American Journal of Pathology, Vol. 149, No. 2, August 1996

Detection of t(2;5)(p23;q35) Translocation by Reverse Transcriptase Polymerase Chain Reaction and *in Situ* Hybridization in CD30-Positive Primary Cutaneous Lymphoma and Lymphomatoid Papulosis

Marie Beylot-Barry,* Laurence Lamant,† Béatrice Vergier,* Anne de Muret,‡ Sylvie Fraitag,§ B. Delord,* Pierre Dubus,* Loïc Vaillant,‡ Michèle Delaunay,* Gaetan Mac Grogan,‖ Claire Beylot,* Antoine de Mascarel,* Georges Delsol,† and Jean-Philippe Merlio*

From the Departments of Pathology, Histology, and Dermatology, CHU de Bordeaux et Université de Bordeaux II, Bordeaux, and the Departments of Pathology, CHU de Purpan,† Toulouse, CHU de Tours,‡ Tours, Hôpital Necker,§ Paris, and Institut Bergonié,‖ Bordeaux, France*

The t(2;5) generates a chimeric NPM-ALK transcript encoded by the nucleophosmin NPM gene fused to the anaplastic lymphoma kinase gene ALK. Using a reverse transcriptase nested polymerase chain reaction assay we have detected NPM-ALK transcripts within CD30$^+$ primary cutaneous lymphoma and lymphomatoid papulosis (LP). The t(2;5) was identified in 4 out of 9 CD30$^+$ anaplastic lymphomas and in 1 out of 4 CD30$^+$ pleomorphic lymphomas. Moreover, the t(2;5) was detected in 3 out of 10 LPs. All NPM-ALK-positive lymphomas and 1 NPM-ALK-positive LP exhibited a clonal rearrangement of the T cell receptor γ-chain gene. The t(2;5) was detected in 2 cases of LP without other evidence for a clonal lymphoid population. To identify cells carrying the t(2;5) translocation, we used immunohistochemistry to detect the ALK-encoded p80 protein and* in situ *hybridization for the specific detection of NPM-ALK transcripts. Both p80 protein and NPM-ALK transcripts were expressed by anaplastic or large CD30$^+$ lymphoma cells with positive NPM-ALK amplification. The presence of t(2;5) in a subset of CD30$^+$ cutaneous lymphoma and LP may indicate a common pathogenesis with a subset of anaplastic nodal lymphoma. (Am J Pathol 1996, 149:483–492)

Primary cutaneous large cell lymphomas (CLCLs) are a heterogeneous group of lymphomas regarding clinical and morphological features and prognosis.[1] Several studies have suggested that CD30 expression by more than 75% of tumor cells may define a subset of CLCL with good prognosis.[1–3] Moreover CD30$^+$ cutaneous lymphoproliferations comprise slowly progressive lymphoproliferative disorders with various evolution ranging from spontaneous regression to systemic dissemination. Lymphomatoid papulosis (LP) represents the benign counterpart of this spectrum.[4] Its common course is benign, but between 5 and 20% of patients will have associated lymphomas, including mycosis fungoides, T-cell immunoblastic lymphoma, or anaplastic lymphoma.[5–8]

Immunophenotypic and molecular genetic studies have shown that the overall majority of cutaneous CD30$^+$ lymphomas are of T cell or null cell origin,[9] which is analogous to systemic anaplastic large cell lymphoma (ALCL).[10] Cutaneous large cell CD30$^+$ lymphomas are clonal, as assessed by antigen receptor gene rearrangement studies.[11,12] The prevalence of monoclonality in LP is close to that observed in cutaneous T cell lymphomas.[13–17] However, a clonal rearrangement of the T cell receptor does not predict the subsequent development of systemic lymphoma.[13,16] Therefore, the molecular events that

Supported by grants from the Délégation à la Recherche Clinique, Ministère de la Santé et Ligue contre le Cancer, Comité de Gironde.

Accepted for publication April 18, 1996.

Address reprint requests to Dr. Marie Beylot-Barry, Laboratoire d'Histologie-Embryologie, UFR III Université de Bordeaux II, 146 rue Léo Saignat, 33076 Bordeaux cedex, France.

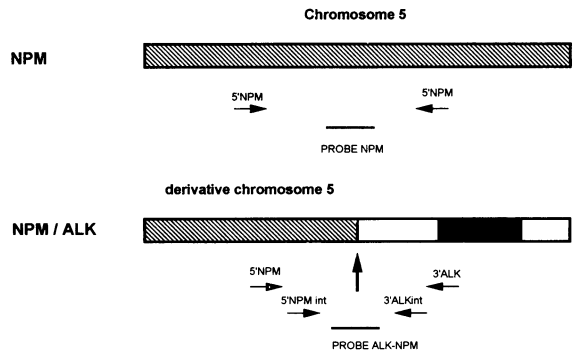


Figure 1. *Schema of the NPM gene on chromosome 5 and the chimeric gene NPM-ALK derived from the der(5) chromosome after translocation with chromosome 2. The position of the breakpoint is indicated by an* arrow. *The position of the oligonucleotide primers and probe used for RT-PCR analysis are shown (see sequences in the text). The region encoding for the tyrosine kinase domain is indicated by a solid box.*

promote the malignant transformation of LP as well as the poor evolution of a subset of $CD30^+$ CLCL remain to be defined.

Conventional cytogenetics in childhood lymphomas have characterized the t(2;5)(p23;q35) translocation since 1985.[18] This translocation has been detected by cytogenetic methods in 30 to 40% of nodal ALCLs.[19–22] However, no t(2;5) has been identified in $CD30^+$ CLCL or LP by cytogenetics.[23]

The t(2;5) breakpoint consists of the fusion of the nucleolar phosphoprotein gene NPM on 5q35, which is ubiquitously expressed,[24,25] with a new protein tyrosine kinase gene named anaplastic lymphoma kinase (ALK), which is located on 2p23.[26] The NPM-ALK fusion protein is composed of the amino-terminal domain of NPM and the catalytic domain of ALK.[26] The chimeric NPM-ALK transcript, encoded by the derivative chromosome 5, can be detected by reverse transcriptase polymerase chain reaction (RT-PCR) using NPM and ALK primers (Figure 1). This allowed t(2;5) detection not only in nodal ALCL[27–31] but also in some non-anaplastic or $CD30^-$ lymphomas.[29]

The aim of our study was to assess whether the t(2;5) is present in primary cutaneous lymphoproliferations, especially in $CD30^+$ CLCL and in LP. Although Lopategui et al[28] reported two cases of $CD30^+$ CLCL with positive NPM-ALK amplification, Kadin et al[23] found no NPM-ALK transcript in a series of two $CD30^+$ CLCLs and two LPs. The absence of t(2;5) in $CD30^+$ cutaneous lymphoproliferations seemed to be confirmed by recent studies of four cases of $CD30^+$ CLCL[31] and six cases of LP.[32] These discrepancies led us to use a highly sensitive nested RT-PCR assay for the detection of t(2;5) in cutaneous biopsy specimens that might contain an admixture of tumor cells and non-neoplastic cells. Moreover, we have detected the expression of the ALK-encoded p80 protein by immunohistochemistry and have compared its results with a specific *in situ* hybridization procedure to identify cells expressing the NPM-ALK chimeric transcripts.

Materials and Methods

The 29 primary cutaneous lymphoproliferative disorders were retrieved from the files of the Departments of Pathology of the University Hospitals of Bordeaux, Toulouse Purpan, Paris Necker, and Tours and from the Bergonié Institute. According to Beljaards's definition for primary cutaneous lymphoma,[2] staging procedures (physical examination, total and differential leukocyte count, chest radiograph, abdominal scan, and bone marrow biopsy) confirmed the absence of initial extracutaneous involvement for at least 6 months after diagnosis. No patient with CLCL had a past record of LP. An exception to these rules was made for one patient with cutaneous ALCL (case 11), who had been cured of Hodgkin's disease for 7 years, as previously reported.[33]

A concordant histopathological diagnosis was made by two independent pathologists (G. Delsol and B. Vergier) on hematoxylin and eosin (H&E)-safran sections of fixed paraffin-embedded skin biopsies. A representative snap-frozen skin biopsy was available. Immunohistochemistry was performed on fixed-tissue sections for the detection of the following antigens: CD3, CD20-L26, CD30-BerH2, and epithelial membrane antigen (DAKO, Copenhagen, Denmark). Frozen tissue sections were used to detect the following B- or T-cell-associated antigens: CD3 (DAKO), CD4 (Becton Dickinson, San Jose, CA), CD8 (Becton Dickinson), and CD22 (DAKO). The detection of p80 protein was performed both on frozen and paraffin sections with the primary antibody anti-p80,[34,35] kindly provided by Nichirei Co. (Tokyo, Japan). Deparaffinized sections were immersed in 500 ml of citrate buffer (0.01 mol/L) and submitted to an antigen retrieval procedure with either domestic microwave irradiation[30] or high-pressure cooking for 3 minutes of pressurized time.[36] The anti-p80 antibody was used at a 1:50 dilution for 16 hours at 4°C. Controls included the omission of the primary antibody and the use of nonspecific rabbit serum. A three-stage streptavidin-peroxidase procedure was performed with the LSAB kit (Dako, Trappes, France), as previously described.[37]

Gene Rearrangement Analysis

A study of immunoglobulin and T-cell receptor gene rearrangement was performed using a PCR-based

strategy. The amplification of the third complementary determining region of the immunoglobulin heavy chain gene was performed with primers Ca1 and Ca2 and primers Ca1 and VLJH as previously described.[38,39] The study of the T-cell γ-chain gene configuration was performed using a recently described system based on a multiplex PCR with GC-clamp primers and denaturing gradient gel electrophoresis.[17]

RT-PCR

RNA was extracted from a 500-μm-thick section of frozen material using Trizol reagent (GIBCO-BRL, Gaithersburg, MD). The presence of amplifiable RNA and the reverse transcription step were controlled by the amplification of the ubiquitously expressed NPM transcript using primers 5′NPM (5′-TCCCTTGGGGGCTTTGAAATAACACC-3′) and 3′NPM (5′-GCTACCACCTCCAGGGGCAGA-3′). Two primers, 5′NPM and 3′ALK (5′-CGAGGTGCGGAGCTTGCTCAGC-3′), were used for the standard PCR, and two internal primers, 5′NPM_{int} (5′-CCAGTGGTCTTAAGGTTGAAG-3′) and 3′ALK_{int} (5′-TTGTACTCAGGGCTCTGCAGC-3′), were used for nested PCR (Figure 1). Reverse transcription was performed for 1 hour at 37°C with 2 μg of total RNA using random hexamers and 400 U of Superscript II RT (GIBCO-BRL). The PCR was performed with 300 ng of each primer and 1.5 U of *Taq* polymerase (Promega, Madison, WI) in an automated thermal cycler (Hybaid, United Kingdom). A 5-minute denaturation step at 94°C was followed by 35 cycles of 94°C for 1 minute, 65°C for 1 minute, 72°C for 2 minutes, and a final extension at 72°C for 5 minutes. Nested PCR was performed according to the same procedure with 5 μl of the standard amplification product as template. To avoid possible cross-contamination, the main steps, *ie*, extraction, amplification, electrophoresis, and the nested procedure were performed in different laboratories. PCR results were checked by two independent experiments for each sample. Negative controls included the amplification of the reaction mixture with water as template and the amplification of RNA samples without the reverse transcription step. Positive controls included the SU-DHL1 cell line (gift of Dr. M. Cleary, Stanford) and a secondary cutaneous anaplastic lymphoma carrying the t(2;5) translocation. To evaluate the sensitivity of the NPM-ALK PCR assays, mixing experiments of the SU-DHL1 cell line into reactive lymphocytes were performed as previously described.[30] NPM-ALK products were detected at the dilution of 1 in 10^5 after the one-step PCR assay and 1 in 10^6 after nested amplification. PCR products of the standard PCR and nested PCR assays were subjected to electrophoresis in a 2% agarose gel, transferred onto nylon membranes, and thereafter hybridized with a NPM-ALK junction-specific oligoprobe (5′-GCTCCTGGTGCTTCCGGCGGTACACTACTAAGTGCTCACT-3′).

In Situ *Hybridization*

The detection of either the NPM transcripts or the NPM-ALK transcripts was performed on frozen sections by *in situ* hybridization (ISH), as previously described.[40] Each probe was labeled with [α^{33}P]dATP using terminal transferase (Amersham, UK). Frozen sections were fixed for 10 minutes in 1% paraformaldehyde, washed, and prehybridized in 4X standard saline citrate (SSC) with 1% Denhardt. After acetylation and dehydration, hybridization was performed with 0.2 ng of probe/slide for 15 hours at 42°C. After several washings at high stringency, sections were dehydrated and air dried. Sections were dipped in Ilford (UK) K5 emulsion, exposed for 7 weeks at 4°C, developed, fixed, and counterstained with Meyer's Hemalun. Controls for the specificity of *in situ* labeling were demonstrated by the absence of signal observed with an unrelated oligonucleotide or by omitting the specific oligonucleotide in the hybridization cocktail.

Results

The expression of the NPM-ALK transcript encoded by the t(2;5) was studied by RT-PCR in a series of 13 $CD30^+$ CLCLs, 6 $CD30^-$ CLCLs, and 10 LPs from 29 patients. The main histopathological characteristics of $CD30^+$ cutaneous lymphoproliferations are shown in Table 1. The $CD30^+$ CLCLs were classified into anaplastic (n =9) or pleomorphic (n =4) CLCL, according to Willemze's classification.[41] A total of 10 $CD30^+$ CLCLs exhibited a T-cell phenotype and 3 did not express either T- or B-cell-associated antigens. The 6 $CD30^-$ CLCLs included 2 centroblastic and 1 immunoblastic B cell lymphoma and 2 pleomorphic large cell and 1 lymphoblastic T-cell lymphoma (data not shown). The 10 LPs were classified according to Willemze as type A (7 cases) or type B (3 cases).[42] The lymphoid infiltrate of all cases of type A LP and 2 cases of type B LP had a predominant T-cell phenotype, whereas B and T cells were mixed in 1 case of type B LP. Immunogenotypic study of $CD30^+$ CLCL and LP showed a clonal T-cell receptor γ-chain gene rearrangement in 7 anaplastic

Table 1. *Cytomorphological Features, Phenotype, and Genotype of the Cutaneous CD30⁺ Lymphomas and Lymphomatoid Papulosis: Comparison of the Results of RT-PCR, nested RT-PCR, p80 Immunostaining, and* in Situ *Hybridization for the NPM/ALK Expression*

Cases	Phenotype	PCR TCRγ	RT-PCR	Nested RT-PCR	p80 immunostaining	*In situ* hybridization
Large cell CD30⁺ lymphoma						
Anaplastic						
1	T	Clonal	+	+	+	++
2	T	Clonal	+	+	−	++
3	T	Clonal	−	+	+	+
4	T	Clonal	−	+	+	+
5	Null	Clonal	−	−	−	−
6	T	ND	−	−	−	−
7	T	ND	−	−	−	−
8	Null	Clonal	−	−	−	−
9	T	Clonal	−	−	−	−
Pleomorphic large cells						
10	T	Clonal	−	+	+	+
11	Null	Clonal	−	−	−	−
12	T	ND	−	−	−	−
13	T	ND	−	−	−	−
Lymphomatoid papulosis						
Type A						
14	T	Clonal	−	+	+	ND
15	T	Polyclonal	−	+	ND	ND
16	T	Clonal	−	−	−	−
17	T	Polyclonal	−	−	−	−
18	T	ND	−	−		−
19	T	ND	−	−	−	−
20	T	Clonal	−	−	−	−
Type B						
21	T	Polyclonal	−	+	+	−
22	T	Clonal	−	−	−	−
23	T/B	Clonal	−	−	−	−

TCR, T-cell receptor γ-chain; ND, not done.

CD30⁺ CLCLs, 2 non-anaplastic CD30⁺ CLCLs, and 5 LPs (Table 1).

These cases were studied for the presence of NPM-ALK transcript by a standard RT-PCR assay and after nested amplification. Results were analyzed after ethidium bromide staining of the electrophoresed PCR products (Figure 2A). First, the standard PCR assay allowed the detection of a 187-bp NPM-ALK transcript in 2 out of 29 cases. The 2 t(2;5)-expressing cases were CD30⁺ anaplastic CLCLs. Second, the nested amplification of the first PCR products provided a 138-bp NPM-ALK PCR product for the 2 previously detected cases and for 6 additional ones that included 2 anaplastic CD30⁺

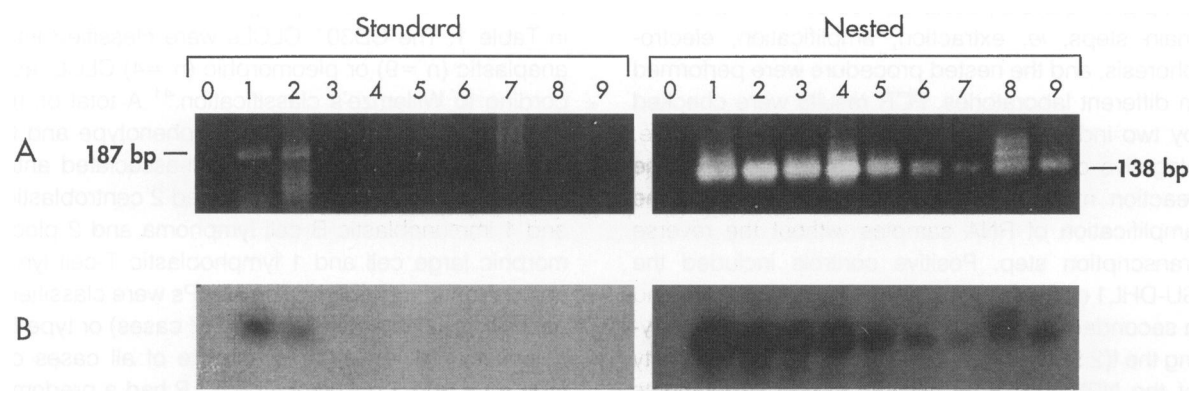


Figure 2. *RT-PCR analysis for the NPM-ALK t(2;5) encoded transcript. Total RNA was extracted from five CD30⁺ primary cutaneous lymphoproliferations (1 to 5), one CD30⁺ secondary cutaneous lymphoproliferation (6), and three LPs (7 to 9) and thereafter reverse transcribed. Amplification by PCR was performed by either a standard or a nested PCR assay. PCR products were separated by electrophoresis and viewed by ethidium bromide staining (***A***). Southern blot was performed with the [α³²P]dATP NPM/ALK fusion probe (***B***). With standard PCR assay, two cases of anaplastic CD30⁺ lymphoma were found to express the NPM-ALK chimeric transcript. Nested PCR assay allowed the detection of NPM-ALK transcript in five CD30⁺ primary cutaneous lymphomas and in three cases of LP.*

CLCLs, 1 pleomorphic large cell CD30$^+$ CLCL, and 3 LPs (Figure 2A). A specific size-identical PCR product was identified in all positive cases. Southern blot hybridization of PCR products with the NPM-ALK-specific probe did not detect any additional cases by comparison with ethidium bromide visualization (Figure 2B).

ISH with the NPM oligoprobe showed a labeling of epithelial cells, leukocytes and lymphoma cells (Figure 3, A and C). Thereafter, results of ISH were interpreted for cases with a positive NPM ISH, which assessed good preservation of RNA for *in situ* labeling. A specific NPM-ALK transcript was observed only in cases with positive RT-PCR amplification (Figure 3, D and F). No labeling was shown on epithelial or inflammatory cells with the NPM-ALK-specific probe (Figure 3B). A strong labeling of large tumor cells was observed in two cases of anaplastic CD30$^+$ CLCL, and a moderate labeling was observed in three cases of CD30$^+$ anaplastic (two cases) or pleomorphic (one case) CLCL as well as on lymphoma cells of the secondary cutaneous CD30$^+$ lymphoma used as positive control. In a given lymphoma, NPM-ALK-positive tumor cells were either of anaplastic or non-anaplastic morphology. Moreover, NPM-ALK transcripts seemed to be equally expressed by tumor cells of a definite lymphoma. Only one case of LP with positive NPM-ALK amplification was studied by ISH, but no cell was labeled by the NPM-ALK-specific probe (case 21).

Immunohistochemistry with the polyclonal anti-p80 antibody showed concordant results on frozen and paraffin sections with a diffuse cytoplasmic staining (Table 1). The pressure cooking technique allowed a more sensitive detection than the microwave procedure. With the latter, only two cases of t(2;5)-bearing CLCL were found to express p80. With the former, a p80 immunoreactivity was observed on lymphoma cells in four out of five t(2;5)-positive CLCLs (Figure 3E) and on large atypical cells in two out of two t(2;5)-positive LPs (Figure 3, G and H). In one t(2;5)-positive CLCL (case 2), no p80 immunoreactivity was observed on frozen and paraffin sections although anaplastic cells were found to express NPM-ALK transcripts by ISH. Although p80 immunostaining was restricted to tumor or atypical cells among the lymphoid cells, p80 expression was not restricted to NPM-ALK-expressing cells in comparison with ISH data (Figure 3, B and G). Indeed, a p80 immunoreactivity was observed both on frozen and paraffin sections over keratinocytes, endothelial cells, and some dendritic cells of the dermis (Figure 3G). Moreover, hair follicle cells and cells of the eccrine sweat glands were labeled (data not shown).

Comparison of Clinical Status and Evolution of CD30$^+$ Lymphoproliferations According to t(2;5) Detection

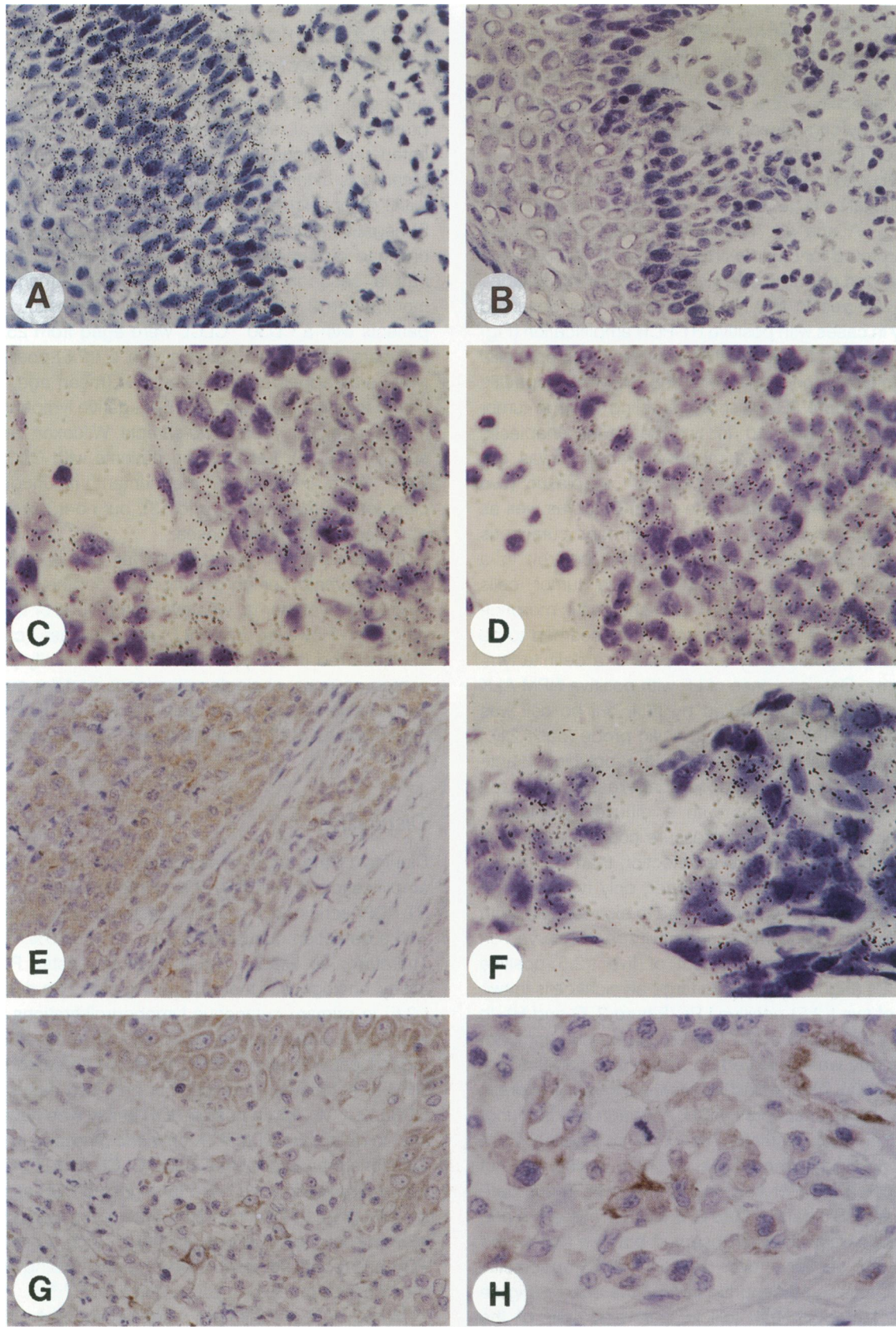
Among the t(2;5)-positive CD30$^+$ CLCLs, no patient presented localized skin lesions, two had disseminated skin lesions, and three had secondary extracutaneous extension (7 months to 3 years after the initial diagnosis). On the other hand, five of the t(2;5)-negative lymphomas presented localized lesions, one had disseminated cutaneous lesions, and two had a secondary extracutaneous involvement (6 months and 2 years after the initial diagnosis). The patients with a CD30$^+$ CLCL were aged from 25 to 88 years, but the six patients with a t(2;5)-positive lymphoma appeared to be younger (mean age, 45 years) than the nine with a t(2;5)-negative lymphoma (mean age, 68 years; two-sample Wilcoxon rank sum, $P = 0.17$). Lastly, three patients with CD30$^+$ CLCL were infected by the human immunodeficiency virus (cases 5, 9, and 13), but no t(2;5) was detected among these cases.

All patients with LP have so far had a typical clinical chronic benign evolution without the occurrence of a lymphoma, whatever the results of t(2;5) detection. However, one t(2;5)-positive case (case 15) occurred in a 5-year-old child who initially presented an unusual single voluminous nodular cutaneous lesion. Thereafter, recurrent typical disseminated lesions of LP were observed in this patient.

Discussion

Cloning of the t(2;5) translocation has allowed the amplification of the NPM-ALK chimeric transcript by RT-PCR technique[26] in CD30$^+$ systemic lymphomas, with a detection rate ranging from 16 to 57.9%.[27–31] Therefore, we used a highly sensitive nested PCR assay, analogous to that of Downing et al,[29] to detect t(2;5) in primary cutaneous lymphoproliferations for which little information is known by conventional cytogenetics. With standard RT-PCR, we detected the NPM-ALK chimeric transcript in only two cases of CD30$^+$ CLCL, and a nested PCR assay was necessary to detect the NPM-ALK chimeric message in six additional cases of both CD30$^+$ CLCL and LP. Our report emphasizes the need for a nested PCR assay to detect t(2;5)-bearing cells that are mixed with reactive cells, as in skin specimens. Such a nested PCR assay was not performed in series dealing with the absence of t(2;5) in CLCL and LP.[23,31,32]

Moreover, our PCR data were confirmed by ISH for the detection of NPM-ALK transcripts and immu-


A
B
C
D
E
F
G
H

nohistochemical detection of p80 protein. ISH allowed the specific identification of the t(2;5)-carrying cells. Moreover, the NPM-ALK transcripts appeared to be uniformly expressed by tumor cells within a definite tumor and not by subsets of tumor cells or bystander cells, in agreement with a recent ISH study of systemic ALCL.[43] The latter study was performed using probes specific for 3′ALK-specific sequences that provide comparable data to p80 immunohistochemical detection. Anti-p80 recognizes a sequence of the putative kinase domain encoded by ALK.[34,35,44] Abnormal NPM-ALK expression in t(2;5)-positive lymphomas leads to p80 immunoreactivity, whereas ALK is not expressed by normal lymphocytes.[26,30,34,35] However, the detection of ALK expression by ISH or immunohistochemistry is not as specific for the t(2;5) as the detection of NPM-ALK transcripts. Indeed, p80 immunoreactivity was observed in one case of nodal ALCL with t(1;2)(q25;p23) translocation.[30] We have observed a p80 immunoreactivity over epithelial, endothelial, and dendritic cells that could correspond to a normal ALK expression within these cells. ISH might distinguish cells expressing either ALK or NPM-ALK transcripts. Moreover, two cases with typical cytogenetic t(2;5) without NPM-ALK expression have been identified.[30,45] In our series, one ALCL with t(2;5) was labeled by ISH for NPM-ALK transcripts, but no p80 immunoreactivity was observed on frozen and paraffin sections. These combined results may indicate that cells carrying the t(2;5) may express neither NPM-ALK transcripts nor protein in some instances.

Samples that were found to contain NPM-ALK transcripts belonged to $CD30^+$ cutaneous lymphoproliferations. The association of t(2;5) with CD30 expression may have biological significance, as interactions between ALK-p80 and CD30 antigen have been suggested by *in vitro* experiments.[44] CD30 expression in primary CLCL has been associated with either a favorable outcome or with spontaneous regression.[2,3] This led to the new classification of CLCL and to distinguishing $CD30^+$ CLCL from both $CD30^-$ CLCL and $CD30^+$ systemic lymphoproliferations.[46–48] Immunohistochemical differences between cutaneous and nodal $CD30^+$ lymphoma have been pointed out, such as the lack of epithelial membrane antigen expression in $CD30^+$ CLCL.[48,49] These data suggest that cutaneous and nodal $CD30^+$ lymphomas are biologically different. Moreover, the presence of the t(2;5) translocation described in systemic $CD30^+$ lymphomas was not found in cutaneous $CD30^+$ lymphomas.[23,31,32] Our study and the report of Lopategui[28] indicate common molecular features between systemic and cutaneous anaplastic lymphomas. The prevalence of t(2;5) translocation in $CD30^+$ lymphomas (42.8%) in our series was close to that of nodal $CD30^+$ lymphomas.[29,30] As for nodal $CD30^+$ lymphoma, the t(2;5) was not specific for anaplastic morphology and was also detected in pleomorphic $CD30^+$ CLCL. Interestingly, all of our CLCLs with t(2;5) were of T cell phenotype or genotype and four out of five expressed epithelial membrane antigen.[11] No NPM-ALK transcript was detected in B or T cell $CD30^-$ CLCL.

These data call into question the absolute distinction between cutaneous and extracutaneous $CD30^+$ lymphomas. The prognosis of $CD30^+$ CLCL may be unfavorable, especially when skin lesions are disseminated,[50] and relapse with systemic involvement may follow spontaneous initial regression.[11] In our series, patients with t(2;5)-positive lymphomas seemed to be younger than patients with t(2;5)-negative lymphomas, as for nodal $CD30^+$ lymphomas.[30,35] They had frequent disseminated skin lesions or secondary extracutaneous extension. Although the prognostic value of t(2;5) in CLCL remains to be evaluated by prospective studies, the t(2;5) may identify a subset of t(2;5)-positive $CD30^+$ primary CLCLs with common molecular and clinical features with t(2;5)-positive $CD30^+$ systemic lymphomas.

LP also belongs to the spectrum of primary cutaneous $CD30^+$ lymphoproliferations, with similar clinical, morphological, and immunophenotypical features between its large atypical cells and those of $CD30^+$ CLCL.[7] Moreover, large atypical $CD30^+$ cells are abundant in type A LP,[42] and the differential diagnosis between skin lesions of LP and cutaneous ALCL may be a difficult challenge in some instances. The presence of t(2;5) in LP is an additional argument for a common pathogenesis between LP and $CD30^+$ CLCL and cannot serve to distinguish the two diseases. Although the presence in LP of a

Figure 3. *ISH with specific NPM* (**A** *and* **C**) *and NPM-ALK* (**B, D,** *and* **F**) *oligoprobes.* **A:** *Note the intense labeling of keratinocytes and inflammatory reactive cells with the NPM probe in a case of LP. H&E stain; magnification, ×250.* **B:** *No signal was observed with the NPM-ALK probe in accordance with no amplification for NPM-ALK for this case. H&E stain; ×250.* **C** *and* **D:** *Large tumor cells of CD30⁺ lymphoma with positive NPM-ALK amplification were labeled both by the NPM* (**C**) *and the NPM-ALK* (**D**) *probes. H&E stain; ×1000.* **E** *and* **F:** *Anaplastic cutaneous lymphoma cells were stained for the p80 protein* (**F**; *×400*) *and labeled for NPM-ALK transcripts* (**E**; *×1000*). **G** *and* **H:** *LP with NPM-ALK amplification. Note the immunostaining for p80 protein of keratinocytes, endothelial cells, and dendritic cells of the dermis* (**G**; *×250*). *Atypical cells with polymorphic convoluted nuclei were also labeled with a moderate cytoplasmic stain* (**G**) *better seen on higher magnification* (**H**; *×1000*).

clonal rearrangement of T-cell receptor genes cannot predict the development of a secondary lymphoma,[16] the clinical relevance of t(2;5) detection for monitoring patients with LP has to be evaluated in large prospective series. In our series, no clonal rearrangement of the T-cell receptor γ-chain gene was found in two cases with t(2;5)-positive LP. This could be explained by a more sensitive detection of clonal lymphoid population when a translocation breakpoint can be amplified by PCR.

Therefore, NPM-ALK amplification by nested RT-PCR may be an efficient tool for the monitoring of diagnosis, staging, and follow-up of a subset of patients with primary cutaneous $CD30^+$ lymphoproliferations. As in nodal ALCL, the search for a t(2;5) would help to subdivide $CD30^+$ primary cutaneous lymphomas into two groups at the time of initial presentation.

Acknowledgments

We thank J. Ferrer and F. Boiteau for expert technical assistance. We also thank the Diagnostics Division of Nicherei Corp. Ltd., Tokyo, for its generous gift of anti-p80 antibody.

References

1. Willemze R, Beljaards RC, Rijaarsdam U: Classification of primary cutaneous large cell lymphomas. Dermatol Clin 1994, 12:361–373
2. Beljaards RC, Kaudewitz P, Berti E, Gianotti R, Neumann C, Rosso R, Paulli M, Meijer CJLM, Willemze R: Primary cutaneous $CD30^+$ large cell lymphoma: definition of a new type of cutaneous lymphoma with a favorable prognosis. Cancer 1993, 71:2097–2104
3. Banerjee S, Heald J, Harris M: Twelve cases of Ki-1 positive anaplastic large-cell lymphoma of the skin. J Clin Pathol 1991, 44:119–125
4. Karp D, Horn T: Lymphomatoid papulosis. J Am Acad Dermatol 1994, 30:379–395
5. Mac Carty M, Vukelja S, Sausville E, Perry J, James W, Jaffe E, Weiss R: Lymphomatoid papulosis associated with Ki-1 positive anaplastic large cell lymphoma. Cancer 1994, 74:3051–3058
6. Beljaards R, Willemze R: The prognosis of patients with lymphomatoid papulosis associated with malignant lymphomas. Br J Dermatol 1992, 126:596–602
7. Kadin M: Lymphomatoid papulosis and associated lymphomas. Arch Dermatol 1993, 129:351–352
8. Wood G, Crooks C, Uluer A: Lymphomatoid papulosis and associated cutaneous lymphoproliferative disorders exhibit a common clonal origin. J Invest Dermatol 1995, 105:51–55
9. Krishnan J, Tomaszewski M, Kao G: Primary cutaneous CD30-positive anaplastic large cell lymphoma: report of 27 cases. J Cutaneous Pathol 1993, 20:193–202
10. Nakamura S, Takagi N, Kojima M, Motoori T, Kitoh K, Osada H, Suzuki H, Ogura M, Kurita S, Oyama A, Ueda R, Takahashi T, Suchi T: Clinicopathologic study of large cell anaplastic lymphoma (Ki-1 positive large cell lymphoma) among the Japanese. Cancer 1991, 68: 118–129
11. Mac Grogan G, Vergier B, Dubus P, Beylot-Barry M, Delaunay MM, Eghbali H, Rivel J, Vital C, De Mascarel A, Bloch B, Merlio JP: $CD30^+$ cutaneous lymphoma: a clinico-pathological and molecular study of 17 cases: Am J Clin Pathol 1996, 105:440–450
12. Sugimoto H, Nakayama F, Yamauchi T, Tokura Y, Iwatsuki K, Takigawa M, Yamada M, Maeda M: Ki-1+ cutaneous lymphoma: Gene rearrangement analysis of tumor cells in tissue and short-term culture of a patient. Arch Dermatol 1988, 124:405–408
13. Whittaker S, Smith N, Jones R, Luzzatto L: Analysis of β, γ, and δ T-cell receptor genes in lymphomatoid papulosis: cellular basis of two distinct histologic subsets. J Invest Dermatol 1991, 96:786–791
14. Weiss L, Wood G, Trela M, Warnke R, Sklar J: Clonal T-cell populations in lymphomatoid papulosis. N Engl J Med 1986, 315:475–479
15. Weiss L, Wood G, Ellisen L, Reynolds T, Sklar J: Clonal T-cell populations in pityriasis lichenoides and variolaformis acuta. Am J Pathol 1987, 126:417–421
16. El-Azhary R, Gibson L, Kurtin P, Pittelkow M, Muller S: Lymphomatoid papulosis: a clinical and histopathological review of 53 cases with leukocyte immunophenotyping, DNA flow cytometry, and T-cell receptor gene rearrangement studies. J Am Acad Dermatol 1994, 30:210–218
17. Theodorou I, Delfau-Larue M, Bigorne C, Lahet C, Cochet G, Bagot M, Wechsler J, Farcet J: Cutaneous T-cell infiltrates: analysis of T-cell receptor-γ gene rearrangement by polymerase chain reaction and denaturing gradient gel electrophoresis. Blood 1995, 86: 305–310
18. Kristoffersson U, Heim S, Heldrup J, Akerman M, Garwicz S, Mitelman F: Cytogenetic studies of childhood non-Hodgkin's lymphomas. Hereditas 1985, 103: 77–84
19. Fischer P, Nacheva E, Mason DY, Sherrington PD, Hoyle C, Hayhoe FGJ, Karpas A: A Ki-1 (CD30)-positive human cell line (Karpas 299) established from a high-grade non-Hodgkin's lymphoma, showing a 2;5 translocation and rearrangement of the T-cell receptor β-chain gene. Blood 1988, 72:234–240
20. Kaneko Y, Frizzera G, Edamura S, Maseki N, Sakurai M, Komada Y, Sakurai M, Tanaka H, Sasaki M, Suchi T, Kikuta A, Wakasa H, Hojo H, Mizutani S: A novel translocation t(2;5)(p23;q35) in childhood phagocytic large T-cell lymphoma mimicking malignant histiocytosis. Blood 1989, 73:806–813
21. Rimokh R, Magaud J, Berger F, Samarut J, Coiffier B, Germain D, Mason DY: A translocation involving a spe-

cific breakpoint (q35) on chromosome 5 is characteristic of anaplastic large cell lymphoma ('Ki-1 lymphoma'). Br J Haematol 1989, 71:31–36

22. Mason DY, Bastard C, Rimokh R, Dastugue N, Huret JL, Kristoffersson U, Magaud JP, Nezelof C, Tilly H, Vannier JP, Hemet J, Warnke R: CD30-positive large cell lymphomas ('Ki-lymphoma') are associated with a chromosomal translocation involving 5q35. Br J Haematol 1990, 74:161–168
23. Kadin M, Peters K, Knoll J: Chromosome abnormalities in lymphomatoid papulosis and cutaneous $CD30^+$ anaplastic large cell lymphoma. United States and Canadian Academy of Pathology Annual Meeting. Toronto, 1995, 657A
24. Chan WY, Liu QR, Borjigin J, Busch H, Rennert OM, Tease LA, Chan PK: Characterization of the cDNA encoding human nucleophosmin and studies of its role in normal and abnormal growth. Biochemistry 1989, 28: 1033–1039
25. Dumbar TS, Gentry GA, Olson MOJ: Interaction of nucleolar phosphoprotein B23 with nucleic acids. Biochemistry 1989, 28:9495–9501
26. Morris S, Kirstein M, Valentine M, Dittmer K, Shapiro D, Saltman D, Look A: Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. Science 1994, 263:1281–1284
27. Builrich F, Morris S, Hummel M, Pileri S, Stein H, Croce C: Nucleophosmin (NPM) gene rearrangements in Ki-1-positive lymphomas. Cancer Res 1994, 54:2873–2877
28. Lopategui J, Sun L, Chan J, Gaffey M, Frierson H, Glackin C, Weiss L: Low frequency association of the t(2;5)(p23;q35) chromosomal translocation with $CD30^+$ lymphomas from American and Asian patients. Am J Pathol 1995, 146:323–328
29. Downing J, Shurtleff S, Zielenska M, Curcio-Brint A, Behm F, Head D, Sandlung J, Weisenburger D, Kossakowska A, Thorner P, Lorenzana A, Ladanyi M, Morris S: Molecular detection of the (2;5) translocation of non-Hodgkin's lymphoma by reverse transcriptase-polymerase chain reaction. Blood 1995, 85:3416–3422
30. Lamant L, Meggetto F, Al Saati T, Brugières L, Bressac de Paillerets B, Dastugue N, Bernheim A, Rubié H, Terrier Lacombe M, Robert A, Brousset P, Rigal F, Schlaifer D, Shiota M, Mori S, Delsol G: High incidence of the t(2;5)(p23;q35) translocation in anaplastic large cell lymphoma and its lack of detection in Hodgkin's disease: comparison of cytogenetic analysis, RT-PCR and p80 immunostaining. Blood 1996, 87:284s
31. Wellmann A, Otsuki T, Vogelbruch M, Clark HM, Jaffe ES, Raffeld M: Analysis of the t(2;5)(p23;q35) translocation by reverse transcription-polymerase chain reaction in $CD30^+$ anaplastic large-cell lymphomas, in other non-Hodgkin's lymphomas of T-cell phenotype, and in Hodgkin's disease. Blood 1995, 86:2321–2328
32. Sarris AH, Luthra R, Papadimitracopoulou V, Dimopoulos MA, McBride JA, Cabanillas F, Morris SW, Deisseroth AB, Pugh WC: A genomic DNA PCR technique demonstrates the presence of the t(2;5) rearrangement in anaplastic large cell lymphoma but not in other non-Hodgkin's lymphomas, Hodgkin's disease, or lymphomatoid papulosis. Blood 1995, 10:715A
33. Beylot-Barry M, Vergier B, Beylot C, DeMascarel A, Doutre MS: Ki1-positive large-cell cutaneous lymphoma mimicking a Stewart-Treves angiosarcoma. Dermatology 1995, 190:77–92
34. Shiota M, Fujimoto J, Takenaga M, Satoh H, Ichinohasama R, Abe M, Nakano M, Yamamoto T, Mori S: Diagnosis of t(2;5)(p23;q35)-associated Ki-1 lymphoma with immunohistochemistry. Blood 1994, 84: 3648–3652
35. Shiota M, Nakamura S, Ichinohasama R, Abe M, Akagi T, Takeshita M, Mori N, Fujimoto J, Miyauchi J, Mikata A, Nanba K, Takami T, Yamabe H, Takano Y, Izumo T, Nagatani T, Mohri N, Nasu K, Satoh H, Katano H, Fujimoto J, Yamamoto T, S M: Anaplastic large cell lymphomas expressing the novel chimeric protein p80 NPM/ALK: a distinct clinicopathologic entity. Blood 1995, 86:1954–1960
36. Norton AJ, Jordan S, Yeomans P: Brief, high-temperature heat denaturation (pressure cooking): a simple and effective method of antigen retrieval for routinely processed tissues. J Pathol 1994, 173:371–379
37. Beylot-Barry M, Vergier B, De Mascarel A, Beylot C, Merlio JP: p53 oncoprotein expression in cutaneous lymphoproliferations: a study of 51 cases. Arch Dermatol 1995, 131:1019–1025
38. Dubus P, Labouyrie E, Bilhou-Nabera C, Belleanée G, Vergier B, Bernard P, De Mascarel A, Merlio JP: Etude de la clonalité B lymphocytaire par la technique d'amplification de gène *in vitro* (PCR) à partir de prélèvements fixés et inclus en paraffine. Ann Pathol 1994, 4:227–233
39. Nizet Y, Martiat P, Vaerman J, Philippe M, Wildmann C, Staelens J, Cornu G, Ferrant A, Michaux J, Sokal G: Follow-up of residual disease (MDR) in B lineage acute leukemias using a simplified PCR strategy: evolution of MDR rather than its detection is correlated with clinical outcome. Br J Haematol 1991, 79:205–210
40. Merlio JP, Ernfors P, Kokaia Z, Middlemas D, Bengzon J, Kokaia M, Smith M, Siesjö B, Hunter T, Lindvall O, Persson H: Increased production of the trKB protein tyrosine kinase receptor after brain insults. Neuron 1993, 10:151–164
41. Willemze R, Beljaards R, Meijer C: Classification of primary cutaneous T-cell lymphomas. Histopathology 1994, 24:405–415
42. Willemze R, Meyer C, Van Vloten W, Scheffer E: The clinical and histological spectrum of lymphomatoid papulosis. Br J Dermatol 1982, 107:131–144
43. Herbst H, Anagnostopoulos J, Heinze B, Dürkop H, Hummel M, Stein H: ALK gene products in anaplastic large cell lymphomas and Hodgkin's disease. Blood 1995, 86:1694–1700
44. Shiota M, Fujimoto J, Semba T, Satoh H, Yamamoto T, Mori S: Hyperphosphorylation of a novel 80 kDa pro-

tein-tyrosine kinase similar to Ltk in a human Ki-1 lymphoma cell line, AMS3. Oncogene 1994, 9:1567–1574
45. Chan W, Elmberger G, Lozano M, Sanger W, Weisenburger D: Large-cell anaplastic lymphoma-specific translocation in Hodgkin's disease (Letter). Lancet 1995, 345:921
46. Willemze R, Beljaards R: Spectrum of primary cutaneous CD30 (Ki-1)-positive lymphoproliferation disorders. J Am Acad Dermatol 1993, 28:973–980
47. Kadin M: Ki-1$^+$/CD30$^+$(anaplastic) large-cell lymphoma: maturation of a clinicopathologic entity with prospects of effective therapy. J Clin Oncol 1994, 12:884–887
48. De Bruin P, Beljaards R, Van Heerde P, Van Der Valk P, Noorduyn LA, Van Krieken JHJM, Kluin-Nelemans JC, Willemze R, Meijer C: Differences in clinical behaviour and immunophenotype between primary cutaneous and primary nodal anaplastic large cell lymphoma of T-cell or null cell phenotype. Histopathology 1993, 23: 127–135
49. Beljaards RC, Meijer CJLM, Scheffer E, Toonstra J, Van Vloten W, Van Der Putte S, Geerts M, Willemze R: Prognostic significance of CD30 (KI-1/Ber-H2) expression in primary cutaneous large-cell lymphomas of T-cell origin. Am J Pathol 1989, 135:1169–1178
50. Kurtin P, DiCaudo D, Habermann T, Chen M, Su D: Primary cutaneous large cell lymphomas: morphologic, immunophenotypic, and clinical features of 20 cases. Am J Surg Pathol 1994, 18:1183–1191