Genomic Profiling of Peripheral T-Cell Lymphoma, Unspecified, and Anaplastic Large T-Cell Lymphoma Delineates Novel Recurrent Chromosomal Alterations

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To characterize genetic alterations in peripheral T-cell lymphoma, not otherwise specified (PTCL NOS), and anaplastic large T-cell lymphoma (ALCL), 42 PTCL NOS and 37 ALCL [17 anaplastic large cell kinase (ALK)-negative ALCL, 9 ALK-positive ALCL, 11 cutaneous ALCL] were analyzed by comparative genomic hybridization. Among 36 *de novo* PTCL NOS, recurrent chromosomal losses were found on chromosomes 13q (minimally overlapping region 13q21, 36% of cases), 6q and 9p (6q21 and 9p21-pter, in 31% of cases each), 10q and 12q (10q23-24 and 12q21-q22, in 28% of cases each), and 5q (5q21, 25% of cases). Recurrent gains were found on chromosome 7q22-qter (31% of cases). In 11 PTCL NOS, high-level amplifications were observed, among them 3 cases with amplification of 12p13 that was restricted to cytotoxic PTCL NOS. Whereas cutaneous ALCL and ALK-positive ALCL showed few recurrent chromosomal imbalances, ALK-negative ALCL displayed recurrent chromosomal gains of 1q (1q41-qter, 46%), and losses of 6q (6q21, 31%) and 13q (13q21-q22, 23%). Losses of chromosomes 5q, 10q, and 12q characterized a group of noncytotoxic nodal CD5+ peripheral T-cell lymphomas. The genetics of PTCL NOS and ALK-negative ALCL differ from other T-NHLs characterized genetically so far, among them enteropathy-type T-cell lymphoma, T-cell prolymphocytic leukemia, and adult T-cell lymphoma/leukemia. (*Am J Pathol* 2004, 164:1837-1848)

Among mature T- and NK-cell lymphomas, peripheral T-cell lymphoma, unspecified (PTCL NOS), and anaplastic large T-cell lymphoma (ALCL) are most frequent.¹ PTCL NOS represent a morphologically and immunohistochemically heterogeneous group of T-cell lymphoma that, by definition, do not correspond to any of the well-

defined entities of mature T-cell lymphoma in the World Health Organization classification.¹ In contrast, ALCL are characterized by anaplastic tumor cell morphology and the expression of CD30.^{1,2} Among ALCL, 60 to 80% of cases carry the translocation t(2;5) or its variants, leading to overexpression of the anaplastic large cell kinase (ALK).^{1,2} Following distinct clinical features, cutaneous and systemic ALCL are distinguished among ALK-negative ALCL.^{1,2}

Although representing the majority of T-cell lymphomas, the genetics of PTCL NOS and ALK-negative ALCL are only poorly characterized so far. Classical cytogenetic studies have demonstrated a high degree of chromosomal complexity. Yet, because of the rarity of these neoplasms, most reports were based on a small number of cases only.³⁻⁷ For the heterogeneous group of PTCL NOS, no characteristic genetic alterations have been defined so far. In addition, the genetic relationship between ALK-negative ALCL and PTCL NOS, the genetics of cutaneous ALCL, and the secondary genetic alterations in ALK-positive ALCL have remained enigmatic so far.

In this study, we investigated 79 cases of PTCL NOS and ALCL by comparative genomic hybridization (CGH), reporting recurrent genetic alterations in PTCL NOS and ALK-negative ALCL for the first time. We show that different T-cell lymphoma entities as defined by the World Health Organization classification show characteristic chromosomal alterations.

Materials and Methods

Specimen Selection and DNA Extraction

More than 600 cases of PTCL NOS and ALCL were selected from the files of the Lymph Node Reference Center at the Department of Pathology, University of Würzburg, Würzburg, Germany, and the Department of Pathology, University of Vienna, Vienna, Austria. All cases were classified as PTCL NOS and anaplastic large cell

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T-cell lymphoma according to the current World Health Organization classification of tumors of hematopoietic and lymphoid tissues.¹ In particular, to distinguish between PTCL NOS and ALK-negative ALCL, the latter diagnosis was only rendered if the lymphoma consisted mostly of large lymphoid cells with abundant cytoplasm, showed pleomorphic, in part embryo-like hallmark nuclei, and showed a very strong expression of CD30 in the majority of cells with a membrane and/or Golgi-pattern of staining. In keeping with the World Health Organization classification of hematopoietic and lymphoid tissues,¹ lymphoma cases that did not show these morphological features and/or only showed weak CD30 positivity in a minority of tumor cells were classified as PTCL NOS.

To assure a sufficient tumor cell content for CGH, 196 cases with <40% nonneoplastic bystander cells were included for DNA extraction. In 11 cases, fresh-frozen material was available for DNA extraction (eight PTCL NOS, three ALK-negative ALCL). In the remaining 185 cases, DNA was extracted from formalin-fixed, paraffin-embedded tissue blocks using the phenol-chloroform extraction method according to previously published protocols.⁸ The length of the extracted DNA was analyzed on an 0.7% agarose gel with suitable DNA length standards. In only 68 cases (38%) could high-molecular DNA be extracted from paraffin blocks and be successfully analyzed by CGH. In the remaining 117 cases, DNA extracted from paraffin-embedded material was not suitable for further genetic analysis by CGH because of DNA degradation (DNA fragment size <1000 bp).

Immunohistochemistry

Immunohistochemical analysis was performed in all cases on formalin-fixed paraffin-embedded tissue sections according to previously published protocols.⁹ Immunostains included markers CD3 (1:400; DAKO, Copenhagen, Denmark), CD4 (1:10; Novocastra, Newcastle upon Tyne, United Kingdom), CD5 (1:20; Novocastra), CD8 (1:30; DAKO), CD20 (1:200; DAKO), CD30 (1:80; DAKO), TIA1 (1:800; Coulter, Hialeah, FL), Granzyme B (GrB-7, 1:20; Sanbio, Germany), Ki 67 (1:300; Novocastra), and ALK1 (mouse monoclonal antibody, 1:50; a kind gift from Karen Pulford, University of Oxford, Oxford, UK).

CGH

CGH was performed at the Department of Pathology, University of Würzburg, on 79 cases (42 PTCL NOS and 37 ALCL) according to a standard protocol.¹⁰ Tumor DNA was labeled by nick translation with biotin-16-dUTP (Roche Diagnostics, Mannheim, Germany). Reference DNA, extracted from placental tissue of a healthy newborn, was labeled with digoxigenin-11-dUTP (Roche Diagnostics). The amount of DNase I (Roche Diagnostics) and DNA polymerase I (Promega, Mannheim, Germany) were adjusted to obtain DNA fragment sizes of ~500 to 1000 bp. After inactivation of DNase I, unincorporated nucleotides were removed by gel filtration on Sephadex

G50-packed columns (Sigma, St. Louis, MO). Equal amounts of tumor and reference DNA (1 μ g each), together with 70 μ g of Cot-1 DNA (Roche Diagnostics) for blocking repetitive sequences, were co-hybridized on commercially available metaphase slides (Vysis, Downers Grove, IL), which had been denatured in 70% deionized formamide/2 $\times$ standard saline citrate/50 mmol/L sodium phosphate, pH 7.0, for 5 minutes at 70°C, followed by dehydration in cold ethanol. After overnight hybridization at 37°C, the slides were washed four times with 50% formamide/2 $\times$ standard saline citrate, pH 7.0, at 42°C and three times at 60°C with 0.1 $\times$ standard saline citrate. Biotin- and digoxigenin-labeled DNA were detected by addition of fluorescein-isothiocyanate avidin (Vector Laboratories, Burlingame, CA) or Cy3-conjugated anti-digoxigenin antibodies (Dianova, Hamburg, Germany), respectively. A 4,6-diamidino-2-phenylindole counterstain was performed for chromosomal banding.

Signals were visualized with a Zeiss Axiophot fluorescence microscope and analyzed with ISIS digital image analysis system (MetaSystems, Altlußheim, Germany). Ratio values of 1.25 and 0.8 were used as upper and lower thresholds for identification of chromosomal gains or losses (Figures 1 and 2). A high-level amplification was defined as an overrepresentation of genetic material with the fluorescence ratio values exceeding 2.0 or based on the observation of strong focal signals in the fluorescein isothiocyanate fluorescence.

Comparison of Genetic Findings in Different Types of T-Cell Lymphoma

CGH findings in PTCL NOS and ALK-negative ALCL were compared to those previously published on enteropathy-type T-cell lymphoma (ETCL), T-cell prolymphocytic leukemia (T-PLL), and aggressive adult T-cell lymphoma/leukemia (ATCL).^{8,11,12} Only genetic imbalances occurring in at least 25% of cases analyzed in any lymphoma entity were included into the comparative study.

Clinical Characteristics and Statistical Analysis

Clinical information was obtained for the patient's history, type and duration of symptoms at initial presentation, and type of treatment. Follow-up information was obtained via clinical records, attending physicians, or autopsy files.

In 36 of the 42 PTCL NOS cases, the material analyzed stemmed from the initial diagnostic specimen obtained at diagnosis and previous to installment of patient treatment. In six cases, only material from recurrent disease could be analyzed (cases 37 to 42; see Table 1). In two cases, lymphoma recurrences could additionally be analyzed and compared to the primary tumor [case 36 (1 year time interval) and case 40 (7 month time interval)].

Regarding the 36 primary PTCL NOS cases, 22 patients were male and 14 were female (Table 1). Age at diagnosis ranged from 27 to 90 years (average, 66 years). Among the 36 patients with primary disease manifestation, 12 were not staged, 4 patients had stage 1, 3 had stage 2, 6 had stage 3, and 11 had stage 4 disease.

Thirty-four of 36 PTCL NOS were primarily nodal lymphomas, only in 2 cases, primary extranodal disease manifestation was observed (subcutaneous fatty tissue of the poplitea and adrenal gland, respectively). Clinical follow-up was available from 30 patients. Of the 30 patients, 23 had already died (survival after diagnosis between 1 to 32 months; average, 10 months). Seven patients were still alive, among them two patients with a follow-up period of longer than 24 months.

Among the six PTCL recurrences, all patients were male, with an average age of 57 years (age range, 35 to 71 years) (Table 1). The time intervals between initial diagnosis and recurrence were 3 months (case 38), 20 months (case 40), 6 years (case 41), and 13 years (case 39), for the remaining cases it was unknown. Except for one patient, all patients had died (survival time after relapse 4 to 32 months; average, 13 months).

Information about patient treatment could be obtained in 28 of 42 patients. Twenty-four patients were treated with CHOP polychemotherapy (combination of cyclophosphomid doxorubicin vincristine and prednisone) only one patient following the IMVP protocol (combination of ifosfamid, methotrexate, and etoposide). Three of the patients did not receive any therapy because of age or advanced disease stage. Two of the patients received additional involved field radiation therapy. One patient underwent allogeneic bone marrow transplantation.

Of nine patients with ALK-positive ALCL (cases 43 to 51), six were male (see Table 2). Age at diagnosis ranged between 11 and 73 years (average, 33 years). All cases were nodal ALCL, in all but one case (case 51, recurrence 18 months after initial diagnosis), the material analyzed stemmed from the initial diagnostic specimen obtained at diagnosis and previous to the treatment of the patient. Among the primary cases, four each were stage 2 and stage 3 disease. Clinical follow-up was available in seven patients, all of them being alive with a follow-up period ranging from 2 to 62 months.

In 13 of 17 ALK-negative systemic ALCL (cases 52 to 64), the material analyzed stemmed from the initial diagnostic specimen obtained at diagnosis and previous to the treatment of the patient (Table 2). Among these, nine patients were male. Age at diagnosis ranged between 7 and 92 (average, 59 years). In only two cases was there extranodal lymphoma manifestation (soft tissue, bone). Among the primary cases, one was stage 1, four were stage 2, two were stage 3, two were stage 4 disease, the remaining patients were not staged. Clinical follow-up was available in 10 patients. Seven patients had already died (survival between 1 and 86 months), whereas three were still alive with a follow-up ranging from 72 to 92 months.

In four patients, only recurrences of ALK-negative ALCL could be analyzed (cases 65 to 68) (Table 2). The time intervals between initial diagnosis and recurrences were 2 years (case 61), 7 years (case 63), and 8 years (case 53), for the remaining case, it was unknown. Information on the treatment regimen was available in seven patients. Six patients were treated with CHOP polychemotherapy, while one patient underwent IMVP chemotherapy followed by autologous bone marrow transplantation.

Of the 11 patients with primary cutaneous ALCL (cases 69 to 79), 8 were male. Age at diagnosis ranged between 29 and 87 years (average, 57 years). In all cases, the material analyzed stemmed from the initial diagnostic specimen previous to treatment. Clinical follow-up was available in eight patients, seven of whom were alive with follow-up periods ranging from 5 to 48 months. In these patients, treatment consisted of local, topic radiotherapy.

Statistical correlation between clinical features, immunophenotype, and genetic alterations, and the comparison of genetic alterations between PTCL NOS, ALK-negative ALCL, ETCL, T-PLL, and adult T-cell lymphoma/leukemia were calculated using Fisher's exact test and Spearman's rank correlation. *P* values below 0.05 were regarded as significant. Survival analysis (impact of the number of genetic imbalances on survival) was performed using Kaplan-Meier statistics; for significance testing, a log-rank test was used.

Results

Immunohistochemical and Clinical Characterization

All cases were classified as PTCL NOS and anaplastic large cell T-cell lymphoma, respectively, according to the current World Health Organization classification of tumors of hematopoietic and lymphoid tissues.¹ The diagnosis of ALK-positive ALCL required unequivocal positivity in immunostains for ALK. To distinguish between PTCL NOS and ALK-negative ALCL, the latter diagnosis required the lymphoma to consist mostly of large lymphoid cells with abundant cytoplasm, to show pleomorphic embryo-like hallmark nuclei, and to display a very strong expression of CD30 in the majority of cells with a membrane and Golgi-pattern of staining. Following the World Health Organization classification of hematopoietic and lymphoid tissues,¹ lymphoma cases that did not show these morphological features and/or only showed weak CD30 positivity in a minority of tumor cells were classified as PTCL NOS.

The clinical and immunohistochemical features of the PTCL NOS and ALCL studied by CGH are summarized in Tables 1 and 2. In brief, 37 of 42 (88%) PTCL expressed CD3, 27 expressed CD4 (64%), 5 expressed CD8 (12%), and 27 expressed CD5 (64%). Eleven cases (26%) displayed a cytotoxic phenotype with expression of TIA1 and/or Granzyme B. Of note, 15 of 42 (36%) PTCL NOS expressed CD30; however, the staining intensity in these cases was generally weak and did not comprise the majority of tumor cells. Furthermore, characteristic hallmark cells were missing. Thus, these cases were diagnosed as PTCL NOS, not as ALK-negative ALCL based on the criteria forwarded by the World Health Organization classification as specified above.¹

CGH of PTCL NOS

Forty-two cases of PTCL NOS were analyzed by CGH, among them 36 primary PTCL NOS and 6 lymphoma

Table 1. Clinical Features, Immunophenotype, and Chromosomal Imbalances in PTCL, Unspecified

	Loc	Age	Sex	Stage	Sur	CD3	CD4	CD5	CD8	CD30	TIA1
1	Nodal	35	F	NS	Lost	+	+	+	—	—	—
2	Nodal	67	F	NS	Lost	—	—	—	—	—	—
3	Nodal	45	F	2a	20	+	—	—	+	—	+
4	Nodal	45	M	4b	7	+	—	+	—	—	—
5	Nodal	27	F	NS	Lost	+	—	—	—	—	+
6	Nodal	59	M	NS	1	+	+	+	—	—	—
7	Nodal	66	M	1a	32	+	—	+	—	—	—
8	Nodal	59	F	3b	25	+	+	+	—	(+)	—
9	Nodal	59	M	4	31	+	—	+	+	—	—
10	Nodal	51	M	3b	5	+	+	—	—	—	+
11	Nodal	63	M	NS	48+	+	—	+	—	—	—
12	Nodal	72	M	NS	3	+	+	+	—	(+)	—
13	Nodal	74	F	1a	Lost	+	+	+	—	(+)	—
14	Nodal	77	F	4b	1	+	+	—	—	+	+
15	Nodal	80	M	4b	3	+	—	—	—	—	+
16	Nodal	83	M	NS	2	—	—	—	—	(+)	+
17	Nodal	66	F	3b	36+	+	—	—	—	—	+
18	Nodal	66	M	3	10	+	+	—	—	(+)	—
19	Nodal	74	F	NS	1	+	+	+	—	—	—
20	Nodal	80	M	NS	Lost	+	+	+	—	—	—
21	Nodal	84	M	4	13	+	+	+	—	+	—
22	Nodal	46	F	4b	15	+	+	+	—	(+)	—
23	Nodal	78	F	2	25	+	+	+	+	—	—
24	Adrenal*	70	F	4	10	+	—	—	—	—	+
25	Nodal	77	M	1	17+	+	—	+	—	—	—
26	Nodal	72	M	1a	5+	+	+	+	—	—	—
27	Nodal	72	M	3	1	+	+	+	—	—	—
28	Nodal	58	M	2a	8+	+	—	+	—	—	—
29	Nodal	59	M	3a	12+	+	+	+	—	+	—
30	Nodal	64	M	4	8+	—	+	+	—	+	—
31	Nodal	66	F	NS	Lost	+	+	—	—	—	—
32	Nodal	90	F	NS	7	+	+	+	—	—	—
33	Nodal	63	M	4	4	—	+	—	—	(+)	—
34	Nodal	81	M	4b	2	+	—	—	—	(+)	—
35	Nodal	82	M	4	5	+	+	+	—	—	—
36	Soft tissue*	80	M	NS	13	+	+	—	+	—	+
36R	Soft tissue										
37R	Nodal	61	M	2a	80+	+	+	+	—	+	+
38R	Nodal	54	M	1	4	+	+	+	+	—	—
39R	Nodal	35	M	NS	11	+	+	+	—	+	—
40R	Nodal	71	M	1a	32	+	+	+	—	—	—
41R	Nodal	56	M	4b	6	+	+	+	—	(+)	—
42R	Cutaneous*	67	M	NS	10	—	—	—	—	—	+

R, disease recurrence; loc, localisation; F, female; M, male; NS, not staged; sur, survival in months.

+, Patient still alive after a follow-up of the months given; immunophenotype; +, strong stain, (+), partial or weak stain; —, negative stain.

*The extranodal PTCL, unspecified, were all negative in EBER hybridization, the nodal PTCL, unspecified, were not tested for EBV association. In case 40, two recurrences were analyzed.

Table 1. *Continued*

GrB	Ki67	Chromosomal gains	Chromosomal losses
–	70%	+1q, +10p13–p14, +11, +14q22–q24	–6q25–q27, –10q26, –13q31–qter
–	60%	+1q32–q42, +2	–Xp, –Xcen–q22
+	70%	+2cen–q22, +6p21–pter, +6p25 (high-level), +9q33–q34	–4cen–p15, –4q, –6q21–qter, –9p21–pter, –13q21–q31, –Xp, –Xcen–q26, –18
–	80%	+10p13–pter, +17	–2q22–24, –5q14–q23, –15q21
+	90%	+7q	–
–	80%	+5q34–35, +7q22–qter	–2q24, –2q36–q37, –4q31–q32, –6q14–q16, –6q25–q27, –9q21, –9q31–32, –10q, –10cen–p13, –12, –13, –18p
–	70%	+3p21, +7p, +7cen–q11, +11, +16p, +17q11–q21	–5q14–q23, –9p, –10, –12q14–q22
–	70%	+2p23–pter, +2p12–13, +3q22–q24, +7q22–qter, +10p12–p13, +18	–2p16–p22, –3cen–p14, –3q27–qter, –4cen–q22, –8cen–p12, –8cen–q23, –10q21–q22, –10q24, –12q13–q23, –13, –16q, –17p
–	70%	–	–2q23–q24, –5q15–q21, –7p13–p21
+	80%	+12p13 (high-level), +12cen–p12, +12cen–q13	–3cen–q21, –5p, –5cen–q31, –6p22–pter, –8cen–q13, –9p21–pter, –10q24–qter, –12q, –13q14–qter, –15q26
–	80%	+2cen–p22 (high-level), +4q25–27, +7, +8q24, +9q34, +14q31–qter, +15q12–q14, +16p, +17q24–qter, +18	–13q14–q21
–	80%	–	–
–	90%	+6p12 (high-level), +15q15 (high-level), +6q22	–6q15–qter, –9p23–pter, –18, –X
+	80%	+1q, +11p15	–6q, –9p21–pter
+	80%	+3p21–pter, +6p25 (high-level), +12p13 (high-level), +11q12–q13, +12q24, +17q, +22q13 (high-level)	–
+	60%	–	–
–	90%	+6p21 (high level), +8q24, +12p13–pter (high level), +14q22–qter (high level), +Xq24–qter	–5q14–q22, –6cen–q22, –10p, –17p
–	90%	–	–4q25–q27, –6cen–q15, –6q24–qter, –8p, –10q, –11, –13q, –17p
–	60%	+1p32–p35, +3p21, +7q, +16p, +17	–2p22–pter, –q35–qter, –9p, –9cen–q21
–	50%	+8q24, +16p, +17cen–q23	–8p21–pter, –9p21
–	40%	+7q, +11q13, +16p, +17q	–10p, –12q14–q22, –13q
–	70%	+1p36 (high-level), +1q23–qter, +2p, +7q22–qter	–3p25–pter, –6q21–q22, –9p, –9cen–q21, –10q23–q24, –12p, –17p
–	70%	+1p36 (high-level), +4p15–pter, +11cen–q13, +16p	–9p21–pter, –10cen–p12, –10q, –12q21–22, –13q
+	70%	+1q, +3p24–pter, +5q34–qter, +6p25, +7, +8q, +9q, +17q	–8p, –11q24–q25, –17p
–	60%	+3p14–p21	–1cen–p31, –8q13–q21, –12q21–q23, –13q14–q32
–	60%	+3p25–pter, +7, +11q25–qter	–1p21–p22, –2p16–pter, –2q22, –3q13–q25, –5q, –6q21, –9p, –10q21–qter, –12, –13cen–q31, –16q
–	80%	+7q, +8q23–q24 (high-level)	–2q22, –5q15–q23
–	40%	+1q32–qter, +7q, +9q33–q34	–5q15–q23, –6q, –9p, –10q21–qter, –12p, –12cen–q22, –13q13–q21, –15q26–qter, –16q23–qter, –17p13, +18q
–	80%	+Xp, +6p24–pter, +18q21	–2q36–q37, –2q22–q24, –5q21–q23, –6q21–qter, –9p, –9cen–q31, –10q, –11q24–qter, –12cen–q12, –12q15–qter, –13, –16q, –18q22–qter
–	70%	+3q25–qter, +5q33, +17cen–q21	–4cen–p14, –4cen–q23, –6q13–q21, –7p15, –8, –13
–	90%	+7, +10p13–pter, +11p, +11cen–q13, +14q21–qter	–
–	80%	+8q24–qter, +9q31–qter	–3, –8p
–	90%	+2p, +p21, +7p, +11p11–p12 (high-level), +16p, +17p	–2q36–qter, –6q16–q24, –8, –10cen–p12, –14q31–qter, –16q, –18
–	60%	+Xq, +5q13 (high-level)	–14q12–q13
–	40%	+3, +8q, +9cen–p13, +9q, +16	–1p21–p31, –6cen–q24
+	90%	+3p21–p24, +9q	–
		+1q41–q42, +3p21–p24, +5q31–qter, +Xq21–qter, +9q, +14q32–qter	–6q26–q27, –15q26–qter
–	60%	+7q	–2q33–qter, –5q21–q23, –8p21–pter, –10
–	50%	+8p, +8q24, +15q22–q24	–
–	70%	+Y, +7	–5q21–q23, –13q14–q21
–	50%	+2p, +6p24–p25, +7, +16p, +17cen–q24	–5q14–q23, –6q23–q25, –9p21, –9cen–q22, –10, –12q21–q22, –16q13–q22
		+2p, +6p24–p25 (high-level), +7, +9q34, +12q24–qter, +16p, +17cen–q24	–5q14–q23, –6q23–q25, –9q21, –9cen–q22, –10, –12q21–q22, –16q13–q22
–	80%	+11q24–qter	–6q15–q21
–	60%	–	–8cen–q23

Table 2. Clinical Features, Immunophenotype, and Chromosomal Imbalances of ALCL

	Dx	Loc	Age	Sex	Stage	Sur	CD3	CD4	CD5	CD8	CD30	TIA1	GrB
43	ALK+ ALCL	Nodal	13	M	3	62+	—	ND	+	ND	+	+	ND
44	ALK+ ALCL	Nodal	32	M	2b	Lost	—	—	—	—	+	—	+
45	ALK+ ALCL	Nodal	73	F	2b	48+	—	+	+	—	+	+	+
46	ALK+ ALCL	Nodal	23	M	2a	Lost	—	—	—	—	+	+	+
47	ALK+ ALCL	Nodal	28	M	3	9+	—	+	+	—	+	+	ND
48	ALK+ ALCL	Nodal	11	M	3b	13+	+	—	—	—	+	+	+
49	ALK+ ALCL	Nodal	50	F	3a	2+	+	+	—	—	+	—	—
50	ALK+ ALCL	Nodal	51	F	2b	6+	—	—	—	—	+	+	—
51R	ALK+ ALCL	Nodal	15	M	4	32+	—	+	—	—	+	+	+
52	ALK— ALCL	Nodal	29	M	2a	86	—	+	—	ND	+	ND	ND
53	ALK— ALCL	Bone	67	F	NS	92+	+	+	—	—	+	—	—
54	ALK— ALCL	Nodal	71	M	2	74+	+	—	—	—	+	—	—
55	ALK— ALCL	Nodal	7	M	3b	72+	—	—	—	—	+	—	—
56	ALK— ALCL	Nodal	88	F	NS	Lost	+	+	+	—	+	—	—
57	ALK— ALCL	Nodal	69	F	4	10	+	+	+	—	+	—	—
58	ALK— ALCL	Nodal	88	M	3	1	+	+	+	—	+	—	—
59	ALK— ALCL	Nodal	45	M	NS	1	+	+	—	—	+	+	+
60	ALK— ALCL	Nodal	92	F	NS	1	+	+	—	—	+	—	—
61	ALK— ALCL	Soft tissue	21	M	1	Lost	—	+	—	—	+	—	+
62	ALK— ALCL	Nodal	50	M	2	Lost	—	+	—	—	+	—	—
63	ALK— ALCL	Nodal	79	M	4a	10	+	+	—	—	+	—	—
64	ALK— ALCL	Nodal	63	M	2b	3	—	+	—	—	—	+	—
65R	ALK— ALCL	Pharynx	65	M	NS	Lost	—	—	—	—	+	—	—
66R	ALK— ALCL	Nodal	34	M	3a	46+	+	+	—	—	+	—	—
67R	ALK— ALCL	Nodal	28	F	4	Lost	—	—	—	—	+	—	+
68R	ALK— ALCL	Nodal	73	F	2b	24	+	+	+	—	+	—	+
69	Cut ALCL	Cutaneous	32	F	1	48+	+	—	—	—	+	—	—
70	Cut ALCL	Cutaneous	73	M	NS	Lost	—	+	—	—	+	—	—
71	Cut ALCL	Cutaneous	29	M	1	24+	—	+	ND	—	+	—	+
72	Cut ALCL	Cutaneous	87	F	1a	23+	+	+	—	—	+	—	—
73	Cut ALCL	Cutaneous	54	M	NS	11	+	+	—	—	+	+	+
74	Cut ALCL	Cutaneous	56	M	1	15+	+	+	—	—	+	—	—
75	Cut ALCL	Cutaneous	82	M	NS	Lost	—	+	+	+	+	—	—
76	Cut ALCL	Cutaneous	41	M	1	7+	+	+	+	—	+	—	+
77	Cut ALCL	Cutaneous	67	F	1	Lost	—	—	—	—	+	—	—
78	Cut ALCL	Cutaneous	57	M	1	8+	+	—	—	—	+	—	—
79	Cut ALCL	Cutaneous	50	M	1	5+	—	+	—	—	+	—	—

R, disease recurrence; loc, localisation; F, female; m, male; NS, not staged; nd, not done; sur, survival in months.

+, Patient still alive after a follow-up of the months given immunophenotype; +, strong stain, (+), partial or weak stain; —, negative stain.

recurrences. Among the primary PTCL NOS (cases 1 to 36), in total, 281 chromosomal imbalances were observed, with losses ($n = 159$) occurring more frequently than gains ($n = 122$) (Table 1; Figure 1). Only one case was negative for genetic imbalances in CGH. The number of chromosomal imbalances among PTCL NOS ranged between 0 and 20 (median, 7 imbalances; average, 7.8 imbalances). The number of losses ranged between 0 and 13 (median, 3 losses; average, 4.4 losses), and the number of gains between 0 and 10 (median, 3 gains; average, 3.4 gains).

The most frequent recurrent losses of chromosomal material were observed at 13q (minimally overlapping region 13q21; 13 cases, 36%), 6q and 9p (6q21 and 9p21-pter; 11 cases each, 31%), 10q and 12q (10q23-24 and 12q21-q22; 10 cases, 28%), and 5q (5q21; 9 cases, 25%) (Figure 1, continuous lines). Further recurrent losses were found on chromosomes 8p (8p21-pter; seven cases, 19%), and on chromosomes 10p and 17p

(10cen-p12 and 17p13; six cases each, 17%). The pattern of losses of chromosome 6q was rather complex; besides a minimally overlapping region of 6q21 lost in 11 cases (31%), material from other chromosomal bands was also frequently lost, among them 6q22 (lost in nine cases, 25%), 6q24-qter/6q15-q16 (lost in eight cases, 22%), and 6q14/6q23 (lost in seven cases, 19%).

Gains of chromosomal material in primary cases of PTCL NOS were most frequently observed on chromosomes 7q (7q22-qter; 11 cases, 31%), 17q (17cen-q21; 9 cases, 25%); 16p (8 cases, 22%); 8q and 9q (8q24 and 9q33-qter; 7 cases each, 19%); and 3p, 1q, and 11q (3p21, 1q32-qter, and 11cen-q13; 6 cases each, 17%) (Figure 1).

In 11 PTCLs, altogether 16 high-level gene amplifications were observed. Recurrent amplifications were detected at 12p13 (three cases), 1p36, and 6p25 (two cases each). Solitary amplifications were observed at 2cen-p22, 5q13, 6p12, 6p21, 8q23-q24, 11p11-p12,

Table 2. *Continued*

EMA	Ki67	Chromosomal gains	Chromosomal losses
+	ND	—	—9p21-pter
+	60%	+1q, +Xp	—10p
+	80%	+6p, +7p21-pter, +7q34-qter, +17q	—11, —13q21-q22; —18q
+	70%	—	—
+	90%	+6p21-p22	—
+	70%	+7p15-pter	—8p21-pter
+	80%	+2p23-pter, +2cen-q33	—
+	80%	+1q	—4q33-qter, —9p23-pter, —10p, —11q22-qter
ND	90%	+1q, 4q31-q34 (high-level), +6p24-p25, +7p21-pter	—6q
ND	60%	+1, +12q24-qter	—
—	90%	—	—
—	70%	—	—
+	70%	—	—
—	90%	+1q	—11p, —13q21-qter, —X
—	70%	+4p16-pter, +6p, +16p12-pter, +17cen-q22, +22	—1cen-p22, —6q16-qter, —8q13-q22, —12q21-22, —18, —Xq
—	90%	+1q, +6p, +6q25-qter	—8q21-q23
+	70%	+1q, +5q35-qter, +6p21, +8q24-qter, 17q12-q21 (high-level)	—2q22-q32, —4q22-q25, —6q21-q22, —6q26-qter, —8p, —13cen-q22, —16q
—	90%	+1q41-qter	—2q21-q22, —7q21-q31, —10
+	90%	+1, +7	—
—	80%	+11p14-pter	—3q, —4q27-qter, —6q16-q22, —11q22-qter, —13q
—	90%	—	—
—	90%	—	—2q23-qter, —6q, —11p14-pter, —11q21-q23
+	70%	—	—4, —6q14-q22, —9p21-pter, —10, —11p, —12, —16q, —18
—	80%	—2q22-q33, —6q, —13q	+7q
—	80%	+17q	—6q16-qter
—	90%	+1p36 (high-level), +2p	—3cen-q26, —Xq
—	70%	—	—
—	90%	+5q34-pter	—6q
—	90%	+7q	—
—	90%	+6p	—8p
(+)	90%	+6p21, +7q34-qter	—
—	70%	—	—
—	90%	—	—
(+)	80%	—	—
—	90%	+4	—
—	80%	+8	—
—	80%	—	—16q

14q22-qter, 15q15, and 22q13. Six cases of PTCL NOS were lymphoma recurrences (cases 37 to 42), among which recurrent chromosomal alterations were observed at similar loci as in the primary PTCL NOS, among them losses of chromosomes 5q (5q21; three cases, 50%) and 10 (two cases, 33%), and gains of 7q (three cases, 50%) (Figure 1, dotted lines). In two cases, follow-up biopsies of recurrent lymphoma could be analyzed. In case 36, both specimens shared a loss of chromosome 3p21-p24 and a gain of 9q, whereas the recurrence additionally showed gains on chromosomes 1q, 5q, 14q, and Xq, and losses on 6q and 15q not present in the initial specimen. In case 40, both specimens shared gains of chromosomes 2p, 6p, 7, 16p, and 17q, and losses of 5q, 6q, 9, 10, 12q, and 16q whereas in the second recurrent specimen, additional gains were observed on 9q and 12q. Of note, the gain on 6p24-p25 showed a much stronger signal in the second recurrence, indicating an increase in copy number between both specimens.

Losses of Chromosomes 5q, 10q, and 12q Characterize a Group of Nodal CD5+ Noncyclophosphamide PTCL NOS

The genetic findings in PTCL NOS were correlated with clinical features and the immunophenotype in multivariate analysis. The group of PTCL NOS showing any losses of 5q/10q/12q was compared to those devoid of these genetic imbalances. This group of lymphomas was distinct from PTCL NOS not showing losses of 5q/10q/12q by a higher frequency of CD5 as well as by negativity for cytotoxic markers Granzyme B and TIA1 ($P < 0.05$). No particular histomorphological characteristics of this group of lymphoma could be identified. Both groups failed to show significant differences with regard to clinical stage at diagnosis; however, when comparing the overall survival of both groups, cases with losses of 5q/10q/12q showed a better overall survival than the remaining T-NHLs (Figure 3). Furthermore, amplifications of

Figure 1. Genetic gains and losses in PTCL NOS ($n = 42$). Summation profile of genetic gains and losses occurring in PTCL NOS as detected by CGH. **Left bar**, loss of chromosomal material; **right bar**, gain of chromosomal material; **thick bars**, high-level amplifications; **continuous lines**, genetic imbalances in PTCL NOS at disease presentation ($n = 36$); **discontinuous dotted lines**, genetic imbalances observed in recurrent PTCL NOS ($n = 6$).

chromosome 12p13 showed a statistically significant correlation with a cytotoxic immunophenotype (expression of TIA1 and/or Granzyme B). Extranodal PTCL NOS more frequently showed gains of 9q33-qter. No statistically significant correlation was observed between the total number of alterations, losses and gains, and clinical features. Similarly, no correlation was observed between losses of 13q, 6q, and 9p and gains of 7q, and immuno-histochemical expression profiles and clinical features.

CGH of ALK-Negative ALCL

Seventeen cases of ALK-negative ALCL were analyzed by CGH, among them 13 primary cases and 4 lymphoma recurrences. Among the 13 primary ALK-negative ALCL (cases 52 to 64), nine cases together showed 49 chromosomal imbalances (20 gains and 29 losses) (Table 2; Figure 2, continuous lines). The number of chromosomal imbalances ranged between 0 and 12 (median, 4 imbalances; average, 3.8 imbalances). The number of losses ranged between 0 and 7 (median, 1; average, 2.2) and the number of gains between 0 and 5 (median, 1; average, 1.5). The most frequent genetic alteration in ALK-negative ALCL at disease manifestation was gain of chro-

mosome 1q (1q41-qter; six cases, 46%). Recurrent losses of chromosomal material were observed on chromosomes 6q (6q21-q22; four cases, 31%) and 13q (13q21-q22; three cases, 23%). A high-level amplification was observed on chromosome 17q (17q12-q21). Among four recurrent ALK-negative ALCL (cases 65 to 68), three cases also showed loss of chromosomal material on 6q (6q21-q22, 75%) (Table 2; Figure 2, dotted lines).

CGH of ALK-Positive ALCL

Nine cases of ALK-positive ALCL were analyzed by CGH (cases 43 to 51), eight of which showed chromosomal gains and losses (range, 0 to 7 chromosomal imbalances) (Table 2). In total, 16 gains and 10 losses were observed (median number of chromosomal imbalances, 2; average, 2.9 per case). Recurrent gains were observed at 1q, 7p, and 6p (three cases each, 33%). Recurrent losses were found on chromosomes 9p, 10p, and 11q (two cases each, 22%). In one case (case 47), a lymphoma recurrence, a high-level amplification was observed at chromosome 4q31-q34.

Figure 2. Genetic gains and losses in ALK-negative ALCL ($n = 17$). Summation profile of genetic gains and losses occurring in ALK-negative ALCL as detected by CGH. **Left bar**, loss of chromosomal material; **right bar**, gain of chromosomal material; **thick bars**, high-level amplifications; **continuous lines**, genetic imbalances in ALK-negative ALCL at disease presentation ($n = 13$); **discontinuous dotted lines**, genetic imbalances observed in recurrent ALK-negative ALCL ($n = 4$).

CGH of Cutaneous ALCL

Eleven cases of primary cutaneous ALCL were analyzed by CGH (cases 69 to 79), seven of which showed chromosomal imbalances (Table 2). In total, only seven gains and three losses were observed (median number of chromosomal imbalances, 1; average, 0.9). Gains involving 6p and 7q were observed in two cases each (18%). No high-level amplifications were observed.

Comparison of Genetics of Different Types of T-NHL

The chromosomal imbalances in PTCL NOS were compared to those of ALK-negative ALCL, and to those of ETCL we had previously published.⁸ In addition, they were compared to CGH results published in the literature on T-PLL and aggressive adult T-cell lymphoma (ATCL) (Table 3).^{11,12}

PTCL NOS and ALK-negative ALCL shared many recurrent genetic alterations (such as loss of 6q and 13q); in statistical analysis, however, they differed genetically with regard to the loss of 9p21-pter that occurred in 31% of PTCL NOS but never in this series in ALCL at disease

presentation (Fisher's, $P = 0.0244$). Furthermore, there was a trend for ALK-negative ALCL to more frequently show gains of chromosome 1q (46% versus 17%; Fisher's, $P = 0.0577$). Including the recurrent cases, PTCL NOS more frequently showed loss of chromosome 5q (33% versus 0%; Fisher's, $P = 0.0126$).

PTCL NOS also displayed characteristic genetic features in comparison to those of ETCL, T-PLL, and ATCL (for all genetic loci P value < 0.05 in Fisher's exact test): Losses of 12q 21-q22 segregated PTCL NOS from T-PLL, ETCL, and ATCL, losses of 5q21-q22, 6q21-q22, 10q23-q24, and 13q21 from ETCL and ATCL. Losses of 9p and gains of 17cen-q21 segregated PTCL NOS from ATCL. Furthermore, PTCL NOS significantly differed from ETCL because of the rare occurrence of gains of 9q; from T-PLL because of the rare occurrence of gains of 8q and 14q, and losses of 8p and 11q; and from aggressive ATCL because of the rarity of gains of chromosomes 3 and 14. Similarly, ALK-negative ALCL was genetically distinct from ETCL, T-PLL, and ATCL: gains of 1q segregated ALCL from T-PLL and ETCL, in addition, ALK-negative ALCL did not show the typical recurrent gains (of 8q, 14q) and losses (of 8p, 11q) of T-PLL, the characteristic recurrent gain of ETCL (gain of 9q), nor did it

Figure 3. PTCL NOS with losses of 5q/10q/12q show a better survival rate. PTCL NOS displaying losses of 5q/10q/12q are characterized by frequent expression of CD5, a noncytotoxic phenotype and show a better survival rate as compared to cases devoid of these alterations (Kaplan-Meier). **Line A**, PTCL NOS with losses of 5q/10q/12q ($n = 16$; median follow-up, 14 months), **line B**, PTCL NOS devoid of losses of 5q/10q/12q ($n = 14$; median follow-up, 6 months).

display the common genetic features of aggressive ATCL (gains of chromosomes 3 and 14q).

Discussion

Among mature T- and NK-cell lymphomas, PTCL NOS and anaplastic large cell lymphoma (ALCL) are most frequent.¹ Yet, apart from ALK-positive ALCL carrying the translocation t(2;5) and its variants, the genetic features of these lymphomas are mostly unknown. We have investigated a series of 79 PTCL NOS and ALCL by CGH, revealing novel and recurrent characteristic chromosomal alterations.

The PTCL in our series consisted of 42 tumor cell-rich lymphomas that were mostly of nodal origin (93% of cases); frequently expressed CD3 (88%), CD4 (64%),

and CD5 (64%); and displayed a cytotoxic phenotype in 26% of cases. In similar lymphoma cases formerly classified as high-grade peripheral T-cell lymphomas, classical cytogenetics, in an albeit very limited number of cases studied, revealed a high degree of chromosomal complexity, polyploidy, and aberrations.³⁻⁶ In concordance, in our series, 43 of 44 cases (98%) of PTCL NOS showed on average more than seven genetic alterations, among them recurrent losses of chromosomes 6q, 13q, 9p, 5q, 12q, and 10q, and gains of chromosome 7q.

Losses of genetic material on chromosomes 6q and 13q are among the most frequent genetic alterations reported for T- and B-NHL.¹³ Similar to the CGH findings in our series, in classical cytogenetics, 20 to 35% of PTCL NOS showed deletions on 6q.³⁻⁶ Similarly, 6q deletions were frequently observed in CGH studies of ATCL, T-PLL, and NK-cell lymphoma/leukemia.^{11,12,14} In analogy to classical cytogenetics of PTCL NOS, 6q21 was the minimally deleted region; however, deletions affecting chromosomal bands 6q14-q16 and 6q23-q24 were also frequently observed (19 to 22% of cases).

Losses of chromosome 13q were observed in more than one third of our series of PTCL NOS, with losses of 13q21 representing the most frequent chromosomal loss among PTCL NOS at initial diagnosis. In previous cytogenetic studies, PTCL NOS showed 13q deletions in 14 to 26% of cases;^{3-6,15} recurrent 13q losses were also reported in CGH studies of ATCL, T-PLL, Sezary syndrome, and NK-cell neoplasms.^{11,12,14,16} The minimal overlapping region in our series of PTCL NOS was chromosomal band 13q21, telomeric to the chromosomal band 13q14 frequently deleted in a variety of B-cell lymphomas but also observed in classical cytogenetic analysis of PTCL NOS. A second minimal overlapping region on 13q was 13q31-qter, corresponding to the minimal overlapping deleted region reported for aggressive ATCL.¹¹

A further recurrent genetic alteration in our series of PTCL NOS was gain of chromosome 7q that we observed in 31% of PTCL NOS at diagnosis, and in 50% of PTCL

Table 3. Genetic Features of T-Cell Lymphomas—Comparison of CGH Findings in PTCL NOS and ALK-Negative ALCL with Previously Published CGH Data of ETCL, T-PLL, and ATCL

Chr.	MOR	PTCL <i>n</i> = 36	ALK-ALCL <i>n</i> = 13	ETCL <i>n</i> = 38 ⁸	T-PLL <i>n</i> = 22 ¹²	ATCL <i>n</i> = 46 ¹¹					
+1q	1q32-qter	6	17%	6	46%	5	13%	0	0%	11	24%
+3p	3p21	6	17%	0	0%	0	0%	1	4%	17	37%
+3p	3p24-pter	5	14%	0	0%	1	3%	1	4%	17	37%
+3q		2	6%	0	0%	0	0%	0	0%	16	35%
−5q	5q21−q22	9	25%	0	0%	1	3%	1	4%	4	9%
−6q	6q21−q22	11	31%	4	31%	3	8%	8	36%	4	9%
+7q	7q22-qter	11	31%	1	8%	8	21%	2	9%	15	33%
−8p	8p21-pter	7	19%	1	8%	8	21%	19	86%	3	7%
+8q	8q24	8	19%	1	8%	4	11%	18	82%	5	11%
−9p	9p21-pter	11	31%	0	0%	7	18%	6	27%	0	0%
+9q	9q33-qter	7	19%	0	0%	20	53%	0	0%	6	13%
−10q	10q23−24	10	28%	1	8%	2	5%	2	5%	3	7%
−11q	11q23-qter	3	8%	2	15%	4	11%	15	68%	0	0%
−12q	12q21−q22	10	28%	1	8%	1	3%	0	0%	1	2%
−13q	13q21	13	36%	3	23%	5	14%	9	41%	2	4%
+14q	14q32	3	8%	0	0%	1	3%	11	50%	15	33%
+17q	17cen-q21	9	25%	2	15%	3	8%	3	14%	2	4%

Chr, chromosome; MOR, minimally overlapping region; n, number of cases.

NOS at disease recurrence. Gains of chromosome 7q along with losses of 6q and 13q have been regarded as common secondary chromosomal alterations that may be associated with lymphoma progression. Of note, in our series, no statistical correlation between clinical features, immunophenotype, or histological type of lymphoma, and gains of 7q and losses of 6q and 13q were observed, giving indirect additional evidence for the possibly secondary nature of these genetic alterations in PTCL NOS.

Recurrent losses on chromosomes 5q, 10q, and 12q have not been reported so far in PTCL NOS. Losses of chromosome 5q, with 5q21 being the minimal overlapping region, were observed in 25% of PTCL NOS at initial diagnosis and in 50% of recurrent PTCL NOS. Although this chromosomal region harbors the adenomatous polyposis tumor suppressor gene locus *APC*, no candidate gene potentially involved in T-cell lymphomagenesis has so far been identified in this chromosomal region. Notably, small interstitial deletions of exactly the same chromosomal region have occasionally been observed in other types of T-NHL. Tsukasaki and colleagues,¹¹ in their series of aggressive ATCL, report small deletions of the minimal overlapping region 5q21-q22 in 4 of 46 cases studied (9%); similarly, in CGH studies of Sezary syndrome, two of seven cases showed loss of chromosome 5, one of them showing a deletion at 5q14-q23, including the minimally deleted region we first describe in PTCL NOS.¹⁶ Thus, genes located in the chromosomal region 5q21-q22 may play an important role in the pathogenesis of T-NHL, in particular in lymphomagenesis of PTCL NOS.

Twenty-eight percent of PTCL NOS showed deletions on chromosome 12q, with 12q21-q22 representing the minimal overlapping region. The chromosomal region 12q21-q22 harbors several genes potentially involved in T-cell lymphomagenesis, among them the proapoptotic genes *TDAG51* (T-cell death-associated gene 51),¹⁷ and *RAIDD/CRAD* (Casp2 and Ripk1 domain-containing adaptor with death domain),^{18,19} and *BTG1* (B-cell translocation gene 1),^{20,21} a gene negatively regulating lymphocyte proliferation.

Recurrent deletions were also observed on chromosome 10q, with a minimally overlapping region at 10q23-q24. Deletions of 10q have only rarely been observed in classical cytogenetics and CGH in T-cell lymphoma.^{3-6,11,12,16} Several genes potentially involved in T-cell lymphomagenesis have been annotated to this minimal overlapping region, among them the tumor suppressor genes *LG1* (leucine-rich gene 1, located at 10q24),²² *MXI1* (max interacting protein 1),²³ and the apoptosis gene *FAS*. Furthermore, the tumor suppressor gene *PTEN* is located at chromosomal band 10q23.3. *PTEN* has been shown to be an important regulator in T-cell homeostasis.^{24,25} T-cell-specific *PTEN* knockout mice develop CD4⁺ T-cell lymphomas at a very high frequency.²⁴ Similarly, mice with heterozygous mutant *PTEN* show an increased incidence of T-cell lymphomas, characterized by loss of heterozygosity of the wild-type allele.²⁵

The significance of the recurrent deletions of chromosomes 5q, 10q, and 12q in PTCL NOS was further highlighted by shared clinical and immunohistochemical features of lymphomas showing losses at either chromosomal location. In multivariate analysis, this group of lymphomas

segregated from the remaining, histologically not distinguishable PTCL NOS by significantly more frequent expression of CD5 and by negativity for cytotoxic markers. In addition, although based only on a limited number of cases, they showed a better overall survival compared to cases without losses of 5q/10q/12q.

Two other notable correlations were observed between genetic and clinical/immunohistochemical features. First, there was a statistically significant correlation between extranodal lymphoma localization and chromosomal gains at 9q, covering the minimal overlapping region previously reported for ETCL.⁸ Thus, our data suggest that at least some of the T-cell lymphomas comprised in the heterogeneous category of PTCL NOS, most notably those with an extranodal disease presentation, may share genetic features with ETCL. Second, all cases with an amplification at 12p13 showed a cytotoxic phenotype and were CD5-negative, delineating another potential genetic subgroup of T-NHL among PTCL NOS.

The distinction of various T-cell lymphoma entities in the World Health Organization classification is primarily based on morphology, immunohistochemistry, and clinical features.¹ Based on those characteristics, three different types of ALCL are distinguished: ALCL expressing ALK, primary systemic ALK-negative ALCL, and primary cutaneous ALCL.² The genetic relationship of ALK-negative ALCL to PTCL NOS is controversial. The detection of the t(2;5) in 60 to 80% of ALCL and its prognostic implications have raised the discussion whether this primary genetic abnormality alone defines this disease entity, while cases lacking the translocation and ALK-expression should be classified separately, eg, as a subgroup of PTCL NOS.^{1,2} The morphological distinction between PTCL NOS and ALCL, Alk-negative is controversial; however, in the current World Health Organization classification, PTCL NOS and ALCL, Alk-negative are kept separate. Following the criteria forwarded by the World Health Organization, to distinguish between PTCL NOS and ALK-negative ALCL, in this series, the latter diagnosis was only rendered if the lymphoma consisted mostly of large lymphoid cells with abundant cytoplasm, showed pleomorphic, embryo-like hallmark nuclei, and showed a very strong expression of CD30 in the majority of cells with a membrane and Golgi-pattern of staining. Following the World Health Organization classification,¹ lymphoma cases devoid of these morphological features and/or only weak or partial CD30 expression were classified as PTCL NOS. Although only based on a limited number of cases, our data support the distinction of PTCL NOS and ALK-negative ALCL: overall, ALK-negative ALCL showed a degree of genetic instability that differed from both PTCL NOS and ALK-positive ALCL (median of 4 imbalances ALK-negative ALCL in contrast to 7.3 in PTCL NOS and 2 in ALK-positive ALCL; $P < 0.05$); in addition, although both entities shared many recurrent genetic imbalances (such as losses of 6q, 10q, 12q, and 13q and gains of 7q), in this series, ALK-negative ALCL differed genetically from PTCL NOS with regard to losses of 5q and 9p. In our series, losses on chromosome 5q (5q21) and 9p (9p21-pter) were never observed in ALK-negative ALCL (and, of note, losses of 5q21 neither in

ALK+ALCL, cutaneous ALCL, or ETCL with ALCL morphology), while these losses were detected in 25% and 31% of PTCL NOS. Given the limited number of cases analyzed, further genetic studies will be required to highlight the genetic differences between ALK-negative ALCL and PTCL NOS.

Reflecting their distinction as distinct T-cell lymphoma entities in the World Health Organization classification, the genetic features of both PTCL NOS and ALK-negative ALCL significantly differ from those of ETCL, T-PLL, and aggressive ATCL.^{8,11,12} Our comparative study shows that PTCL NOS is characterized by losses of 5q that set this lymphoma apart from all other types of T-cell lymphoma investigated genetically so far, and by losses of 12q segregating it from T-PLL, ETCL, and aggressive ATCL. On the other hand, PTCL NOS and ALK-negative ALCL only rarely show the characteristic genetic features of T-PLL (namely, gains of 8q and 14q, and losses of 8p and 11q), of ETCL (gain of 9q), and of aggressive ATCL (gains of chromosome 3).

In summary, our study shows that among the morphologically and immunohistochemically heterogeneous group of PTCL NOS, genetic features may segregate at least one particular group of noncytotoxic nodal CD5+ T-cell lymphoma characterized by chromosomal losses of 5q, 12q, and 10q. Furthermore, although there is a considerable overlap between the genetic features of ALK-negative ALCL and PTCL NOS, losses of chromosome 5q and 9p and gains of chromosome 1q may segregate PTCL NOS from ALK-negative ALCL. PTCL NOS and ALK-negative ALCL are genetically distinct from other T-cell lymphoma entities investigated genetically so far, among them T-PLL, ATCL, and ETCL.

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References

- Jaffe ES, Harris NL, Stein H, Vardiman JW: Tumours of haematopoietic and lymphoid tissues. World Health Organization Classification of Tumours. Lyon, IARC Press, 2001
- Stein H, Foss HD, Durkop H, Marafioti T, Delsol G, Pulford K, Pileri S, Falini B: CD30+ anaplastic large cell lymphoma: a review of its histopathologic, genetic and clinical features. *Blood* 2000, 96:3681–3695
- Schlegelberger B, Himmler A, Gödde E, Grote W, Feller AC, Lennert K: Cytogenetic findings in peripheral T-cell lymphomas as a basis for distinguishing low-grade and high-grade lymphomas. *Blood* 1994, 83:505–511
- Schlegelberger B, Himmler A, Bartles H, Kuse R, Sterry W, Grote W: Recurrent chromosome abnormalities in peripheral T-cell lymphomas. *Cancer Genet Cytogenet* 1994, 78:15–22
- Schlegelberger B, Feller AC: Classification of peripheral T-cell lymphomas: cytogenetic findings support the updated Kiel classification. *Leuk Lymphoma* 1996, 20:411–416
- Lepetre S, Buchonnet G, Stamatoullas A, Lenain P, Duval C, d'Anjou J, Callat MP, Tilly H, Bastard C: Chromosome abnormalities in peripheral T-cell lymphoma. *Cancer Genet Cytogenet* 2000, 117:71–79
- Renedo M, Martinez-Delgado B, Arranz E, Garcia MJ, Urioste M, Martinez-Ramirez A, Rivas C, Cigudosa JC, Benitez J: Chromosomal changes pattern and gene amplification in T cell non-Hodgkin's lymphomas. *Leukemia* 2001, 15:1627–1632
- Zettl A, Ott G, Makulik A, Katzenberger T, Starostik P, Eichler T, Puppe B, Bentz M, Müller-Hermelink HK, Chott A: Chromosomal gains at 9q characterize enteropathy-type T-cell lymphoma. *Am J Pathol* 2002, 161:1635–1645
- Rüdiger T, Ott G, Ott MM, Müller-Deubert SM, Müller-Hermelink HK: Differential diagnosis between classic Hodgkin's lymphoma, T-cell-rich B-cell lymphoma and paraneoplastic lymphoma by paraffin immunohistochemistry. *Am J Surg Pathol* 1998, 22:1184–1191
- Lichter P, Bentz M, Du Manoir S, Joos S: Comparative genomic hybridization. *Human Chromosomes: Principles and Techniques*, ed 2. Edited by RS Verma, A Babu. New York, McGraw-Hill, 1995, pp 191–210
- Tsukasaki K, Krebs J, Nagai K, Tomonaga M, Koeffler P, Bartram CR, Jauch A: Comparative genomic hybridization analysis in adult T-cell leukemia/lymphoma: correlation with clinical course. *Blood* 2000, 97:3875–3881
- Soulhier J, Pierron G, Vecchione D, Garand R, Brizard F, Sigaux F, Stern MH, Aurias A: A complex pattern of recurrent chromosomal losses and gains in T-cell prolymphocytic leukemia. *Genes Chromosomes Cancer* 2000, 31:248–254
- Zhang Y, Matthiesen P, Harder S, Siebert R, Castoldi G, Calasanz MJ, Wong KF, Rosenwald A, Ott G, Atkin NB, Schlegelberger B: A 3-cM commonly deleted region in 6q21 in leukemias and lymphomas delineated by fluorescence in situ hybridization. *Genes Chromosomes Cancer* 2000, 27:52–58
- Siu LP, Wong KF, Chan JKC, Kwong YL: Comparative genomic hybridization analysis of natural killer cell lymphoma/leukemia. *Am J Pathol* 1999, 155:1419–1425
- Rosenwald A, Ott G, Krumdiek AK, Dreyling MH, Katzenberger T, Kalla J, Roth S, Ott MM, Müller-Hermelink HK: A biological role for deletions in chromosomal band 13q14 in mantle cell and peripheral T-cell lymphoma? *Genes Chromosomes Cancer* 1999, 26:210–214
- Karenko L, Kähkönen M, Hyytinen ER, Lindlöf M, Ranki A: Notable losses at specific regions of chromosome 10q and 13q in the Sezary syndrome detected by comparative genomic hybridization. *J Invest Dermatol* 1999, 112:392–395
- Neef R, Kuske MA, Prols E, Johnson JP: Identification of the human PHLDA1/TDAG51 gene: down-regulation in metastatic melanoma contributes to apoptosis resistance and growth deregulation. *Cancer Res* 2002, 62:5920–5929
- Ahmad M, Srinivasula SM, Wang L, Talanian RV, Litwack G, Fernandes-Alnemri T, Alnemri ES: CRADD, a novel human apoptotic adaptor molecule for caspase-2 and FasL/tumor necrosis factor receptor-interacting protein RIP. *Cancer Res* 1997, 57:615–619
- Hofmann WK, de Vos S, Tsukasaki K, Wachsmann W, Pinkus GS, Said JW, Koeffler HP: Altered apoptosis pathways in mantle cell lymphoma detected by oligonucleotide microarray. *Blood* 2001, 98:787–794
- Rouault JP, Rimokh R, Tessa C, Paranhos G, French M, Duret L, Garocci M, Germain D, Samarut J, Magaud JP: BTG1, a member of a new family of antiproliferative genes. *EMBO J* 1992, 11:1663–1670
- Sasajima H, Nakagawa K, Yokosawa H: Antiproliferative proteins of the BTG/Tob family are regulated by the ubiquitin-proteasome system. *Eur J Biochem* 2002, 269:3596–3604
- Chernova OB, Somerville RPT, Cowell JK: A novel gene, LGI1, from 10q24 is rearranged and downregulated in malignant brain tumors. *Oncogene* 1998, 17:2873–2881
- Wechsler DS, Hawkins AL, Li X, Jabs EW, Griffin CA, Dang CV: Localization of the human Mxi1 transcription factor gene (MXI1) to chromosome 10q24–q25. *Genomics* 1994, 21:669–672
- Suzuki A, Yamaguchi MT, Ohteki T, Sasaki T, Kaisho T, Kimura Y, Yoshida R, Wakeham A, Higuchi T, Fukumoto M, Tsubata T, Ohashi PS, Koyasu S, Penninger JM, Nakano T, Mak TW: T cell-specific loss of PTEN leads to defects in central and peripheral tolerance. *Immunity* 2001, 14:523–534
- Suzuki A, de la Pompa JL, Stambolic V, Elia AJ, Sasaki T, del Barco Barrantes I, Ho A, Wakeham A, Ito A, Khoo W, Fukumoto M, Tak TW: High cancer susceptibility and embryonic lethality associated with mutation of the PTEN tumor suppressor gene in mice. *Curr Biol* 1998, 8:1169–1178