Molecular characterization of chronic-type adult T-cell leukemia/lymphoma: discovery of molecular biomarkers for acute transformation ポスター発表	Yoshida N, Karube K, Utsunomiya A, Tsukasaki K, Imaizumi Y, Taira N, Uike N, Umino A, Arita K, Suguro M, Tsuzuki S, Kinoshita T, Nakamura S, Ohshima K, Seto M.	American society of Hematology Meeting on Lymphoma Biology (Colorado, USA)	2014年 8月10日～ 13日	国外
ATLの予後因子：患者側、腫瘍細胞側因子とdose intensity. ポスター発表	上運天綾子、北中明、山下清、外山孝典、前田宏一、松岡均、河野浩、佐藤誠一、石崎淳三、下田和哉	第111回日本内科学会講演会（東京）	2014年 4月11日 ～13日	国内

2．学会誌・雑誌等における論文掲載

掲載した論文（発表題目）	発表者氏名	発表した場所（学会誌・雑誌等名）	発表した時期	国内・外の別
Recent advances in treatment of adult T-cell leukemia- lymphoma.	Utsunomiya A, Choi I, Chihara D, Seto M	Cancer Science	2015 (in press)	国内
Primary Central Nervous System Lymphoma in Miyazaki, Southwestern Japan, a Human T-Lymphotropic Virus Type-1 (HTLV-1)-Endemic Area: clinicopathological review of 31 cases.	Maekawa K, Moriguchi-Goto S, Kamiunten A, Kubuki Y, Shimoda K, Takeshima H, Asada Y, Marutsuka K.	J Clin Exp Hematop.	2014; 54: 179-185.	国外
Molecular characterization of chronic-type adult T-cell leukemia/lymphoma.	Yoshida N, Karube K, Utsunomiya A, Tsukasaki K, Imaizumi Y, Taira N, Uike N, Umino A, Arita K, Suguro M, Tsuzuki S, Kinoshita T, Ohshima K, Seto M	Cancer Res	2014；74：6129-6138	国外
Detection of the G17V RHOA Mutation in Angioimmunoblastic T-Cell Lymphoma and Related Lymphomas Using Quantitative Allele-Specific PCR.	Nakamoto-Matsubara R, Sakata-Yanagimoto M, Enami T, Yoshida K, Yanagimoto S, Shiozawa Y, Nanmoku T, Satomi K, Muto H, Obara N, Kato T, Kurita N, Yokoyama Y, Izutsu K, Ota Y, Sanada M, Shimizu S, Komeno T, Sato Y, Ito T, Kitabayashi I, Takeuchi K, Nakamura N, Ogawa S, Chiba S.	PLoS One.	2014；9：e109714	国外
Japan Clinical Oncology Group (JCOG) prognostic index and characterization of long-term survivors of aggressive adult T-cell leukaemia-lymphoma (JCOG0902A).	Fukushima T, Nomura S, Shimoyama M, Shibata T, Imaizumi Y, Moriuchi Y, Tomoyose T, Uozumi K, Kobayashi Y, Fukushima N, Utsunomiya A, Tara M, Nosaka K, Hidaka M, Uike N, Yoshida S, Tamura K, Ishitsuka K, Kurosawa M, Nakata M, Fukuda H, Hotta T, Tobinai K, Tsukasaki K.	Br J Haematol	2014；166：739-748	国外

Loss of NDRG2 expression activates PI3K-AKT signalling via PTEN phosphorylation in ATLL and other cancers.	Nakahata S, Ichikawa T, Maneesaay P, Saito Y, Nagai K, Tamura T, Manachai N, Yamakawa N, Hamasaki M, Kitabayashi I, Arai Y, Kanai Y, Taki T, Abe T, Kiyonari H, Shimoda K, Ohshima K, Horii A, Shima H, Taniwaki M, Yamaguchi R, Morishita K.	Nat Commun.	2014；5：3393.	国外
Somatic RHOA mutation in angioimmunoblastic T cell lymphoma.	Sakata-Yanagimoto M, Enami T, Yoshida K, Shiraishi Y, Ishii R, Miyake Y, Muto H, Tsuyama N, Sato-Otsubo A, Okuno Y, Sakata S, Kamada Y, Nakamoto-Matsubara R, Tran NB, Izutsu K, Sato Y, Ohta Y, Furuta J, Shimizu S, Komeno T, Sato Y, Ito T, Noguchi M, Noguchi E, Sanada M, Chiba K, Tanaka H, Suzukawa K, Nanmoku T, Hasegawa Y, Nureki O, Miyano S, Nakamura N, Takeuchi K, Ogawa S, Chiba S.	Nat Genet.	2014；46：171-175	国外

Ⅴ.　研究成果の刊行物・別刷

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Review Article

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

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Key words

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 ($3{\cdot}5 \times 10^9$/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).

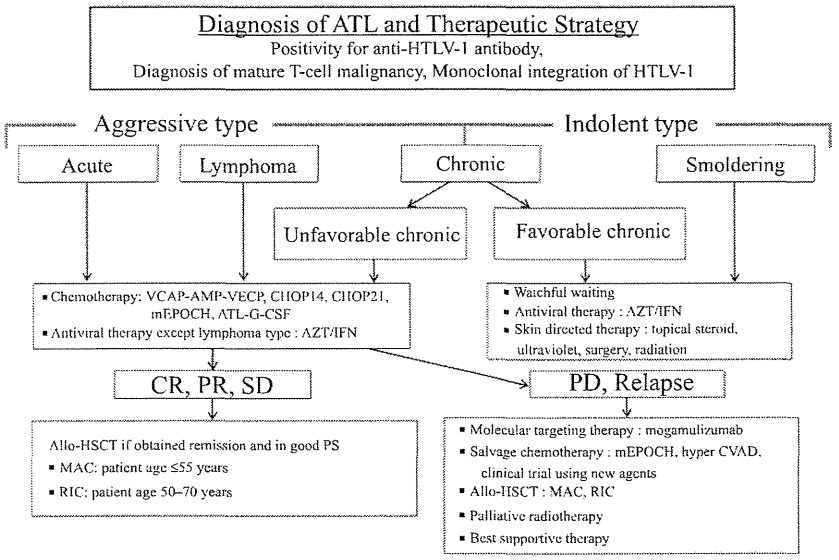

Fig. 1. Treatment algorithm for adult T-cell leukemia-lymphoma (ATL) patients. ATL diagnosis is based on anti-HTLV-1 antibody positivity in the serum, the presence of mature T-cell malignancy, and the Southern blot detection of monoclonal integration of HTLV-1 proviral DNA in the tumor cells. ATL treatment 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

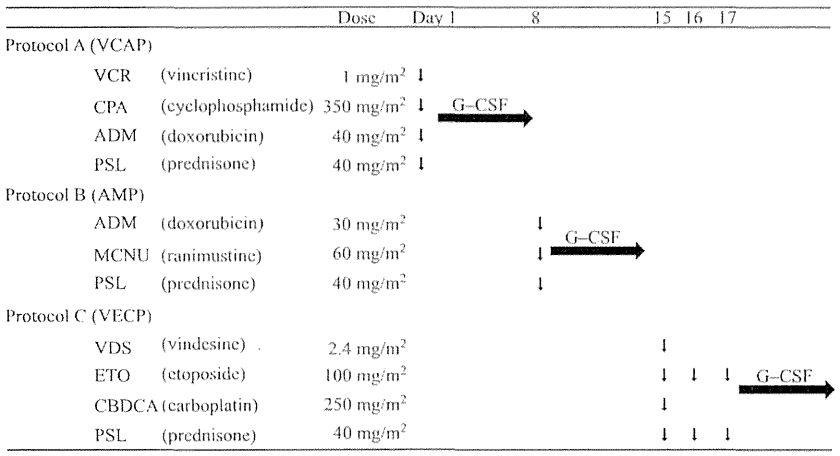

Fig. 2. The VCAP-AMP-VECP regimen. A, B and C are repeated every 28 days for 6 cycles. Cytarabine (40 mg), methotrexate (15 mg) and prednisone (10 mg) are administered intrathecally before cycles 2, 4 and 6. VCAP-AMP-VECP, vincristine, cyclophosphamide, doxorubicin and prednisone (VCAP)-doxorubicin, 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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Original Article

Primary Central Nervous System Lymphoma in Miyazaki, Southwestern Japan, a Human T-Lymphotropic Virus Type-1 (HTLV-1)-Endemic Area: Clinicopathological Review of 31 Cases

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Primary central nervous system lymphoma (PCNSL) is a rare and aggressive brain tumor. The aim of this study was to clarify the prevalence of T-cell-type PCNSL (T-PCNSL) in a human T-lymphotropic virus type-1 (HTLV-1)-endemic area of Southwestern Japan. We retrospectively investigated 31 PCNSL cases diagnosed between 1996 and 2013 at the University of Miyazaki Hospital. These cases accounted for 4.4% of all nodal or extranodal malignant lymphomas. Histologically, most of these cases were diagnosed as diffuse large B-cell lymphoma, while only two cases were considered to be low-grade and high-grade B-cell lymphoma (not otherwise specified). No T-PCNSL was found in this series. In addition, Epstein-Barr virus-encoded RNAs were not detected by *in situ* hybridization in any of the cases. Overall, no T-PCNSL cases were found in 18 years in a region with a high frequency of HTLV-1 seropositivity, namely, Southwestern Japan. This suggests that PCNSL and lymphomas of other anatomical sites are biologically distinct. [*J Clin Exp Hematop 54(3) : 179-185, 2014*]

Keywords: B-cell lymphoma, human T-lymphotropic virus type 1, primary central nervous system lymphoma, T-cell lymphoma

INTRODUCTION

Primary central nervous system (CNS) lymphoma (PCNSL) is defined as a lymphoma arising in the brain, spinal cord, or leptomeninges in the absence of lymphoma outside of the nervous system at the time of diagnosis. PCNSL is a rare tumor that comprises approximately 1.5% to 3% of all brain tumors and 1% of all non-Hodgkin lymphomas; however, the incidence of PCNSL has recently increased within populations of immunocompromised individuals with human immunodeficiency virus infection and in immunocompetent elderly individuals.[1] The vast majority of PCNSLs have a B-cell origin (B-PCNSL), particularly diffuse large B-cell lymphoma (DLBCL) in most Western countries, with only 2-3% of PCNSLs being derived from T cells.[2] In contrast, a higher prevalence of T-PCNSL was reported in Asia: 8-14% in Japan and 16.7% in Korea.[3] However, in our experience, the reported prevalence of 8-14% in Japan seems to be too high. Therefore, we postulated that some specific phenomenon may contribute to the high PCNSL prevalence in Japan and investigated clinical and histological findings of PCNSL at the University of Miyazaki Hospital, in a human T-lymphotropic virus type-1 (HTLV-1)-endemic area. We also investigated the association of Epstein-Barr virus (EBV) with PCNSL, which may account for the high prevalence of PCNSL in Japan.[4]

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MATERIALS AND METHODS

Patient characteristics

We searched the archives of the University of Miyazaki Hospital for newly diagnosed nodal or extranodal malignant lymphomas between 1996 and 2013, as after this time, an electronic medical chart system was introduced in our hospital, and detailed clinical information has become available. We extracted the cases of newly diagnosed PCNSL as well as

all cases of nodal or extranodal lymphoma. The following information was requested: 1) clinical status before treatment; 2) history of presence or absence of immunocompromised status including a cancer, congenital immune deficiency, acquired immune deficiency syndrome, and immunosuppressive therapy; 3) tumor location; 4) nature of adjuvant therapy (radiation and/or chemotherapy); 5) evaluation of clinical status at the end of the first treatment; and 6) outcome as of Nov. 2013. Cases were excluded if any clinical information was not available.

Histological studies

All biopsies and surgical samples were fixed in 10% buffered formalin and embedded in paraffin. Sections (4 μm) were routinely stained with hematoxylin and eosin (H&E). The histological subclassification of lymphomas was based on the revised WHO Classification of Tumours of the Haematopoietic and Lymphoid Tissues.[3] We examined the growth pattern of the lymphomas and recorded the presence of perivascular cuffing and infiltration in the vascular wall.

Immunohistochemistry and in situ *hybridization*

All of the cases were evaluated immunohistochemically. To determine whether each PCNSL was of the B- or T-cell lineage, immunohistochemical staining was performed using antibodies against CD79a (1:200, heat treatment (HT), DakoCytomation, Denmark), CD20 (1:400, DakoCytomation), and cytoplasmic CD3 (cCD3) (1:200, DakoCytomation) in all 31 cases. For a more detailed examination of B-cell-derived PCNSLs, we evaluated the immunoreactivity using antibodies against CD10 (Diluted, Nichirei Bioscience Inc., Japan), BCL2 (1:50, DakoCytomation), BCL6 (1:200, HT, Dako Cytomation), IRF4/MUM1 (1:100, HT, DakoCytomation), and CD5 (1:100, HT, DakoCytomation). To evaluate the proliferation activity of lymphoma cells in all cases, an antibody against Ki-67 (1:50, HT, DakoCytomation) was used. Ki-67 labeling index was defined as the percentage of Ki-67-positive cells among the total lymphoma cells and was evaluated in the hotspots. Immunohistochemical stains were performed on a Leica Autostainer (Bond III, Leica Biosystems, Germany) using standard methods. *In situ* hybridization (ISH) for EBV-encoded small RNAs (EBERs) was conducted on paraffin sections from all cases using the EBV Probe ISH Kit (Leica Biosystems).

Ethical issues

This study was approved by the Local Ethics Committee (2013-132), and informed consent was obtained from all patients.

RESULTS

In total, 710 cases of newly diagnosed nodal or extranodal malignant lymphomas were detected between 1996 and 2013 at the University of Miyazaki Hospital. Thirty-one cases (4.4%) of these 710 lymphomas originated in the CNS. The

Table 1. Patient Characteristics for 31 cases of primary central nervous system lymphoma (PCNSL)

Characteristics	No. of patients	%
Age		
> 60	22	71
< 60	9	29
Sex		
M	17	55
F	14	45
Tumor location		
Supratentrial space	27	87
frontal	8	26
temporal	3	10
occipital	1	3
basal ganglia	2	6
multifocal	9	29
others	4	13
Cerebellum	1	3
Pituitary	2	6
Occular	1	3
Symptoms		
Hemiplegia	8	26
Headache	5	16
Amnesia	4	13
Dysopia	4	13
Disorientation	3	10
Disturbed consciousness	3	10
Convulsion	2	6
Fatigue	1	3
Eye-pain	1	3
Immunocompromised status		
Yes	7	23
No	24	77
Therapy		
Radiation (R)	2	6
Chemotherapy (C)	13	42
R + C	14	45
Surgery + C	1	3
Non-Tx	1	3
Effect of the first therapy		
CR	9	29
PR	15	48
SD	2	6
NE	2	6
Unknown	3	10
Outcome		
Alive with disease	7	23
Alive without disease	5	16
Dead of disease	7	23
Dead of other disease	1	3
Unknown	11	35

R, radiation; C, chemotherapy; Tx, therapy; CR, complete remission; PR, partial remission; SD, stable disease; NE, not evaluable

principal clinical characteristics of the patients at the time of diagnosis are summarized in Table 1. The patients included 17 males and 14 females. There were no pediatric cases, with a median age at diagnosis of 67 years (range: 21-85 years), with 71% (22/31) being over 60 years of age. Twenty-seven cases (87%) involved the supratentorial space, including 8 cases in the frontal lobe (26%), 3 in the temporal lobe (10%), 2 in the basal ganglia (6%), and 1 in the occipital lobe (3%). Two cases involved the sellar region (6%), and 1 case (3%) originated from either the cerebellum or the ocular region. Multifocal lesions were detected in 9 cases (29%). Most of the patients experienced neurological symptoms, especially hemiparesis (26%) and headache (16%), and four patients presented with amnesia and dysopia (13%). Seven patients (23%) had a history of an immunocompromised status, including diabetes mellitus (4 cases), cancer (2 cases), and hemodialysis (1 case). The result of serologic study for HTLV-1 was negative in all patients. Thirteen patients (42%) were treated by chemotherapy alone, 14 patients (45%) by chemotherapy followed by radiation, and 2 patients received radiation alone. Additionally, one patient received chemotherapy followed by complete surgical resection, and another patient died before treatment was initiated. The effectiveness of the initial treatment was assessed by magnetic resonance imaging. The initial treatment resulted in complete remission in 9 patients (29%), partial remission in 15 patients (48%), and stable disease (SD) in two patients (6%), while 2 patients (6%) could not be evaluated. As of Nov. 2013, 7 patients (23%) survived with disease, 5 patients (16%) survived and were disease-free, and 7 patients (23%) had died of the disease. Follow-up data were unavailable in 11 patients.

Histologically, all of the cases showed a perivascular infiltrative pattern (Fig. 1A), but diffuse proliferation was also noted (Fig. 1B). Lymphoma cells infiltrated the walls of medium-sized to large vasculature (Fig. 1C). In all cases except 2, the lymphoma cells were medium-sized to large, with centroblast- or immunoblast-like nuclei (Fig. 2). In two cases, the lymphoma cells were small to medium-sized with relatively uniform round nuclei that proliferated in a perivascular infiltrative pattern (Fig. 3). Reactive gliosis, necrosis, and/or hemorrhage were observed in some cases.

Immunohistochemically, while lymphoma cells were positive for CD79a and CD20 in all cases, CD10 expression was observed in approximately 13%, BCL2 in 78%, BCL6 in 78%, and IRF4/MUM1 in 87% (Fig. 4). The histological subtypes and the results of the immunohistochemical study are listed in Table 2. Small lymphocytes of varying densities, which were present in the tumor or in the tumor periphery, may have been reactive lymphocytes, which were immunopositive for cCD3 or CD5. In contrast, the lymphoma cells were negative for cCD3 in all cases. In only one case, the lymphoma cells were positive for CD5 (Case 29), and this was one of the cases in which initial treatment resulted in SD.

According to the WHO classification system,[3] we diagnosed 29 cases as DLBCL: 23 of these (79%) were subclassified as the non-germinal center B-cell type (non-GCB) and 6 cases as the GCB type (21%). The GCB cases seemed to be associated with a better prognosis than the non-GCB ones, which is similar to findings in systemic or other organ lesions described in a previous study.[5] The Ki-67 labeling index in

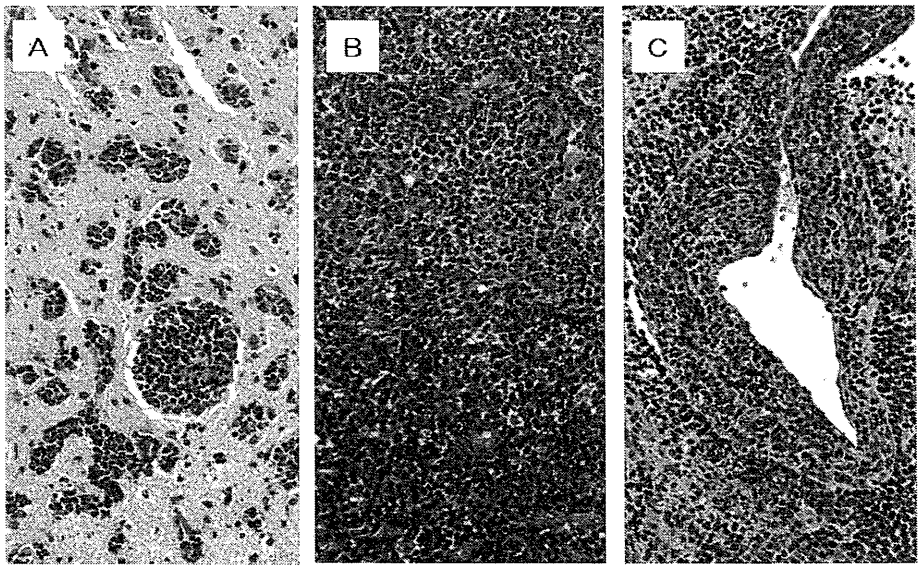

Fig. 1. Invasive pattern of lymphoma cells. Perivascular (*1A*), diffuse (*1B*), and vascular wall (*1C*). (*1A*) Case 15, and (*1B*) and (*1C*) Case 11, H&E stain, × 100.

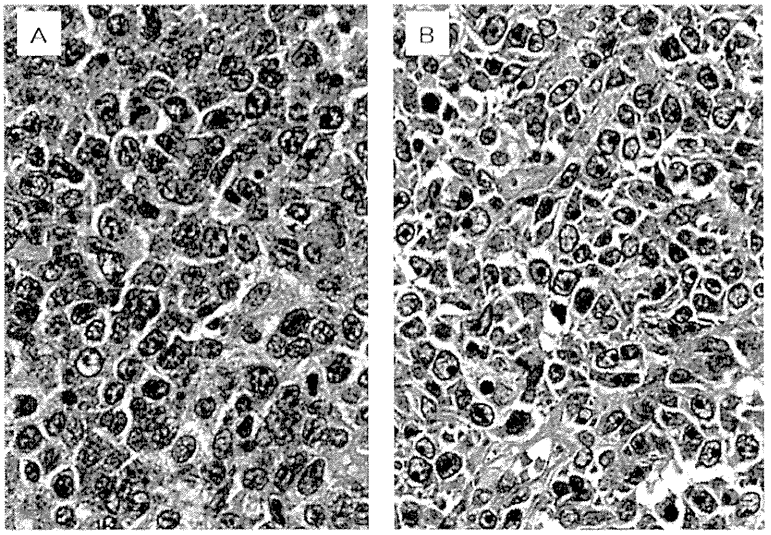

Fig. 2. High magnification of lymphoma cells. Immunoblastic (*2A*) and centroblastic (*2B*) lymphoma cells. (*2A*) Case 11 and (*2B*) Case 18, H&E stain, × 400.

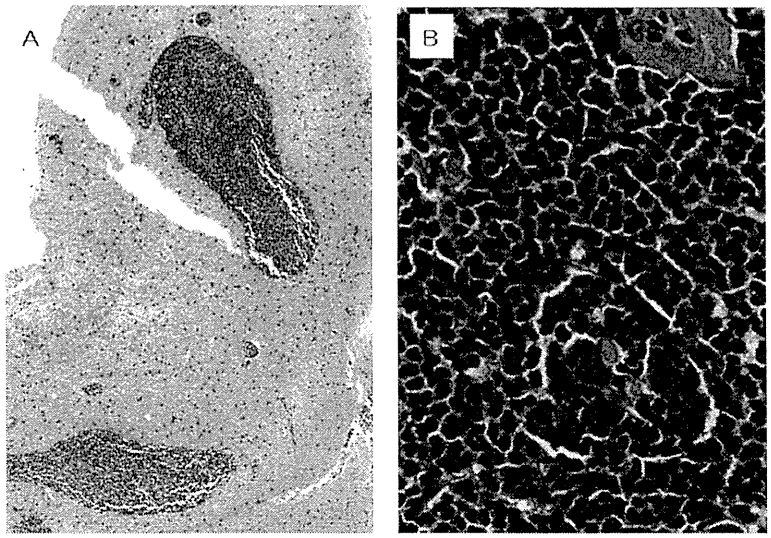

Fig. 3. Low-grade B-cell lymphoma in case 25. Perivascular infiltration of lymphoma cells (*3A*) and small lymphoma cells under high magnification (*3B*). H&E stain, (*3A*) × 100, (*3B*) × 400.

all cases was more than 70% in DLBCL. Two cases in which the lymphoma cells were small or medium in size were diagnosed as unclassifiable low-grade B-cell lymphoma with a low Ki-67 labeling index (4.3%) or unclassifiable high-grade B-cell lymphoma with a high Ki-67 labeling index (81%). No positive signal was detected with EBER-ISH in any of the cases (Table 2).

DISCUSSION

In the WHO Classification of Tumours of the CNS, PCNSL is defined as a lymphoma arising in the CNS without extra-CNS lesions present at the time of diagnosis.[6] However, in the WHO Classification of Tumours of the Haematopoietic and Lymphoid Tissues, PCNSL is listed as a subtype of DLBCL that occurs as an intracranial or intraocular lesion, excluding intravascular large B-cell lymphoma and lymphomas that arise in the dura or in the orbital region.[3] PCNSLs other than DLBCL are rare, although such cases have been reported.[7,8] In addition to B-PCNSL, the WHO Classification of Tumours of the CNS also states that T-PCNSLs constitute approximately 2-5% of all PCNSLs in Western countries and 8-14% in Japan.[6] These data of a high prevalence of T-PCNSL listed in the WHO Classification of Tumours of the CNS[6] were cited from a report by Hayabuchi *et al.* as 8.5% among 234 PCNSLs assessed using a panel of T/B-cell markers[9] and a report by Hayakawa *et al.* as 14% among 21 PCNSLs assessed by immunohistochemical techniques.[10] In other East Asian countries, the T-PCNSL prevalence was reported as 16.7% in Korea[11] and 1% in Taiwan.[12] Using the criteria described in the WHO Classification of Tumours of the Haematopoietic and

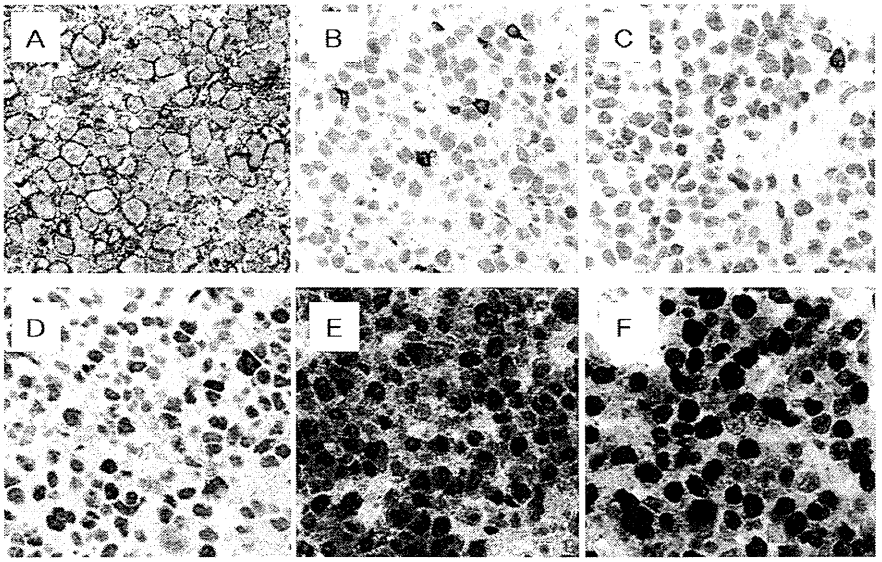

Fig. 4. The results of immunohistochemical staining in case 20. CD20 (*4A*), cCD3 (*4B*), CD10 (*4C*), BCL6 (*4D*), MUM1 (*4E*), and Ki-67 (*4F*). Lymphoma cells were positive for CD20, BCL6, and MUM1, but negative for cCD3 and CD10. Ki-67-positive index was over 80%.

Table 2. Immunohistochemistry, *in situ* hybridization for Epstein-Barr virus-encoded small RNAs (EBER-ISH) and histological subtype

Case	CD79a	CD20	cCD3	CD10	BCL2	BCL6	MUM1	CD5	EBER-ISH
1	+	+	−	−	−	−	−	ND	−
2	+	+	−	−	+	+	+	ND	−
3	+	+	−	−	+	−	+	ND	−
4	+	+	−	−	+	−	+	ND	−
5	+	+	−	−	+	+	+	ND	−
6	+	+	−	−	−	−	−	ND	−
7	+	+	−	−	+	+	−	ND	−
8	+	+	−	−	+	+	+	ND	−
9	+	+	−	+	+	+	+	ND	−
10	+	+	−	−	−	+	+	ND	−
11	+	+	−	−	+	−	+	ND	−
12	+	+	−	−	+	−	+	ND	−
13	+	+	−	+	+	+	+	ND	−
14	+	+	−	−	+	+	+	−	−
15	+	+	−	−	+	+	+	−	−
16	+	+	−	−	−	+	+	−	−
17	+	+	−	+	+	+	+	−	−
18	+	+	−	−	−	+	+	−	−
19	+	+	−	−	−	+	−	−	−
20	+	+	−	−	+	+	+	−	−
21	+	+	−	−	+	+	+	−	−
22	+	+	−	−	+	+	+	−	−
23	+	+	−	−	+	+	+	−	−
24	+	+	−	−	+	+	+	−	−
25	+	+	−	−	+	−	+	−	−
26	+	+	−	−	+	+	+	ND	−
27	+	+	−	−	+	+	+	−	−
28	+	+	−	+	−	+	+	−	−
29	+	+	−	−	+	+	+	+	−
30	+	+	−	−	+	+	+	−	−
31	+	+	−	−	+	+	+	−	−

ND, not done

Lymphoid Tissues, we diagnosed 29 cases as primary DLBCL of the CNS and the other two cases as low- and high-grade B-cell lymphoma (not otherwise specified). There were no T-PCNSL cases in this study, even though these cases were from an HTLV-1-endemic region in Southwestern Japan, where 6.6 to 12.1% of the population are seropositive for HTLV-1.[13] Shibamoto *et al.* reported that T-PCNSL in Japan decreased by 8.5% during 1985-1994, by 5.2% during 1995-1999, and by 1.7% during 2000-2004, although these authors did not discuss the reason for this.[14] Moreover, other reports described that T-PCNSLs constituted 8 or 14% of PCNSLs, which may be outdated, but no detailed examinations of the histological findings were described.[9,10] Additionally, Dulai *et al.* indicated that T-PCNSL may not be recognized unless examinations are performed to detect T-cell receptor gene rearrangements for CNS lesions composed of a polymorphous but predominant T-cell infiltrate, because of a high degree of overlap in morphologic and immunophenotypic features between T-PCNSL and reactive infiltrate.[15] Conversely, a lesion of reactive T-cell infiltrate in the CNS can be misdiagnosed as T-PCNSL unless detection of T-cell receptor gene rearrangements is performed. There have been only a few reports of adult T-cell leukemia/lymphoma (ATLL) in PCNSL,[2,16] and no T-PCNSL including ATLL was detected in the present study, which was performed in an HTLV-1-endemic area. In fact, the annual incidence of new-onset T-cell lymphoma in our hospital was approximately 35% (unpublished data), and most of these cases were ATLL. Considering these data, the current incidence of T-PCNSL in Japan might not be so high. Thus, it is necessary to ascertain the "true" incidence of T-PCNSL and the relationship between T-PCNSL and HTLV-1 in a worldwide study including Japan and other HTLV-1-endemic areas. Furthermore, in a study reviewing 47 autopsy cases of ATLL in Miyazaki Prefecture, Japan, CNS involvement developed in 4 of 35 cases examined (11.4%).[17] The relatively high reported prevalence of T-PCNSL, therefore, might be due to CNS invasion by lymphomas that originated outside of the CNS.

Histologically, all cases showed a diffuse and perivascular infiltration pattern. Regarding the relationship between blood vessels and lymphoma cells, certain morphological features are important for PCNSL diagnosis, such as "perivascular cuffs and angiocentric infiltration pattern".[1,3,9] However, these textbooks do not mention vessel wall invasion. In our study, we detected vessel wall invasion by lymphoma cells in tissue samples including large blood vessels in 88% of the cases. Perivascular lymphocytic infiltration itself is also found in non-neoplastic CNS diseases such as inflammatory diseases or demyelinating disorders. Thus, invasion of the vessel wall may be a characteristic feature of PCNSL and can be a diagnostic indicator to differentiate a lymphoma from inflammatory or demyelinating disorders.

Hans *et al.* showed that non-GCB was associated with a poor prognosis compared with GCB in systemic DLBCL.[5] In addition, Camilleri-Broet *et al.* suggested that the activated B-cell-like immunophenotype of PCNSL was also associated with a poor prognosis.[18] Thus, the GCB cases of PCNSL might have a good prognosis compared with the non-GCB cases. We were unable to evaluate the prognostic difference between GCB and non-GCB due to the small number of cases.

CD5-positive DLBCL is associated with female predominance, older age at diagnosis, higher serum lactate dehydrogenase level, and poor prognosis.[19,20] Imai *et al.* reported that 12 from 40 CNS DLBCL cases (30%) were revealed as CD5-positive and they showed poorer prognosis than the CD5-negative cases.[21] In our series, only one female case was CD5-positive from 29 DLBCLs (3%), and her age at diagnosis was 85 years old, with no data on the serum LDL level. The therapeutic effect of the initial treatment was SD in this case and alive with disease after a very short follow-up period. In another study of systemic DLBCL excluding PCNSL, Ennishi *et al.* reported that 11 from 121 cases (9%) were revealed as CD5-positive by flow cytometry, whereas only 7 (6%) cases showed positive staining for CD5 (4C7, Novocastra) immunohistochemically.[22] Ennishi *et al.* pointed out that CD5 paraffin immunohistochemistry is less sensitive and they concluded that flow cytometric analysis or frozen-section immunohistochemistry needs to be carried out for the detection of CD5 in DLBCL. We consider that the very low proportion of CD5-positive cases in this study arose from these methodological conditions.

EBV-positive DLBCL can occur among immunocompetent elderly patients over 50 years of age. In this study, there were no EBV-positive cases, although this study included 22 (71%) elderly cases aged over 60. Actually, we experienced a case of EBV-positive PCNSL that had suffered from ATLL,[23] but we excluded it from the present study because this was just a consultation case from another hospital. Oyama *et al.* hypothesized that the malignant transformation of B cells occurs because of a decrease in immune function associated with aging.[24,25] Thus, it is suggested that the incidence of EBV-positive PCNSL may be higher in a study with a larger number of cases.

In summary, we reported PCNSL diagnosed over a period of 18 years (1996-2013) at our hospital. Most of our results are in accordance with previous reports, with the exception of the two PPL cases. However, no T-PCNSL cases were found in this study, despite it being in an HTLV-1-endemic area of Japan. To determine the true prevalence of T-PCSNL, it is necessary to conduct further research, including multicenter studies.

DISCLOSURE STATEMENT

There is no conflict of interest.

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Cancer Research

Molecular Characterization of Chronic-type Adult T-cell Leukemia/Lymphoma

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Abstract

Adult T-cell leukemia/lymphoma (ATL) is a human T-cell leukemia virus type-1–induced neoplasm with four clinical subtypes: acute, lymphoma, chronic, and smoldering. Although the chronic type is regarded as indolent ATL, about half of the cases progress to acute-type ATL. The molecular pathogenesis of acute transformation in chronic-type ATL is only partially understood. In an effort to determine the molecular pathogeneses of ATL, and especially the molecular mechanism of acute transformation, oligo-array comparative genomic hybridization and comprehensive gene expression profiling were applied to 27 and 35 cases of chronic and acute type ATL, respectively. The genomic profile of the chronic type was nearly identical to that of acute-type ATL, although more genomic alterations characteristic of acute-type ATL were observed. Among the genomic alterations frequently observed in acute-type ATL, the loss of *CDKN2A*, which is involved in cell-cycle deregulation, was especially characteristic of acute-type ATL compared with chronic-type ATL. Furthermore, we found that genomic alteration of *CD58*, which is implicated in escape from the immunosurveillance mechanism, is more frequently observed in acute-type ATL than in the chronic-type. Interestingly, the chronic-type cases with cell-cycle deregulation and disruption of immunosurveillance mechanism were associated with earlier progression to acute-type ATL. These findings suggested that cell-cycle deregulation and the immune escape mechanism play important roles in acute transformation of the chronic type and indicated that these alterations are good predictive markers for chronic-type ATL. *Cancer Res; 74(21); 6129–38. ©2014 AACR.*

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Introduction

Adult T-cell leukemia/lymphoma (ATL) is a human T-cell leukemia virus type-1 (HTLV-1)–induced neoplasm (1, 2). Four clinical subtypes of ATL have been classified on the basis of clinical manifestation: acute, lymphoma, chronic, and smoldering (3). Among these subtypes, chronic-type ATL shows characteristic manifestations such as increased abnormal lymphocytes in peripheral blood, lactate dehydrogenase (LDH) levels up to twice the normal upper limit, and absence of hypercalcemia. Chronic-type ATL is relatively rare and its frequency is estimated to be 8% to 18% of ATL cases (3). Previous reports regard the chronic type as indolent ATL compared with acute/lymphoma types, which show an aggressive clinical course (3, 4). However, a recent study of indolent ATL demonstrated that about half of the patients with chronic-type ATL progress to acute-type ATL within approximately 18 months from diagnosis and subsequent death (4). This finding suggests that patients with chronic-type ATL also had a poor prognosis. High LDH, high blood urea nitrogen, and low albumin levels have been identified as poor prognostic factors for chronic-type ATL, and patients with chronic-type ATL with these poor prognostic factors therefore need to be treated by intensive chemotherapy as in the case of patients with aggressive ATL (5).

Disruptions of *CDKN2A*, *CDKN2B*, and *TP53* have been reported as candidate genes that play important roles in acute

transformation of chronic-type ATL (6–12). However, these acute transformation–related genetic alterations have been identified only by focusing on genes that were previously shown to be involved in tumor progression of other malignancies. Therefore, these genetic alterations may be indicative of acute transformation in some cases, although the molecular mechanism of acute transformation remains to be fully elucidated. Identification of the molecular characteristics of chronic-type ATL using unbiased and genome-wide methods can provide further insights to elucidate the acute transformation mechanisms in chronic-type ATL. However, the molecular pathogenesis of chronic-type ATL has long remained unknown due to its rarity (13).

In the present study, high-resolution oligo-array comparative genomic hybridization (aCGH) and gene expression profiling (GEP) were applied to 27 cases of chronic-type ATL in an effort to determine the molecular pathogenesis. The same approaches were used with 35 cases of acute-type ATL, and we then compared the molecular characteristics of chronic- and acute-type ATL to investigate the molecular mechanism of acute transformation.

Materials and Methods

Patient samples

We collected and analyzed 27 cases of chronic-type ATL and 35 cases of acute-type ATL (Table 1 and Supplementary Table S1 in Supplementary Data). These samples were obtained from patients at Imamura-Bunin Hospital (Kagoshima, Japan), Nagasaki University School of Medicine (Nagasaki, Japan), Heart Life Hospital (Nakagusukuson, Japan), and Kyushu Cancer Center (Fukuoka, Japan). In accordance with Shimoyama criteria, the diagnoses were made by expert hematologists (A. Utsunomiya, K. Tsukasaki, Y. Imaizumi, N. Taira, and N. Uike; ref. 3). Samples and medical records used in our study were approved by the Institute Review Board of the Aichi Cancer Center (Nagoya, Japan). Informed consent was obtained according to the Declaration of Helsinki from all patients. DNA and RNA used in this study were extracted from purified CD4-positive cells as previously reported (14). For the cumulative incidence of acute transformation, events were defined as acute transformation or any treatment for ATL.

Copy number analysis by aCGH and GEP

We performed aCGH analysis on all samples using 400K aCGH (Agilent, Cat. # G4448A; Agilent Technologies) and 44K aCGH (Agilent, Cat. # G4413A) slides (Supplementary Table S1). Thirteen acute-type cases analyzed in a previous study were included (14). Procedures for DNA digestion, labeling, hybridization, scanning, and data analyses were performed according to the manufacturer's protocols (www.agilent.com). Raw data were transferred to the Genomic Workbench v5.0 software (Agilent Technologies) for further analysis as described previously (14–16). Among these identified alterations, we focused on minimal common regions (MCR). MCRs are defined as alterations that encompass less than 3 protein-coding genes among all samples analyzed in this study (17). Copy number variations/polymorphisms (CNV) were identified using a database (HS_hg18_CNV-20120403, Agilent), which was obtained from Database of Genomic Variants (http://projects.tcag.ca/variation/) in April 2012 and then excluded from further analyses as described previously (16). We also performed aCGH analysis on matched normal DNA samples that were available and confirmed that the identified MCRs were not CNVs (Supplementary Fig. S1A).

For analysis of GEP, the Whole Human Genome 44K Oligo-microarray Kit (Agilent, Cat. # G4112F) was used for the hybridization of labeled RNA. The total RNA of 13 chronic samples and 21 acute samples was analyzed. The experimental protocol used reflected the manufacturer's protocol (www.agilent.com) as previously reported (15, 16). Using the results of GEP, gene set enrichment analysis (GSEA) was performed as previously described (15, 16, 18).

The detailed description of these analyses can be found in Supplementary Methods. The microarray data were submitted to ArrayExpress and assigned accession numbers E-MTAB-1808 (aCGH) and E-MTAB-1798 (GEP).

Mutation analyses of CD58 and β2-microglobulin

The exons 1–4 of *CD58* and 1 and 2 of *β2-microglobulin* (*B2M*), whose mutations were identified in peripheral T-cell lymphomas (PTCL; ref. 19), were amplified from gDNA using PCR. PCR primers used are detailed in the previous study (20). Twenty-six acute-type and 26 chronic-type ATL samples, for which adequate DNA was available, were analyzed. Direct sequencing of PCR products was performed through capillary electrophoresis using the ABI3100 sequencer (Applied Biosystems).

Flow cytometry

Analysis of cell surface CD58 in ATL cell lines was performed using anti-CD58 PE antibody (AlCD58, Beckman Coulter).

Table 1. Patient information at sampling

Subtype	No. of samples	Median age (range), y	Median WBC (range), u/L	Median LDH (range), IU/L	Median calcium (range), mg/dL	Median albumin (range), g/dL	Median BUN (range), mg/dL
Chronic type	27	61 (42–81)	1,1400 (6,000–22,100)	233 (155–465)	9.3 (8.4–10.2)	4.2 (3.0–4.8)	15.5 (7.4–26.4)
Acute type	35	57 (32–85)	2,1700 (4,100–224,800)	688 (203–2,223)	9.3 (7.7–17.4)	3.8 (2.6–4.5)	NA

Abbreviations: BUN, blood urea nitrogen; NA, not available; WBC, white blood cells.