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).

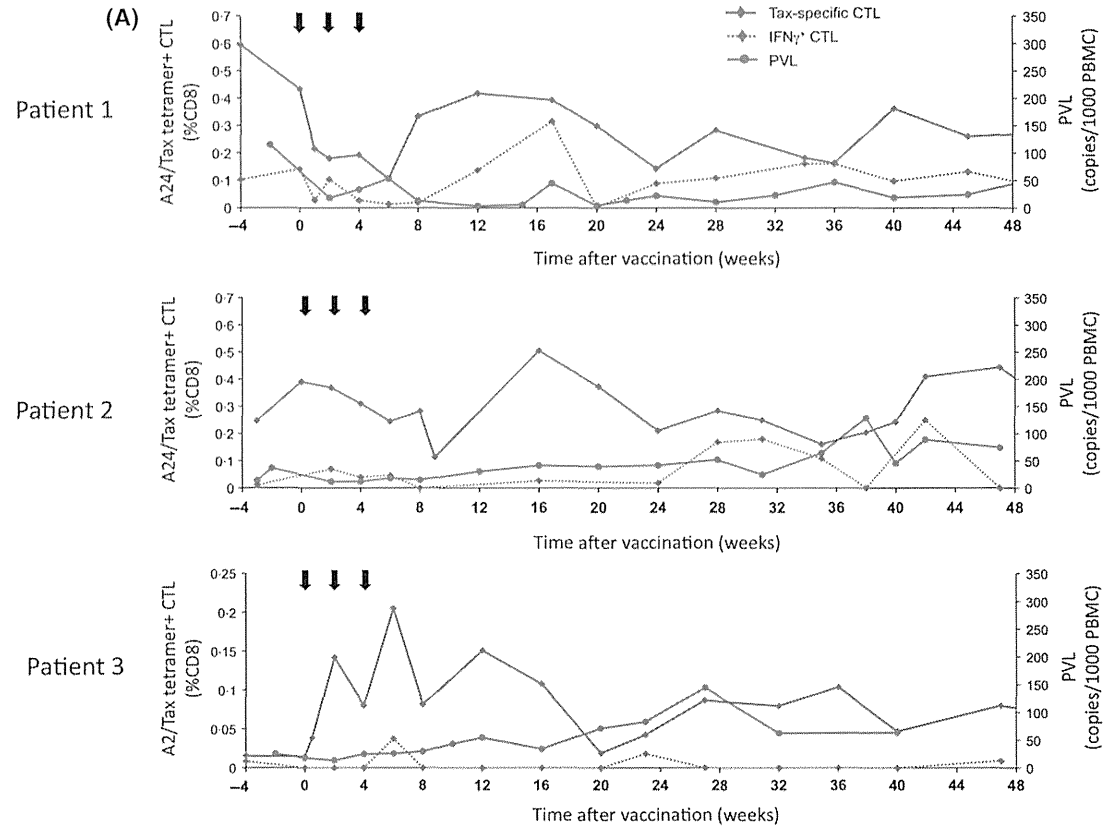
In all three patients, the level of the proviral load in the peripheral blood mostly remained below 100 copies per 1000 PBMCs at least for 1 year after vaccination, with the exception of sporadic small spikes (Fig 3A).

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



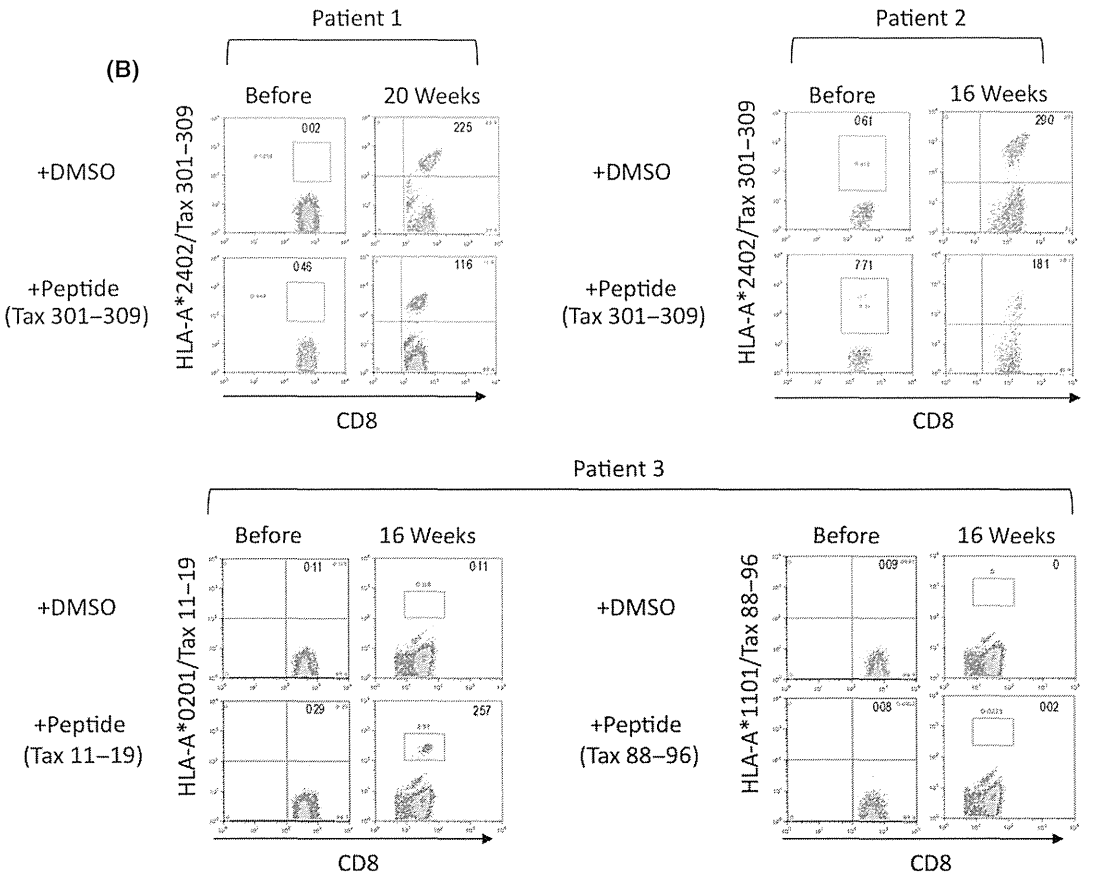


Fig 3. Immunological responses in the three patients after the Tax-DC vaccine therapy. (A) Long-term kinetics of the Tax-specific cytotoxic T cells (CTLs; % CD8+ cells, blue solid line), and γ-interferon (IFN-γ)-producing Tax-specific CTLs (% CD8+ cells, blue broken line), and human T cell leukaemia 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.

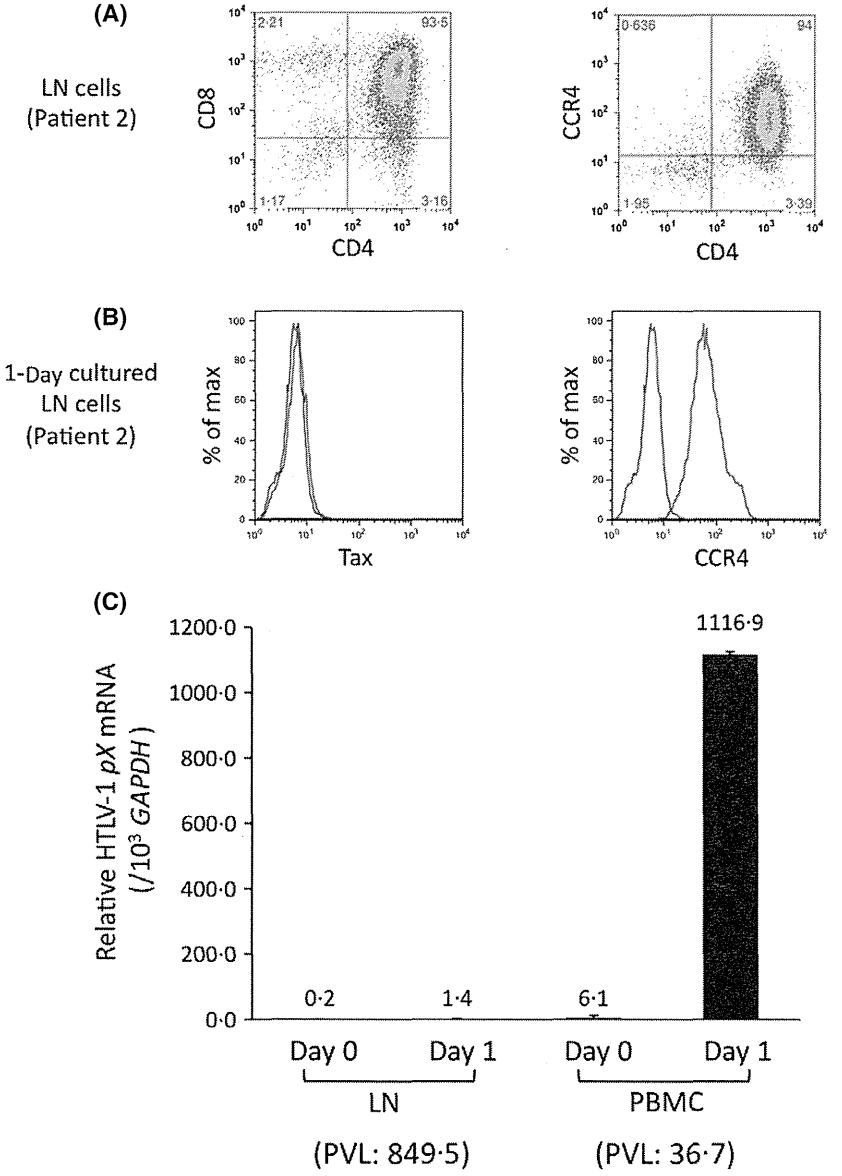


Fig 4. Absence of HTLV-I expression in the lymph node cells obtained from Patient 2. Cells were isolated from a biopsy specimen of the inguinal lymph node (LN) from Patient 2 at 9 weeks after the initiation of the Tax-DC vaccine therapy and subjected for characterization. (A) The cell surface phenotype 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.

References

De Keersmaecker, B., Allard, S.D., Lacor, P., Schots, R., Thielemans, K. & Aerts, J.L. (2012) Expansion of polyfunctional HIV-specific T cells upon stimulation with mRNA electroporated dendritic cells in the presence of immunomodulatory drugs. *Journal of Virology*, **86**, 9351–9360.

Fuessel, S., Meye, A., Schmitz, M., Zastrow, S., Linne, C., Richter, K., Lobel, B., Hakenberg, O.W., Hoelig, K., Rieber, E.P. & Wirth, M.P. (2006) Vaccination of hormone-refractory prostate cancer patients with peptide cocktail-loaded dendritic cells: results of a phase I clinical trial. *The Prostate*, **66**, 811–821.

Gill, P.S., Harrington, W. Jr, Kaplan, M.H., Ribeiro, R.C., Bennett, J.M., Liebman, H.A., Bernstein-Singer, M., Espina, B.M., Cabral, L., Allen, S., Kornblau, M.D., Pike, M.C. & Levine, A.M. (1995) Treatment of adult T-cell leukemia-lymphoma with a combination of interferon alfa and zidovudine. *New England Journal of Medicine*, **332**, 1744–1748.

Hanabuchi, S., Ohashi, T., Koya, Y., Kato, H., Hasegawa, A., Takemura, F., Masuda, T. & Kannagi, M. (2001) Regression of human T-cell leukemia virus type I (HTLV-I)-associated lymphomas in a rat model: peptide-induced T-cell immunity. *Journal of the National Cancer Institute*, **93**, 1775–1783.

Harashima, N., Kurihara, K., Utsunomiya, A., Tanosaki, R., Hanabuchi, S., Masuda, M., Ohashi, T., Fukui, F., Hasegawa, A., Masuda, T., Takaue, Y., Okamura, J. & Kannagi, M. (2004) Graft-versus-Tax response in adult T-cell

leukemia patients after hematopoietic stem cell transplantation. *Cancer Research*, **64**, 391–399.

Harashima, N., Tanosaki, R., Shimizu, Y., Kurihara, K., Masuda, T., Okamura, J. & Kannagi, M. (2005) Identification of two new HLA-A*1101-restricted tax epitopes recognized by cytotoxic T lymphocytes in an adult T-cell leukemia patient after hematopoietic stem cell transplantation. *Journal of Virology*, **79**, 10088–10092.

Hermine, O., Bouscary, D., Gessain, A., Turlure, P., Leblond, V., Franck, N., Buzyn-Veil, A., Rio, B., Macintyre, E., Dreyfus, F. & Bazarbachi, A. (1995) Brief report: treatment of adult T-cell leukemia-lymphoma with zidovudine and interferon alfa. *New England Journal of Medicine*, **332**, 1749–1751.

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

Hishizawa, M., Imada, K., Kitawaki, T., Ueda, M., Kadowaki, N. & Uchiyama, T. (2004) Depletion and impaired interferon-alpha-producing capacity of blood plasmacytoid dendritic cells in human T-cell leukaemia virus type I-infected individuals. *British Journal of Haematology*, **125**, 568–575.

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

Ishida, T. & Ueda, R. (2011) Immunopathogenesis of lymphoma: focus on CCR4. *Cancer Science*, **102**, 44–50.

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

Ishida, T., Hishizawa, M., Kato, K., Tanosaki, R., Fukuda, T., Takatsuka, Y., Eto, T., Miyazaki, Y., Hidaka, M., Uike, N., Miyamoto, T., Tsudo, M., Sakamaki, H., Morishima, Y., Suzuki, R. & Utsunomiya, A. (2013) Impact of graft-versus-host disease on allogeneic hematopoietic cell transplantation for adult T cell leukemia-lymphoma focusing on preconditioning regimens: nationwide retrospective study. *Biology of Blood and Marrow Transplantation*, **19**, 1731–1739.

Jacobson, S., Shida, H., McFarlin, D.E., Fauci, A.S. & Koenig, S. (1990) Circulating CD8+ cytotoxic T lymphocytes specific for HTLV-I pX in patients with HTLV-I associated neurological disease. *Nature*, **348**, 245–248.

Jones, K.S., Petrow-Sadowski, C., Huang, Y.K., Bertolette, D.C. & Ruscetti, F.W. (2008) Cell-free HTLV-1 infects dendritic cells leading to transmission and transformation of CD4(+) T cells. *Nature Medicine*, **14**, 429–436.

Kannagi, M., Shida, H., Igarashi, H., Kuruma, K., Murai, H., Aono, Y., Maruyama, I., Osame, M., Hattori, T., Inoko, H. & Harada, S. (1992) Target epitope in the Tax protein of human T-cell leukemia virus type I recognized by class I major histocompatibility complex-restricted cytotoxic T cells. *Journal of Virology*, **66**, 2928–2933.

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

Kurihara, K., Harashima, N., Hanabuchi, S., Masuda, M., Utsunomiya, A., Tanosaki, R., Tomonaga, M., Ohashi, T., Hasegawa, A., Masuda, T., Okamura, J., Tanaka, Y. & Kannagi, M. (2005) Potential immunogenicity of adult T cell leukemia cells in vivo. *International Journal of Cancer*, **114**, 257–267.

Lee, B., Tanaka, Y. & Tozawa, H. (1989) Monoclonal antibody defining tax protein of human T-cell leukemia virus type-I. *The Tohoku Journal of Experimental Medicine*, **157**, 1–11.

Linette, G.P., Zhang, D., Hodi, F.S., Jonasch, E.P., Longerich, S., Stowell, C.P., Webb, I.J., Daley, H., Soiffer, R.J., Cheung, A.M., Eapen, S.G., Fee, S.V., Rubin, K.M., Sober, A.J. & Haluska, F.G. (2005) Immunization using autologous dendritic cells pulsed with the melanoma-associated antigen gp100-derived G280-9V peptide elicits CD8+ immunity. *Clinical Cancer Research*, **11**, 7692–7699.

Makino, M., Wakamatsu, S., Shimokubo, S., Arima, N. & Baba, M. (2000) Production of functionally deficient dendritic cells from HTLV-I-infected monocytes: implications for the dendritic cell defect in adult T cell leukemia. *Virology*, **274**, 140–148.

Nagayama, H., Sato, K., Morishita, M., Uchimaru, K., Oyaizu, N., Inazawa, T., Yamasaki, T., Enomoto, M., Nakaoka, T., Nakamura, T., Maekawa, T., Yamamoto, A., Shimada, S., Saida, T., Kawakami, Y., Asano, S., Tani, K., Takahashi, T.A. & Yamashita, N. (2003) Results of a phase I clinical study using autologous tumour lysate-pulsed monocyte-derived mature dendritic cell vaccinations for stage IV malignant melanoma patients combined with low dose interleukin-2. *Melanoma Research*, **13**, 521–530.

Ohashi, T., Hanabuchi, S., Kato, H., Tateno, H., Takemura, F., Tsukahara, T., Koya, Y., Hasegawa, A., Masuda, T. & Kannagi, M. (2000) Prevention of adult T-cell leukemia-like lymphoproliferative disease in rats by adoptively transferred T cells from a donor immunized with human T-cell leukemia virus type 1 Tax-coding DNA vaccine. *Journal of Virology*, **74**, 9610–9616.

Okamura, J., Utsunomiya, A., Tanosaki, R., Uike, N., Sonoda, S., Kannagi, M., Tomonaga, M., Harada, M., Kimura, N., Masuda, M., Kawano, F., Yufu, Y., Hattori, H., Kikuchi, H. & Saburi, Y. (2005) Allogeneic stem-cell transplantation with reduced conditioning intensity as a novel immunotherapy and antiviral therapy for adult T-cell leukemia/lymphoma. *Blood*, **105**, 4143–4145.

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

Rende, F., Cavallari, I., Corradin, A., Silic-Benussi, M., Toulza, F., Toffolo, G.M., Tanaka, Y., Jacobson, S., Taylor, G.P., D'Agostino, D.M., Bangham, C.R. & Ciminale, V. (2011) Kinetics and intracellular compartmentalization of HTLV-1 gene expression: nuclear retention of HBZ mRNAs. *Blood*, **117**, 4855–4859.

Takamori, A., Hasegawa, A., Utsunomiya, A., Maeda, Y., Yamano, Y., Masuda, M., Shimizu, Y., Tamai, Y., Sasada, A., Zeng, N., Choi, I., Uike, N., Okamura, J., Watanabe, T., Masuda, T. & Kannagi, M. (2011) Functional impairment of Tax-specific but not cytomegalovirus-specific CD8+ T lymphocytes in a minor population of asymptomatic human T-cell leukemia virus type 1-carriers. *Retrovirology*, **8**, 100.

Takeda, S., Maeda, M., Morikawa, S., Taniguchi, Y., Yasunaga, J., Nosaka, K., Tanaka, Y. & Matsuoka, M. (2004) Genetic and epigenetic inactivation of tax gene in adult T-cell leukemia cells. *International Journal of Cancer*, **109**, 559–567.

Tanosaki, R., Uike, N., Utsunomiya, A., Saburi, Y., Masuda, M., Tomonaga, M., Eto, T., Hidaka, M., Harada, M., Choi, I., Yamanaka, T., Kannagi, M., Matsuoka, M. & Okamura, J. (2008) Allogeneic hematopoietic stem cell transplantation using reduced-intensity conditioning for adult T cell leukemia/lymphoma: impact of antithymocyte globulin on clinical outcome. *Biology of Blood and Marrow Transplantation*, **14**, 702–708.

Thomas-Kaskel, A.K., Zeiser, R., Jochim, R., Robbel, C., Schultze-Seemann, W., Waller, C.F. & Veelken, H. (2006) Vaccination of advanced prostate cancer patients with PSCA and PSA peptide-loaded dendritic cells induces DTH responses that correlate with superior overall survival. *International Journal of Cancer*, **119**, 2428–2434.

Tsukasaki, K., Hermine, O., Bazarbachi, A., Ratner, L., Ramos, J.C., Harrington, W. Jr, O'Mahony, D., Janik, J.E., Bittencourt, A.L., Taylor, G.P., Yamaguchi, K., Utsunomiya, A., Tobinai, K. & Watanabe, T. (2009) Definition, prognostic

factors, treatment, and response criteria of adult T-cell leukemia-lymphoma: a proposal from an international consensus meeting. *Journal of Clinical Oncology*, **27**, 453–459.

Tsukasaki, K., Tobinai, K., Hotta, T. & Shimoyama, M. (2012) Lymphoma study group of JCOG. *Japanese Journal of Clinical Oncology*, **42**, 85–95.

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

Ueda, Y., Itoh, T., Nukaya, I., Kawashima, I., Okugawa, K., Yano, Y., Yamamoto, Y., Naitoh, K., Shimizu, K., Imura, K., Fuji, N., Fujiwara, H., Ochiai, T., Itoi, H., Sonoyama, T., Hagiwara, A., Takesako, K. & Yamagishi, H. (2004) Dendritic cell-based immunotherapy of cancer with carcinoembryonic antigen-derived, HLA-A24-restricted CTL epitope: clinical outcomes of 18 patients with metastatic gastrointestinal or lung adenocarcinomas. *International Journal of Oncology*, **24**, 909–917.

Utsunomiya, A., Miyazaki, Y., Takatsuka, Y., Hanada, S., Uozumi, K., Yashiki, S., Tara, M., Kawano, F., Saburi, Y., Kikuchi, H., Hara, M., Sao, H., Morishima, Y., Kodera, Y., Sonoda, S. & Tomonaga, M. (2001) Improved outcome of adult T cell leukemia/lymphoma with allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplantation*, **27**, 15–20.

Wierecky, J., Muller, M.R., Wirths, S., Halder-Oehler, E., Dorfel, D., Schmidt, S.M., Hantschel, M., Brugger, W., Schroder, S., Horger, M.S., Kanz, L. & Brossart, P. (2006) Immunologic and clinical responses after vaccinations with peptide-pulsed dendritic cells in metastatic renal cancer patients. *Cancer Research*, **66**, 5910–5918.

Wu, L., Adams, M., Carter, T., Chen, R., Muller, G., Stirling, D., Schafer, P. & Bartlett, J.B. (2008) lenalidomide enhances natural killer cell and monocyte-mediated antibody-dependent cellular cytotoxicity of rituximab-treated CD20+ tumor cells. *Clinical Cancer Research*, **14**, 4650–4657.

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CHEST

Chest HRCT findings in acute transformation of adult T-cell lymphoma/leukemia

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Abstract

Objectives To assess chest high-resolution computed tomography (HRCT) findings in patients with acute transformation of adult T cell leukaemia/lymphoma (ATLL).

Methods We retrospectively identified 72 consecutive patients at our institution with ATLL between October 2000 and March 2014. The cases included acute type (n=20), lymphoma type (n=21), smouldering type (n=24) and chronic type (n=7). Sixteen (7 men, 9 women; aged 36–85 years, mean 63.3 years) of 31 patients (24 with smouldering and seven with chronic type; 51.6 %) developed acute transformation of ATLL, and had undergone chest HRCT examinations. Parenchymal abnormalities, enlarged lymph nodes, pericardial effusion, pleural effusion and skin lesions were evaluated on HRCT.

Results Chest HRCT of 15 of the 16 patients showed abnormal findings, including ground-glass opacity (GGO) (n=8), consolidation (n=5), interlobular septal thickening (n=5) and nodules (n=5). Pleural effusion was found in five patients, lymph node enlargement in 10 patients and multiple skin thickening in two patients.

Conclusions Almost all patients with acute transformation of ATLL had abnormal findings on chest HRCT, which consisted mainly of lymph node enlargement, GGO, interlobular septal thickening, nodules and bilateral pleural effusions.

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Key points

- *The recognition of CT findings of acute transformation is important*
- *Almost all patients with acute transformation have abnormal findings on HRCT*
- *Characteristic CT features are present in acute transformation of indolent ATLL*

Keywords Computed tomography · Chest · Adult T cell leukemia/lymphoma · Acute transformation · HTLV-1

Abbreviations

ATLL	Adult T cell leukaemia/lymphoma
BAL	Bronchoalveolar lavage
GGO	Ground-glass opacity
HRCT	High-resolution computed tomography
HTLV-1	Human T-lymphotropic virus type 1
LDH	Lactate dehydrogenase
TBLB	Transbronchial lung biopsy

Introduction

Human T-lymphotropic virus type 1 (HTLV-1), which is prevalent in southwestern Japan and the Caribbean basin [1], is aetiologically associated with adult T cell leukaemia/lymphoma (ATLL). The diversity in clinical features and prognosis of patients with ATLL has led to its categorization into four subtypes: acute, lymphoma, chronic and smouldering. These are defined by organ involvement, lactate dehydrogenase (LDH) and calcium values. In patients with acute type and lymphoma type (aggressive ATLL), intensive chemotherapy is usually recommended. Patients with aggressive ATLL

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have a very poor prognosis owing to intrinsic chemoresistance and frequent infectious complications because of immune deficiency. Patients with chronic type and smouldering type (indolent ATLL), however, have a better prognosis than those with aggressive ATLL, and watchful waiting until disease progression has been recommended. However, the long-term prognosis of patients with indolent ATLL is not good without a plateau phase in the survival curve [2]. There are several reports documenting acute transformation to aggressive type in up to 40 % of patients with indolent ATLL [2].

Okada et al. reported thoracic computed tomography (CT) findings based on radiological features in 87 patients with ATLL [3]. These CT findings mainly consisted of ground-glass opacity (GGO), centrilobular nodules, and thickening of the bronchovascular bundles in the periphery. Recently, Hanaka et al. reported a case of ATLL with rapid progression of pulmonary areas of GGO and multiple nodules, resulting from acute transformation of chronic ATLL [4]. To our knowledge, no other English-language studies on CT features in patients with acute transformation of ATLL have been published. The purpose of this study was to assess chest high-resolution CT (HRCT) findings in patients with acute transformation of ATLL that may be of clinical significance.

Materials and methods

Patients

Our institutional review board approved this retrospective study and waived the requirement for informed consent.

On the basis of the patient population at our institution, we retrospectively identified 72 consecutive patients with ATLL between October 2000 and March 2014. The patients consisted of 20 with acute type, 21 with lymphoma type, 24 with smouldering type and seven with chronic type. Of the 31 patients with smouldering type or chronic type, 16 (seven men, nine women; aged 36–85 years, mean 63.3 years; 51.6 %) developed acute transformations by serological and clinical findings, and had undergone chest HRCT. At the same time, there were no patients diagnosed with infectious disease by serological tests and clinical findings.

ATLL was diagnosed by positive HTLV-1 antibody and the presence of abnormal lymphocytes with convoluted nuclei (ATLL cells) in the peripheral blood or histological findings compatible with a diagnosis of ATLL in biopsied tissue.

The diagnosis for acute transformation of ATLL was established by fulfilling the diagnostic criteria for acute type or lymphoma type of ATLL. The periods between the diagnosis of smouldering type or chronic type and acute exacerbation of ATLL were 1– 124 months (mean 25.8 months). The patients presented with several symptoms, such as general fatigue in eight, fever in seven, eruption in seven, lymph node enlargement in six, chest pain in one and joint pain in one.

CT examinations

HRCT examinations were performed with a variety of scanners, volumetrically with a multi-detector CT with 1-mm reconstruction from the apex of the lung to the diaphragm. The images were obtained with the patient in the supine position at full inspiration. Images were captured at window settings that allowed viewing of the lung parenchyma (window level −600 HU; window width 1,500 HU) and the mediastinum (window level 10–30 HU; window width 300 HU).

A pulmonary CT was performed within 1 day to 1 month (mean 18.5 days) after the onset of fever, general fatigue, lymph node enlargement and other abnormal conditions. Intravenously administered contrast material was used in six patients.

CT image interpretation

Two chest radiologists (with 27 and 12 years of experience in interpretation of chest CT images), who were aware of the underlying diagnoses, retrospectively and independently interpreted the CTs. Conclusions were reached by consensus.

CT images were evaluated for the presence and extent of abnormalities, including GGO, consolidation, bronchial wall thickening, centrilobular nodules, intralobular reticular opacity, nodules, cavity, interlobular septal thickening and lymph node enlargement. The presence or absence of pleural effusion and pericardial effusion was also recorded. In addition, the combination of abnormalities was assessed. Radiological features were defined according to the glossary of terms established by the Fleischner Society [5].

The distribution of parenchymal disease was also noted. We assessed whether abnormal findings were located unilaterally or bilaterally. If the main lesion was predominantly located in the inner third of the lung, the disease was classified as centrally distributed. If the main lesion was predominantly located in the outer third of the lung, the disease was classified as peripherally distributed. If the lesions showed no predominant distribution, the disease was classified as randomly distributed. Additionally, zonal predominance was classified as upper, lower or random. Upper-lung zone predominance indicated that most abnormalities were observed at a level above the tracheal carina, while lower-zone predominance indicated that most abnormalities were located below the upper zone. When abnormalities showed no clear zonal predominance, the lung disease was classified as randomly distributed.

Follow-up examinations after treatment were also assessed.

Results

CT patterns

Chest CTs showed abnormalities in 15 of the 16 patients (93.8 %) with acute transformation of ATLL (Table 1). In nine of the 15 patients (60.0 %), parenchymal abnormal findings were found, in which GGO (n=8; 53.3 %; Figs. 1 and 2) was the most frequently observed abnormality, followed by consolidation (n=5; 33.3 %; Figs. 1 and 2), interlobular septal thickening (n=5; 33.3 %; Figs. 1, 2, and 3) and nodules (n=5; 33.3 %; Fig. 1). Intralobular reticular opacity (n=3; 20.0 %) and cavity (n=1; 6.7 %) were also observed. Centrilobular nodules could not be found.

Pleural effusions were identified in five of the 15 patients (33.3 %); four were bilateral pleural effusions (26.7 %) and one was unilateral pleural effusion (6.7 %). In one of the five patients (20.0 %), parenchymal abnormalities were not observed.

Mediastinal and/or axillary lymph node enlargements were observed in 10 patients (66.7 %): mediastinum lymph node enlargement alone in one (6.7 %); axillary lymph node enlargement alone in three (20.0 %; Fig. 4) and both in six (40.0 %). Enlarged lymph nodes were found in the paratracheal, tracheobronchial and subcarinal regions. Hilar lymph node enlargement was not seen in the patients.

Thickening of the skin was found in two patients (13.3 %; Fig. 5), in multiple regions such as the anterior and posterior chest wall. In these two patients, there were no abnormal findings in the lung parenchyma.

Table 1 CT findings in 15 patients

Findings	No. of patients
Ground-glass opacity	8 (53.3)
Consolidation	5 (33.3)
Interlobular septal thickening	5 (33.3)
Nodule/mass	5 (33.3)
Intralobular reticular opacity	3 (20.0)
Bronchial wall thickening	2 (13.3)
Cavity	1 (6.7)
Centrilobular nodules	0 (0)
Pericardial effusion	0 (0)
Pleural effusion	5 (33.3)
Unilateral	1 (6.7)
Bilateral	4 (26.7)
Lymph node enlargement	10 (66.7)
Mediastinum	7 (46.7)
Axillary	9 (60.0)
Skin lesions	2 (13.3)

Data in parentheses are percentages

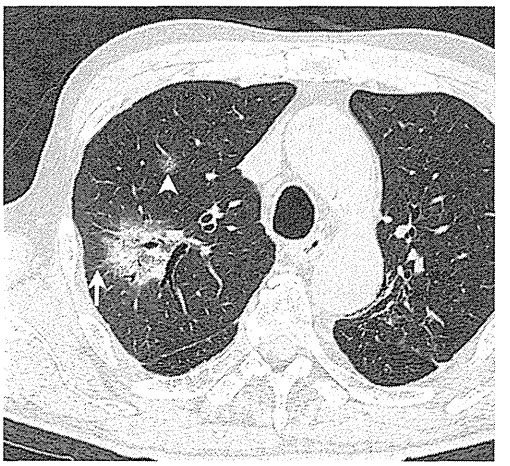

Fig. 1 Acute transformation (acute type) in a 67-year-old male patient with smouldering type ATLL, 21 days after the onset of fever. Transverse CT image (1-mm thickness) at the level of the aortic arch shows mass-like consolidation with surrounding GGO and air bronchogram and interlobular septal thickening (*arrow*). Small nodule is present in the right upper lobe (*arrowhead*)

The most frequently observed combination of abnormalities was GGO and interlobular septal thickening (n=5; 33.3 %; Figs. 1 and 2), followed by GGO and lymph node enlargement (n=5; 33.3 %), GGO and consolidation (n=4; 26.7 %; Figs. 1 and 2), GGO and nodules (n=4; 26.7 %; Fig. 1), GGO and pleural effusion (n=4; 26.7 %) and pleural effusion and lymph node enlargement (n=4; 26.7 %).

Disease distribution

Among the nine patients with parenchymal abnormalities, abnormal findings were found bilaterally in all of the patients,

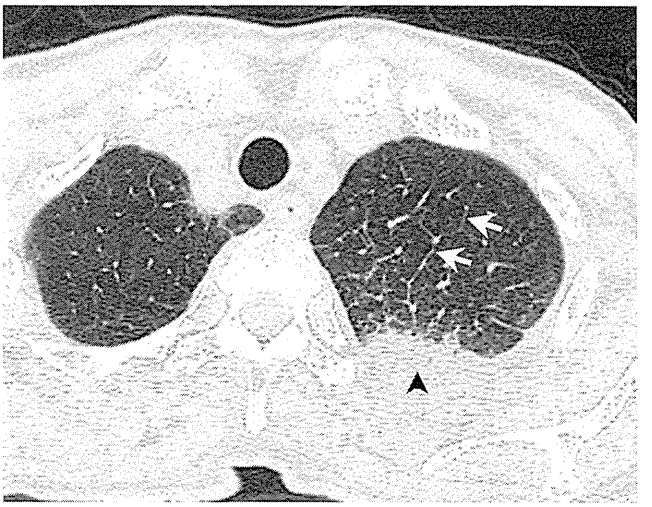

Fig. 2 Acute transformation (acute type) in a 77-year-old female patient with smouldering type ATLL, 7 days after the onset of fever and general fatigue. Transverse CT image (1-mm thickness) at the level of the left upper lobe shows consolidation (*arrowhead*), GGO and interlobular septal thickening (*arrows*)

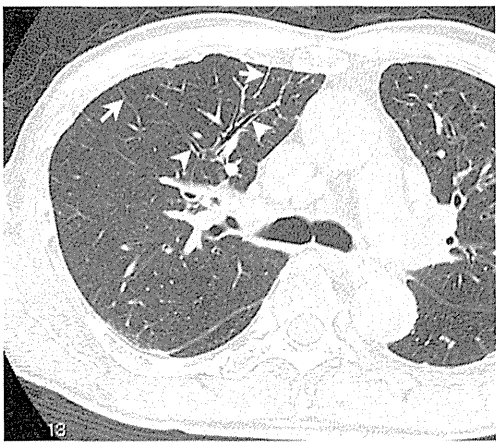

Fig. 3 Acute transformation (acute type) in a 69-year-old male patient with smouldering type ATLL, 10 days after the onset of fever. Transverse CT image (1-mm thickness) at the level of the tracheal carina shows interlobular septal thickening (*arrows*) and bronchial wall thickening (*arrowheads*)

and were randomly distributed in eight of the patients (88.9 %). The remaining patient showed a peripheral distribution (11.1 %).

The predominant zonal distribution was the upper zone in two patients (22.2 %), the lower zone in one patient (11.1 %) and a random distribution in six patients (66.7 %).

Follow-up study

All 15 patients underwent chemotherapy. In 11 patients, abnormal findings improved on follow-up CT examinations or chest radiographs, whereas in the remaining four patients abnormal findings worsened and the patients died.

There were no significant differences in the initial HRCT patterns and distribution of disease between the improved patients and the deceased patients.

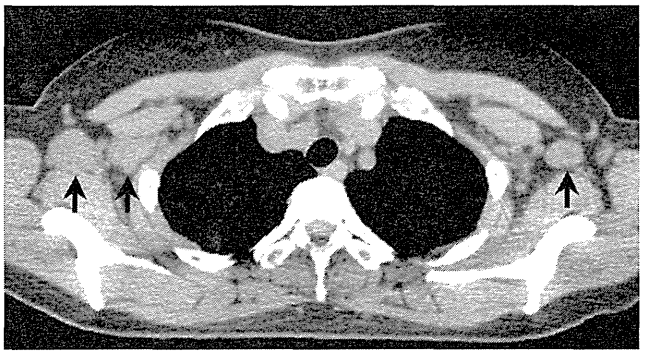

Fig. 4 Acute transformation (acute type) in a 43-year-old female patient with chronic type ATLL, 14 days after the onset of general fatigue and lymph node enlargement of the neck and axillary regions. Transverse CT image (1-mm thickness) at the level of the axillary regions shows multiple axillary lymph node enlargement (*arrows*)

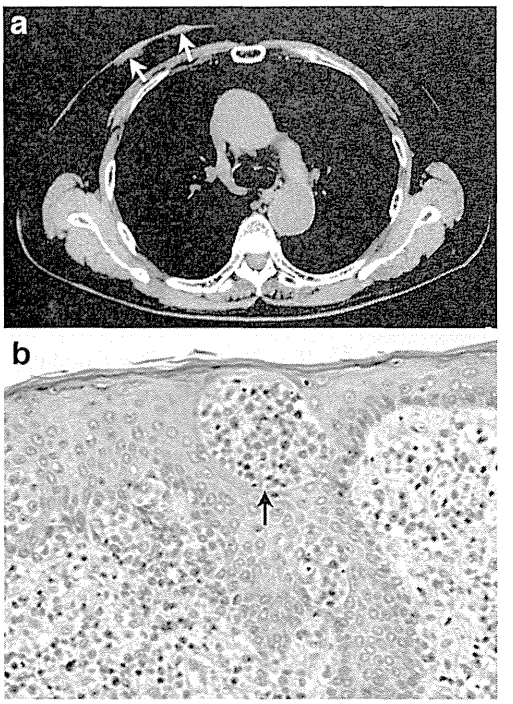

Fig. 5 Acute transformation (lymphoma type) in a 73-year-old female patient with smouldering type ATLL, 20 days after the onset of eruptions and lymph node enlargement of the inguinal regions. **a** Transverse CT image (1-mm thickness) at the level of the tracheal carina shows multiple thickening of the skin (*arrows*). **b** Photomicrograph of the biopsy specimen obtained from the thickened skin of the right chest wall shows a microabscess (*arrow*) and dens dermal infiltration by lymphoid cells with nuclear atypia

Pathological findings

Skin biopsies were performed in two patients with thickening of the skin. The pathological findings showed a microabscess and dens dermal infiltration by lymphoid cells with nuclear atypia.

Transbronchial lung biopsy (TBLB), surgical lung biopsy or bronchoalveolar lavage was not performed because patients were in poor general health and needed to undergo immediate chemotherapy. Additionally, postmortem studies in four deceased patients were not undertaken.

Discussion

ATLL was first described in 1977 as a distinct clinicopathological entity with a suspected viral aetiology [6]. Subsequently, HTLV-1 was isolated as a carcinogenic pathogen. HTLV-1 infects approximately 15–20 million people worldwide, with endemic areas in southwestern Japan, the Caribbean and Africa. After prolonged latency periods (10–30 years), approximately 0.5–5.0 % of HTLV-1-infected individuals will develop ATLL [7–9]. The three major routes of HTLV-1

transmission are mother-to-child via breast-feeding, sexual intercourse and blood transfusions. HTLV-1 infection early in life, in the form of breast-feeding, is crucial in the development of ATLL. ATLL is defined as a neoplastic clonal growth of HTLV-1-infected cells. ATLL can present in various clinical manifestations by involving many organs, including lungs, skin, gastrointestinal tract, the central nervous systems and bones. The diverse clinical features of this disease have led to its subclassification as acute, lymphoma, chronic and smouldering subtypes [10]. The first is the smouldering type, in which more than 5 % of T lymphocytes are abnormal, the peripheral blood contains less than 4,000 lymphocytes per microlitre, there is no hypercalcaemia, LDH level is up to 1.5-fold the normal upper limit, there is no lymphadenopathy and no involvement of extranodal organs except the skin or lungs. This type is the least common, accounting for approximately 5 % of ATLL. The second is the chronic type, in which lymphocytosis occurs (defined as T lymphocytosis greater than 4,000/μL), LDH is less than twice the normal upper limit, no hypercalcaemia is present and there is possible lymph node, liver, spleen, skin and lung involvement. This type accounts for approximately 15–20 % of ATLL. The third is the lymphoma type, in which histologically proven lymph node enlargement is present but there is no lymphocytosis (defined as less than 1 % abnormal T lymphocytes). This type accounts for approximately 20 % of ATLL. Patients frequently have an elevated LDH level and can have hypercalcaemia. The fourth is the acute type, for patients not classified into any of the above three types, and occurs in approximately 55–60 % of ATLL. Patients with the acute type present with systemic symptoms, organomegaly, lymphadenopathy and an elevated LDH level. The white blood cell count is usually greater than 20,000/mL.

The median survival time is 6.2 months for the acute type, 10.2 months for the lymphoma type and 24.3 months for the chronic type; 62.8 % of patients with the smouldering type were still alive after 4 years [10].

Because ATLL patients present with a variety of clinical symptoms and course, it is important for the clinician to differentiate between aggressive ATLL (acute type and lymphoma type), necessitating immediate treatment, and indolent ATLL (chronic type and smouldering type) that does not require specific treatment. In the last two types, the disease has an indolent initial course but frequently progresses to acute type ATLL. Ishitsuka et al. reported that, in 26 patients with smouldering type ATLL, 10 patients (38.5 %) developed acute transformation to acute or lymphoma type ATLL, and died despite chemotherapy [11]. Takasaki et al. evaluated the long-term prognosis of 60 Japanese patients with indolent ATLL [2]. Among the patients, 44 (73.3 %) progressed to aggressive ATLL (all were acute type), and 41 (68.3 %) died. The median time to acute transformation was 18.8 months (range 0.3 months to 17.6 years).

In our study, 16 of 31 patients (24 with smouldering and seven with chronic type; 51.6 %) developed acute transformation of ATLL (14 patients with acute type, two with lymphoma type) and four of these patients (25 %) died. The mean time between the diagnosis of smouldering or chronic type and acute exacerbation of ATLL was 25.8 months (range 1–124 months).

There have been several reports of the mechanisms of acute transformation of ATLL. Tsukasaki and colleagues compared the gene-expression profiles of four pairs of chronic and acute ATLL samples using oligonucleotide microarrays to elucidate the differences in gene expression during progression to acute ATLL [12]. They identified 203 genes that were commonly up-regulated in acute- vs. chronic-phase samples, and an additional 91 commonly down-regulated genes. Some of the up-regulated genes were located in amplified regions identified by comparative genomic hybridization in the corresponding chronic/acute ATLL samples. Tsukasaki et al. concluded that distinct sets of genes that are known to be critical in cellular transformation and/or activation are up- or down-regulated during the transition to the acute phase of ATLL.

A close relationship between strongyloidiasis and HTLV-1 has also been reported [13–18]. Nakada et al. studied the prevalence of HTLV-1 antibody in sera from *Strongyloides* carriers and controls [13]. In their study, 99 of 166 *Strongyloides* carriers (59.6 %) had HTLV-1 antibodies but 595 of 2,962 controls (20.1 %) had HTLV-1 antibody. Conversely, the detection rate of faecal larvae among the *Strongyloides*-seropositive patients was significantly higher in patients with the HTLV-1 antibody than in those without the HTLV-1 antibody. These findings suggest an effect of a concurrent HTLV-1 infection on the course and intensity of *Strongyloides* infection [14].

Nakada et al. reported that 36 patients with strongyloidiasis were seropositive for HTLV-1, and that 14 of these patients (38.9 %) had monoclonal integration of HTLV-1 proviral DNA in their blood lymphocytes [15]. They concluded that, although the immunodeficiency caused by HTLV-1 could predispose patients to hyperinfestation by *Strongyloides*, parasitic and retroviral infestations might be important co-factors leading to the development of ATLL. Nonetheless, the mechanisms of acute exacerbation of ATLL remain unclear. In the present study, *Strongyloides stercoralis* infection in patients with acute transformation of ATLL was not examined.

With regard to the radiological findings in ATLL, Okada et al. reported thoracic CT findings in 87 patients with ATLL, of which 60 patients (69.0 %) had abnormal findings [3]. They consisted mainly of GGO (62 %), centrilobular nodules (42 %), bronchovascular bundles thickening (37 %), interlobular septal thickening (28 %) and nodules (22 %). Moreover, pleural effusion and lymph node enlargement were found frequently in 37 % and 45 %, respectively. In 46 patients, CT–pathology correlation was performed using surgical or

autopsy specimens. Pathologically, these CT findings corresponded to atypical lymphocyte infiltration along the interstitium and the alveolar spaces. As for CT findings in acute transformation of ATLL, there is only one case report [4]. Hanaka et al. reported a case of a 48-year-old Japanese man with acute transformation from ATLL chronic type [4] in which CT findings were correlated with pathological findings using TBLB specimens. The chest CT images showed GGO and nodules in both lungs. Pathologically, the extent of GGO corresponded to the partial infiltration of ATLL cells and foam cells into the alveolar walls, and the nodules corresponded to thickening of the alveolar walls, mild alteration of alveolar structures and overt precipitation of fibrin.

Distinguishing between acute transformation of ATLL and infection in patients with indolent ATLL is important because the treatment strategies are quite different. In the clinic, the diagnosis of pneumonia is established by the isolation of causative pathogens from sputum, bronchoalveolar lavage, urine or blood, along with respiratory symptoms and abnormal findings on chest radiographs. Additionally, the elevation of the serum level of β-D-glucan or antigen for each type of fungus antigen in combination with HRCT findings is suggestive of fungal infection and *Pneumocystis jirovecii* pneumonia.

Increased numbers of ATLL cells, elevated LDH level, hypercalcaemia and lymphadenopathy were findings suggestive of acute transformation in patients with indolent ATLL. Bronchoalveolar lavage fluid or transbronchial lung biopsy specimens provide clues to the differential diagnosis of pulmonary manifestation of ATLL acute transformation versus infections; however, patients with ATLL often have poor general status and it is difficult to undergo a transbronchial lung examination. Moreover, immediate treatment should be started. This was the case in the present study.

Recently, Okada and colleagues reported HRCT findings in 749 pneumonia patients (385 with community-acquired pneumonia and 364 with nosocomial pneumonia: 86 *Streptococcus pneumoniae*, 211 *Haemophilus influenzae*, 109 *Moraxella catarrhalis*, 83 *Staphylococcus aureus*, 80 *Klebsiella pneumoniae*, 33 *Streptococcus milleri*, 35 *Pseudomonas aeruginosa*, 40 *Chlamydia pneumoniae*, 42 *Mycoplasma pneumoniae* and 30 seasonal influenza virus); this included 136 patients with malignancy [19–27]. In those reports, the CT findings of interlobular septal thickening, lymph node enlargement and nodules were found in 2.9–15.0 % (mean 9.5 %), 0–18.2 % (mean 4.3 %) and 0–18.2 % (mean 7.9 %), respectively. In the present report of patients with acute transformation of ATLL, these CT findings were found in 33.3 %, 66.7 % and 33.3 %, respectively. These frequencies were higher in patients with acute transformation of ATLL than in patients with pneumonia. Moreover, thickened skin and axillary lymph node enlargement could not be found in patients with each type of pneumonia. In patients with cytomegalovirus pneumonia and *Pneumocystis jirovecii* pneumonia, HRCT finding of nodules is often seen; however, in cytomegalovirus pneumonia, interlobular septal thickening, lymph node enlargement and thickening of the skin are rarely seen [28, 29]. In *Pneumocystis jirovecii* pneumonia, interlobular septal thickening or lymph node enlargement is relatively rarely seen [30].

Therefore, the HRCT of interlobular septal thickening, lymph node enlargement (especially in axillary regions) and thickened skin lesions, which may be non-specific findings, are suggestive of acute transformation in patients with indolent ATLL, along with clinical findings.

It should be noted that there are several limitations to the present study. First, this was a retrospective study and CT image interpretation was performed by consensus. Second, this study included only a small number of patients. Differences in HRCT findings between acute type and lymphoma type in acute transformation of ATLL could not be assessed. Third, the differences between acute transformation of ATLL and infection in indolent ATLL patients were not studied on HRCT. Therefore, it might be difficult to generalize the HRCT findings observed in this study as 'characteristic findings'. Fourth, no correlation with pathological findings of the lungs was possible because patients were in poor general health. Finally, the HRCT images were obtained using different protocols.

In summary, almost all of the patients with acute transformation of ATLL had abnormal findings on chest HRCT, which mainly consisted of lymph node enlargement, GGO, interlobular septal thickening, nodules and bilateral pleural effusion. In particular, lymph node enlargement in axillary regions, interlobular septal thickening and thickened skin lesions are suggestive of acute transformation in patients with indolent ATLL, along with clinical findings.

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References

1. Gibbs WN, Lofters WS, Campbell M et al (1987) Non-Hodgkin lymphoma in Jamaica and its relation to adult T-cell leukemia-lymphoma. Ann Intern Med 106:361–368
2. Takasaki Y, Iwanaga M, Imaizumi Y et al (2010) Long-term study of indolent adult T-cell leukemia-lymphoma. Blood 115:4337–4343

3. Okada F, Ando Y, Kondo Y, Matsumoto S, Maeda T, Mori H (2004) Thoracic CT findings of adult T-cell leukemia or lymphoma. Am J Roentgenol 182:761–767
4. Hanaka M, Yatera K, Itoh C et al (2013) Case of adult T-cell leukemia/lymphoma with rapid progression of pulmonary areas of ground-glass attenuation and multiple nodules. Respir Investig 51: 41–45
5. Hansell DM, Bankier AA, MacMahon H, McLoud TC, Muller NL, Remy J (2008) Fleischner Society: glossary of terms for thoracic imaging. Radiology 246:697–722
6. Uchiyama T, Todoi J, Sagawa K, Takatsuki K, Uchio H (1977) Adult T-cell leukemia: clinical and hematologic features of 16 cases. Blood 50:481–492
7. Weber J (1990) HTLV-1 infection in Britain. Br Med J 301:71–72
8. Dixon AC, Dixon PS, Nakamura JM (1989) Infection with the human T-lymphotropic virus type 1: a review for clinicians. Wes J Med 151:632–637
9. [No authors listed] (1988) HTLV-1 comes of age. Lancet 1(8579): 217–219
10. Shimoyama M (1991) Diagnostic criteria and classification of clinical subtypes of adult T-cell leukaemia-lymphoma: a report from the Lymphoma Study Group (1984-87). Br J Haematol 79:428–437
11. Ishitsuka K, Ikeda S, Utsunomiya A et al (2008) Smouldering adult T-cell leukaemia/lymphoma: a follow-up study in Kyushu. Br J Haematol 143:442–444
12. Tsukasaki K, Tanosaki S, DeVos S et al (2004) Identifying progression-associated genes in adult T-cell leukemia/lymphoma by using oligonucleotide micoroarrays. Int J Cancer 109:875–881
13. Nakada K, Kohakura M, Komoda H, Hinuma Y (1984) High incidence of HTLV-1 antibody in carriers of *Strongyloides stercoralis*. Lancet 17:633
14. Sato Y, Shiroma Y (1989) Concurrent infections with *Strongyloides* and T-cell leukemia virus and their possible effect on immune responses of host. Clin Immunol Immnopathol 52:214–224
15. Sato Y, Toma H, Takara M, Kiyuna S, Shiroyama Y (1990) Seroepidemiological studies in the concomitance of strongyloidiasis with T-cell leukemia viral infection in Okinawa, Japan. Jpn J Parasitol 39:376–383
16. Stewart DM, Ramanathan R, Mahanty S, Fedorko DP, Janik JE, Morris JC (2011) Dissseminated *Strongyloides stercoralis* infection in HTV-1-associated adult T-cell leukemia/lymphoma. Acta Haematol 126:63–67
17. Nakada K, Yamaguchi K, Furugen S et al (1987) Monoclonal integration of HTLV-1 proviral DNA in patients with strongyloidiasis. Int J Cancer 40:145–148
18. D'Incan M, Combemale P, Verrier B et al (1994) Transient adult T-cell leukemina/lymphoma picture during varicella infection in an HTLV-1 carrier. Leukemia 8:682–687
19. Okada F, Ando Y, Wakisaka M, Matsumoto S, Mori H (2005) *Chlamydia pneumoniae* pneumonia and *Mycoplasma pneumoniae* pneumonia: comparison of clinical findings and CT findings. J Comput Assist Tomogr 29:626–632
20. Okada F, Ando Y, Tanoue S et al (2012) Radiological findings in acute *Haemophilus influenzae* pulmonary infection. Br J Radiol 85: 121–126
21. Okada F, Ando Y, Nakayama T et al (2011) Pulmonary thin-section CT findings in acute *Moraxella catarrhalis* pulmonary infection. Br J Radiol 84:1109–1114
22. Morikawa K, Okada F, Ando Y et al (2012) Methicillin-resistant *Staphylococcus aureus* and methicillin-susceptible *S. aureus* pneumonia: comparison of clinical and thin-section CT findings. Br J Radiol 85:e168–e175
23. Okada F, Ando Y, Matsushita S et al (2012) Thin-section computed tomography findings of patients with acute *Streptococcus pneumoniae* pneumonia with and without concurrent infection. Br J Radiol 85:e357–e364
24. Okada F, Ando Y, Honda K et al (2009) Clinical and pulmonary thin-section CT findings in acute *Klebsiella pneumoniae* pneumonia. Eur Radiol 19:809–815
25. Okada F, Ando Y, Honda K et al (2010) Acute *Klebsiella pneumoniae* pneumonia alone and with concurrent infection: comparison of clinical and thin-section CT findings. Br J Radiol 83:854–860
26. Okada F, Ono A, Ando Y et al (2013) High-resolution CT findings in *Streptococcus milleri* pulmonary infection. Clin Radiol 68:e331–e337
27. Ono A, Okada F, Takata S et al (2014) A comparative study of thin-section CT findings between seasonal influenza virus pneumonia and *Streptococcus pneumoniae* pneumonia. Br J Radiol. doi:10.1259/bjr.20140051
28. Franquet T (2011) Imaging of pulmonary viral pneumonia. Radiology 260:18–39
29. Gasparetto EL, Ono SE, Escuissato D, Marchiori E, Roldan L, Marques HL (2004) Cytomegalovirus pneumonia after bone marrow transplantation: high resolution CT findings. Br J Radiol 77:724–727
30. Kuhlman JE, Kavuru M, Fishman EK, Siegelman SS (1990) Pneumocystis carinii pneumonia: spectrum of parenchymal CT findings. Radiology 175:711–714

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Treatment of Patients with Adult T Cell Leukemia/Lymphoma with Cord Blood Transplantation: A Japanese Nationwide Retrospective Survey

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Graft-versus-adult T cell leukemia/lymphoma

A B S T R A C T

Allogeneic bone marrow and peripheral blood stem cell transplantations are curative treatment modalities for adult T cell leukemia/lymphoma (ATLL) because of the intrinsic graft-versus-ATLL effect. However, limited information is available regarding whether cord blood transplantation (CBT) induces a curative graft-versus-ATLL effect against aggressive ATLL. To evaluate the effect of CBT against ATLL, we retrospectively analyzed data from 175 patients with ATLL who initially underwent single-unit CBT. The 2-year overall survival (OS) rate was 20.6% (95% confidence interval [CI], 13.8% to 27.4%). A multivariate analysis revealed that the development of graft-versus-host disease (GVHD) was a favorable prognostic factor for OS (hazard ratio, .10; 95% CI, .01 to .94; $P = .044$). Furthermore, the 2-year OS (42.7%; 95% CI, 28.1% to 56.6%) of patients with grade 1 to 2 acute GVHD was higher than that of patients without acute GVHD (24.2%; 95% CI, 11.2% to 39.8%; $P = .048$). However, the cumulative incidence of treatment-related mortality (TRM) was high (46.1%; 95% CI, 38.2% to 53.7%), and early death was particularly problematic. In conclusion, CBT cures patients with ATLL partly through a graft-versus-ATLL effect. However, novel interventions will be required, particularly in the early phase, to reduce TRM and optimize GVHD.

INTRODUCTION

Adult T cell leukemia/lymphoma (ATLL), an aggressive peripheral T cell neoplasm caused by the human T cell lymphotropic/leukemia virus type-1, has an extremely poor prognosis [1]. Intensive chemotherapy and autologous stem cell transplantation have not been shown to improve this prognosis [2,3]. As a curative treatment, allogeneic hematopoietic stem cell transplantation (allo-HSCT) can confer long-term remission via a graft-versus-ATLL effect in a proportion of patients with ATLL [4-7]. Recent reports have demonstrated that allo-HSCT using bone marrow (BM) or peripheral blood stem cells (PBSC) from a related or unrelated donor can effectively treat ATLL, yielding a 3-year overall survival rate

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(OS) of approximately 30% [8-16]. However, patients with ATLL typically lack a suitable HLA-identical sibling donor because both the recipients and donors are typically elderly and because the aggressive ATLL tumor burden reduces the available time to find a suitable unrelated donor within the Japan Marrow Donor Program. Umbilical cord blood, which can serve as an alternative to BM or PBSC as a source of stem cells, has been used primarily to treat children; however, the number of unrelated-donor cord blood transplantation (CBT) procedures used to treat adult patients with ATLL is increasing in Japan. The rapid availability of CBT may provide a great advantage for patients who require urgent allo-HSCT to treat aggressive ATLL [17].

Currently, the outcome of CBT in patients with acute leukemia is comparable to that of other graft sources [18,19]; however, there are few reports on the outcomes of CBT in patients with ATL [20,21]. Moreover, it is difficult to draw firm conclusions regarding the efficacy of this procedure because of the small number of cases. Therefore, to evaluate the role of CBT for ATLL in a larger and more recent cohort, we performed a nationwide retrospective study of patients with ATLL who underwent CBT as the initial allo-HSCT.

PATIENTS AND METHODS

Data Collection

We analyzed nationwide survey data from the Japan Society for Hematopoietic Cell Transplantation regarding patients with ATLL who had undergone an initial CBT between March 2001 and December 2009 (n = 175). This analysis included the patients' clinical characteristics, such as the age at transplantation, gender, disease status at transplantation, date of transplantation, time from diagnosis to transplantation, conditioning regimens, and number of infused cells. The number of mismatches was counted with respect to HLA-A, HLA-B (low-resolution typing), and DRB1 (high-resolution typing). The present study was approved by the data management committees of the Japan Society for Hematopoietic Cell Transplantation as well as the institutional ethics committee of the Kyushu University Graduate School of Medical Sciences.

Definitions

OS was defined as the time from transplantation until death, and patients who remained alive at the time of the last follow-up were censored. The causes of death were reviewed and categorized as either ATLL-related or transplantation-related mortality (TRM). *ATLL-related mortality* was defined as death caused by a relapse or progression of ATLL, whereas *TRM* was defined as any death related to transplantation other than ATLL-related mortality, according to the judgment of each institution. The patients were divided into 2 groups according to the conditioning regimen: full-intensity conditioning (FIC) and reduced-intensity conditioning (RIC). FIC and RIC were defined according to the proposals of Giralt et al. [22] and Bacigalupo et al. [23], respectively, with slight modifications. In the present study, conditioning regimens that included ≥5 Gy of total body irradiation (TBI) in a single fraction or ≥8 Gy of TBI in multiple fractions, oral busulfan (BU) at >8 mg/kg, intravenous BU at >6.4 mg/kg, or melphalan (Mel) at >140 mg/m^2 were considered FIC; all others were classified as RIC.

Statistical Analysis

Descriptive statistics were used to summarize the variables related to patient demographics and transplantation characteristics. The probability of the OS time was estimated according to the Kaplan-Meier method. To evaluate the influences of confounding factors on acute graft-versus-host disease (GVHD) and survival, the log-rank test and proportional hazards modeling were used for the univariate and multivariate analyses, respectively. The Cox proportional hazard model was used for the multivariate analyses of OS in which all independent variables were incorporated in the model, followed by the use of a stepwise selection method [24]. Fine and Gray proportional hazard modeling was used to estimate the effects of the same variables used in the multivariate analysis for OS on the cumulative incidence rates of TRM and ATLL-related mortality [25,26]. In these regression models, the occurrence of GVHD was treated as a time-dependent covariate [27]. In the analysis of acute GVHD, patients were assigned to the "no acute GVHD group" at the time of transplantation and transferred to the "acute GVHD group" at the onset of the maximum grade of acute GVHD. The landmark method was used to evaluate the effects of GVHD on OS and the cumulative incidence of disease-associated and treatment-related deaths among patients who remained alive at 60 days for acute GVHD and at 100 days for chronic GVHD after transplantation. Factors associated with at least borderline significance ($P \leq .10$) in the univariate analysis were subjected to a multivariate analysis using a backward stepwise covariate selection. All P values were 2-tailed, and P values $\leq .05$ were considered statistically significant. All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University), a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0) [28].

RESULTS

Patient Characteristics

The characteristics of 175 ATLL patients who received a single CBT are shown in Table 1. The median age at CBT was 55 years (range, 27 to 79 years). The cohort comprised 70 women and 105 men with the following ATLL statuses at CBT: complete remission (CR; n = 50), not in CR (n = 116), and unknown (n = 9). The conditioning regimen intensity was classified as FIC in 63 (36%) patients and RIC in 128 (62%) patients. FIC was further subdivided into 2 groups as follows: TBI (n = 47) or non-TBI (n = 15). RIC was also subdivided into 3 groups as follows: fludarabine (Flu) + Mel (n = 75), Flu + BU (n = 15), and other types (n = 15). Cyclosporine and tacrolimus were administered for prophylaxis to 90 (51%) and 77 patients (44%), respectively. Cyclosporine-based prophylaxis was subdivided into 3 groups as follows: (1) cyclosporine

Table 1
Patient Characteristics at Cord Blood Transplantation

Variables	No. of Patients (n = 175)
Age at transplantation, median (range), yr	55 (27-79)
Gender	
Male	105
Female	70
Disease status at transplantation	
CR	50
Not in CR	116
Unknown	9
Conditioning regimen	
FIC	63
RIC	108
Unknown	4
GVHD prophylaxis	
Cyclosporine-based	90
Tacrolimus-based	77
Unknown	8
Time from diagnosis to transplantation, d	
<200	94
≥200	75
Unknown	6
Year of transplantation	
<2005	71
≥2005	104
HLA matching*	
0 mismatched loci	5
1 mismatched locus	36
2 mismatched loci	73
≥3 mismatched loci	42
Unknown	19
ABO matching	
Matched	56
Minor mismatched	49
Major mismatched	69
Unknown	1
Nucleated cells infused per 10^7/kg, median (range)	2.58 (.36-5.34)
CD34-positive cells infused per 10^5/kg, median (range)	.85 (.07-5.39)

* Number of mismatches was counted among HLA-A, -B (low-resolution typing), and DRB1 (high-resolution typing).

alone (n = 33), (2) cyclosporine + short-term methotrexate (MTX) (n = 45), and (3) cyclosporine + mycophenolate mofetil (MMF; n = 12). Tacrolimus-based prophylaxis was subdivided into 4 groups as follows: (1) tacrolimus alone (n = 37), (2) tacrolimus + short-term MTX (n = 32), (3) tacrolimus + MMF (n = 5), (4) and tacrolimus + prednisolone (n = 3). Ninety-four patients (54%) received CBT < 200 days after diagnosis. One hundred twenty-four (71%) patients underwent CBT with 2 HLA-mismatched loci. The numbers of infused nucleated and CD34-positive cells were 2.58×10^7/kg (range, .36 to 5.34×10^7/kg) and $.85 \times 10^5$/kg (range, .07 to 5.39×10^5/kg), respectively. Engraftment evaluation was possible in 125 patients (71%) within a median interval of 19 days after CBT (range, 7 to 46 days). Among the survivors, the median follow-up duration was 22.5 months (range, 0 to 74.5 months).

Prognostic Factors for Survival

The OS rates of 175 patients with ATLL who received CBT were 30.2% (95% confidence interval [CI], 23.0% to 37.4%) at 1 year and 20.6% (95% CI, 13.8% to 27.4%) at 2 years (Figure 1A). The cumulative incidence rates of ATLL-related mortality and TRM at 2 years were 31.9% (95% CI, 24.8% to 39.3%) and 46.4% (95% CI, 38.5% to 54.0%), respectively (Figure 1B). The following confounding factors affected survival: age, gender, disease status at transplantation, days from diagnosis to transplantation, date of transplantation, age at transplantation, conditioning regimen, number of infused nucleated and CD34-positive cells, ABO compatibility, HLA compatibility, GVHD prophylaxis, and the development of acute GVHD. A univariate analysis revealed that higher OS ($P < .05$) correlated with CR at transplantation, minor ABO incompatibility, the addition of other agents to calcineurin inhibitors (MTX or MMF), and the development of acute GVHD (Table 2). A multivariate analysis was performed to further examine the effects of an age <55 years, the development of acute GVHD as a time-dependent covariate coincident with CR at transplantation, minor ABO incompatibility, and the addition of other agents to calcineurin inhibitors (Table 3). Compared with the absence of GVHD, the development of acute GVHD was associated independently with higher OS (hazard ratio [HR], .10; 95% CI, .01 to 0.94; $P = .044$).

Effects of Acute GVHD on Survival

To further validate the effect of acute GVHD on OS, we examined survival according to the acute GVHD grade in a landmark analysis. The median time to onset of acute GVHD of any grade after transplantation was 21 days (range, 5 to 100 days). Acute GVHD occurred in 80 patients (46%) as

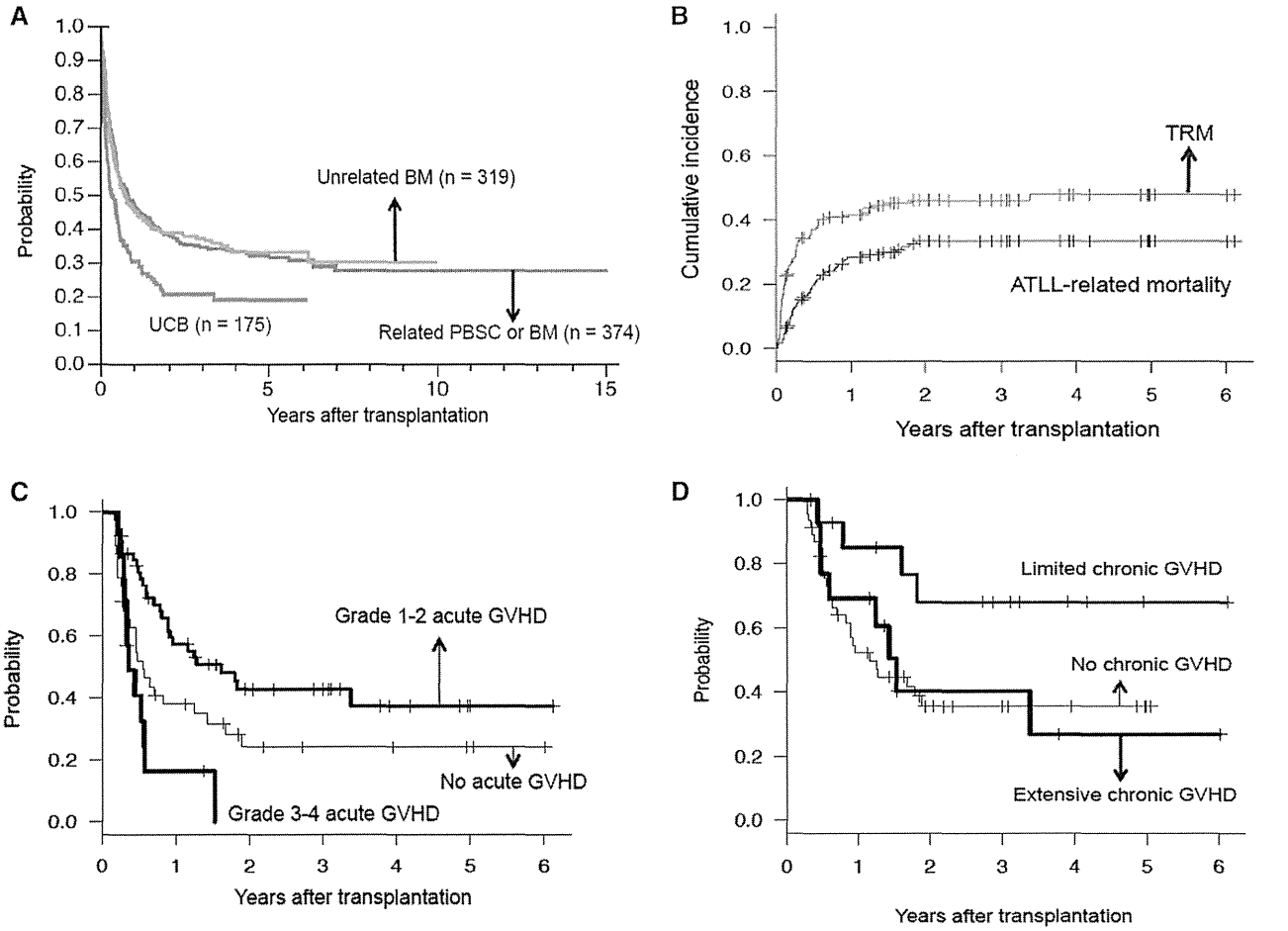

Figure 1. Survival, adult T cell leukemia/lymphoma (ATLL)-related mortality rates, and transplantation-related mortality (TRM) rates of patients receiving cord blood transplantation (CBT). (A) Kaplan-Meier curves of the estimated overall survival rates (OS) of ATLL patients treated with CBT. UCB, umbilical cord blood; PBSC, peripheral blood stem cells; BM, bone marrow, GVHD, graft-versus-host disease. (B) Cumulative incidence curves of ATLL-related mortality and TRM in patients treated with CBT. (C) Landmark plots of OS to determine the effects of acute GVHD. (D) Landmark plots of OS to determine the effects of chronic GVHD.

Table 2
Univariate Analysis of Risk Factors for Overall Survival

Variables		No.	OS		
			Two-Year OS (%)	95% CI	*P* Value
Age 1	<60 yr	134	23.0	15.0-31.0	.080
	≥60 yr	41	12.0	6.0-22.4	
Age 2	<55 yr	85	25.4	15.0-35.8	.100
	≥55 yr	90	15.6	7.0-24.2	
Sex	Female	70	22.3	11.5-33.1	.453
	Male	105	19.4	10.8-28.0	
Disease status at transplantation	CR	50	40.3	25.5-55.1	.003
	Not in CR	116	14.3	7.1-21.7	
Time from diagnosis to transplantation	<200 d	94	22.4	12.8-32.0	.752
	≥200 d	75	19.9	9.7-30.1	
Yr of transplantation	<2005	71	17.6	8.2-27.0	.160
	≥2005	104	23.1	13.5-31.5	
Conditioning regimen	FIC	63	20.2	9.8-30.6	.740
	RIC	108	20.2	11.8-28.6	
Infused nucleated cell dose ($\times 10^7$/kg)	<2	19	10.8	0-29.3	.290
	≥2	145	22.6	14.9-30.3	
Infused CD34 cell dose ($\times 10^5$/kg)	<1	97	23.3	13.9-32.7	.396
	≥1	66	19.1	8.0-30.2	
ABO matching	Matched	56	12.8	3.4-22.2	.024
	Minor mismatched	49	30.5	15.5-45.5	
	Major mismatched	69	20.5	9.9-31.1	
HLA matching	0 mismatched	5	30.0	0-77.4	.525
	1 mismatched	36	21.6	5.6-37.6	
	2 mismatched	73	24.6	14.3-35.9	
	≥3 mismatched	42	18.1	3.9-32.3	
GVHD prophylaxis 1	Cyclosporine-based	90	21.9	12.5-31.4	.710
	Tacrolimus-based	77	20.3	10.0-30.4	
GVHD prophylaxis 2 (cyclosporine/tacrolimus + other drug)	No	70	12.4	4.8-20.0	.003
	Yes	97	32.7	21.1-44.3	
Acute GVHD	No	59	16.8	5.7-27.9	<.0001
	Yes	80	29.4	18.2-40.6	

follows: grade 1, n = 23 patients; grade 2, n = 37 patients; grade 3, n = 14 patients; and grade 4, n = 6 patients. There was no significant difference in OS between patients with grades 1 and 2 GVHD ($P = 1.00$), in contrast to the difference between patients with grades 1 and 3 GVHD ($P = .013$). Moreover, based on the previous national survey analysis of the effect of acute GVHD on survival in patients with ATLL [5,15], the effect of acute GVHD on OS in the present study was evaluated using landmark plots (landmark day 60) according to the following 3 categories: (1) no acute GVHD (n = 38), (2) grade 1 to 2 acute GVHD (n = 53), and (3) grade 3 to 4 acute GVHD (n = 14). The 2-year OS rates for patients according to the acute GVHD grade were as follows: 24.2% (95% CI, 11.2% to 39.8%) without acute GVHD; 42.7% (95% CI, 28.1% to 56.6%) with grade 1 to 2 GVHD; and 0% with grade 3 to 4 GVHD (Figure 1C). These analyses demonstrated that the development of grade 1 to 2 acute GVHD was associated with higher OS compared with the absence of acute GVHD ($P = .048$), whereas the development of grade 3 to 4 acute GVHD was associated with lower OS compared with that in patients with grade 1 to 2 acute GVHD ($P = .0003$). The cumulative 2-year ATLL-related mortality rates according to the GVHD grades were as follows: 32.6% (95% CI, 19.7% to 46.1%) for grade 1 to 2 acute GVHD; 29.8% (95% CI, 8.2% to 55.6%) for grade 3 to 4 acute GVHD; and 45.9% (95% CI, 29.0% to 61.3%) for no acute GVHD. There was a trend toward a lower risk of relapse or progression in those who developed grade 1 to 2 acute GVHD relative to those without GVHD. Among patients with non-CR at transplantation, there was also a trend toward higher 2-year OS (36.7%; 95% CI, 18.7% to 54.9%) in those who developed grade 1 to 2 acute GVHD than in those without GVHD (15.6%; 95% CI, 3.4% to 35.9%). These data suggested a graft-versus-ATLL effect induced by CBT.

Table 3
Multivariate Analysis of Risk Factors for OS

Variables	OS		
	HR	95% CI	*P* Value
Age, yr			
<55	1		
≥55	1.15	.63-2.09	.652
Disease status at transplantation			
CR	1		
Not in CR	1.38	.73-2.63	.190
ABO matching			
Matched	1		
Minor mismatched	.56	.25-1.24	.152
Major mismatched	.77	.39-1.48	.337
GVHD prophylaxis (cyclosporine/ tacrolimus + other drug)			
No	1		
Yes	.76	.42-1.38	.365
Acute GVHD (time-dependent covariate)			
No	1		
Yes	.10	.01-.94	.044

Effects of Chronic GVHD on Survival

Chronic GVHD was evaluated in 74 patients who survived for at least 100 days after transplantation. Chronic GVHD occurred in 28 patients (37%) with a median time to onset of 115 days (range, 73 to 1287 days) after CBT. The effect of chronic GVHD on OS was evaluated using landmark plots (landmark day 100), and the 2-year OS results were as follows: no chronic GVHD (n = 46), 35.6% (95% CI, 21.0% to 50.0%); limited chronic GVHD (n = 15), 68.1% (95% CI, 35.4%

to 86.8%); and extensive chronic GVHD (n = 13), 40.4% (95% CI, 13.4% to 66.4%) (Figure 1D). There was a trend toward a higher OS among patients with limited chronic GVHD, but there were no significant differences relative to patients without chronic GVHD ($P = .10$) and those with extensive chronic GVHD ($P = .12$).

Cause of Death

At the last follow-up, 46 patients remained alive and 129 were deceased. The median follow-up time among the survivors was 22.5 months (range, 0 to 74.5 months). Disease progression (n = 52) was the leading cause of death. Infection was the cause of death in 40 patients (31%; bacterial, n = 27 patients; fungal, n = 3; viral, n = 8; and others, n = 2). Viral infection-related deaths were caused by the following pathogens: cytomegalovirus, n = 3; adenovirus, n = 2; human herpesvirus-6, n = 2; and varicella-zoster virus, n = 1. Among the 27 patients who succumbed to bacterial infection, 16 died before engraftment at a median of 17 days after CBT (range, 7 to 38 days). Among the 20 patients who developed severe acute grade 3 to 4 GVHD, 2 remain alive without disease progression. However, 9 of the 20 patients died of GVHD, 5 of disease progression, and 4 of infection.

The Fine and Gray proportional hazards model was applied to identify the variables affecting ATLL-related mortality and TRM. The pretransplantation variables included age, gender, disease status at CBT, days from diagnosis to transplantation, age at transplantation, conditioning regimen, number of infused nucleated cells, ABO compatibility, HLA compatibility, and GVHD prophylaxis. The following pretransplantation factors associated with a higher risk of ATLL-related mortality were identified in a multivariate analysis: not in CR at CBT (HR, 3.37; 95% CI, 1.12 to 10.2; $P = .032$) and an age > 55 years at CBT (HR, 2.32; 95% CI, .98 to 5.48; $P = .054$). The following pretransplantation factors were associated with a marginally higher risk of TRM: lower number of infused nucleated cells ($\geq 2 \times 10^7$/kg versus $< 2 \times 10^7$/kg; HR, .56; 95% CI, .30 to 1.02; $P = .059$) and GVHD prophylaxis with a calcineurin inhibitor alone (additional agents plus calcineurin inhibitors versus calcineurin inhibitors alone; HR, .60; 95% CI, .34 to 1.07; $P = .064$).

DISCUSSION

We present here the results of the largest retrospective study of ATLL patients receiving CBT; these results have extended our knowledge relative to that gained from other studies, which were limited by the numbers of cases [15,20,21]. Because graft source selection is strongly influenced by the donor availability, it is difficult to directly compare the outcomes of CBT with those of other allo-HSCT modalities. Nevertheless, the outcome of CBT for ATLL in the previous nationwide survey, with a 3-year OS rate of 17%, was clearly unsatisfactory because the study period corresponded with the developmental phase of CBT in adult patients [15]. Recent improvements in the outcome of CBT have been expected after optimization of the number of cells used for CBT and the improved HLA-compatibility of cord blood units [29-31]. Consequently, a recent nationwide survey data of adults with acute non-ATLL leukemia revealed no differences in the outcome of CBT in comparison with those of other allo-HSCT modalities [18,19]. However, the updated data (through December 2009) indicated that CBT for ATLL remained associated with a poorer 3-year OS of 20.6%, compared with OS of 34.4% among the 374 patients who received related BM or PBSC and 37.1% among the 319 patients who received unrelated BM ($P < .0001$) (Figure 1A). Therefore, the aim of the present study focused on the feasibility of CBT in the context of a larger cohort of patients with ATLL.

In the present study, 2 important findings were identified regarding CBT for ATLL. First, CBT cured patients with ATLL partly through a graft-versus-ATLL effect. Second, the high rate of TRM (approximately 50%) remains a significant problem. The OS curve for ATLL patients who received CBT reached a plateau by 3 years, suggesting long-term survival of selected patients, although the outcome of CBT for ATLL (3-year OS, 20%) did not compare favorably with those of other allo-HSCT modalities. Regarding the prognostic factors affecting survival, our present univariate analysis identified the 5 following significant variables associated with higher OS: (1) age, (2) disease status at transplantation, (3) ABO compatibility, (4) addition of agents such as MTX or MMF to calcineurin inhibitors for GVHD prophylaxis, and (5) development of acute GVHD. Further, the multivariate analysis revealed that the development of acute GVHD was independently associated with better OS relative to the absence of acute GVHD. A landmark analysis showed that the development of grade 1 to 2, or so called mild-to-moderate acute GVHD, was associated with better OS when compared with the absence of acute GVHD. There was also a trend toward a lower risk of relapse or progression with the development of acute GVHD when compared with the absence of GVHD and better OS in patients with limited chronic GVHD. Taken together, these data suggest the presence of a curative graft-versus-ATLL effect conferred by CBT.

However, it is typically difficult for physicians to optimize the effects of acute GVHD to prevent disease progression via graft-versus-ATLL. Therefore, a more realistic attempt would be the control of pretransplantation factors that might affect the CBT outcome and, thus, enhance the benefit of allo-HSCT. The multivariate analysis performed herein with respect to ATLL-related deaths identified disease status at CBT as the most important factor. ATLL usually resists conventional chemotherapy and must be treated soon after diagnosis because of the rapid proliferation of tumor cells, which generates a high tumor burden [2,3]. In the future, novel agents, such as mogamulizumab, a humanized anti-CCR4 monoclonal antibody, might improve CBT-associated survival by decreasing the tumor burden before transplantation [32-35]. Another possibility for improving survival might be reducing the time from diagnosis to transplantation while patients with ATLL remain chemosensitive. Moreover, CBT provides a considerable advantage for patients who require urgent allo-HSCT to combat aggressive ATLL.

In the present study, we have shown that CBT is feasible and curative. However, the high rate of TRM remained a significant problem. Bacterial infection caused the highest incidence of death (21%) during the neutropenic period. The infusion of lower numbers of nucleated cells ($< 2 \times 10^7$/kg), which is usually associated with delayed engraftment, was marginally associated with TRM. Neutrophil recovery is slower in patients treated via CBT, and immunosuppressed patients with ATLL might be at an increased risk of developing more frequent opportunistic infections [36]. Improved supportive care to prevent bacterial infection is required after CBT for patients experiencing a prolonged neutropenic period. The ongoing development of better graft engineering [37] or double-CBT [38] might facilitate rapid neutrophil recovery and, thus, help to reduce the TRM rate in CB recipients.

The present study has several limitations. First, our results concerning the effect of chronic GVHD on survival should be interpreted with caution because the relatively small number of patients who developed chronic GVHD did not allow us to evaluate the effect of this condition on survival in a multivariate analysis. Instead, we were limited to performing a landmark analysis of OS according to the severity of chronic GVHD. Certainly, we detected a trend toward higher OS in patients with limited chronic GVHD when compared with patients without chronic GVHD, suggesting the possible presence of a graft-versus-ATLL effect. However, these results might be biased because of insufficient statistical power. Our future studies will assess the effect of chronic GVHD on the outcome of CBT for the treatment of ATLL after a long-term follow-up. Although the present study employed, to our knowledge, the largest cohort of CBT-treated patients to date and our results demonstrated that CBT is a feasible and effective treatment, this was a retrospective analysis. Therefore, this finding requires confirmation in prospective studies. To establish reliable criteria for CBT administration, a prospective multicenter clinical trial is underway in Japan to evaluate the safety and efficacy of CBT combined with Flu, Mel, and low-dose TBI (4 Gy) along with GVHD prophylaxis (tacrolimus and MMF [39]).

In conclusion, CBT is feasible and effective for patients with ATLL and acts via a graft-versus-ATLL effect. However, the outcome of CBT is unsatisfactory when compared with those of other allo-HSCT modalities. The high rate of TRM must be reduced, and the development of novel strategies is required to further improve the outcome of CBT.

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REFERENCES

1. Uchiyama T, Yodoi J, Sagawa K, et al. Adult T-cell leukemia: clinical and hematologic features of 16 cases. *Blood*. 1977;50:481-492.
2. Tsukasaki K, Maeda T, Arimura K, et al. Poor outcome of autologous stem cell transplantation for adult T cell leukemia/lymphoma: a case report and review of the literature. *Bone Marrow Transplant*. 1999;23:87-89.
3. Tsukasaki K, Utsunomiya A, Fukuda H, et al. VCAP-AMP-VECP compared with biweekly CHOP for adult T-cell leukemia-lymphoma: Japan Clinical Oncology Group Study JCOG9801. *J Clin Oncol*. 2007;25:5458-5464.
4. 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;64:391-399.
5. Kanda J, Hishizawa M, Utsunomiya A, et al. Impact of graft-versus-host disease on outcomes after allogeneic hematopoietic cell transplantation for adult T-cell leukemia: a retrospective cohort study. *Blood*. 2012;119:2141-2148.
6. 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;120:1734-1741.
7. Itonaga H, Tsushima H, Taguchi J, et al. Treatment of relapsed adult T-cell leukemia/lymphoma after allogeneic hematopoietic stem cell transplantation: the Nagasaki Transplant Group experience. *Blood*. 2013;121:219-225.
8. 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*. 2001;27:15-20.
9. Kami M, Hamaki T, Miyakoshi S, et al. Allogeneic haematopoietic stem cell transplantation for the treatment of adult T-cell leukemia/lymphoma. *Br J Haematol*. 2003;120:304-309.
10. Fukushima T, Miyazaki Y, Honda S, et al. Allogeneic hematopoietic stem cell transplantation provides sustained long-term survival for patients with adult T-cell leukemia/lymphoma. *Leukemia*. 2005;19:829-834.
11. Nakase K, Hara M, Kozuka T, et al. Bone marrow transplantation from unrelated donors for patients with adult T-cell leukemia/lymphoma. *Bone Marrow Transplant*. 2006;37:41-44.
12. Kato K, Kanda Y, Eto T, èt al. Allogeneic bone marrow transplantation from unrelated human T-cell leukemia virus-I-negative donors for adult T-cell leukemia/lymphoma: retrospective analysis of data from the Japan Marrow Donor Program. *Biol Blood Marrow Transplant*. 2007;13:90-99.
13. Shiratori S, Yasumoto A, Tanaka J, et al. A retrospective analysis of allogeneic hematopoietic stem cell transplantation for adult T cell leukemia/lymphoma (ATL): clinical impact of graft-versus-leukemia/lymphoma effect. *Biol Blood Marrow Transplant*. 2008;14:817-823.
14. Yonekura K, Utsunomiya A, Takatsuka Y, et al. Graft-versus-adult T-cell leukemia/lymphoma effect following allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2008;41:1029-1035.
15. 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;116:1369-1376.
16. Choi I, Tanosaki R, Uike N, et al. Long-term outcomes after hematopoietic SCT for adult T-cell leukemia/lymphoma: results of prospective trials. *Bone Marrow Transplant*. 2011;46:116-118.
17. Takizawa J, Aoki S, Kurasaki T, et al. Successful treatment of adult T-cell leukemia with unrelated cord blood transplantation. *Am J Hematol*. 2007;82:1113-1115.
18. Atsuta Y, Morishima Y, Suzuki R, et al. Comparison of unrelated cord blood transplantation and HLA-mismatched unrelated bone marrow transplantation for adults with leukemia. *Biol Blood Marrow Transplant*. 2012;18:780-787.
19. Atsuta Y, Suzuki R, Nagamura-Inoue T, et al. Disease-specific analyses of unrelated cord blood transplantation compared with unrelated bone marrow transplantation in adult patients with acute leukemia. *Blood*. 2009;113:1631-1638.
20. Nakamura T, Oku E, Nomura K, et al. Unrelated cord blood transplantation for patients with adult T-cell leukemia/lymphoma: experience at a single institute. *Int J Hematol*. 2012;96:657-663.
21. Fukushima T, Itonaga H, Moriuchi Y, et al. Feasibility of cord blood transplantation in chemosensitive adult T-cell leukemia/lymphoma: a retrospective analysis of the Nagasaki Transplantation Network. *Int J Hematol*. 2013;97:485-490.
22. Giralt S, Ballen K, Rizzo D, et al. Reduced-intensity conditioning regimen workshop: defining the dose spectrum. Report of a workshop convened by the center for international blood and marrow transplant research. *Biol Blood Marrow Transplant*. 2009;15:367-369.
23. Bacigalupo A, Ballen K, Rizzo D, et al. Defining the intensity of conditioning regimens: working definitions. *Biol Blood Marrow Transplant*. 2009;15:1628-1633.
24. Scrucca L, Santucci A, Aversa F. Competing risk analysis using R: an easy guide for clinicians. *Bone Marrow Transplant*. 2007;40:381-387.
25. Scrucca L, Santucci A, Aversa F. Regression modeling of competing risk using R: an in depth guide for clinicians. *Bone Marrow Transplant*. 2010;45:1388-1395.
26. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med*. 1999;18:695-706.
27. Cortese G, Andersen PK. Competing risks and time-dependent covariates. *Biom J*. 2010;52:138-158.
28. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant*. 2013;48:452-458.
29. Laughlin MJ, Eapen M, Rubinstein P, et al. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. *N Engl J Med*. 2004;351:2265-2275.
30. Rocha V, Labopin M, Sanz G, et al. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *N Engl J Med*. 2004;351:2276-2285.

31. Sanz MA. Cord-blood transplantation in patients with leukemia—a real alternative for adults. *N Engl J Med.* 2004;351:2328-2330.
32. 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;28:1591-1598.
33. 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;30:837-842.
34. Ito Y, Miyamoto T, Chong Y, et al. Successful treatment with anti-CC chemokine receptor 4 MoAb of relapsed adult T-cell leukemia/lymphoma after umbilical cord blood transplantation. *Bone Marrow Transplant.* 2013;48:998-999.
35. Kato K, Miyamoto T, Numata A, et al. Diffuse panbronchiolitis after humanized anti-CCR4 monoclonal antibody therapy for relapsed adult T-cell leukemia/lymphoma. *Int J Hematol.* 2013;97:430-432.
36. Itonaga H, Taguchi J, Fukushima T, et al. Distinct clinical features of infectious complications in adult T cell leukemia/lymphoma patients after allogeneic hematopoietic stem cell transplantation: a retrospective analysis in the Nagasaki transplant group. *Biol Blood Marrow Transplant.* 2013;19:607-615.
37. de Lima M, McNiece I, Robinson SN, et al. Cord-blood engraftment with ex vivo mesenchymal-cell coculture. *N Engl J Med.* 2012;367:2305-2315.
38. Rocha V, Crotta A, Ruggeri A, et al. Double cord blood transplantation: extending the use of unrelated umbilical cord blood cells for patients with hematological diseases. *Best Pract Res Clin Haematol.* 2010;23:223-229.
39. Uchida N, Wake A, Nakano N, et al. Mycophenolate and tacrolimus for graft-versus-host disease prophylaxis for elderly after cord blood transplantation: a matched pair comparison with tacrolimus alone. *Transplantation.* 2011;92:366-371.