median survival times of patients with acute-type ATL treated with first-line AZT/IFN-α and chemotherapy were 12 and 9 months, respectively. However, achievement of CR with first-line AZT/IFN-α therapy resulted in a prolonged survival time of more than 10 years in 70% of the study population and 75% of the acute-type ATL subgroup. Patients with lymphoma-type ATL did not benefit from AZT/IFN-α therapy; the median survival times of these patients treated with first-line AZT/IFN-α and chemotherapy were 12 and 15 months, respectively. Finally, first-line AZT/IFN-α therapy in chronic- and smoldering-type ATL resulted in 100% OS at a median follow-up time of 5 years. Although the results for AZT/IFN-α in indolent ATL seem to be promising compared with the results seen with watchful waiting until disease progression recently reported from Japan,[45] the possibility of selection bias cannot be ruled out. In conclusion, these results suggest that treatment of ATL using AZT/IFN-α results in high response and CR rates particularly in acute, chronic, and smoldering types of ATL, resulting in prolonged survival in a significant proportion of patients. Although this is a retrospective analysis, the results seem to be promising, and further studies comparing AZT/IFN-α and chemotherapy in acute ATL are warranted.

alloHSCT. alloHSCT is now considered a promising treatment of young patients with aggressive ATL. Despite higher treatment-related mortality in a retrospective multicenter analysis, the estimated 3-year OS rate of 45% is promising, possibly reflecting a graft-versus-ATL effect.[46] A phase I trial of alloHSCT with reduced-intensity conditioning for ATL also revealed promising results. Minimal residual disease after alloHSCT detected by proviral load was much less compared with that after chemotherapy or AZT/IFN-α therapy, suggesting the presence of a graft-versus-ATL effect as well as graft-versus-HTLV-1 activity.[47] It remains uncertain which type of alloHSCT (myeloablative or reduced-intensity conditioning) is most suitable for the treatment of ATL. However, myeloablative alloHSCT, but not reduced-intensity conditioning alloHSCT, might be considered for the treatment of patients with progressive disease (PD) at relapse as well as at onset. Furthermore, selection criteria with respect to response to previous treatments, sources of stem cells, and HTLV-1 viral status of the donor remain to be determined.

Required Pretreatment Evaluation

The diagnosis of ATL is based on HTLV-1 seropositivity and histologically and/or cytologically proven peripheral T-cell malignancy as described in the WHO classification.[4] In uncertain cases, Southern blot hybridization for monoclonal integration of HTLV-1 provirus is useful for the diagnosis, although the sensitivity is to detect the presence of approximately 5% or more monoclonal ATL cells in peripheral-blood mononuclear cells or fresh biopsy.[6]

Traditionally, patients with indolent ATL (ie, the chronic or smoldering type) have been managed similarly to patients with CLL, with a watchful waiting policy until disease progression.[6,8,9] In the consecutive trials for aggressive ATL by Japan Clinical Oncology Group (JCOG)–Lymphoma Study Group, previously untreated patients with aggressive ATL (ie, acute-, lymphoma-, or unfavorable chronic-type ATL) were eligible for participation.[36] Unfavorable chronic-type ATL was defined by at least one of the following three factors: a low serum albumin, high LDH, or high blood urea nitrogen concentration. Unfavorable chronic-type ATL had an unfavorable prognosis similar to acute- or lymphoma-type ATL when treated with chemotherapy.[6] In those trials, other eligibility criteria included no prior chemotherapy, age of 15 to 69 years, and Eastern Cooperative Oncology Group PS of 0 to 3 or 4 as a result of hypercalcemia.[6,36] Eligibility criteria for organ function were also described.[6,36]

Supportive Care

Sulfamethoxazole-trimethoprim and antifungal agents were recommended for the prophylaxis of *Pneumocystis jiroveci* pneumonia and fungal infections, respectively, in the JCOG trials.[6,36] Although cytomegalovirus infection commonly occurs in ATL patients, ganciclovir is not routinely recommended for prophylaxis. In addition, in patients not receiving chemotherapy, antifungal prophylaxis may not be critical. Prophylaxis with anti-*Strongyloides* agents, such as ivermectin or albendazole, should be considered to avoid systemic infection in patients with a history of past and/or present exposure to the parasite in the tropics. Treatment with corticosteroids and proton pump inhibitors may precipitate fulminant *Strongyloides* infestation and warrants testing before these agents are used in endemic areas. It is suggested that *Strongyloides* infection may increase the risk of subsequent development of ATL. Therefore, in HTLV-1 carriers, although not yet demonstrated, prophylaxis of *Strongyloides* may reduce the risk of ATL development.[48-50] Hypercalcemia associated with aggressive ATL should be managed with treatment of the disease, hydration, and bisphosphonate therapy.[6,8]

RESPONSE CRITERIA

The complex presentation of ATL, often with both leukemic and lymphomatous components, makes response assessment difficult; however, response criteria are mandatory to ensure uniform interpretation of clinical trials (Table 2). Most current ATL trials use response criteria proposed by JCOG that have been applied since 1991.[6,36] At the international consensus meetings, a modification of the JCOG criteria was suggested, reflecting the criteria for CLL and NHL that had been published later (Table 2).[51,52] CR was defined as disappearance of all clinical, microscopic, and radiographic evidence of disease. Specific lymph node requirements include that all nodes must have regressed to normal size (≤ 1.5 cm in their greatest transverse diameter) and previously involved nodes that were 1.1 to 1.5 cm must have decreased to ≤ 1.0 cm.[51] Because HTLV-1 carriers frequently have a small percentage of abnormal lymphocytes with polylobated nuclei, so-called flower cells, in peripheral blood, provided that less than 5% of such cells remained, CR was judged to have been attained if the absolute lymphocyte count, including flower cells, was less than 4×10^9/L.[36,52] A designation of unconfirmed CR was adopted to include patients with a ≥ 75% reduction in tumor size but with a residual mass after treatment, as previously reported for NHL.[47] These patients must also have an absolute lymphocyte count, including flower cells, of less than 4×10^9/L. Partial response (PR) was defined as a ≥ 50% reduction in the sum of the products of the greatest diameters of measurable disease without the appearance of new lesions. In addition, PR was required to satisfy a 50% or greater reduction in absolute abnormal lymphocyte counts in peripheral blood. PD in peripheral blood was defined by a ≥ 50% increase from nadir in the count of flower cells and an absolute lymphocyte count, including flower cells, of ≥ 4×10^9/L. PD or relapsed disease in the other lesions was defined as a ≥ 50% increase from nadir in the sum of the products of measurable disease or the appearance of new lesions excluding skin. Stable disease

Table 2. Response Criteria for Adult T-Cell Leukemia-Lymphoma

Response	Definition	Lymph Nodes	Extranodal Masses	Spleen, Liver	Skin	Peripheral Blood	Bone Marrow
Complete remission*	Disappearance of all disease	Normal	Normal	Normal	Normal	Normal†	Normal
Uncertified complete remission*	Stable residual mass in bulky lesion	≥ 75% decrease‡	≥ 75% decrease‡	Normal	Normal	Normal†	Normal
Partial remission*	Regression of disease	≥ 50% decrease‡	≥ 50% decrease‡	No increase	≥ 50% decrease	≥ 50% decrease	Irrelevant
Stable disease*	Failure to attain complete/partial remission and no progressive disease	No change in size	No change in size	No change in size	No change in size	No change	No change
Relapsed disease or progressive disease	New or increased lesions	New or ≥ 50% increase§	New or ≥ 50% increase§	New or ≥ 50% increase	≥ 50% increase	New or ≥ 50% increase‖	Reappearance
Not assessable							

*Require each criterion to be present for a period of at least 4 weeks.
†Provided that < 5% of flower cells remained, complete remission was judged to have been attained if the absolute lymphocyte count, including flower cells, was $< 4 \times 10^9$/L.
‡Calculated by the sum of the products of the greatest diameters of measurable disease.
§Defined by ≥ 50% increase from nadir in the sum of the products of measurable disease.
‖Defined by ≥ 50% increase from nadir in the count of flower cells and an absolute lymphocyte count, including flower cells, of $> 4 \times 10^9$/L.

was defined as failure to attain CR/PR or PD. CR, unconfirmed CR, PR, and stable disease require each criterion for a period of at least 4 weeks.

Recently, revised response criteria were proposed for lymphoma. New guidelines were presented incorporating positron emission tomography (PET), especially for assessment of CR.[53] It is well known and described in the criteria that several kinds of lymphoma, including peripheral T-cell lymphomas, are variably [^{18}F]fluorodeoxyglucose avid.[53] No report described the PET results in response assessment of ATL until now. The usefulness of PET or PET/CT should be evaluated in response assessment of ATL in a prospective study. Meanwhile, PET or PET/CT should be used for evaluation of response when the tumorous lesions are fluorodeoxyglucose avid at diagnosis.

ISSUES FOR FUTURE INVESTIGATIONS IN ATL

Targeted Therapy

Several new agents against ATL are now under investigation. A promising targeted therapy for ATL is the combination of arsenic trioxide and IFN-α, which targets both Tax and the nuclear factor-κB pathway.[54-56] This combination exhibits clinical efficacy in relapsed/refractory ATL patients[57] and is currently being evaluated in untreated patients. Monoclonal antibodies against several molecules expressed on the surface of ATL cells and other lymphoid malignant cells, such as CD25, CD2, CD52, and chemokine receptor 4, have been promising in recent clinical trials. Histone deacetylase inhibitors such as vorinostat (suberoylanilide hydroxamic acid), romidepsin, and panobinostat (LBH589) have also been promising in preclinical and/or clinical studies against T-cell malignancies including ATL. Pralatrexate, a novel antifolate, and forodesine, a purine nucleotide phosphorylase inhibitor, are potential new agents with potent preclinical activity in T-cell malignancies including ATL. Other potential therapies for ATL under investigation include the combination of the proteasome inhibitor bortezomib with high-dose CHOP chemotherapy[58] and antiangiogenic therapy, such as anti–vascular endothelial growth factor monoclonal antibodies[59] or antitransferrin receptor.[60] Microarray analysis has identified survivin, β-catenin, syk, and lyn as potential targets for therapy.[61]

Prevention

Two steps should be considered for the prevention of HTLV-1–associated ATL. The first step is the prevention of HTLV-1 infection. This has been established in some HTLV-1 endemic areas in Japan by screening for HTLV-1 among blood donors and refraining from breast feeding among pregnant women who are carriers. The second step is the prevention of ATL development among HTLV-1 carriers. This has not been established partly because only approximately 5% of HTLV-1 carriers develop the disease in their lifetime and the risk factors remain unknown. Therefore, a cohort study of HTLV-1 carriers (Joint Study of Predisposing Factors for ATL Development) is ongoing nationwide in Japan.

Clinical Trials

Clinical trials have been paramount to the recent advances in ATL treatment, including assessment of chemotherapy, AZT/IFN-α, and alloHSCT, as described earlier. We have proposed a strategy for ATL treatment stratified by subclassification and prognostic factors. However, future clinical trials should be incorporated to ensure that the consensus is continually updated to establish evidence-based practice guidelines.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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Array Comparative Genomic Hybridization Analysis of PTCL-U Reveals a Distinct Subgroup with Genetic Alterations Similar to Lymphoma-Type Adult T-Cell Leukemia/Lymphoma

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Abstract

Purpose: Peripheral T-cell lymphoma, unspecified (PTCL-U) comprises histopathologically and clinically heterogeneous groups. The purpose of this study was to identify subgroups with distinct genetic, histopathologic, and prognostic features.

Experimental Design: We used array comparative genomic hybridization (CGH) for high-resolution analysis of 51 PTCL-U patients and the array data for examining possible correlations of histopathologic and clinical features. Moreover, we compared the genetic, histopathologic, and prognostic features of the PTCL-U cases with those of 59 cases of lymphoma-type adult T-cell leukemia/lymphoma (ATLL).

Results: We identified 32 regions with frequent genomic imbalance, 1 region with high copy number gain at 14q32.2, and 1 region with homozygous loss at 9p21.3. Gains of 7p and 7q and loss of 9p21.3 showed a significant association with poor prognosis. PTCL-U cases with genomic imbalance showed distinct histopathologic and prognostic features compared with such cases without alteration and a marked genetic, histopathologic, and prognostic resemblance to lymphoma-type ATLL.

Conclusions: The array CGH enabled us to identify the frequently altered genomic regions with strong prognostic power among PTCL-U cases. A correlative analysis using the array CGH data disclosed a subgroup in PTCL-U with genomic alterations and with histopathologic and clinical relevance. In addition to histopathologic similarity, the strong genetic and prognostic resemblance between PTCL-U cases with genomic imbalance detected by array CGH and lymphoma-type ATLL seems to support the notion that the former may constitute a distinct PTCL-U subgroup.

Peripheral T-cell lymphoma (PTCL), unspecified (U) is the most commonly encountered group among PTCLs in western countries (1–3). This category consists of the cases that do not belong to any of the recognized subtypes of PTCLs in the WHO classification (4). PTCL-U comprises histologically and clinically heterogeneous groups, which makes it difficult to assess conventional therapies accurately and to develop new therapeutic targets. The first priority is thus to identify the subgroups of PTCL-U. However, the molecular basis of their clinical heterogeneity is still poorly understood. Several reports of conventional cytogenetic studies including comparative genomic hybridization (CGH; refs. 5, 6) and array CGH (7) mentioned some recurrent aberrations but did not identify the genetic hallmarks for the categorization of distinct subgroups.

Adult T-cell leukemia/lymphoma (ATLL) is a human T-lymphotropic virus type I-mediated neoplasm and the most commonly occurring PTCL group in Japan (4, 8). Among its four clinical subtypes (9), the lymphoma type presents with prominent lymphadenopathy. The histomorphologic feature of lymphoma-type ATLL is pleomorphic nuclei of a size known to be very similar to that of a part of PTCL-U patients (10–12). Because these two disease entities cannot be satisfactorily classified by means of histology, the final diagnosis of lymphoma-type ATLL depends on the confirmation of the monoclonal integration of the human T-lymphotropic virus type I provirus into cellular DNA. It is well known that the prognosis of lymphoma-type ATLL and some PTCL-U patients is extremely poor. These findings suggest that some common mechanisms underlie the tumorigenesis of lymphoma-type ATLL and some PTCL-U patients, but no clear evidence has

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Translational Relevance

PTCL-U comprises histopathologically and clinically heterogeneous groups. Clarification of the subgroups in PTCL-U is therefore of great importance, but the molecular basis of the clinical heterogeneity is still poorly understood. Our group previously studied the genomic imbalance of several types of lymphomas by array CGH and provided evidences that DNA copy number changes could be used to distinguish disease entities and subtypes. In the present study, we analyzed 51 PTCL-U patients and detected a distinct subgroup in PTCL-U with potential pathologic and clinical relevance. Interestingly, the genome profile of this group was similar to that of lymphoma-type ATLL. These new findings should help improve our understanding of the clinical heterogeneity of PTCL-U and provide a molecular basis for establishing disease categories.

been presented. We previously used array CGH to identify some genomic imbalances in lymphoma-type ATLL and identified part of the molecular basis for the ATLL subtypes (13).

In the study reported here, we used array CGH to analyze copy number alterations in 51 PTCL-U in an attempt to identify subgroup(s). We then used the array data to examine correlations of histopathologic and clinical features. Moreover, we compared the genetic, histopathologic, and prognostic features of PTCL-U cases with those of 59 cases of lymphoma-type ATLL to determine whether they have any genetic features in common.

Patients, Materials, and Methods

Patients. We studied a total of 51 PTCL-U and 59 lymphoma-type ATLL cases, whose frozen tumor samples, clinical data, and follow-up data were available under a protocol approved by the Institutional Review Board of the Aichi Cancer Center. As for lymphoma-type ATLL, 49 of these cases were previously reported on by our group in an array CGH study (13) and 10 new ones were analyzed for this study, for which informed consent was obtained in accordance with the Declaration of Helsinki. The patients were selected from those hospitalized between 1985 and 2004 at Fukuoka University School of Medicine, University of the Ryukyus School of Medicine, and Imamura Bun-in Hospital and their affiliated hospitals. In accordance with WHO classification criteria, the clinical features, hematologic characteristics, histology, and immunophenotypes were reviewed by two expert hematopathologists (K.O. and S.N.). T-cell phenotype was diagnosed by the expression of one or more pan-T-cell antigens (CD45RO, CD2, CD3, and CD7) and T-cell-associated antigens (CD4 or CD8) and the absence of pan-B-cell antigens (CD19 and CD20). All PTCL-U cases enrolled in this study were serologically negative for human T-lymphotropic virus type I. For the diagnosis of ATLL, monoclonal integration of human T-lymphotropic virus type I proviral DNA into the cells was confirmed by means of Southern blotting analysis. The lymphoma-type ATLL is differentiated from the other clinical subtypes by prominent lymphadenopathy without peripheral blood involvement as reported previously by us (13). Cases of angioimmunoblastic T-cell lymphoma, anaplastic large cell lymphoma, enteropathy-associated T-cell lymphoma, nasal-type natural killer cell/T-cell lymphoma, and primary cutaneous T-cell lymphoma were excluded from this study. Any controversial cases were reassessed by the two pathologists until a consensus diagnosis was obtained. All samples were reviewed by an expert hematopathologist (K.O.) for their morphologic characteristics, including nuclear size, nuclear atypia, capillary proliferation, eosinophil/plasma cell proliferation, and lymphoepitheloid cell proliferation. Nuclear size was determined as small, medium, large, or pleiomorphic nuclei. The cases with very large cells with large nuclei were designated anaplastic nuclei. For immunohistochemical analysis, antibodies to CCR3, CCR4, and CXCR3 (Pharmingen) were used in paraffin-embedded samples. When a case showed >30% of tumor cells stained positively for CCR3, CCR4, or CXCR3, it was defined as positive. Clinical and biological information was obtained from clinical records and is summarized in Table 1. Clinical follow-up data were available for 51 PTCL-U and 37 lymphoma-type ATLL patients.

DNA samples. DNA samples were extracted from frozen tissues of biopsies or peripheral blood samples by using standard proteinase K/phenol-chloroform DNA isolation method. The biopsy tissues were obtained from lymph nodes of 46 PTCL-U and 59 lymphoma-type ATLL cases and from an extranodal areas in 4 PTCL-U patients (cases 14, 20, 42, and 50). The data for the biopsy site of 1 PTCL-U patient (case 44) were missing. Normal DNA was obtained from the blood of a healthy male donor.

Southern blot analysis. The rearrangements of TCR genes Cβ1, Cβ2, and Jγ were examined as reported previously (10). Bands were quantified using Adobe Photoshop (Adobe Systems) and ImageJ software.[6] The putative percentage of tumor cells was calculated by comparing densities obtained from a germ-line band and a rearrangement band. When it was difficult to judge whether a very faint increase in intensity was caused by a rearranged band or background noise, we designated all such cases as "germ-line band only" to avoid pseudo-positive results. In case 27, the discrepancy in the estimated percentage of tumor cells between pathologic and Southern blot findings might be due to the difference in the number of tumor cells between samples used for paraffin-embedded tissue and DNA preparation (Fig. 1).

Array CGH. Except for the array glasses, DNA preparation, labeling, array fabrication, hybridization, normalization, and analysis were done as described previously (13–18). The array CGH glasses used in our study were ACC version 4.0 [for the 49 cases of lymphoma-type ATLL, which were published previously by Oshiro et al. (13)] and version 5.0 (for the 51 cases of PTCL-U and 10 cases of lymphoma-type ATLL, which were newly analyzed in this study). About 5% of the BAC/PAC clones that produced unreliable data in our previous analyses were changed to version 5.0. The array consisted of 2,304 BAC/PAC clones spotted in duplicate. These clones came from libraries RP11 and RP13 for BAC clones and RP1 and RP3 to RP5 for PAC clones and were obtained from the BAC/PAC Resource Center at the Children's Hospital Oakland Research

[6] http://rsb.info.nih.gov/ij

Table 1. Clinical, biological, and histopathologic characteristics of PTCL-U patients

	Total, *n* (%)	Genomic imbalance (+), *n* (%)	Genomic imbalance (-), *n* (%)	*P**
Total	51	29	22	
Clinical and biological characteristics				
Age (y)				
≤60	20 (40)	7 (25)	13 (59)	0.0209
>60	30 (60)	21 (75)	9 (41)	
Sex				
Men	32 (63)	20 (69)	12 (55)	0.3835
Women	19 (37)	9 (31)	10 (45)	
Clinical stage				
I-II	15 (29)	8 (28)	7 (32)	0.7657
III-IV	36 (71)	21(72)	15 (68)	
Lactate dehydrogenase level				
Normal	24 (47)	12 (41)	12 (55)	0.405
Elevated	27 (53)	17 (59)	10 (45)	
Performance status				
0-1	14 (30)	8 (32)	6 (27)	0.76
2-4	33 (70)	17 (68)	16 (73)	
Extranodal involvement				
0-1	44 (86)	24 (83)	20 (91)	0.6841
>1	7 (14)	5 (17)	2 (9)	
Bone marrow involvement				
Involved	3 (7)	1 (4)	2 (10)	0.5772
Not involved	42 (93)	24 (96)	18 (90)	
International prognostic index				
Low	7 (15)	3 (13)	4 (18)	0.0237
Low intermediate	8 (17)	1 (4)	7 (32)	
High intermediate	23 (50)	13 (54)	10 (45)	
High	8 (17)	7 (29)	1 (5)	
Prognostic index for PTCL-U				
Low	15 (32)	5 (20)	10 (45)	0.0328
High	32 (68)	20 (80)	12 (55)	
Histopathologic characteristics				
Nuclear size				
Small	1 (2)	1 (3)	0 (0)	0.0001
Medium	17 (34)	3 (10)	14 (67)	
Large	4 (8)	1 (3)	3 (14)	
Anaplastic	7 (14)	5 (17)	2 (10)	
Pleomorphic	21 (42)	19 (66)	2 (10)	
Nuclear atypia				
Positive	28 (55)	23 (79)	5 (23)	0.0001
Negative	23 (45)	6 (21)	17 (77)	
Capillary proliferation				
Positive	17 (33)	8 (28)	9 (41)	0.3772
Negative	34 (67)	21 (72)	13 (59)	
Eosinophil/plasma cell proliferation				
Positive	9 (18)	4 (14)	5 (23)	0.474
Negative	42 (82)	25 (86)	17 (77)	
Lymphoepithelioid cell proliferation				
Positive	11 (22)	1 (3)	10 (45)	0.0004
Negative	40 (78)	28 (97)	12 (55)	
CCR3				
Positive	20 (0)	2 (8)	18 (82)	<0.0001
Negative	28 (100)	24 (92)	4 (18)	
CCR4				
Positive	17 (35)	16 (62)	1 (5)	<0.0001
Negative	31 (65)	10 (38)	21 (95)	
CXCR3				
Positive	22 (46)	8 (31)	14 (64)	0.041
Negative	26 (54)	18 (69)	8 (36)	

NOTE: Percentages were calculated based on the number of cases that could be evaluated.
*P values were calculated with the use of the χ^2 test or the Fisher's exact test.

Institute.[7] The names of the clones and their chromosome locations are listed in Supplementary Information 1. The BAC/PAC clones were aligned with each of the chromosomes based on data from Ensembl Genome Data Resources (release 40)[8] or the National Center for Biotechnology Information (Build 36.1).[9] The locations of all the clones to be used for

[7] http://bacpac.chori.org/

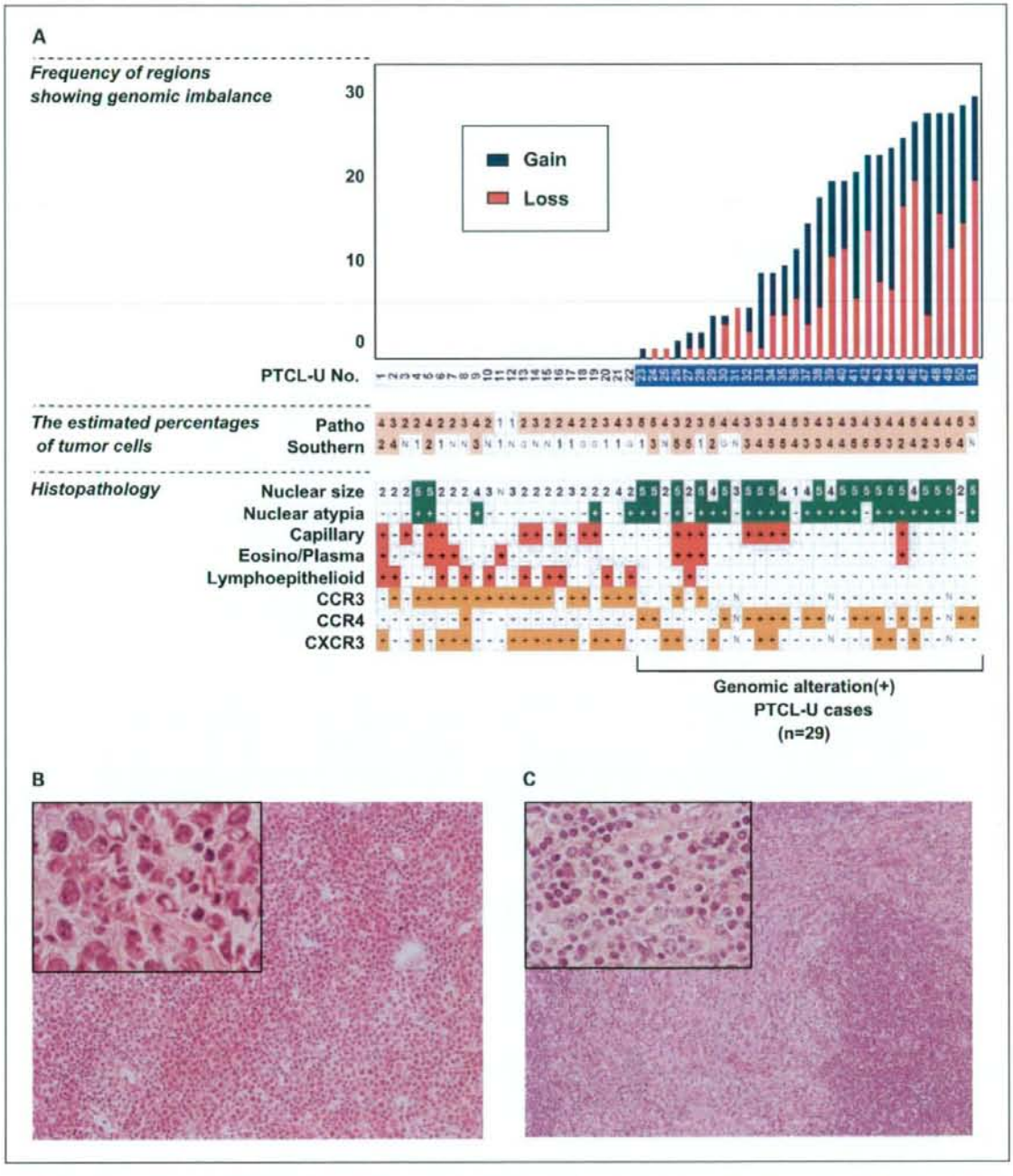

Fig. 1. Frequency of regions showing genomic imbalance and histopathologic characteristics of PTCL-U patients. *A, top,* frequencies of the regions with genomic imbalance (*Y axis*) for 51 individual cases in PTCL-U (*X axis*). *Green columns,* frequency of gain regions; *red columns,* frequency of loss regions. *Middle,* estimated percentages of tumor cells in individual cases. *Patho,* estimated percentage of tumor cells evaluated by an expert hematopathologist (K.O.); Southern, estimated percentage of tumor cells evaluated by Southern blot analysis. The estimated percentages of tumor cells were divided into five groups (pentiles): 1, 0% to 20%; 2, 20% to 40%; 3, 40% to 60%; 4, 60% to 80%; and 5, 80% to 100%. *G,* germ-line bands without rearrangement by Southern blot analysis; *N,* data not available. *Bottom,* patients' histopathologic characteristics; *left,* eight histopathologic variables. *Numbers,* nuclear size, with 1 to 5 indicating small, medium, large, anaplastic, and pleomorphic nuclei, respectively. For the remaining variables, +, positive; -, negative. Histomorphologic features of representative cases in PTCL-U (*B*) with genomic imbalance (case 38) and (*C*) those without (case 6; H&E staining; original magnification, ×100). *Insets,* high-power photos of cytomorphology (H&E staining; original magnification, ×400).

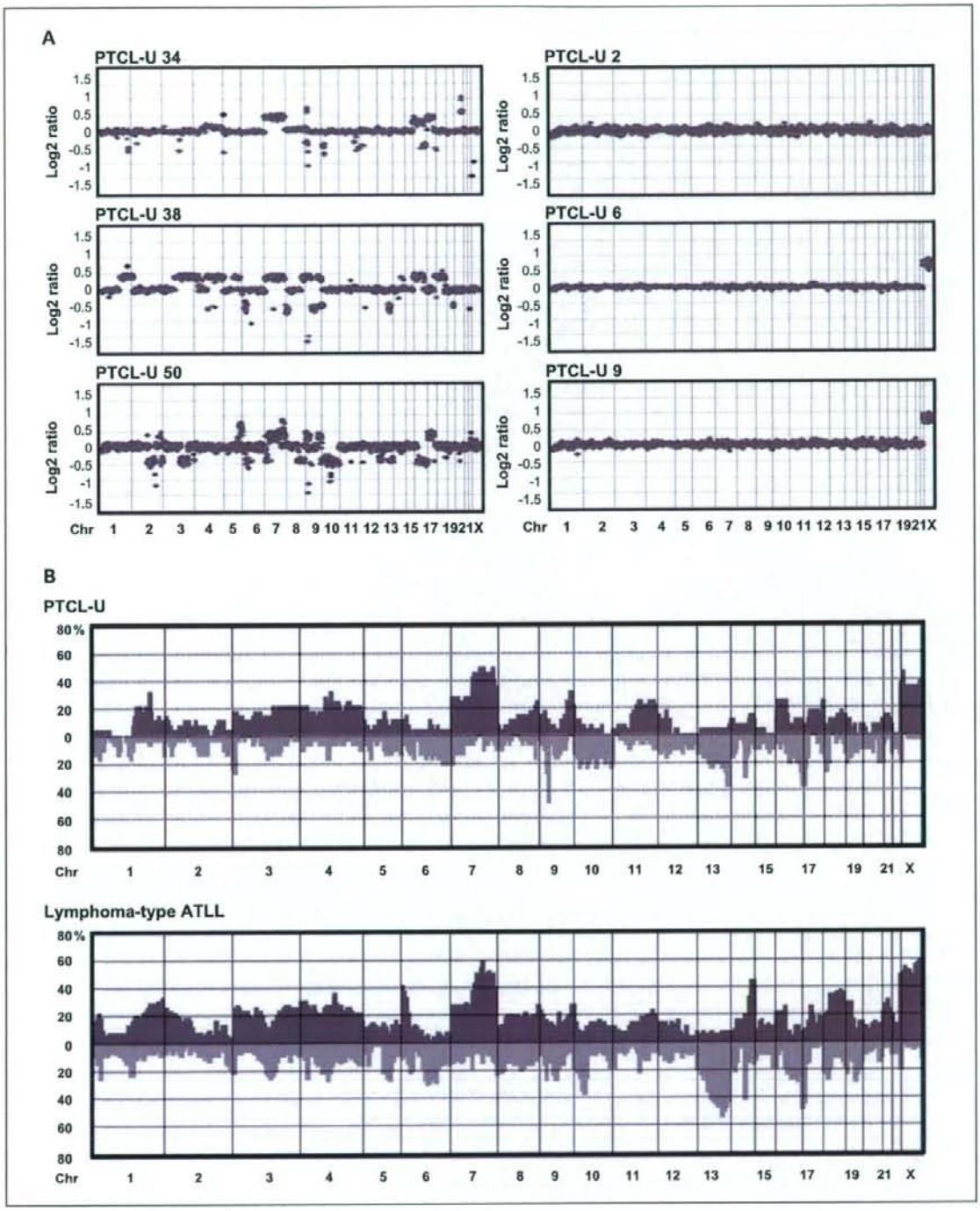

Fig. 2. Array CGH data of PTCL-U. *A*, representative whole-genome DNA copy number profiles of individual cases. *Left*, profiles were derived from three individual cases with genomic alteration; *right*, cases without genomic alteration. *B*, genome-wide frequency plots of copy number alterations identified in 29 PTCL-U (*top*) and 59 lymphoma-type ATLL (*bottom*) samples.

array CGH were confirmed by fluorescence *in situ* hybridization. Clones that showed unreliable data with 17 unrelated normal individuals were excluded from the data analysis, as were clones of the sex chromosomes. For the analysis including cases of lymphoma-type ATLL, the clones that were seen in both versions 4.0 and 5.0 were used. This resulted in a total of 2,138 clones (covering 2,867 and 1.34 Mb resolutions) being subjected to further analysis. The thresholds for gain and loss with 5% false discovery rate were +0.184 and -0.165 for PTCL-U and +0.153 and -0.129 for lymphoma-type ATLL (see Supplementary Information 2). We defined a region of gain or loss as (*a*) three contiguous clones showing gain ($\log_2$ ratio = +0.2 to +1.0) or loss ($\log_2$ ratio = -1.0 to -0.2) or (*b*) clones, if not contiguous, showing high copy number gain ($\log_2$ ratio > +1.0) or homozygous loss ($\log_2$ ratio < -1.0; ref. 14). When two regions were located at an interval of a single BAC showing normal $\log_2$ ratio, we considered the two regions to be one region. A frequent region was defined as a region seen in 5 cases for PTCL-U and 12 cases for lymphoma-type ATLL. Our array CGH could detect single copy amplifications in a sample with at least 20% tumor cells, which could be accurately determined by experiments mixing male and female DNA samples (18).

Statistical analysis. All data were analyzed with the Stat View 5.0 software package (SAS Institute). For statistical analysis, differences in frequency of the genetic, clinical, or pathomorphologic factors were evaluated by means of χ^2 analysis, Fisher's exact test, or Student's *t* test. Patient overall survival data were analyzed with the Kaplan-Meier method and compared by means of the log-rank test. The Cox proportional hazards regression model was used for all univariate and multivariate analyses. All reported *P* values are two-sided, and $P < 0.05$ was considered statistically significant.

Results

Frequency of regions of genomic alteration. With the array CGH, various DNA gains and losses were detected in 29 of the 51 PTCL-U samples (cases 23-51; total regions: average, 15.6; range, 1-31; gains: average, 8.0 regions; range, 0-24; losses: average, 7.6 regions; range, 0-21; Figs. 1A and 2A). In 22 cases (cases 1-22), no genomic alterations were found (Figs. 1A and 2A). Because our array CGH may have failed to detect genomic imbalance in samples with <20% of tumor cells (14, 18), we correlated the frequency of genomic alterations with tumor cell burden as evaluated by an expert hematopathologist (K.O.). In addition, we estimated the percentage of tumor cells from the bands produced by Southern blot analysis (Fig. 1A, *middle*). Of the 29 cases showing genomic imbalance, 23 (79.3%) contained >20% tumor cells. On the other hand, of the remaining 22 cases, 4 (18.2%) with >20% tumor cells did not have any genomic alterations (cases 1, 2, 5, and 9). These results indicate that the tumor burden in PTCL-U is heterogeneous. Most of the 22 cases without genomic imbalance did not have a tumor burden large enough to be detected by array CGH.

Frequently altered regions in PTCL-U. Frequencies of DNA gains and losses for all BAC clones are shown in Fig. 2B (*top*).

Table 2. Frequent regions of genomic imbalance in PTCL-U patients

Chromosome	Mb*	*n*	Overall survival, *P* (log-rank)
Gain †			
1q24.2	1.04	5	0.5641
1q25.1-25.2	2.07	5	0.7829
3q25.1-26.1 ‡	10.96	6	0.4865
3q26.2-27.1 ‡	13.83	6	0.4865
3q27.3-29 ‡	8.18	5	0.8829
4p16.2-15.2 ‡	22.06	6	0.1124
4q21.1-21.21 ‡	3.99	5	0.8629
4q21.23-28.1 ‡	38.42	7	0.6313
4q28.3-31.23 ‡	16.25	7	0.9391
4q32.1	2.14	5	0.51
4q32.3-35.1	18.74	6	0.9545
7p ‡	62.95	10	0.0045
7q ‡	93.93	16	0.0223
9q31.3-32	3.18	6	0.0183
9q33.2-34.2 ‡	11.18	6	0.0183
11q14.1	5.91	5	0.9946
11q14.3-tel	43.05	7	0.7588
16p13.2-12.2	16.65	7	0.5338
Loss †			
1p31.2-13.1	2.14	5	0.093
2q37.3	2.27	6	0.8978
6q14.3 ‡	0.05	5	0.8271
9p21.3 ‡	1.70	16	0.0146
10p14-13	5.96	5	0.9649
10p12.31-11.22 ‡	11.89	6	0.7708
10q11.21-21.1	10.37	6	0.3118
10q23.2-23.31	4.63	5	0.58
10q24.2-26.3	33.30	6	0.4637
13q14.11-14.2 ‡	4.05	5	0.9565
13q21.1-34 ‡	59.66	11	0.1853
16q12.1-21 ‡	7.90	5	0.7311
17p13.3-11.2 ‡	16.97	11	0.4375
18p11.31d-11.22b ‡	5.72	6	0.1902

*Affected regions are indicated by megabases.
† Copy number change of X chromosome was excluded.
‡ An asterisk indicates the altered region frequently shown in the cases of lymphoma-type ATLL.

In 29 cases showing genomic alteration, we detected 32 regions (>15%; 5 cases) with frequent genomic imbalance as listed in Table 2. Regions (>15%; 5 cases) of frequent gain were detected on 1q24.2, 1q25.1-25.2, 3q25-26.1, 3q26.2-27.1, 3q27.3-29, 4p16.2-15.2, 4q21.1-21.21, 4q21.23-28.1, 4q28.3-31.23, 4q32.1, 4q32.3-35.1, 7p, 7q, 9q31.3-32, 9q33.2-34.2, 11q14.1, 11q14.3-tel, and 16p13.2-12.2 and regions of frequent loss on 1p31.2-13.1, 2q37.3, 6q14.3, 9p21.3, 10p14-13, 10p12.31-11.22, 10q11.21-21.1, 10q23.2-23.31, 10q24.2-26.3, 13q14.11-14.2, 13q21.1-34, 16q12.1-21, 17p13.3-11.2, and 18p11.31d-11.22b (Fig. 2B; Table 2). Gains at 7p and 7q and loss at 9p21.3 were frequent and found to have strong prognostic power (Table 2).

Three consecutive BAC clones at 14q32.2 were identified as regions of recurrent (≥2 cases) high-level amplification (Table 3). RP11-179F4 (gain: 3 cases; high-level amplification: 2 cases) was found to contain *BCL11B*, which has been implicated in lymphoid malignancy (13, 19, 20). Three consecutive BAC clones at 9p21.3 were identified as regions of recurrent homozygous loss (Table 3). RP11-336O12 (loss: 5 cases;

[8] http://www.ensembl.org/
[9] http://www.ncbi.nlm.nih.gov

Table 3. List of BAC clones showing recurrent high copy number gain and homozygous loss in PTCL-U patients

Clone name	Cytogenetic position	Start (bp)	End (bp)	Gain/loss cases*	Amplification/ homozygous loss†	Gene‡
Gain						
RP11-1127D7	14q32.2	9832135	9848315	3	2	—
RP11-179F4	14q32.2	9867021	9882729	3	2	*BCL11B*
RP11-450C22	14q32.2	9896938	9916093	4	2	*SETD3, CCNK*
Loss						
RP11-336O12	9p21.3	2032100	2047878	5	2	*MLLT3*
RP11-380P16	9p21.3	2120977	2133626	8	3	*IFNA17, IFNA5, KLHL9*
RP11-149I2	9p21.3	2189825	2200141	14	4	*MTAP, CDKN2A, CDKN2B*

*Number of patients with gain/loss. Gain was defined as $\log_2$ ratio = +0.2 to +1.0 and loss as $\log_2$ ratio = -1.0 to -0.2.
†Number of patients with amplification/homozygous loss. Amplification was defined as $\log_2$ ratio > +1.0 and homozygous loss as $\log_2$ ratio < -1.0.
‡All of the genes located on the BAC/PAC clones.

homozygous loss: 2 cases) was found to contain *MLLT3/AF9*, which has been associated with acute leukemia (21). RP11-149I2, where the *CDKN2A/p16* and *CDKN2B/p15* tumor suppressor genes are located, was the most frequently detected region of loss (14 cases including four with homozygous loss).

Histopathologic characteristics. We investigated the histomorphologic features of 29 cases with genomic imbalance. In terms of nuclear size, many cases (19 of 29; 65.5%) showed pleomorphology in their nuclei (Fig. 1A, *bottom*, and B; Table 1). Most cases (23 of 29; 79.3%) featured nuclear atypia (Fig. 1A, *bottom*, and B; Table 1). The proliferation of lymphoepithelioid cell was infrequent (1 of 29 cases; 3.4%) (Fig. 1A, *bottom*; Table 1). We noticed that the PTCL-U cases with genomic imbalance tended to show homogeneous morphologic features, whereas many previous studies have reported that PTCL-U cases featured morphologically heterogeneous diseases (1–4). This prompted us to further investigate the histomorphologic features of the remaining 22 PTCL-U cases without genomic imbalance.

PTCL-U cases without genomic imbalance showed histomorphologic features distinct from those with genomic imbalance (Fig. 1A, *bottom*, and C; Table 1). In terms of nuclear size, cases without genomic imbalance featured significantly more cases with medium size nuclei: 3 of 29 (10.3%) versus 14 of 21 (66.7%) cases ($P < 0.0001$; Fig. 1A, *bottom*, and C; Table 1). Lymphoepithelioid cell proliferation was detected significantly more frequently in cases without genomic imbalance than in those with such imbalance: 1 of 29 cases (3.4%) versus 10 of 22 (45.5%) cases ($P = 0.0004$; Fig. 1A, *bottom*, and C; Table 1).

In addition, the two groups showed highly significant differences in terms of prognostic importance, because cases with genomic imbalance were found to be significantly associated with shorter overall survival time ($P = 0.0042$; Fig. 3).

Univariate analysis also revealed that the presence of genomic imbalance was an unfavorable prognostic factor ($P = 0.006$; hazard ratio, 2.97; confidence interval, 1.36867-6.46343; Supplementary Table S1), whereas multivariate analysis failed to show independence of its prognostic power (Supplementary Table S1). International prognostic index, nuclear atypia, and CCR3 were shown as unfavorable prognostic factors by univariate analysis (international prognostic index: $P = 0.021$, hazard ratio = 3.12, confidence interval = 1.19-8.23; nuclear atypia: $P = 0.0028$, hazard ratio = 3.44, confidence interval = 1.54-7.78; CCR3: $P = 0.026$, hazard ratio = 2.49, confidence interval = 1.11-5.55; Supplementary Table S1).

The chemokine receptors CCR3, CCR4, and CXCR3 were recently mentioned as prognostic factors in PTCL-U (11, 12). We therefore conducted a correlative analysis of our PTCL-U groups with the expressions of CCR3, CCR4, and CXCR3 (Fig. 1A, *bottom*; Table 1). Cases with genomic imbalance frequently showed statistically significant expression of CCR4: 16 of 26 (61.5%) versus 1 of 22 (4.5%) cases ($P < 0.0001$;

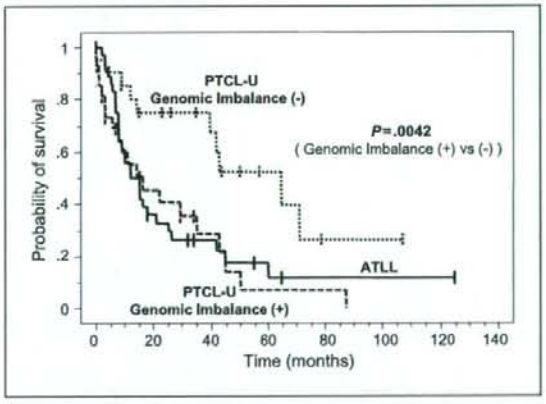

Fig. 3. Kaplan-Meier overall survival curves for PTCL-U with and without genomic imbalance and for lymphoma-type ATLL. The log-rank test was used for comparative significance testing of PTCL-U cases with and without genomic imbalance. Follow-up data were available for 29 cases in PTCL-U with genomic imbalance, 22 in PTCL-U without, and 37 in lymphoma-type ATLL. The median overall survival from the time of diagnosis was 15 mo for PTCL-U (range, 0-108 mo) and 17 mo for lymphoma-type ATLL (range, 0-125 mo). The information of initial therapy regimen was available for 14 cases of PTCL-U with and 12 cases of PTCL-U without genomic imbalance as well as for 24 cases of lymphoma-type ATLL. In addition, most cases were treated with anthracycline-based chemotherapy (10 of 14 of PTCL-U cases with and 11 of 12 of those without genomic imbalance as well as 22 of 24 of ATLL cases). PTCL-U genomic imbalance (+), PTCL-U with genomic imbalance; PTCL-U genomic imbalance (-), PTCL-U without genomic imbalance.

Fig. 1A, *bottom*; Table 1). Cases without genomic imbalance, on the other hand, frequently showed the statistically significant expression of CCR3 and CXCR3: CCR3, 2 of 26 (7.7%) versus 18 of 22 (81.8%) cases ($P < 0.0001$) and CXCR3, 8 of 26 (30.8%) versus 14 of 22 (63.6%) cases ($P = 0.041$; Fig. 1A, *bottom*; Table 1).

Comparison of genomic profiles of PTCL-U and lymphoma-type ATLL. Most of PTCL-U cases with genomic imbalance showed pleomorphic nuclei and the expression of CCR4 (Fig. 1A, *bottom*, and B; Table 1), whereas the presence of pleomorphic nuclei and the expression of CCR4 was the distinguishing histomorphologic feature of lymphoma-type ATLL. We also compared the genomic profile of PTCL-U with that of the 59 lymphoma-type ATLL cases and found that they were very similar to each other (Fig. 2B). Seventeen of the 32 regions of frequent genomic imbalance in PTCL-U were also found in lymphoma-type ATLL (53.1%; Table 2). Interestingly, the overall survival curve of PTCL-U cases with genomic imbalance was also similar to that of lymphoma-type ATLL (Fig. 3).

Discussion

The high-resolution analysis using array CGH employed in this study identified 32 regions of frequent (>5 cases) genomic imbalance in PTCL-U. Twenty-five of these 32 regions (78.1%) were consistent with those reported in previous studies (5–7). In addition, we were able to identify 7 regions (4p16.2-15.2 gain, 7p gain, 2q37.3 loss, 10p14-13 loss, 10p12.31-11.22 loss, 16q12.1-21 loss, and 18p11.31d-11.22b loss) not reported previously. This study also disclosed the poor prognosis for gains of 7p and 7q and loss of 9p21.3 in PTCL-U patients. These regions were most frequently detected, which suggests that these genomic alterations are deeply involved in the tumorigenesis of PTCL-U.

Three consecutive BAC clones with high-level amplification were detected at 14q32.2 and those with homozygous loss at 9p21.3. Two cases showed high-level amplification on RP11-179F4 at 14q32.2 where *BCL11B* was located. We reported previously that lymphoma-type ATLL cases also showed high-level amplification at 14q32.2, although *BCL11B* was not highly expressed (13). The expression level in PTCL-U cases would need to be investigated in future study. Loss at RP11-149I2 (9p21.3) showed the highest frequency (14 cases including four with homozygous loss), indicating that the responsible gene is located in the homozygous loss region at 9p21.3. It has been reported that the *CDKN2A/p16* and *CDKN2B/p15* tumor suppressor genes located on RP11-149I2 have important roles in the tumorigenesis of other types of lymphoma including ATLL (17, 22), which suggests that *CDKN2A/p16* and *CDKN2B/p15* are likely candidates as the responsible genes.

The two groups, one with and the other without genomic imbalance, showed good correlation of their histopathologic and clinical features. Previous studies have identified several groups in PTCL-U by using gene expression profiling, chemokine receptors, and cytotoxic molecules (11, 12, 23–27). However, the genetic background of these groups is still poorly understood. In this context, it should be noted that detailed analysis by array CGH enabled us to elucidate the genetic features of the group with genomic imbalance. Zettl et al. studied genomic alterations of PTCL-U cases in western countries by using conventional CGH and reported that PTCL-U with any loss of chromosome 5q, 10q, and 12q correlated with good prognosis and the immunophenotype (6). No such correlation was found in our study possibly because of genetic heterogeneity due to differences in geographic regions and in racial predisposition. In fact, losses at 5q or 12q were only infrequently detected in our study.

Most of the PTCL-U cases with genomic imbalance histomorphologically showed pleomorphic nuclei and nuclear atypia. These findings were very similar to those for lymphoma-type ATLL, making a histomorphologic diagnosis difficult. The expression of CCR4, which is a chemokine receptor and closely associated with lymphoma-type ATLL (28), was a distinctive feature of the PTCL-U cases with genomic imbalance. Moreover, lymphoma-type ATLL and PTCL-U cases with genomic imbalance showed similar overall survival curves. It is of special interest that PTCL-U cases with genomic imbalance share many regions of genomic alteration with lymphoma-type ATLL. Ours is the first report to show correlation between PTCL-U and lymphoma-type ATLL in terms of not only clinical features and histopathology but also genetics. This strong correlation between PTCL-U cases with genomic imbalance detected by array CGH and lymphoma-type ATLL would support the notion that the former are distinct from PTCL-U cases without genomic imbalance. The significant prognostic difference between PTCL-U cases with and without genomic imbalance also underscores the distinct features of the former.

PTCL-U cases without genomic imbalance comprised cases with a small tumor burden and/or those with no copy number alterations in their tumor DNA. We cannot deny that the percentage of the former may be much higher, but at present it is difficult to accurately determine whether this is indeed the case. Thus, although the genetic background of PTCL-U cases without genomic imbalance thus remains to be clarified, it is important to note that the similarity of the genome profiles of the cases with genomic alteration and the lymphoma-type ATLL was clearly shown in this study. The incidence of proliferation of lymphoepithelioid cells was significantly higher in PTCL-U cases without than in those with genomic imbalance. PTCL-U cases in which genomic imbalance was not detected by array CGH and the cases designated as lymphoepithelioid cell variant in accordance with the WHO classification (4) share several features including the presence of lymphoepithelioid lesions with a small tumor burden and relatively good prognosis.

PTCL-U cases with and without genomic imbalance may be categorized as high-grade and low-grade T-cell lymphomas, respectively, according to the updated Kiel classification (29). This classification includes several morphologic subtypes, which the REAL/WHO classification collectively refers to as PTCL-U because of the lack of clear evidence of distinctiveness in clinicopathologic features and diagnostic reproducibility (4, 30). In our study, however, PTCL-U cases with and without genomic imbalance clearly correlated not only with the morphology and prognosis but also with the expression of chemokine receptors. Moreover, the strong genetic, pathologic, and prognostic resemblance between PTCL-U cases with genomic imbalance detected by array CGH and lymphoma-type ATLL highlights the distinctive features of the former. Our findings may thus provide a new insight into the classification of PTCL-U.

In conclusion, the use of array CGH enabled us to identify the frequently altered genomic regions with strong prognostic power. A correlative analysis using the array CGH data disclosed a subgroup in PTCL-U, featuring genomic alterations, which showed histopathologic and clinical relevance. The histopathologic characteristics of PTCL-U cases with genomic imbalance were very similar to those of lymphoma-type ATLL. In addition to histopathology, strong genetic and prognostic resemblance between PTCL-U cases with genomic imbalance and lymphoma-type ATLL were disclosed. Comparison of the PTCL-U subgroup and lymphoma-type ATLL in terms of their genetic and prognostic characteristics may help identify their shared mechanism of lymphomagenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Dr. Shinobu Tsuzuki for discussions and encouragement throughout this study and Hiroko Suzuki and Yumiko Kasugai for outstanding technical assistance.

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original article

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The International Prognostic Index predicts outcome in aggressive adult T-cell leukemia/lymphoma: analysis of 126 patients from the International Peripheral T-cell Lymphoma Project

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Background: The International Peripheral T-cell Lymphoma Project was organized to better understand the T-cell and natural killer (NK) cell lymphomas, and our task is to present the clinicopathologic correlations and therapeutic results for adult T-cell leukemia/lymphoma (ATL).

Patients and methods: Among 1153 patients with T-cell or NK cell lymphomas, 126 patients (9.6%) with ATL were represented in this project. All were categorized as aggressive ATL, i.e. acute or lymphoma type, and 87% fell into the lymphoma type.

Results: The median age was 62 years and the male to female ratio was 1.2 : 1. Significant prognostic factors for overall survival (OS) by univariate analysis were the presence of B symptoms ($P = 0.018$), platelet count $<150 \times 10^9$/l ($P = 0.065$), and the International Prognostic Index (IPI; $P = 0.019$). However, multivariate analysis indicated that only the IPI was an independent predictor of OS. Combination chemotherapy including anthracyclines was given as the initial therapy in 109 of the 116 patients (94%) who received treatment, and the overall and complete response rates were 70% and 34%, respectively. However, there was no survival benefit for those receiving an anthracycline-containing regimen.

Conclusion: Patients with aggressive ATL have a poor clinical outcome and the IPI is a useful model for predicting outcome in ATL of the lymphoma type.

Key words: ATL, leukemia, lymphoma, T-cell, prognostic index, international

introduction

Adult T-cell leukemia/lymphoma (ATL) is a peripheral T-cell malignancy caused by a retrovirus, human T-cell lymphotropic virus type I (HTLV-1) [1, 2], and is now regarded as a tumor derived from regulatory T cells which express FoxP3 [3, 4]. ATL is diagnosed based on its characteristic clinicopathologic features and the presence of integrated HTLV-1 provirus in the DNA of the tumor cells. ATL has characteristic cytological features with atypical 'flower cells' in the peripheral blood and pleomorphic lymphoma cells in tissue sections [1, 2, 5] and can be divided into four clinical subtypes, i.e. smoldering, chronic, acute, and lymphoma types. The acute type is the most common variant, presenting with disseminated disease and having a highly aggressive clinical course. In contrast, the smoldering type has an indolent clinical course with only a small percentage of leukemic cells and occasional skin involvement. The chronic type also has an indolent clinical course, but with a higher percentage of leukemic cells, slowly progressive skin disease, mild lymphadenopathy, and hepatosplenomegaly. The lymphoma type usually presents with disseminated disease including prominent lymph node enlargement, but with few leukemic cells [1, 2, 5]. The aggressive forms of ATL, including the acute and lymphoma types, are usually treated with combination chemotherapy, but the prognosis is poor with a median survival of <1 year

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compared with other forms of peripheral T-cell lymphoma (PTCL) [6–9].

In the past decade, there have been significant advances in our understanding of the biology of malignant lymphoma and some progress in treatment as well. However, our understanding of PTCL in general is far behind that of the B-cell lymphomas. Therefore, the International Peripheral T-cell Lymphoma Project was organized to assess the clinical applicability and reproducibility of the World Health Organization classification of peripheral T-cell and natural killer (NK) cell lymphomas, as well as to evaluate therapeutic outcomes and identify prognostic factors [10]. This report describes the clinicopathologic correlations and therapeutic results for patients with ATL from the International Peripheral T-cell Lymphoma Project.

patients and methods

We collected previously untreated patients with *de novo* peripheral T-cell or NK/T-cell lymphoma, excluding mycosis fungoides and Sézary syndrome, who were diagnosed from 1 January 1990 to 31 December 2002, in 22 centers in 13 countries around the world (Appendix 1) [10]. The diagnosis of ATL was based on histologic features and the presence of either positive HTLV-1 serology or monoclonal integration of HTLV-1 provirus [2, 9]. All cases were reviewed by four expert hematopathologists, and a consensus diagnosis was made by the agreement of three or four experts. We collected clinical data and laboratory findings including HTLV-1 serology, leukocyte count, and absolute lymphocyte count, as well as initial treatment and subsequent therapy. Treatment outcome was determined by overall survival (OS) and failure-free survival (FFS). OS was defined as the time from diagnosis to death from any cause, with surviving patient follow-up being censored at the last contact date. FFS was defined as the time from diagnosis to first progression, relapse after response, or death from any cause. Follow-up of patients not experiencing any of these events was censored at the date of last contact. OS and FFS were calculated by the method of Kaplan and Meier, and time to event distributions were compared using the log-rank test. Comparisons of clinical and prognostic factors were carried out using the chi-square or Fisher's exact test. Multivariate analysis was carried out with a Cox hazards regression model using stepwise selection.

results

In this project, 1314 cases were collected from North America, Europe, and Asia, and a diagnosis of PTCL or NK/T-cell lymphoma was confirmed in 1153 cases. ATL was diagnosed in 126 patients (9.6%) and was rare in North America (2.0%) and Europe (1.0%), but frequent in Asia (25%) among all PTCL patients. All the Asian cases were from Japan.

There are four clinical subtypes of ATL, i.e. smoldering, chronic, acute, and lymphoma types, in Shimoyama's classification [5]. In this study, smoldering and chronic ATL were excluded. The lymphoma type of ATL is defined by a lymphocyte count of <4000/μl in Shimoyama's classification [5]. Thus, 104 patients (87%) were classified as the lymphoma type and the rest as acute type (13%).

The clinical characteristics of the 126 ATL patients are shown in Table 1. There were 69 males and 57 females, with a median age of 62 years. Major signs and symptoms included lymphadenopathy (77%), fatigue (32%), anorexia (26%), skin eruption (23%), abdominal pain (23%), splenomegaly (13%), and hepatomegaly (10%). Overall, 90% of the patients had advanced stage disease by the Ann Arbor classification, 31% had B symptoms, and 23% were nonambulatory. Bone marrow infiltration and two or more sites of extranodal involvement were seen in 28% and 34% of the cases, respectively. In addition, 20 patients (17%) had a platelet count of $<150 \times 10^9$/l. We were able to evaluate 124 patients according to the International Prognostic Index (IPI) [11] and only 18.5% were in the good prognosis category (IPI = 0/1).

Chemotherapy was given to 116 patients, and combination chemotherapy including an anthracycline was given as an initial therapy to 109 patients (94.0%). Autologous or allogeneic hematopoietic stem-cell transplantation (HSCT) was carried out in 17 patients, including 10 patients as initial therapy and

Table 1. Clinical characteristics of 126 patients with aggressive ATL

Age (years)		
Median (range)	62	(20–92)
Sex		
Male/female	69/57	1.2 : 1
Stage		
I/II	12	9.6%
III	22	17.6%
IV	91	72.8%
B symptoms		
No	87	69.0%
Yes	39	31.0%
Performance status		
Ambulatory	97	77.0%
Nonambulatory	29	23.0%
Largest mass		
<5 cm	74	65.5%
≥5 cm	39	34.5%
Bone marrow involvement		
No	87	71.9%
Yes	34	28.1%
Nodal/extranodal disease		
Nodal only	37	31.4%
Nodal and extranodal	72	61.0%
Extranodal only	9	7.6%
Extranodal sites		
0–1	83	65.9%
≥2	43	34.1%
Serum LDH		
≤Normal	74	59.7%
>Normal	50	40.3%
Absolute lymphocyte count		
<4000/μl	104	86.7%
≥4000/μl	16	13.3%
IPI scores		
0/1	23	18.5%
2	41	33.1%
3	40	32.3%
4/5	20	16.1%

ATL, adult T-cell leukemia/lymphoma; LDH, lactate dehydrogenase; IPI, International Prognostic Index.

seven in first relapse. The response to initial therapy is shown in Table 2. The overall and complete response rates were 70% and 34%, respectively. However, majority of the patients (82%) have died, mostly from lymphoma or the complications of therapy. Only 5% of the patients were in complete remission at the time of death, and the median FFS and OS were only 0.6 and 0.8 years, respectively (Figure 1).

Table 3 shows the significant prognostic factors by the univariate analysis. Adverse prognostic factors for OS were the presence of B symptoms ($P = 0.018$), a platelet count $<150 \times 10^9$/l ($P = 0.065$), and a high (≥3) IPI score ($P = 0.019$; Figure 2A). Unexpectedly, bone marrow involvement, an elevated absolute lymphocyte count (≥4000/μl; Figure 3), elevated serum lactate dehydrogenase (LDH), hypercalcemia, and combination chemotherapy without an anthracycline (Figure 4) had no influence on OS. The IPI score was the only significant predictor of survival in multivariate analysis. Figure 5 shows OS according to the IPI in the lymphoma type of ATL (lymphocytes < 4000/μl). The IPI predicted for OS in the lymphoma type of ATL ($P = 0.04$), but not in the acute type (not shown; $P = 0.24$). Based on these results, the IPI is a useful model for predicting outcome in ATL of the lymphoma type.

Table 2. Response to initial therapy and clinical course in aggressive ATL

Treatment response	CR	32 (28%)	
	CRu	7 (6%)	CR + PR 81 (70%)
	PR	42 (36%)	
	NR	35 (30%)	
Recurrence of disease			71 (88%)
Alive 23 (18%)/dead 103 (82%)			
Causes of death	Lymphoma		74 (75%)
	Toxicity		10 (10%)
	Infection		2 (2%)
	Myelodysplasia		1 (1%)
	Other		8 (8%)
	Unknown		8 (8%)
Remission at death			5 (5%)

ATL, adult T-cell leukemia/lymphoma; CR, complete response; CRu, complete response unconfirmed; PR, partial response; NR, no response.

discussion

The International Peripheral T-cell Lymphoma Project was organized to better understand the T-cell and NK cell lymphomas [10]. ATL is endemic in southwest Japan, the Caribbean Islands, countries surrounding the Caribbean Basin, and parts of Central Africa and South America [12]. In the present study, one half of the patients were registered from Fukuoka, located in southwest Japan, and 30% from the rest of Japan, but none from the rest of Asia. Also, 20% of the patients were registered from Europe or North America including two patients from the Vancouver site and highlighting a previously unknown HTLV-1 endemic region. Both of these patients were indigenous American Indians [13].

The present study reveals that the IPI has predictive value for patients with aggressive ATL, particularly those with the lymphoma type. A previous nationwide study in Japan revealed five adverse prognostic factors for ATL by multivariate analysis: (i) age over 40 years; (ii) low performance score; (iii) hypercalcemia; (iv) elevated serum LDH level; and (v) the number of lesions [14]. These prognostic factors are similar to those in the IPI. This previous study in Japan reported on 818 ATL patients diagnosed from 1984 to 1987 [5, 14], with 56.5% having the acute type, 19.1% the lymphoma type, 18.6% the chronic type, and 5.5% with the smoldering type of ATL. Thus, the acute type is the most common subtype of ATL in Japan [5, 7, 8, 14]. However, in the present study, 87% of the patients had the lymphoma type and the rest had the acute type. The IPI has been shown to predict outcome in patients with PTCL [15], but cases of ATL were not included in that study. Although the IPI was developed for patients with aggressive B-cell lymphoma treated with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) or CHOP-like regimens [11], our study shows that the IPI is also useful for predicting outcome in aggressive ATL, especially the lymphoma type.

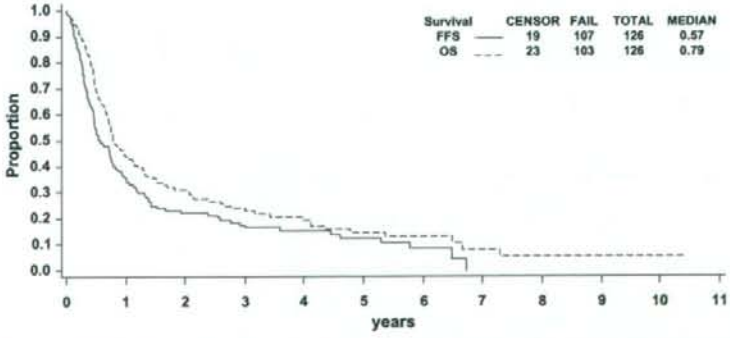

Figure 1. Overall survival (OS) and failure-free survival (FFS) of 124 patients with the aggressive adult T-cell leukemia/lymphoma.

The frequency of bone marrow involvement was only 28% in the present study, but was 85% in the previous Japanese report [14]. This difference is most likely due to the differences in ATL subtypes in the two studies. Although few reports have described the frequency of bone marrow involvement in the various subtypes of ATL, Kinoshita [16] reported that 60 of 65 patients (92.3%) with the acute type, but only 7 of 40 patients (17.5%) with the lymphoma type, had bone marrow involvement. Recently, Takasaki et al. [17] reported that visceral organ involvement, including the bone marrow, was a prognostic factor in ATL. Thrombocytopenia ($<100 \times 10^9$/l) and monocytosis ($\geq 0.8 \times 10^9$/l) were also found to be significant adverse prognostic factors by multivariate analysis. In contrast, we could identify no significant prognostic factors other than the IPI by multivariate analysis. However, Takasaki et al. [17] analyzed 168 ATL patients consisting of 75% with the acute type, 9% with the lymphoma type, 15% with the chronic type, and 4% with the smoldering type. Therefore, the proportions of the various subtypes in that paper were quite different from those in the present study, making meaningful comparisons difficult. Furthermore, in a recent report, the acute and the lymphoma types of ATL were found to be genomically different [18]. Thus, future studies of ATL should include separate analyses of prognostic factors for each of the ATL subtypes.

Table 3. Prognostic factors in aggressive ATL by univariate analysis

Factors	*n*	Median OS (years)	
B symptoms			
No	87	1.12	$P = 0.018$
Yes	39	0.66	
Platelet count ($\times 10^9$/l)			
≥150	101	0.85	$P = 0.065$
<150	20	0.67	
IPI score			
0/1	23	2.07	$P = 0.019$
2	41	1.32	
3	40	0.71	
4/5	20	0.73	

ATL, adult T-cell leukemia/lymphoma; OS, overall survival; IPI, International Prognostic Index

The clinical outcome in our series of aggressive ATL was extremely poor, with a median OS of only 0.8 years. Combination chemotherapy including an anthracycline was given to most of our patients, but did not improve the survival significantly. These data confirm that CHOP and CHOP-like regimens are not effective in aggressive ATL [19, 20]. Tsukasaki et al. [21] recently reported the results of a phase III trial (JCOG 9801) comparing the safety and efficacy of vincristine, cyclophosphamide, doxorubicin, and prednisone (VCAP); doxorubicin, ranimustine, and prednisone (AMP); and vindesine, etoposide, carboplatin, and prednisone (VECP) (VCAP–AMP–VECP) versus biweekly CHOP in the aggressive types of ATL. The 3-year OS of those receiving VCAP–AMP–VECP therapy was only 24%, which was similar to our study. Allogeneic HSCT for ATL appears to be effective in some patients [22, 23], but there are inherent problems including

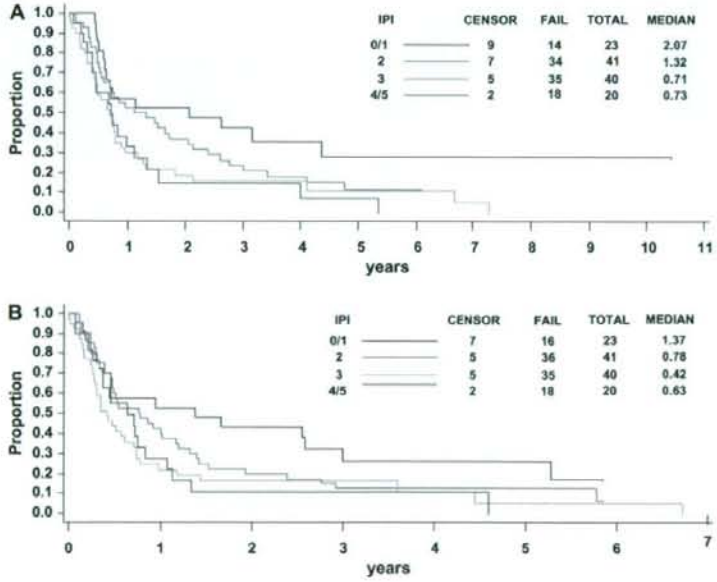

Figure 2. Overall survival (A) and failure-free survival (B) of patients with aggressive adult T-cell leukemia/lymphoma according to the International Prognostic Index (A, $P = 0.019$; B, $P = 0.14$).

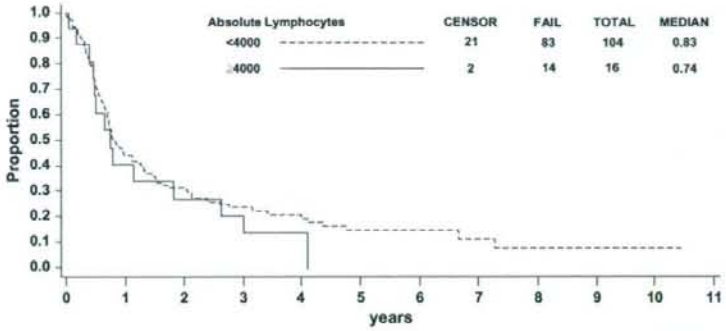

Figure 3. Overall survival of patients with aggressive adult T-cell leukemia/lymphoma according to the absolute lymphocyte count ($P = 0.44$).

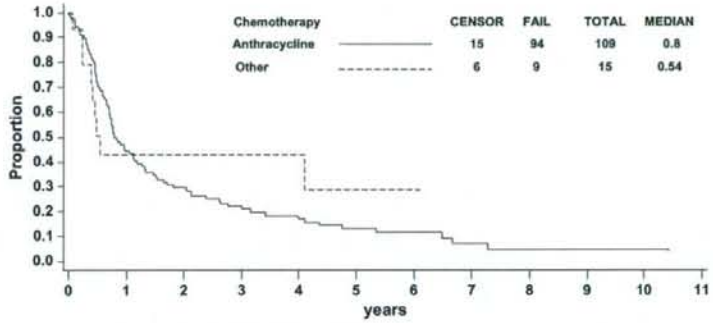

Figure 4. Overall survival of patients with aggressive adult T-cell leukemia/lymphoma according to treatment with or without an anthracycline ($P = 0.63$).

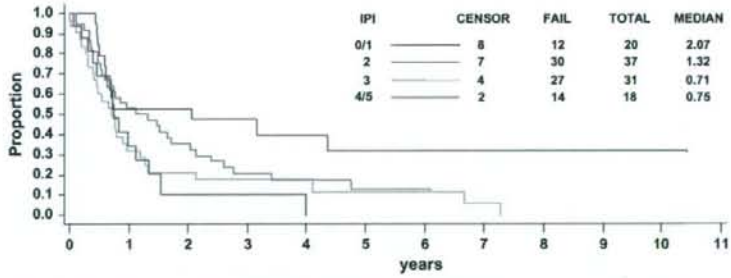

Figure 5. Overall survival of patients with the lymphoma type of adult T-cell leukemia/lymphoma according to the International Prognostic Index ($P = 0.04$).

high transplantation-related mortality, the use of HTLV-1-seropositive donors, and, most importantly, the proper selection of patients who will benefit from this therapy.

Unfortunately, we found no clues that might improve the treatment of ATL from this study. Nonetheless, further international collaboration using novel strategies is necessary to develop better therapies for this difficult disease. This study from the International Peripheral T-cell Lymphoma Project is the first report describing the results of an international cooperative study of ATL and thus represents an important first step in the international effort to improve the treatment and outcome of patients with ATL and other forms of PTCL.

Appendix 1 Participating sites and physicians

British Columbia Cancer Agency	Vancouver, Canada	Kerry Savage, MD; Joseph Connors, MD; Randy Gascoyne, MD; Mukesh Chhanabhai, MD
National Cancer Institute	Bethesda, MD	Wyndham Wilson, MD; Elaine Jaffe, MD
University of Nebraska Medical Center	Omaha, NE	James Armitage, MD; Julie Vose, MD; Dennis Weisenburger, MD; James Anderson, PhD; Fred Ullrich, MS; Martin Bast, BS
Massachusetts General Hospital	Boston, MA	Ephraim Hochberg, MD; Nancy Harris, MD
Los Angeles County Hospital, University of Southern California	Los Angeles, CA	Alexandra Levine, MD; Bharat Nathwani, MD
Arizona Cancer Center	Tucson, AZ	Thomas Miller, MD; Lisa Rimsza, MD
University of Barcelona Hospital	Barcelona, Spain	Emili Montserrat, MD; Armando Lopez-Guillermo, MD; Elias Campo, MD
Spanish National Cancer Center	Madrid, Spain	Marta Cuadros, MD; Javier Alvarez Ferreira, MD; Beatriz Martinez Delgado, MD
Norwegian Radium Hospital	Oslo, Norway	Harold Holte, MD; Jan Delabie, MD
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