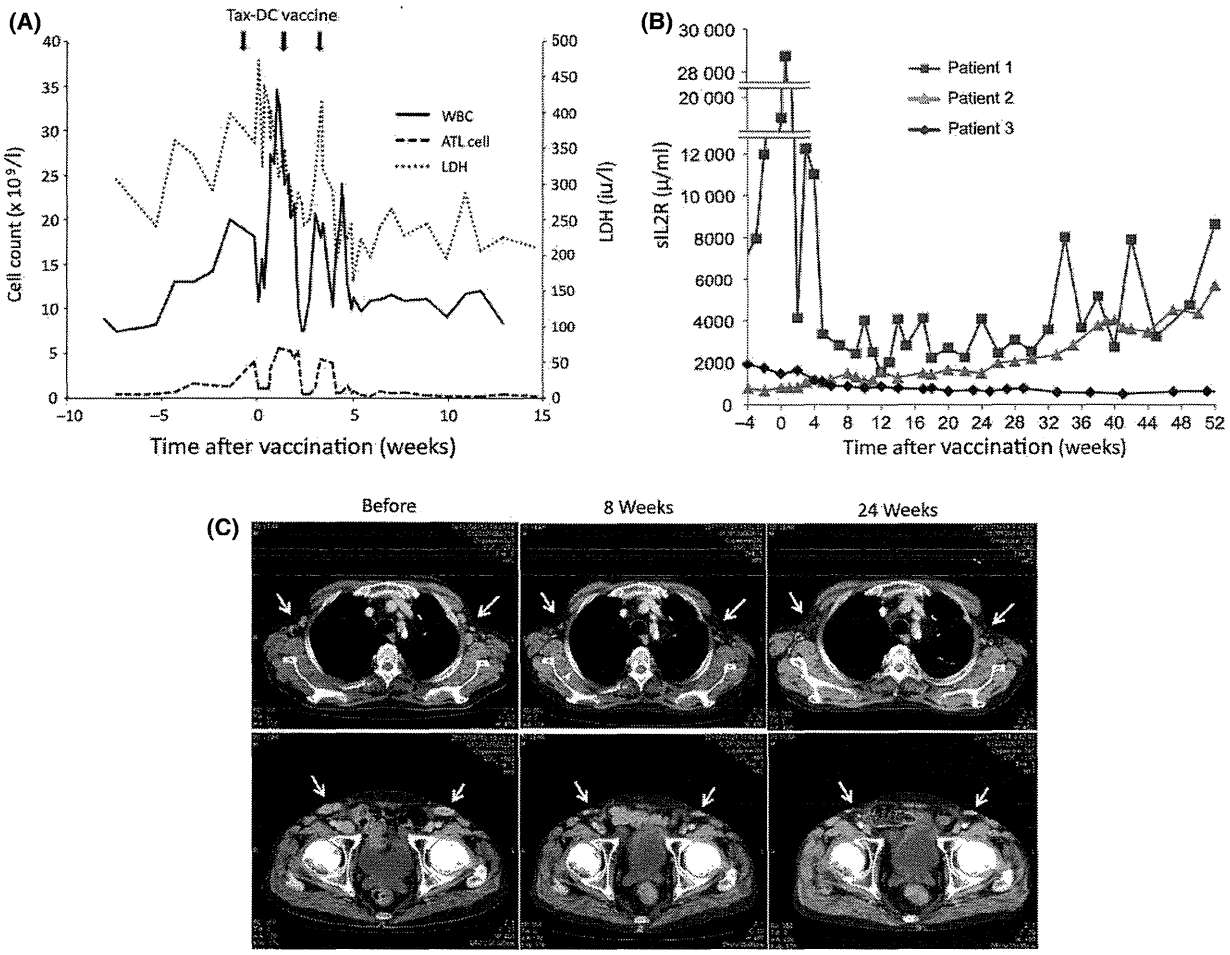


Fig 2. Clinical courses of the patients after the Tax-DC vaccine therapy. (A) Changes in the peripheral white blood cell count (WBC, solid line), ATL cell count (dashed line) and lactate dehydrogenase (LDH) level (fine dotted line) during the initial 15 weeks in Patient 1. The arrows indicate the days of Tax-DC vaccine administration. (B) Kinetics of the sIL2R levels in the sera obtained from Patients 1 (red), 2 (green) and 3 (blue) during the long-term observation period after the initiation of the Tax-DC vaccine therapy. (C) Computerized tomography images of the axillary (top) and inguinal (bottom) lymph nodes (arrows) of Patient 1 before and 8 and 24 weeks after the initiation of the Tax-DC vaccine therapy.

Patient 3 had HLA-A*02:01 and A*11:01. Although peptides of CTL epitopes for both HLA alleles were available, we chose the Tax11-19 peptide for HLA-A2 because HLA-A2 has a higher frequency in Japanese individuals. After each vaccination, the patient developed a low-grade fever and dermatitis (grade 2); however, no other severe adverse events were noted. She achieved a PR with an improvement in the KPS 8 weeks after the initiation of the Tax-DC vaccine therapy. Thereafter, the level of sIL2R returned to normal (Fig 2B). The patient subsequently achieved a CR at 6 months and has remained in this status for more than 19 months after the completion of the Tax-DC vaccine therapy.

Immunological responses after the Tax-DC vaccine therapy

In Patient 1, Tax-specific $CD8^+$ CTLs (HLA-A*24:02/Tax301-309 $tetramer^+$) were detectable prior to vaccination, and their frequency in peripheral $CD8^+$ cells transiently decreased during the Tax-DC vaccine administration, then recovered and maintained a constant level with some fluctuation (Fig 3A). The IFN-γ production from Tax-specific CTL also fluctuated. It is noteworthy that a vigorous proliferative response of Tax-specific CTLs was observed *in vitro* in the PBMC sample obtained at 20 weeks after the initiation of the Tax-DC vaccine therapy (Fig 3B), in which the proportion of HLA-A*24:02/Tax301-309 $tetramer^+$ cells in $CD8^+$ cells increased up to 22·5% within 2 weeks of the culture. A mild proliferative response of CTLs was also observed at 12 weeks. Samples obtained from the same patient prior to vaccination lacked such strong responses, implying a functional improvement in CTLs after the Tax-DC vaccine therapy.

Similar to that observed in Patient 1, a markedly increased level of spontaneous *in vitro* proliferative responses of Tax-specific CTLs was observed in the PBMC samples obtained from Patient 2 at 16 weeks after the initiation of the Tax-DC vaccine therapy, although the CTLs of this patient had exhibited a proliferative response prior to vaccination to a lesser degree (Fig 3B). The IFN-γ producing response of the CTL in this patient slightly improved after vaccination and showed some peaks at later time points.

As the size of the lymph nodes in Patient 2 did not improve within the first 8 weeks, a biopsy of the inguinal lymph node was performed at 9 weeks during the study period. The tumour cells isolated from the lymph node were $CD4^+$ $CD8^+$ $CCR4^+$ (Fig 4A) and possessed HTLV-I proviruses (849·5 copies/1000 cells). However, HTLV-I Tax proteins or mRNA expression was not induced in the lymph node cells after a short-term *in vitro* culture, whereas the viral expression was inducible in the PBMC sample of the same patient before vaccination (Fig 4B,C).

Tax-specific CTLs were below detectable levels prior to vaccination in Patient 3. However, 2 weeks after the initiation of the vaccine therapy with Tax 11–19 peptide-pulsed DCs, $CD8^+$ Tax-specific CTLs became detectable with HLA-A*0201/Tax11-19 tetramers, but not HLA-A*1101/Tax88-96 tetramers (Fig 3A). Although the IFN-γ producing response was barely detectable because of the low CTL frequency, an *in vitro* proliferative response of Tax-specific CTLs was observed in the PBMC samples obtained from Patient 3 most clearly at 16 weeks of the Tax-DC vaccine therapy, upon stimulation with Tax11-19 peptides, but not Tax 88–96 peptides (Fig 3B).

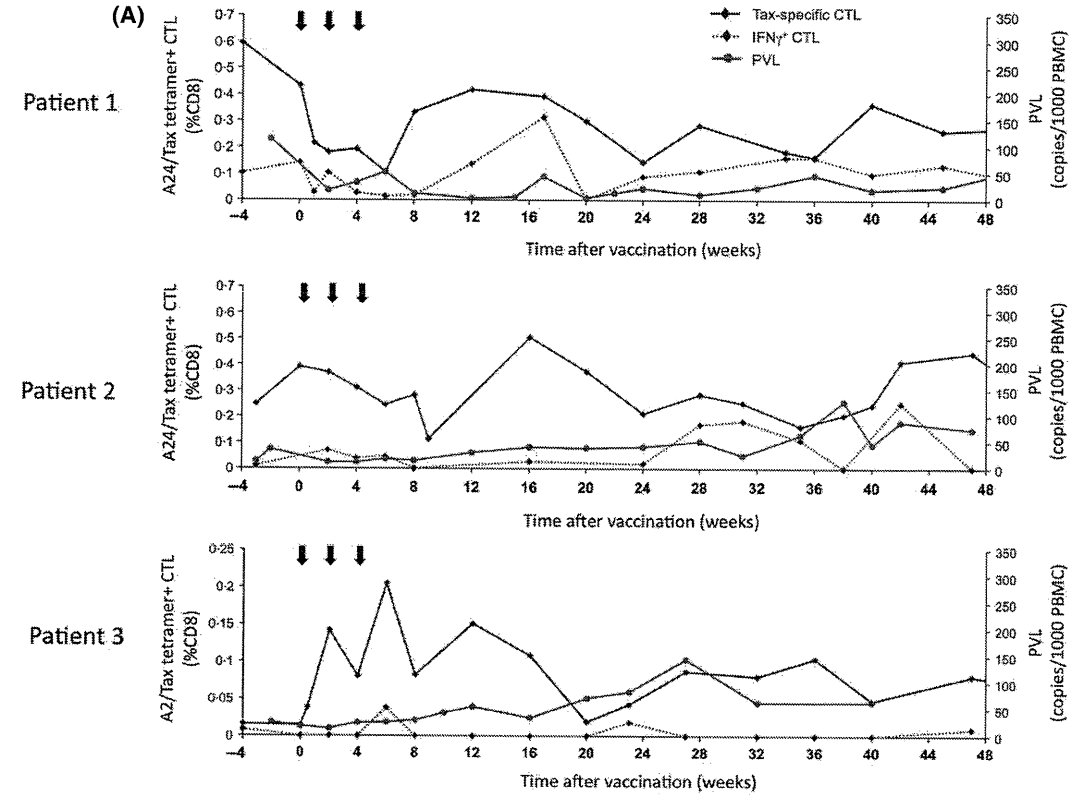
In all three patients, the level of the proviral load in the peripheral blood mostly remained below 100 copies per 1000 PBMCs at least for 1 year after vaccination, with the exception of sporadic small spikes (Fig 3A).

Discussion

Although various therapeutic trials have been conducted, the prognosis of ATL remains dismal. According to the simplified ATL prognostic index (ATL-PI) (Katsuya *et al*, 2012), the median survival time is only 4·6, 7·0 and 16·2 months, while the 2-year overall survival rate is 6%, 17% and 37%, for patients in high-, intermediate- and low-risk groups, respectively. According to the ATL-PI, Patients 1 and 2 were classified as intermediate-risk, while Patient 3 was classified as high-risk. Therefore, it is quite unique and surprising that all three patients remained in a favourable condition, without the need for any additional anti-tumour therapy, for at least 24, 14 and 19 months respectively, after only three administrations of the Tax-DC vaccine. In particular, Patients 1 and 3 obtained PR by 8 weeks after the initiation of the Tax-DC vaccine therapy.

Although these results are exciting, we cannot completely rule out the persisting effects of lenalidomide and/or mogamulizumab, which were previously administered in each patient prior to the Tax-DC vaccine therapy. These previous treatments may also have positively contributed to the present results via their immunomodulatory effects. According to recent reports, mogamulizumab has been shown to decrease the level of $CCR4^+$ regulatory T cells (Ishida & Ueda, 2011), and lenalidomide has immunomodulatory effects indirectly enhancing the activity of natural killer and T cells (Wu *et al*, 2008; De Keersmaecker *et al*, 2012).

The biopsy specimen of a residual surface lymph node from Patient 2 contained HTLV-I proviruses, although the viral expression was not inducible in the isolated cells even after *in vitro* culture (Fig 4). In general, induction of Tax expression after short-term culture is observed in approximately 50% of ATL cases (Kurihara *et al*, 2005). In the other 50% of ATL cases, the ATL cells lack the ability to express Tax, presumably due to the genomic and epigenetic changes in the HTLV-I proviruses (Takeda *et al*, 2004). Given that the viral expression was inducible in PBMCs of Patient 2 obtained prior to vaccination, the absence of viral induction



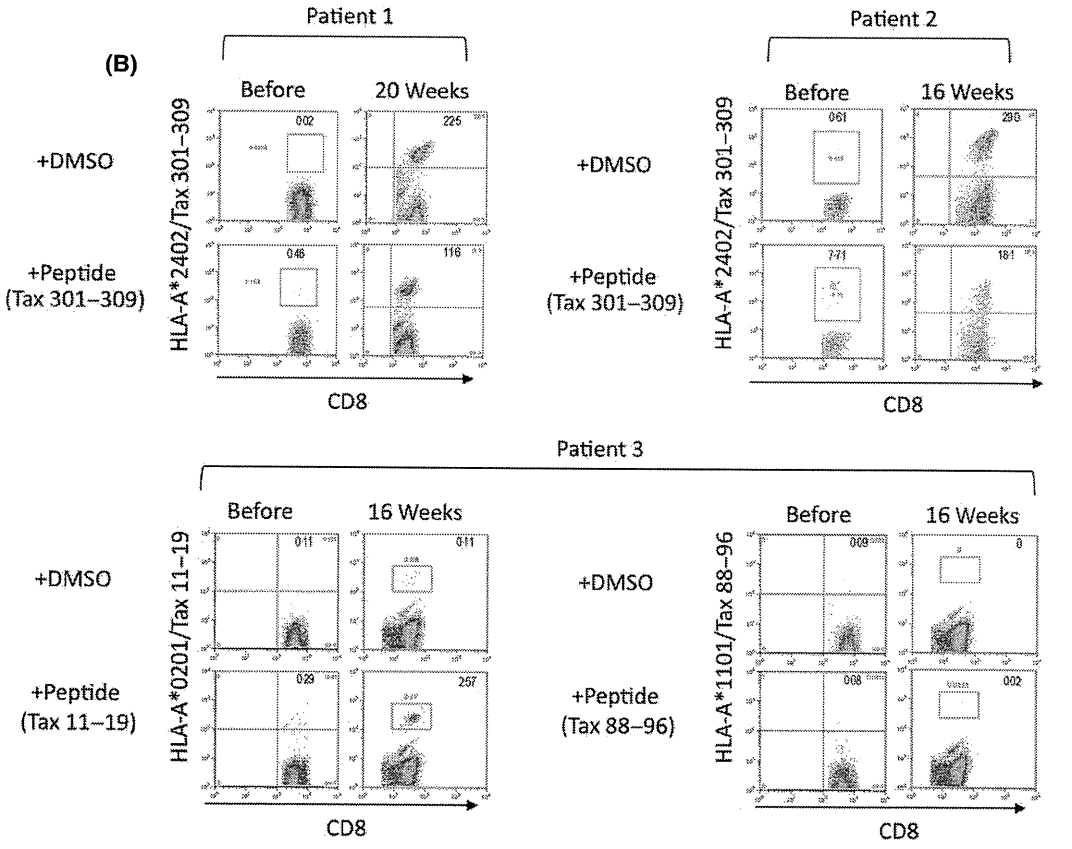


Fig 3. Immunological responses in the three patients after the Tax-DC vaccine therapy. (A) Long-term kinetics of the Tax-specific cytotoxic T cells (CTLs; % CD8+ cells, blue solid line), and γ-interferon (IFN-γ)-producing Tax-specific CTLs (% CD8+ cells, blue broken line), and human T cell leukaemia virus type-I proviral load (HTLV-I PVL) [copies/1000 peripheral blood mononuclear cells (PBMCs), red] in the peripheral blood of the three patients. Each arrow indicates administration of the vaccine. (B) The proliferative ability of the Tax-specific CTLs was evaluated using flow cytometry following incubation of the PBMCs for 13–15 d *in vitro* with cognate Tax peptide (100 nmol/l) or dimethyl sulfoxide (DMSO) in the presence of 10 u/ml of recombinant human IL2. The cells were stained with HLA/Tax tetramer-PE, anti-human CD8-PE-Cy5 mAb and anti-human CD3-FITC mAb. The values represent the percentage of tetramer$^+$ cells/CD3$^+$ CD8$^+$ cells.

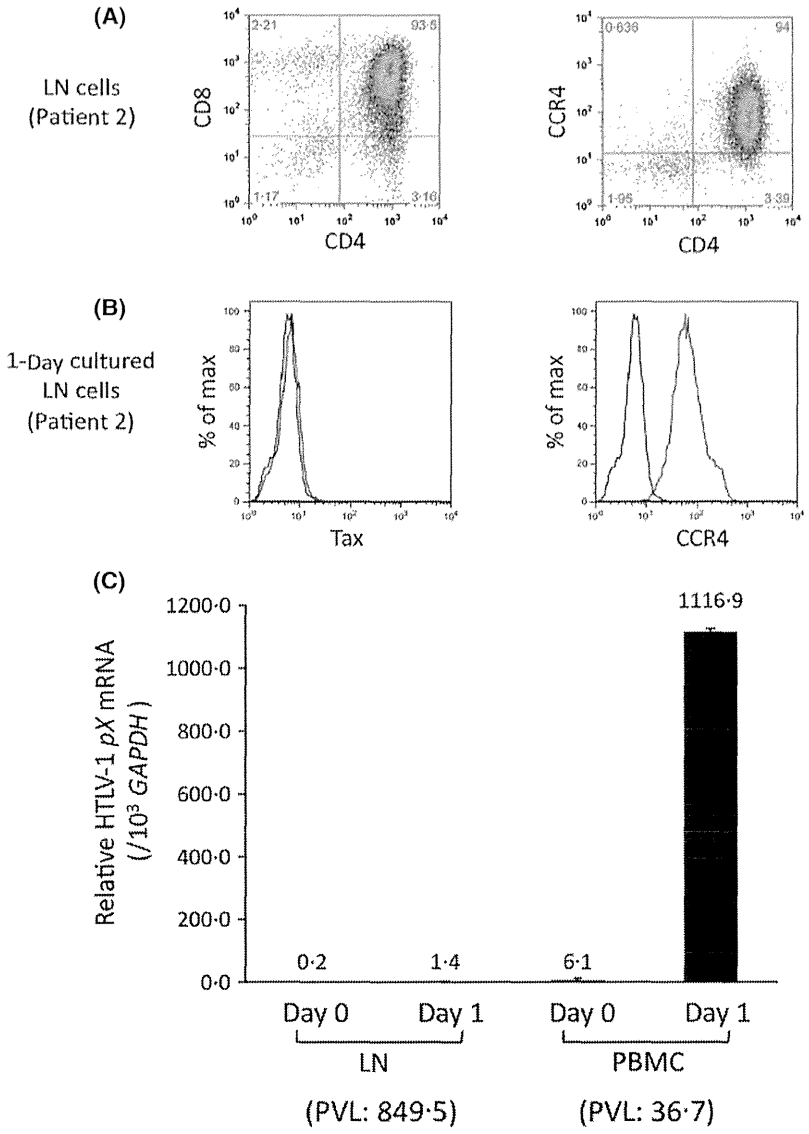


Fig 4. Absence of HTLV-I expression in the lymph node cells obtained from Patient 2. Cells were isolated from a biopsy specimen of the inguinal lymph node (LN) from Patient 2 at 9 weeks after the initiation of the Tax-DC vaccine therapy and subjected for characterization. (A) The cell surface phenotype of the LN cells immediately after isolation was analysed following staining with the indicated mAbs. (B) The intracellular Tax and CCR4 expression levels (red) in the LN cells after a 1-d culture *in vitro* were analysed following fixation of the cells with methanol. The blue histogram indicates the results of control antibody staining. (C) The HTLV-I *pX* mRNA expression levels in the LN cells before and after a 1-d culture *in vitro* were evaluated by quantitative reverse transcription polymerase chain reaction. The viral mRNA expression in peripheral blood mononuclear cells (PBMCs) obtained from the same patient before vaccination was similarly analysed as a positive control. The relative values standardized by *GAPDH* mRNA copy numbers were indicated as the means and standard deviations of duplicate samples. The proviral load (PVL) in the samples (copies/1000 cells) is indicated in parenthesis.

in the lymph node cells suggests that these tumour cells had escaped from Tax-specific CTLs.

Intriguingly, the Tax-specific CTLs demonstrated a vigorous proliferative response *in vitro* in all three patients at approximately 16–20 weeks after the initiation of the Tax-DC vaccine therapy. In particular, in Patients 1 and 2, the CTLs proliferated spontaneously without stimulation (Fig 3B). Similar phenomena have been reported in patients with HTLV-I-Associated Myelopathy/Tropical Spastic Paraparesis (Jacobson *et al*, 1990; Takamori *et al*, 2011) and occasionally in ATL patients post-HSCT (Harashima *et al*, 2005), interpreted to be the result of a normal CTL response against HTLV-I-infected cells *in vivo*. In the present study, although it is unclear whether the Tax-DC vaccine newly induced CTLs or simply activated pre-existing CTLs, Tax-specific CTLs appear to survey infected cells, at least for several months after the Tax-DC vaccine therapy, in responding to the dynamic activity of HTLV-I-infected cells *in vivo*.

In Patient 3, the Tax-specific CTLs emerged after vaccination and exhibited a clear proliferative response that peaked at 16 weeks. This response was preferentially directed toward the HLA-A2-restricted Tax epitope used for the therapy, not the HLA-A11-restricted epitope, suggesting the contribution of the Tax-DC vaccine therapy to CTL induction.

Although active CTL responses were observed in the first several months in all three patients, the responses diminished thereafter. At later time points (6 months or later) the sIL2R levels gradually increased in Patients 1 and 2 (Fig 2B). This finding suggests the need for a boosting vaccination or additional treatment to decrease the degree of immune suppression in order to maintain long-lasting anti-tumour effects.

In conclusion, the Tax-DC vaccine therapy is a safe and feasible treatment for ATL patients in stable condition. The promising clinical outcomes observed in the present study imply that the Tax-DC vaccine therapy has the potential to be an effective second-line treatment for ATL, although the anti-tumour effects of this vaccine therapy must be confirmed in further clinical trials with an increased number of patients. To our knowledge, this is the first clinical report to show the significance of a therapeutic vaccine targeting viral antigens as a new treatment modality for HTLV-I-induced malignancies. Given that Tax-specific CTL responses are impaired in patients with smouldering types of ATL and also in a small subset of asymptomatic HTLV-I carriers (Takamori *et al*, 2011), the vaccine therapy may be beneficial in these populations as well. The present study thus provides important information in a new era of anti-ATL immune therapies with the potential to be extended for prophylaxis of the disease in the future.

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Authorship

Y.S. designed the study, prepared the protocol, administered the Tax-DC therapy in patients and analysed the data. A.H. designed the study, prepared the protocol, established the method of Tax-DC preparation and analysed the data. T.I. administered the Tax-DC therapy in the patients. A.S., R.T., A.T., I.C., T.F., O.M. and T.T. participated in the protocol preparation. M.M. performed the provirus analysis. N.W. and A.T. performed the flow cytometric analysis. S.T. and K.A. supervised the institutional cell processing. M.K. proposed the initial idea and concept, designed the study, prepared the protocol and analysed the data. N.U. and J.O. supervised and coordinated the clinical and basic studies. M.K., Y.S., A.H. and J.O. wrote the manuscript. All co-authors approved the final version of the manuscript.

Disclosure

Tokyo Medical and Dental University holds a patent for the Tax epitope for HLA-A*11:01, of which M. Kannagi and R. Tanosaki are included in the inventors. This epitope was not used for a vaccine in the present study. S. Takaishi receives grants and personal fees from the MEDINET Co. Ltd., outside the submitted work.

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Review Article

Recent advances in the treatment of adult T-cell leukemia-lymphomas

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Adult T-cell leukemia-lymphoma, allogeneic hematopoietic stem cell transplantation, antiviral therapy, chemotherapy, molecular targeted therapy

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Recent advances in treatment for adult T-cell leukemia-lymphoma (ATL) are reviewed herein. It is currently possible to select a therapeutic strategy for ATL and predict prognosis by classification of patients by clinical subtypes and clinicopathological factors. Although the overall survival (OS) of patients with ATL has increased marginally because of advances in chemotherapy, further prolongation of survival might be difficult with conventional chemotherapy alone. Promising results have been reported for antiviral therapy using zidovudine and interferon-α, and, indeed, antiviral therapy is currently the standard treatment for patients with ATL in western countries. Remarkably, the 5-year OS rates are 100% for both the smoldering-type and chronic-type ATL. Recently, treatments for ATL have included allogeneic hematopoietic stem cell transplantation and molecular targeted therapies. Furthermore, the anti-CCR4 monoclonal antibody mogamulizumab has been shown to have marked cytotoxic effects on ATL cells, especially in the leukemic type of ATL. In the lymphoma type of ATL, the response rate may be improved by combining mogamulizumab with chemotherapy. It should be recognized that prevention of infection from carriers of human T-cell leukemia virus type-I and transfer of the virus from mother to infant are crucial issues for the eradication of ATL.

Adult T-cell leukemia-lymphoma (ATL) is a mature T-cell neoplasm caused by human T-cell leukemia virus type-I (HTLV-1).[1] Following the initial report by Uchiyama *et al.*,[2] many key discoveries concerning the mechanism of leukemogenesis of ATL have been made in association with the HTLV-1 *tax* and HTLV-1 *basic leucine zipper factor* genes.[3,4] Several clinical manifestations of ATL are known and may be classified into four clinical subtypes based on the presence of organ involvement and the results of blood work-up.[5] This classification is currently used as the basis for therapeutic strategies.

Therapeutic interventions, including intensive chemotherapy for aggressive ATL, are not associated with satisfactory outcomes, mainly because ATL cells are often resistant to chemotherapeutic agents;[6] moreover, patients with ATL frequently suffer from a number of opportunistic infections.[5] We reported for the first time that allogeneic hematopoietic stem cell transplantation (allo-HSCT) improved overall survival (OS) in ATL patients.[7]

In Europe and USA, antiviral therapy has been frequently applied for ATL patients since the therapeutic efficacy of zidovudine (AZT) and interferon-α (IFN) has been demostrated.[8,9] More recently, the mechanism of action of AZT combined with IFN (AZT/IFN) has been partially elucidated.[10] Antiviral therapy has received greater attention in Europe and USA than in Japan. Finally, new molecular targeted agents are under investigation in patients with ATL.

Herein, we review current treatments for ATL, along with previous and future therapies.

Epidemiology

Approximately 10–20 million people are infected with HTLV-1 worldwide; endemic areas include Central Africa, South America, the Caribbean basin, Iran, south-western Japan and Melanesia.[11] In Japan, approximately 1.1 million individuals are infected with HTLV-1[12] and approximately 1000 HTLV-1 carriers develop ATL each year.[13]

In late 2000, a decrease in the prevalence of HTLV-1 carriers has been observed in the Kyushu district (south-western island of Japan, an endemic area for ATL); however, the prevalence is increasing in several regions in the non-endemic areas.[12] The age-standardized incidence rates of ATL in the Honshu region of Japan and the USA, both of which are considered non-endemic areas, are increasing significantly, although no changes in incidence have been observed in the Kyushu district.[14] These results suggest that HTLV-1 is spreading through the migration of carriers from endemic to non-endemic areas. The mortality (per 100 000 person-years)

of patients with ATL decreased from 1.86 (95% confidence interval [CI]: 1.84–1.87) to 1.41 (95% CI: 1.40–1.43) in Kyushu during the period of 2000–2009, and from 0.22 (95% CI: 0.22–0.23) to 0.16 (95% CI: 0.16–0.17) in other areas of Japan from 2003–2009, and these trends are statistically significant.[13] The number of allo-HSCT performed in Japan has increased since 2000.[13] A significant inverse correlation was observed between the decreasing mortality trend and the increasing number of allo-HSCT procedures. The decreasing trend in mortality observed in ATL patients might be associated with allo-HSCT.[13]

Diagnosis and Clinical Subtype

A diagnosis of ATL is made by anti-HTLV-1 positivity in sera and by confirming the presence of a mature T-cell malignancy. The identification of monoclonal integration of HTLV-1 proviral DNA in tumor cells by Southern blot analysis is required to confirm a diagnosis of ATL.

Adult T-cell leukemia-lymphoma is divided into four clinical subtypes (acute, lymphoma, chronic and smoldering) according to leukemic manifestation in the blood, organ involvement, serum lactate dehydrogenase (LDH) levels and corrected serum calcium levels (Table 1).[5] Chronic type is divided into two subtypes: the unfavorable chronic type with at least one poor prognostic factor and the favorable chronic type with no poor prognostic factors. Poor prognostic factors include three factors, including serum LDH > upper limit of normal (ULN), serum blood urea nitrogen > ULN and serum albumin < lower limit of normal.[15]

Prognostic Factors and Stratification

The Lymphoma Study Group has identified five prognostic factors: age, total number of involved lesions, serum calcium level, serum LDH level and performance status (PS).[16] When ATL is stratified into three different groups (i.e. low risk group and high risk group based on the combination of prognostic factors, and extremely high risk group with high levels of serum calcium), OS is clearly different between the three groups. Nonetheless, these stratifications are not practical clinically as the classification system is rather complicated. In order to provide a more clinically useful system, Shimoyama devised a new clinical classification scheme for the four subtypes mentioned above.[5]

Several research groups in Japan have reported other factors that may also influence OS in ATL patients. These include deletion of *p16*, lung resistance-related protein and multi-drug resistance associated protein genes, eosinophilia, and expression of CC chemokine receptor 4 (CCR4) and serum interleukin (IL)-5.[17]

Recently, the Ann Arbor clinical stage, PS, and three continuous variables, age, serum albumin and soluble interleukin-2 receptor, were identified as independent prognostic factors in a multicenter retrospective analysis of 807 patients with newly diagnosed, acute-type and lymphoma-type ATL. Based on these results, Katsuya *et al.*[18] propose a prognostic index for acute-type and lymphoma-type ATL.

Treatment of Adult T-cell Leukemia-lymphoma

The current treatment strategy for patients with ATL is shown in Figure 1. Treatment is based on the clinical subtype. Patients with aggressive ATL, such as acute, lymphoma or chronic types, with at least one poor prognostic factor should receive early chemotherapy. In the USA and Europe, antiviral therapy using AZT/IFN is the standard treatment for leukemic-type ATL. In Europe, chemotherapy is the first-line therapy for lymphoma-type ATL, because OS with antiviral therapy alone is very short.[19]

Table 1. Diagnostic criteria for clinical subtype of adult T-cell leukemia-lymphoma

	Smoldering	Chronic§	Lymphoma	Acute
Anti-HTLV-1 antibody	+	+	+	+
Lymphocyte ($\times 10^9$/L)	<4	≥4¶	<4	†
Abnormal T-lymphocytes	≥5%	+††	≤1%	+††
Flower cells of T-cell marker	Occasionally	Occasionally	No	+
LDH	≤1·5N	≤2N	†	†
Corrected Ca (mmol/L)	<2·74	<2·74	†	†
Histology-proven lymphadenopathy	No	†	+	†
Tumor lesion				
Skin	‡	†	†	†
Lung	‡	†	†	†
Lymph node	No	†	Yes	†
Liver	No	†	†	†
Spleen	No	†	†	†
CNS	No	No	†	†
Bone	No	No	†	†
Ascites	No	No	†	†
Pleural effusion	No	No	†	†
GI tract	No	No	†	†

†No essential qualification except terms required for other subtype(s). ‡No essential qualification if other terms are fulfilled, but histology-proven malignant lesion(s) is required in case abnormal T-lymphocytes are less than 5% in peripheral blood. §Chronic type is divided into two subtypes: the unfavorable chronic type with at least one poor prognostic factor and the favorable chronic type with no poor prognostic factors. Poor prognostic factors include three factors: serum LDH > upper limit of normal (ULN), serum BUN > ULN and serum albumin < lower limit of normal. ¶Accompanied by T-lymphocytosis (3·5 × 10^9/L or more). ††In case abnormal T-lymphocytes are less than 5% in peripheral blood, histology-proven tumor lesion is required. Ca, calcium; CNS, central nervous system; GI, gastrointestinal; HTLV-1, human T-cell leukemia virus type-I; LDH, lactate dehydrogenase; N, normal upper limit. Source: Shimoyama (1991).

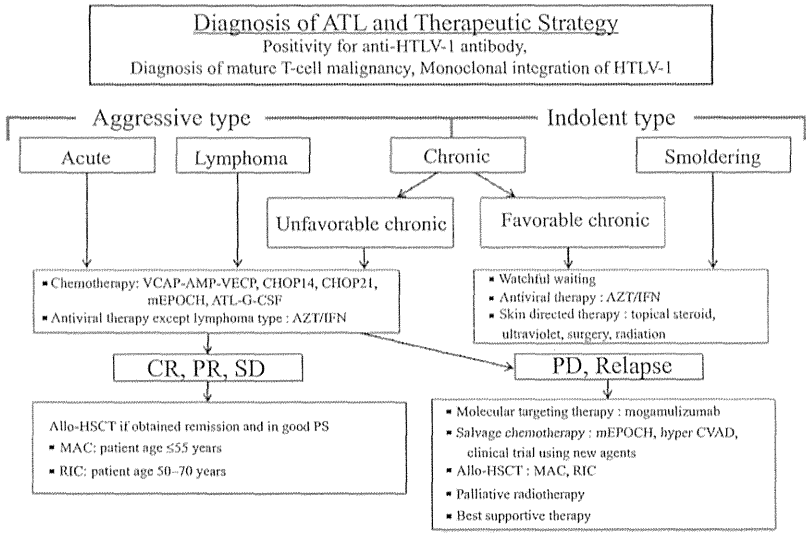

Fig. 1. Treatment algorithm for adult T-cell leukemia-lymphoma (ATL) patients. ATL diagnosis is based on anti-HTLV-1 antibody positivity in the serum, the presence of mature T-cell malignancy, and the Southern blot detection of monoclonal integration of HTLV-1 proviral DNA in the tumor cells. ATL treatment is usually determined according to the clinical subtypes and prognostic factors. The presence of an aggressive-type ATL (acute, lymphoma and chronic types with poor prognostic factors) or indolent-type ATL (chronic and smoldering types without poor prognostic factors) is critical when making treatment decisions. Patients with an aggressive-type (acute, lymphoma and unfavorable chronic type) generally receive immediate combination chemotherapy or antiviral therapy with zidovudine and interferon-α (AZT/IFN), except for those with the lymphoma type. The international consensus meeting primarily recommends the VCAP-AMP-VECP regimen. Other therapeutic regimens include CHOP14, CHOP21, mEPOCH and ATL-G-CSF. The patients undergo further treatment with allogeneic hematopoietic stem cell transplantation, which is particularly effective in young patients with good performance statuses, and those who have achieved remission before transplantation. In Japan, patients with an indolent-type ATL without any skin lesions are usually followed up under a watchful waiting policy until the disease transforms to an aggressive type. Antiviral therapy is frequently performed for favorable chronic and smoldering ATL patients in non-Japanese nations, and skin directed therapy is applied for smoldering ATL with skin manifestations. allo-HSCT, allogeneic hematopoietic stem cell transplantation; ATL-G-CSF, combination chemotherapy consisting of vincristine, vindesine, doxorubicin, mitoxantrone, cyclophosphamide, etoposide, ranimustine, and prednisone with granulocyte-colony stimulating factor support; AZT/IFN, zidovudine and interferon-α; CHOP, cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP14 is performed every 2 weeks and CHOP21 is performed every 3 weeks); CR, complete remission; hyper CVAD, cyclophosphamide, vincristine, doxorubicin, and dexamethasone; MAC, myeloablative conditioning; mEPOCH, etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin (EPOCH) with modifications; PD, progressive disease; PR, partial remission; PS, performance status; RIC, reduced intensity conditioning; SD, stable disease; VCAP-AMP-VECP, vincristine, cyclophosphamide, doxorubicin and prednisone (VCAP)-doxorubicin, ranimustine and prednisone (AMP)-vindesine, etoposide, carboplatin and prednisone (VECP).

Chemotherapy

Several chemotherapy combinations have been investigated for ATL patients in Japan, although the median OS range was only 6–8.5 months.[20] The Japan Clinical Oncology Group-Lymphoma Study Group (JCOG-LSG) has conducted a number of clinical trials for ATL patients in Japan, with complete response (CR) rates of 17–43% and median OS of 5–13 months in prospective multicenter studies.[21] The JCOG-LSG conducted a randomized clinical trial in patients with aggressive ATL in which a VCAP-AMP-VECP regimen (Fig. 2) was compared to a biweekly doxorubicin, cyclophosphamide, vincristine and prednisone (CHOP14) regimen.[22] The VCAP-AMP-VECP regimen reduced one course of VCAP-AMP-VECP from the original LSG15 regimen and added cytarabine to the intrathecal administration of methotrexate and prednisone as a prophylaxis against central nervous system (CNS) relapse. The CR rate and median OS of the VCAP-AMP-VECP regimen and CHOP14 regimen were 40% (95% CI: 27.6–54.2) versus 25% (95% CI: 14.5–37.3), and 13 versus 11 months, respectively. The CR rate of the VCAP-AMP-VECP regimen was significantly higher than that of CHOP14. In terms of the OS, there was no significant difference in the two groups (hazard ratio [HR] = 0.751, 95% CI: 0.50–1.13).[22] The VCAP-AMP-VECP regimen is considered a standard chemotherapeutic regimen for aggressive ATL in Japan.

Stem Cell Transplantation

In general, autologous HSCT has not been successful because of ATL relapses or infectious complications.[23] We and other Japanese researchers have reported that allo-HSCT could improve the outcome of ATL,[7] mainly using conventional myeloablative regimens (MAC); however, high transplant-related mortality poses a challenge (Table 2).

Therefore, allo-HSCT with reduced intensity conditioning regimens (RIC) was prospectively evaluated. Okamura *et al.*[24] report the safety and feasibility of allo-HSCT with RIC using peripheral blood stem cells from an HLA-matched sibling donor in older patients with ATL who achieved remission after chemotherapy. A total of 29 patients were registered, and the 5-year OS rate was 34% (95% CI: 18–51), indicating the potential curability of the disease.[25] Unrelated bone marrow (uBM) and cord blood transplantation with RIC were also prospectively evaluated as alternative strategies to allo-HSCT; follow up is currently under way.

By 2012, more than 1000 ATL patients had received various types of allo-HSCT. Currently, approximately 120 ATL patients undergo allo-HSCT each year in Japan.[26] Based on the incidence rate,[27] approximately 10% of ATL patients receive allo-HSCT each year. Several related aspects have been reported in a nationwide retrospective study. Based on the stem cell sources, the 3-year OS rate was highest for patients with related HLA-matched donors (41%, 95% CI: 33–49), followed

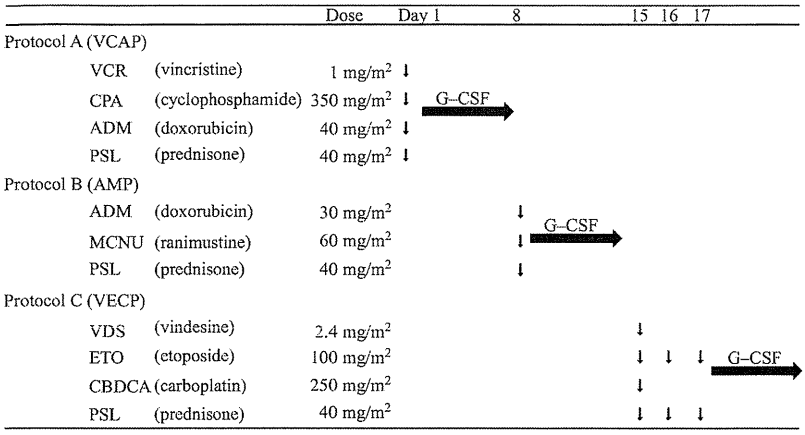

Fig. 2. The VCAP-AMP-VECP regimen. A, B and C are repeated every 28 days for 6 cycles. Cytarabine (40 mg), methotrexate (15 mg) and prednisone (10 mg) are administered intrathecally before cycles 2, 4 and 6. VCAP-AMP-VECP, vincristine, cyclophosphamide, doxorubicin and prednisone (VCAP)-doxorubicin, ranimustine and prednisone (AMP)-vindesine, etoposide, carboplatin and prednisone (VECP); G-CSF, granulocyte-colony stimulating factor.

by those with uBM (39%, 95% CI: 29–49).[28] In terms of the effect of acute graft-versus-host-disease (GVHD) on OS, grade I/II acute GVHD was significantly associated with a longer OS.[29] Regarding the effect of the conditioning regimen intensity on OS, although no significant difference was observed in the OS between MAC and RIC, a trend for superior OS was observed with RIC in older patients.[30] Bazarbachi *et al.*[31] report the results from the European Group for Blood and Marrow Transplantation's Lymphoma Working Party, and allo-HSCT might salvage ATL patients in non-Japanese patients.

Immunotherapy

Anti-tumor immune system activity has also been recognized in ATL patients who have received allo-HSCT.[29] Cytotoxic T-cells that targeted the HTLV-1 specific tax protein were detected in patients who were in remission after allo-HSCT.[32]

The discontinuation of immunosuppressive agents or donor lymphocyte infusions was effective in some ATL patients who relapsed after allo-HSCT; many of them developed GVHD subsequently.[33,34] The graft versus (Gv)-ATL effect, in particular the graft versus-tax (Gv-tax) effect after allo-HSCT, has been reported in ATL patients.[32] Therefore, immunotherapy targeting the tax protein may be effective in patients whose tumor cells express the tax protein. Indeed, a vaccine targeting tax was shown to induce anti-tumor activity in a mouse model.[35] Based on these findings, the anti-ATL vaccine, where the tax peptide is pulsed into autologous dendritic cells, was administered to three previously treated ATL patients as a clinical trial; the treated patients exhibited clinical effects without any serious adverse events except for a slight fever and transient skin reaction.[36] These results suggest that further improvements in immunotherapy are warranted.

Antiviral Therapy

Antiviral therapy using AZT/IFN was initially described by Gill *et al.*[8] and Hermine *et al.*[9] Gill *et al.* report an overall response rate (ORR) of 58% for 19 ATL patients, including 7 previously treated patients. Although a high ORR was achieved, the median OS of only 4.8 months in 12 of the previously untreated patients was considered unsatisfactory.[8] Subsequently, several follow-up studies of antiviral therapy using AZT/IFN have been conducted for ATL patients in Europe; however, the median OS could only be prolonged by 6–18 months.[37]

Bazarbachi *et al.* conducted a meta-analysis of antiviral therapy for ATL patients; they report that the median OS achieved with antiviral therapy was superior to that achieved with combination chemotherapy for ATL patients, especially for the leukemic subtypes, such as the smoldering, chronic and acute types of ATL.[19] Remarkably, a 5-year OS rate of 100% was achieved in patients with chronic and smoldering types of ATL with this antiviral therapy. It was concluded that antiviral therapy using AZT/IFN was the gold standard for the leukemic subtypes of ATL, although patients with the lymphoma type showed less benefit from antiviral therapy than from combination chemotherapy.[19,38] Takasaki *et al.*[39] report that the prognosis of indolent-type ATL in Japan is worse than that reported previously.[5] Bazarbachi *et al.*[19] report excellent results with antiviral therapy; therefore, it is important to verify the efficacy of antiviral therapy for Japanese ATL patients. Because the national health insurance system in Japan has not yet approved the use of these two drugs in the treatment of ATL patients, a randomized phase III clinical trial was recently initiated by the JCOG-LSG for treating indolent-type ATL with antiviral therapy consisting of AZT and IFN versus watchful waiting. This clinical trial will provide conclusive information regarding the optimal standard treatment for indolent-type ATL.

Molecular Targeted Therapy

Anti-CCR4 antibody therapy. The overexpression of CCR4 has been reported in tumor cells of various lymphoid neoplasms. The ratio of expression varies among different disease entities and is higher in mature T-cell and NK-cell neoplasms. Approximately 90% of ATL cases are CCR4-positive.[40] CCR4 expression has also been shown to affect the prognosis of ATL patients; multivariate analysis revealed that CCR4 positivity was a significant unfavorable prognostic factor.[40]

Mogamulizumab (KW-0761) is a first-in-class defucosylated humanized anti-CCR4 monoclonal antibody that has been generated by protein engineering;[41] mogamulizumab shows highly potent ADCC activity because of its high affinity of binding to effector cells, including NK cells.

Based on the phase I study, a phase II study for CCR4-positive relapsed ATL was conducted in Japan wherein 1.0 mg/kg of mogamulizumab was intravenously administered once a

Table 2. Summary of published reports on allogeneic hematopoietic stem cell transplantation in ATL

Reference	Patient Number	Median age (range)	Sex M/F	Subtype	Donor	Donor HTLV-1 Ab	Stem cell source	Disease Status at SCT	Conditioning regimen	Cause of death	Outcome
Utsunomiya (BMT, 2001)	10	45 (33–51)	7/3	Acute: 8 Lymphoma: 1 Other: 1	MSD: 9 MUD: 1	Neg: 7 Posi: 3	BM: 8 PB: 1 BM + PB: 1	CR: 4 PR: 5 NR: 1	MAC: 10	TRM: 4	Median leukemia-free survival 17.5+ M (range 3.7–34.4+)
Kami (BJH, 2003)	11	47 (15–59)	7/4	Acute: 5 Lymphoma: 4 Other: 2	MSD: 9 PMRD: 1 MUD: 1	Neg: 9 Posi: 2	BM: 7 PB: 3 BM + PB: 1	CR: 6 PR: 1 PD: 4	MAC: 9 RIC: 2	TRM: 7	1Y-OS 54.5 ± 30.0%
Fukushima (Leukemia, 2005)	40	44 (28–53)	22/18	Acute: 30 Lymphoma: 10	MSD: 27 PMRD: 5 NUD: 8	Neg: 27 Posi: 9 NE: 4	BM: 21 PB + 19	CR: 15 PR: 13 NC: 3 PD: 9	MAC: most cases	TRM: 16 Unk: 1 ATL: 4	3Y-0S 45.3%
Kato (BBMT, 2007)	33	49 (24–59)	18/15	Acute: 20 Lymphoma: 7 NE: 6	MUD: 33	Neg: 33	BM: 33	CR + PR: 15 NR: 14 NE: 4	MAC: 27 RIC: 6	TRM: 9 ATL: 2 NE: 3	1Y-OS 49.5%
Shiratori (BBMT, 2008)	15	57 (41–66)	3/12	Acute: 6 Lymphoma: 8 Other: 1	MSD: 10 MRD: 5	Neg: 13 Posi: 2	BM: 8 PB: 4 BM + PB: 3	CR: 9 PR: 5 PD: 1	MAC: 5 RIC: 10	TRM: 2 ATL: 2	3Y-OS 73.3%
Nakase (BMT, 2006)	8	49 (45–59)	2/6	Acute: 5 Lymphoma: 3	MUD: 3 PMUD: 5	Neg: 8	BM: 8	CR: 6 Non-CR: 2	MAC: 5 RIC: 3	TRM: 2 ATL: 1	Median OS 20M (range 0-43)
Nakamura (IJH, 2012)	10	51 (31–64)	6/4	Acute: 9 Lymphoma: 1	PMUD: 10	Neg: 10	UCB	CR: 2 PR: 4 SD: 1 PD: 3	MAC: 6 RIC: 4	ATL: 4 Sepsis: 1 GVHD+ATL: 1	2Y-OS: 40% (95% CI 67-12)
Fukushima (IJH, 2013)	27	52 (41–63)	18/9	Acute: 17 Lymphoma: 10	MUD: 1 PMUD: 26	Neg: 27	UCB	CR: 5 PR: 11 RIF: 5 REL: 6	MAC: 9 RIC: 18	TRM: 10 ATL: 9	3Y-OS: 27.4%
Bazarbachi (BMT, 2014)	17	47 (21–58)	9/8	Acute: 5 Lymphoma: 10 Chro/Smold: 2	MSD: 6 MUD: 7 UnK: 1 PMRD: 3	ND	ND	CR: 9 PR: 4 PD: 4	MAC: 4 RIC: 13	ATL: 8 GVHD: 2 Sepsis: 1	3Y-OS: 34.3%

ATL, adult T-cell leukemia-lymphoma. BBMT, *Biology of Blood and Marrow Transplantation*; BJH, *British Journal of Haematology*; BMT, bone marrow transplantation; Chro/Smold, chronic /smoldering; CR, complete remission; GVHD, graft-versus-host disease; IJH, *International Journal of Hematology*; M, month; MAC, myeloablative conditioning; MSD, HLA-matched sibling donor; MUD, HLA-matched unrelated donor; NC, no change; ND, not described; NE, not evaluable; Neg, negative; NR, no response; OS, overall survival; PD, progressive disease; Posi, positive; PMRD, HLA partially matched related donor; PMUD, HLA partially matched unrelated donor; PR, partial remission; RIC, reduced intensity conditioning; SCT, stem cell transplantation; SD, stable disease; TRM, transplant-related mortality; UCB, unrelated cord blood; UnK, unknown.

week for 8 weeks; of the 26 patients evaluable for efficacy assessment, the ORR was 50% (95% CI: 30–70), and response rates according to disease lesions were 100% for blood, 63% for skin, and 25% for nodal and extranodal lesions. The median progression-free survival and OS were 5.2 and 13.7 months, respectively.[42] Subsequently, a randomized clinical trial was conducted for evaluating VCAP-AMP-VECP treatment with or without mogamulizumab in newly diagnosed CCR4-positive aggressive ATL patients in Japan. Combination therapy with VCAP-AMP-VECP plus mogamulizumab demonstrated a CR rate of 52% (95% CI: 33–71), which was 19% higher than treatment with VCAP-AMP-VECP alone (33%, 95% CI: 16–55).[43]

Although mogamulizumab was very effective for relapsed ATL, adverse drug reactions, including infusion reaction (89%) and skin rash (63%), were frequently observed in the phase II study. Severe skin rash was observed occasionally, and one patient developed Stevens-Johnson syndrome during the phase II study.[42]

Molecular targeted therapy with small molecules. Recently, molecular targeted therapy with small molecules, such as tyrosine kinase inhibitors, angiogenesis inhibitors and proteasome inhibitors, has been applied for various malignancies. The proteasome inhibitor bortezomib has been reported to suppress the growth of ATL cell lines and freshly isolated ATL cells;[44] a trial for relapsed or refractory ATL patients is currently under way in Japan to investigate the clinical efficacy of bortezomib.

Supportive Therapy

Hypercalcemia associated with disease progression and opportunistic infections caused by immunodeficiency are problematic events in ATL patients.[5] Patients with hypercalcemia need immediate treatment with hydration, antidiuretics, calcitonin, steroid hormones and bisphosphonates. Furthermore, urgent chemotherapy using anti-cancer agents for ATL is needed in severe cases of hypercalcemia.

As CNS relapse is known to occur frequently in ATL patients, the intrathecal administration of the anti-cancer agents methotrexate, cytarabine and prednisone is required for prophylaxis.[22]

Opportunistic infections from bacteria, fungi, virus, protozoans and parasites are frequently observed in ATL patients, and appropriate treatment is needed.[5] In Japan, prophylactic treatment includes the use of fluconazole for *Candida*, itraconazole for *Aspergillus* and trimethoprim-sulfamethoxazole for *Pneumocystis jirovecii*.

Recent Findings of Genomic Heterogeneity of Adult T-cell Leukemia-lymphoma Cells

The initial pathogenic event for ATL is HTLV-1 integration; however, additional genetic alterations in ATL have also been implicated in its pathogenesis.[45] Umino *et al.*[46] recently reported the clonal heterogeneity of ATL tumor cells involving different genomic alterations; they demonstrated that these cells originated from a common cell. It was suggested that approximately 70% of ATL cases undergo clonal evolution, and that genetic instability may attribute to the accumulation of genomic alterations. The existence of multiple clones with genomic instability is one factor that renders ATL cells resistant to conventional chemotherapy. Even if a proportion of cells are killed by chemotherapy, there is always the possibility that a new resistant clone will emerge. Therefore, it is feasible to use allo-HSCT that can cure ATL patients by eliminating the HTLV-1-integrated recipient ATL clones through immune reaction, and by replacing the hematopoietic system with the donor type. Whole genome sequencing revealed that carriers have 10^3 to 10^4 orders of distinct clones with different HTLV-1 integration sites, and that most clones harbored one copy of HTLV-1.[47] This indicates that HTLV-1 carriers potentially have 10^3 to 10^4 malignant clones. If the number of pre-malignant cells increases, there is a greater possibility that malignant transformation can occur. Therefore, it is important to reduce the number of pre-malignant cells in carriers of HTLV-1 in order to prevent the development of ATL.

Prevention of Adult T-cell Leukemia-lymphoma Development

An ongoing nationwide prospective investigation (Joint Study on Predisposing Factors of ATL Development) was initiated in 2001 to identify HTLV-1 carriers with the highest risk of developing ATL. Four risk factors have been associated with ATL development in HTLV-1 carriers, including age ≥40 years, high HTLV-1 proviral loads in peripheral blood mononuclear cells, family history of ATL, and any clinical signs or symptoms.[48] Although it is obviously very important to prevent the development of ATL in HTLV-1 carriers with any of these risk factors, there are currently no available means towards this end.

The prevention of HTLV-1 infection is also of utmost importance because ATL is caused by HTLV-1 infection. HTLV-1 infection is thought to be transmitted by breastfeeding from the mother to infant, sexual intercourse or blood transfusion. The incidence of ATL development in HTLV-1 carriers differs according to the route of infection.[49] A nationwide prospective study is currently under way in Japan using three different nursing methods: cessation of breastfeeding, short nursing periods and ordinary nursing.

Future Directions

Histone deacetylase (HDAC) inhibitors, such as vorinostat (suberoylanilide hygroxamic acid: SAHA), panobinostat (LBH-589) and MS-275, have been recognized for their abilities to inhibit HTLV-1-infected cell lines and freshly isolated ATL cells.[50] Clinical use of these HDAC inhibitors for the treatment of ATL patients is expected.

Furthermore, various combinations of treatment, including chemotherapy, allo-HSCT, immunotherapy and molecular targeted therapies may help to cure a higher proportion of ATL patients in the future.

Conclusions

Although new therapeutic options are gradually improving the curability of ATL, treatment remains challenging for ATL patients. Nevertheless, to increase the ATL cure rate, rigorous investigation is necessary for optimizing therapeutic combinations, prevention of ATL development in HTLV-1 carriers, and reduction in the number of HTLV-1 carriers.

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Disclosure Statement

Dr Utsunomiya has received honoraria from Kyowa Hakko Kirin. The other authors have no competing interests to declare.

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