Amplification of Genomic DNA Demonstrates the Presence of the t(2;5)(p23;q35) in Anaplastic Large Cell Lymphoma, But Not in Other Non-Hodgkin’s Lymphomas, Hodgkin’s Disease, or Lymphomatoid Papulosis

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Anaplastic large cell lymphoma (ALCL) is a distinct clinicopathologic variant of intermediate grade non-Hodgkin’s lymphomas (NHL) composed of large pleomorphic cells that usually express the CD30 antigen and interleukin (IL)-2 receptors, and is characterized by frequent cutaneous and extranodal involvement. With variable frequency ALCL bear the t(2;5)(p23;q35) chromosomal translocation that fuses the nucleophosmin (NPM) gene on chromosome 5q35 to a novel protein kinase gene, Anaplastic Lymphoma Kinase (ALK), on chromosome 2p23. We determined the frequency of this translocation with a novel DNA polymerase chain reaction (PCR) technique using 0.5 µg of genomic DNA, 5’-primers derived from the NPM gene and 3’-primers derived from the ALK gene and hybridization with internal probes. The presence of amplifiable DNA in the samples was tested in the inclusion in the PCR reaction of oligonucleotide primers designed to amplify a 3016-bp fragment from the β-globin locus. NPM-ALK fusion amplicons were detected using DNA isolated either from all three ALCL cell lines tested, or from all four primary ALCL tumors known to contain the t(2;5)(p23;q35) translocation. Nested amplicons were detected by hybridization in 100% of specimens diluted 104-fold and in 20% of those diluted 105-fold. We subsequently examined archival genomic DNA from 20 patients with ALCL, 39 with diffuse large cell, 2 with mantle cell, 20 with peripheral T cell, 13 with low-grade NHL, 31 with Hodgkin’s disease (HD), and 6 with lymphomatoid papulosis. Fusion of the NPM and ALK genes was detected in three of 18 patients with ALCL who had amplifiable DNA (17%, 95% confidence intervals 4% to 41%), but not in any patients with other NHL, HD, or lymphomatoid papulosis. The amplicon sizes were different in all cell lines and patients reflecting unique genomic DNA breakpoints. We conclude that with genomic DNA-PCR the rearrangement of the NPM-ALK loci is restricted to patients with ALCL. Further studies are needed to determine the prognostic significance of the NPM-ALK rearrangement, to determine whether its detection can aid in the differential diagnosis between ALCL, Hodgkin’s disease, and lymphomatoid papulosis, and to establish the usefulness of the genomic DNA PCR in the monitoring of minimal residual disease in those patients whose tumors bear the t(2;5).

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and has led some to consider them extremes on a continuous spectrum of malignant lymphoid neoplasms. This morphologic overlap, the clinical and morphologic similarity between lymphomatoid papulosis and ALCL, especially the primary cutaneous form, and the report that a common T-cell clone was involved in cutaneous T-cell lymphoma, HD, and LP that developed over time in one patient has suggested the existence of a biologic relationship between these disorders.

Cytogenetic analysis showed the presence of the t(2;5)(p23;q35) chromosomal translocation with variable frequency in ALCL. With cell lines derived from patients with ALCL, Morris et al at St Jude Children’s Research Hospital demonstrated that the t(2;5)(p23;q35) chromosomal translocation fuses sequences from a nuclear phosphoprotein gene, called nucleophosmin (NPM), that is located on chromosome 5q35 to sequences of a novel gene, designated Anaplastic Lymphoma Kinase (ALK), that is located on chromosome 2p23. The NPM gene is highly conserved and its protein product is involved in the late stages of ribosomal assembly. The ALK gene codes for a novel transmembrane protein kinase that has sequence homology to the β-chain of the insulin receptor, the β-chain of the insulin-like growth factor-1 receptor, the leukocyte tyrosine kinase, and the Drosophila homologue Sevenless. The t(2;5)(p23;q35) translocation results in the formation of a fusion protein consisting of amino-terminal sequences derived from the NPM gene fused to carboxy-terminal cytoplasmic sequences from the ALK gene, including the consensus protein tyrosine kinase residues. The breakpoint locations predicted that this fusion protein would lack the extracellular and transmembrane domains of ALK and would thus be expected to be located intracellularly. In fact, such a cytoplasmic protein kinase was independently isolated as a hyperphosphorylated phosphotyrosine containing protein of Mr 80,000 daltons from a cell line bearing the t(2;5)(p23;q35) translocation and was subsequently demonstrated to be the product of the NPM-ALK gene fusion. Antibodies raised against the kinase domain of the ALK reacted with lymphoma cells in biopsies of all three patients with ALCL bearing a t(2;5)(p23;q35) translocation.

We now report the frequency of the t(2;5)(p23;q35) translocation among adult patients with non-Hodgkin’s lymphoma (NHL), HD, and lymphomatoid papulosis seen at the University of Texas M.D. Anderson Cancer Center (MDACC). We used a newly developed polymerase chain reaction (PCR) assay that uses genomic DNA, 5′-primers derived from the NPM gene, and 3′-primers derived from the ALK gene.

MATERIALS AND METHODS

Reagents and enzymes. All chemicals were reagent grade or better. Restriction endonucleases were obtained from Gibco-BRL (Gaithersburg, MD) and AmpliTaq polymerase from Perkin Elmer-Cetus (Foster City, CA). Antibodies for immunostaining were obtained from DAKO (Carpinteria, CA) and included monoclonal antibodies against CD15 (Leu M-1), CD20 (L26), CD30 (Ber-H2), CD45 (leukocyte common antigen), CD45R0 (UCHL-1), epithelial membrane antigen (EMA), and polyclonal anti-CD3.

Cells and tissues. The t(2;5)(p23;q35)-positive cell lines SUP-M2, SU-DHL-1 and UCONN-L23,4,5 were obtained from St Jude Children’s Research Hospital and were the source of the high molecular weight genomic DNA that was used as a positive control in DNA-PCR amplification experiments. The genomic DNA of four additional patients with ALCL, known to contain the t(2;5)(p23;q35) gene rearrangement were obtained from St Jude Children’s Research Hospital (S.M.). Patients referred to the University of Texas MDACC for diagnosis and treatment of malignant lymphoma were the subjects for this study if archival genomic DNA was available for analysis. Surgical biopsies were obtained for routine diagnostic studies after signed informed patient consent was obtained according to standard institutional guidelines and high molecular weight genomic DNA was extracted from tissue not used for standard diagnostic tests. Immunohistochemical studies were performed on paraffin sections using conventional avidin-biotin-peroxidase methodology. Pathologic classification of NHL was performed according to the REAL classification, of ALCL according to the criteria summarized by Pileri et al, and of HD according to Lukes and Butler. The diagnosis of lymphomatoid papulosis was based on previously described combined clinicopathologic criteria.

Restriction analysis. DNA aliquots were digested with the appropriate restriction enzymes (Hind III, BamHI, Bgl II), blotted onto nylon filters, and hybridized with radioactive probes using standard methods. Because genomic DNA fragments located immediately adjacent to the chromosome 2 breakpoint within the ALK gene were not available at the time of the study, filters were probed with p6-3/1.2S, which is a 1.2 kb Sac I fragment of the NPM gene within 7 kb centromeric of the NPM breakpoint in cell lines SU-DHL-1 and SUP-M2. This fragment detects NPM gene rearrangements in genomic DNA digested with many restriction enzymes. We also used probe p2I-3/3E, which is a 3-kb EcoR I fragment of the NPM gene located approximately 15 kb telomeric of the breakpoint in cell lines SU-DHL-1 and SUP-M2. This probe detects rearranged NPM fragments in genomic DNA restricted with Bgl II. As positive control, we used DNA from the SUP-M2 cell line derived from a patient with ALCL with the t(2;5)(p23;q35) chromosomal translocation, and as control normal human genomic DNA. Because both hybridization probes contained human genomic repetitive DNA sequences, the probes and filters were preannealed with cold competitor DNA.

Genomic DNA PCR (DNA-PCR). This was performed using a modification of a previously described method. We used as outer 5′-end primer oligonucleotide NPM-A, derived from the NPM gene and as 3′-end primer oligonucleotide ALK-A derived from the ALK gene (Fig 1). As a control for the presence of amplifiable DNA able to generate large amplicons, we included a second primer pair that would generate 3016 fragment from the β-globin region. The 5′-prime oligonucleotide was 5′-GAGAAGCCAAAGCACGTTTACA-3′, and the 3′-prime oligonucleotide was 5′-GTGGATGATGCTCCCTACTCC-3′. The final reaction mix contained 0.5 μg of genomic DNA, 10 mmol/L Tris-HCl, pH8.3, 250 μmol/L of each deoxynucleotide triphosphate, 50 mmol/L KCl, 1.5 mmol/L MgCl2, and 50 ng of each primer in a total volume of 50 μL overlain with 35 μL of mineral oil. The reaction tubes were heated at 80°C for 5 minutes before the addition in each tube of 1.5 U of AmpliTag polymerase. After hot start with the enzyme, the samples were denatured for an additional minute at 94°C, and then were subjected to 35 cycles of PCR by denaturing at 94°C for 40 seconds, annealing at 64°C for 1 minute, and extending at 72°C for 4 minutes. The last elongation step lasted for 10 minutes. One microliter of the initial PCR product was used as a template for the nested PCR reaction that was prepared as before, but used as nested 5′-end primer oligonucleotide NPM-B, derived from the NPM gene, and as nested 3′-end primer oligonucleotide ALK-B, derived from the ALK gene (Fig 1). Following a 1-minute denaturation at 92°C, the reaction mix was

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subjected to 20 cycles of amplification, each consisting of a 40-
second denaturation step at 92°C, a 1-minute annealing step at 54°C,
and a 3-minute elongation step at 72°C. The last elongation step
was 10 minutes. The amplified products (18 μL) were analyzed by
electrophoresis on a 0.8% agarose gel, stained with ethidium bromide,
visualized, and photographed under ultraviolet light. The gels
were subsequently transferred manually to Sure Blot membranes
(Oncor, Gaithersburg, MD) in alkaline conditions according to the
instructions provided by the manufacturer. The membranes were
hybridized with a T4 nucleotide kinase end-labeled
NPM-ALK junctional oligonucleotide probe, 5‘-AGCACCTTTAGTAGTGACGC
GGA-3’, and labeled fragments were detected by autoradiography.

Clinical information. The records of all patients were retrieved
and reviewed to establish the clinical presentation, laboratory and
immunologic characteristics of the tumors, the Ann Arbor stage91
and the International Prognostic Index score.92 Final stage was
rearranged in a multidisciplinary conference where all clinical, radio-
logic, and laboratory data were reviewed by participating hematolo-
gists, medical oncologists, radiotherapists, and radiologists. The 95%
confidence intervals were calculated with the BICSI software (De-
partment of Biomathematics, University of Texas MDACC, Hous-
ton, TX).

RESULTS
To establish the PCR assay, we first amplified genomic DNA isolated from three cell lines derived from patients
with ALCI shown to carry the t(2;5)(p23;q35) by classical
cytogenetics, restriction enzyme analysis, and reverse tran-
scriptase PCR.28 Amplification of 0.5 μg of genomic DNA
gave rise to amplicons of 1,603, 1,677, and 1,715 bases (Fig
2A, lanes 9 to 14), with comparable yield by either outer
primer or nested PCR. The different sizes of these amplicons
were confirmed by DNA sequencing that demonstrated dif-
ferent molecular breakpoints in each (data not shown). With
serial dilution of 0.5 μg of genomic DNA (corresponding
approximately to 60,000 to 80,000 cells) from the UCONN-
L2 cell line into normal genomic DNA, it was demonstrated
that nested amplicons were consistently detected by autoradi-

Fig 1. Schematic of the normal NPM and ALK proteins and of the
NPM-ALK fusion protein produced by the t(2;5). Downward-pointing
arrows represent the positions at which the NPM and ALK cDNAs
are interrupted by the t12;51 translocation and are joined to create
NPM-ALK. These translocations are known to occur within intron
sequences, but because the genomic organization of these two genes
is not known, we do not show the introns here. The metal binding
domain (MB), acidic amino acid cluster (ACI), and nuclear localization
sequences (N) of NPM are indicated, as is the transmembrane (TM)
segment of ALK. The approximate positions on the cDNA of the outer
(NPM-A and ALK-A) and inner (NPM-B and ALK-B) primer pairs used
in these studies is designated under NPM-ALK, and their complete
sequences are shown at the bottom of the figure. The fused NPM-
ALK genomic locus the NPM and ALK sequences will be separated
by an intron containing different proportions of NPM and ALK intron
sequences. The length of this intron will vary depending on the pre-
cise breakpoint location, but its length cannot exceed the sum of the
lengths of the corresponding NPM and ALK introns, thus setting an
upper limit on the length of the expected amplicons.

Fig 2. Detection of NPM-ALK rearrangement in cell lines and test
patients genomic by DNA-PCR. High molecular weight genomic DNA
(0.5 μg) was subjected to plain amplification or nested amplification
as described in Materials and Methods, followed by product separa-
tion on 0.8% TAE agarose gels. (A) Etidium bromide staining.
Odd-numbered lanes depict amplification with outer primer pairs,
even-numbered lanes depict nested amplification. Lanes 1 and 2, test
patient 1; lanes 3 and 4, test patient 2; lanes 5 and 6, test patient 3;
lanes 7 and 8, test patient 4; lanes 9 and 10, cell line UCONN-L2; lanes
11 and 12, SU-DHL-1; lanes 13 and 14, SUP-M2. (B) Autoradiography.
Genomic DNA (0.5 μg) derived from the UCONN-L2 cell line was
diluted with normal genomic DNA as indicated at the top of each
lane, then subjected to nested PCR amplification, agarose gel electro-
phoresis, transfer to membranes, hybridization, and autoradior-
ography. The Haelll digest of the φX174 DNA was used as molecular
weight markers and was loaded in the left lane with the correspon-
ding sizes in bases marked on the left side of the figure. The hybridiza-
tion reaction was performed with the junctional oligonucleotide and
also with a probe for the detection of the φX174 DNA.
Table 1. Molecular Detection of Rearrangement Involving the NPM and ALK Genes in Patients With Lymphoma

<table>
<thead>
<tr>
<th>Histology</th>
<th>Restriction Analysis</th>
<th>Genomic DNA PCR</th>
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<tr>
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<td>NPM Rearranged</td>
<td>t(2:5) Frequency</td>
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<tr>
<td>ALCL</td>
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<tr>
<td>Other NHL</td>
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</tr>
<tr>
<td>Hodgkins Disease</td>
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<tr>
<td>Lymphomatoid papulosis</td>
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Abbreviation: CI, confidence interval.

* Includes 39 patients with diffuse large cell, 20 with peripheral T cell, 2 with mantle cell, 5 with follicular, 4 with mucosa-associated, and 4 with small lymphocytic lymphomas. Three patients with diffuse large cell lymphoma reflected Richter's transformation of chronic lymphocytic leukemia.

ography (Fig 2B) when tumor DNA was diluted 104-fold. However, a dilution of 105-fold, corresponding to 0.6 to 0.8 genomes per reaction, generated detectable nested amplicons in only 20% of PCR reactions, as would be expected from Poisson statistics (data not shown). The genomic DNA-PCR assay was subsequently validated using high molecular weight DNA from four patients from St Jude Children's Research Hospital who had ALCL known to carry the t(2;5)(p23;q35) rearrangement on the basis of classical cytogenetics, restriction enzyme analysis, and reverse transcriptase PCR. Amplification of DNA generated four different amplicons of 446, 1123, 1534, and 1665 bases from these patients (Fig 2A, lanes 1 to 8). Amplification with outer primers generated amplicons that were easily detectable by ethidium bromide staining in two (Fig 2A, lanes 3 and 5), and faintly detectable amplicons in the remaining two patients (Fig 2A, lanes 1 and 7). However, amplification with nested primers generated amplicons that were easily detected with ethidium bromide staining in all four test patients (Fig 2A, lanes 2, 4, 6, and 8). However, hybridization easily detected all four amplicons produced with either the outer primer pair or the nested PCR (data not shown).

We subsequently used this nested PCR assay followed by hybridization and archival genomic DNA to determine if the t(2;5)(p23;q35) was present among 94 patients with NHL, 31 with HD, and 6 with lymphomatoid papulosis. The t(2;5) was detected by PCR in three of 18 patients with ALCL who had amplifiable DNA with an overall frequency of 17% and with 95% confidence intervals (CI) of 4% to 41%. The rearrangement was not detected in any patient with other NHL histology, HD, or lymphomatoid papulosis with either the PCR technique or by restriction enzyme analysis (Table 1).

The clinical and laboratory characteristics of the 20 patients with ALCL are listed in Table 2. The 13 males and seven females ranged from 9 to 87 years in age and were all negative for antibodies to human immunodeficiency viruses. Two patients presented with primary cutaneous form, 16

Table 2. Clinical and Laboratory Characteristics of Patients With ALCL and Detection of NPM-ALK Rearrangements

<table>
<thead>
<tr>
<th>Patient</th>
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<th>IPI Score</th>
<th>TCR-β</th>
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<th>CD30</th>
<th>EMA</th>
<th>IP</th>
<th>NPM Locus</th>
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</table>

Abbreviations: U, unstageable; NA, not available; ND, not done; R, rearranged; G, germline; TCR, T-cell receptor; IG, immunoglobin heavy chain; CLL, chronic lymphocytic leukemia; IPI, International Prognostic Index; EMA, epithelial membrane antigen; IP, immunophenotype.
with the classical nodal ALCL, and two developed ALCL in the background of treated small lymphocytic lymphoma and chronic lymphocytic leukemia, both of B-cell phenotype. The lymphoma cells were CD30-positive in 17 (85%), and were of T-cell lineage in 12 (60%), B-cell lineage in five (25%), and null in three patients (15%). In the two patients with preexistent low-grade B-cell lymphoproliferative disorders, the ALCL were null cell in one and T cell in the other (patients 8 and 10, respectively). Five patients (25%) were initially diagnosed as HD (patients 1, 4, 5, 14, and 19) and four (patients 1, 4, 5, and 14) were actually treated as HD before referral to MDACC where the diagnosis was changed to ALCL based on morphology and immunophenotype. Patient 2 was initially diagnosed and treated as seminoma with platinum, etoposide, and bleomycin without response. The diagnosis was subsequently changed to HD and he was treated with mechlorethamine, vincristine, procarbazine, and prednisone and he achieved a complete remission that lasted 6 months. He was then referred to MDACC where the diagnosis was changed to ALCL. Patient 19 was initially diagnosed as HD, but was referred for an opinion to MDACC where the diagnosis was changed to ALCL, Hodgkin’s-related variant, before any therapy was given. The changes in diagnosis were also supported in three of these five patients by clonal rearrangement of the immunoglobulin genes in one (Table 2, patient 4), of the β-chain of the T-cell receptor in two (patients 5 and 19). All diagnostic revisions were completed before to the analysis of NPM-ALK rearrangement.

The rearrangement of the NPM locus was detectable with restriction analysis of genomic DNA using probes derived from the NPM gene in two patients of 17 and 37 years of age (Table 2, patients 2 and 7). Nested amplification of genomic DNA generated amplicons of 1,190 and 2,200 bases, confirming the NPM-ALK rearrangement (Table 2, patients 2 and 7 respectively, and Fig 3, lanes 2 and 7). One 9-year-old patient without detectable NPM rearrangement by restriction analysis exhibited an NPM-ALK amplicon of 900 bases by nested DNA PCR (Table 2 patient 5, and Fig 3, lane 5). The sizes of these amplicons were clearly distinguishable from each other and from the amplicons generated from the cell lines or from the four validation patients (Fig 1A). All three patients with the NPM-ALK rearrangement presented with nodal disease, two were both T cell and CD30-positive (patients 2 and 5), whereas the third was null cell and CD30-negative (patient 7). Pathologically two patients had classical and one monomorphic ALCL. Overall, in this group of 18 patients with ALCL and amplifiable DNA, the NPM-ALK rearrangement was observed in 17% (95% CI of 4% to 41%). If the two patients with the primary cutaneous ALCL and the one with preexisting low grade lymphoma are excluded, the translocation was detected in three of 15 patients with nodal ALCL for an overall frequency of 20% and 95% CI extending from 4% to 48%. Similarly, among these 15 patients with primary nodal ALCL, the translocation was seen in three of nine who were younger than 40 years (33%, 95% CI 7% to 70%). The diagnosis of ALCL was correctly established at presentation in all three patients bearing the NPM-ALK gene rearrangement. One of them relapsed after 5 months and expired after 11 months from disseminated lymphoma despite appropriate initial and salvage therapy (Table 2, patient 2). The other two remain in unmaintained complete remission at 27 and 50 months after presentation (Table 2, patients 5 and 7).

**DISCUSSION**

We report the frequency of the NPM-ALK chromosomal translocation in patients with lymphoma and lymphomatoid papulosis with a nested PCR technique using genomic DNA, 5'-primers derived from the NPM gene and 3'-primers derived from the ALK gene. This technique detects the NPM-
ALK rearrangement without the requirement for gene trans-
scription implicit in reverse transcriptase-based PCR tech-
niques and can be performed using genomic DNA that is
routinely prepared in most pathology laboratories without
the need for special tissue processing for extraction of
DNA-PCR generates amplicons of different size for each
patient. This is a reflection of different molecular
introns. As restriction analysis has demonstrated tight clus-
tering of these breakpoints, it is likely that the DNA
PCR will detect all or essentially all NPM-ALK rearrangements,
and will be much easier to perform that the more cumbersome reverse transcriptase PCR. In addition, in contrast to
the reverse transcriptase PCR that generates amplicons of
same size for essentially all patients, the genomic DNA-PCR generates amplicons of different size for each
patient. This is a reflection of different molecular breakpoints, and provides an additional internal control
against positive amplifications resulting from sample cross-
contamination in the laboratory.

In our series, the molecular recombination of NPM and
ALK genes resulting from the t(2;5)(p23;q35) chromosomal translocation was confined to patients with ALCL, in whom the frequency was 17% with 95% confidence intervals
ranging from 4% to 41%. In our hands, the rearrangement was
limited to those with nodal ALCL where its frequency was
20% with 95% CI ranging from 4% to 46% and was
restricted to those with T cell or null cell immunophenotype.
We did not detect the rearrangement in nonanaplastic large cell lymphomas, other NHLs, HD, or lymphomatoid pa-
pulosis. In ALCL, the reported frequency of NPM-ALK rearrangement as determined by RT-PCR has ranged from 12%
to 80%2,3,3,3,16,41,42,47-51,54 (Table 3). The figure of 54% re-
ported by investigators at St Jude Children’s Research Hos-
pital50 is probably skewed because they included in this
group patients with a cytogenetically known t(2;5)(p23;q35)
who were predominantly pediatric, in whom classical cyto-
genetic analysis suggests a higher frequency of the translo-
ation.15,24-27 One small study of patients with ALCL has re-
ported a frequency of 80% for NPM-ALK rearrangements,49
which is much higher than the frequency reported in pediatric or adult ALCL by either cytogenetics or by molecular meth-
ods by other investigators (Table 3). However, because of
the small number of patients examined, the 95% CI ranged
from 28% to 99% and it is not clear whether this higher apparent frequency is statistically higher than most results
reported in Table 3. The frequency of the NPM-ALK gene
rearrangement among all 425 reported ALCL patients is
33%, with 95% CI extending from 29% to 38% (Table 3).
The presence of the t(2;5) has also been reported in pediatric peripheral T-cell lymphoma,55 T-cell lymphomas of small irregular lymphocytes with few CD30 positive cells,7 and in
nonanaplastic CD30-positive or negative pediatric NHL.27
However, it is not yet clear whether this truly indicates the
presence of the rearrangement outside the spectrum of ALCL
or simply variations in the pathologic subclassification of
NHL and ALCL, especially its monomorphic variant.47

A Japanese series of patients with ALCL has suggested
that the NPM-ALK rearrangement (detected immunocyto-
chemically) was associated with better 5-year survival.52
However, positive patients were younger and had lower rates
of extranodal involvement than negative patients. These con-
siderations, coupled with the absence of data on initial and
postrelapse therapy, on the distribution of other prognostic
factors, and on the presence of human T-lymphotropic virus-
I (HTLV-I) antibodies among the patients analyzed, makes
the prognostic significance of the NPM-ALK rearrangement
an issue that requires further investigation. The exact role
of the t(2;5)(p23;q35) translocation in the pathogenesis of
ALCL remains to be fully defined and larger numbers of
patients are needed to determine its prognostic significance.

The NPM-ALK gene rearrangement was not detected in
patients with HD by us (95% confidence interval 0% to 13%) and seven other series (see Table 4), but has been detected in four series with frequencies ranging from 5% to 85%.39,45-49
Orscheshek et al49 reported the highest frequency of 85%.

Table 3. Frequency of NPM-ALK Rearrangement in ALCL

<table>
<thead>
<tr>
<th>Author</th>
<th>Method</th>
<th>Patients</th>
<th>Positive</th>
<th>% Positive</th>
<th>95% CI</th>
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<td>RT-PCR</td>
<td>28</td>
<td>15</td>
<td>54*</td>
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<tr>
<td>Lopetagou et al54</td>
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<td>4</td>
<td>12</td>
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<tr>
<td>Nagay et al56</td>
<td>RT-PCR</td>
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<td>2</td>
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<td>2-40</td>
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<td>Wellman et al57</td>
<td>RT-PCR</td>
<td>24</td>
<td>11</td>
<td>46</td>
<td>16-57</td>
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<tr>
<td>Yee et al58</td>
<td>RT-PCR</td>
<td>17</td>
<td>5</td>
<td>29</td>
<td>10-56</td>
</tr>
<tr>
<td>Waggot et al59</td>
<td>DNA-PCR Southern</td>
<td>5</td>
<td>5</td>
<td>100*</td>
<td>40-100</td>
</tr>
<tr>
<td>Bullrich et al60</td>
<td>Southern</td>
<td>16</td>
<td>2</td>
<td>13</td>
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</tr>
<tr>
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<td>5</td>
<td>4</td>
<td>80</td>
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<td>Shiota et al62,63</td>
<td>IM, RT-PCR</td>
<td>105</td>
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<tr>
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<td>Sarris et al (present series)</td>
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<td>141</td>
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Abbreviations: C, cytogenetic analysis; RT, reverse transcriptase; ISH, in-situ hybridization; IM, immunocytochemistry.
* Only cases that were morphologically ALCL.
† Excludes the five cases of Waggot et al59 because this was not a prevalence study.
DETECTION OF t(2;5) WITH GENOMIC DNA PCR

which was similar to the frequency of 80% reported by that group in unselected cases of ALCL.49 This extraordinarily high frequency is clearly outside the CI reported by most investigators for HD (Table 4) and remains unexplained. The frequency of the NPM-ALK rearrangement among all 368 screened cases of HD is 5%, with 95% CIs extending from 3% to 8%, and is much lower than the frequency of 33% for all reported ALCL patients (Tables 3 and 4). However, it is unclear whether all the reported HD patients bearing the NPM-ALK rearrangement had in fact HD, because the distinction of ALCL from HD can be difficult at times.2,11,22,37 This was evident in our series, because 25% of our patients with ALCL were initially diagnosed as having HD. Pathology review at MDACC resulted in revision of these diagnoses to ALCL based on morphologic criteria, immunophenotyping, and the detection of clonal rearrangements of the immunoglobulin and/or the T-cell receptor β-chain loci.

We conclude that the nested genomic DNA-PCR technique is useful for the rapid detection of the NPM-ALK rearrangement, that this rearrangement is confined to patients with ALCL, and is absent in other NHL, HD, and lymphomatoid papulosis. Further studies are needed to determine the prognostic significance of NPM-ALK rearrangement, to resolve whether its detection can aid in the differentiation of ALCL from HD or lymphomatoid papulosis, and to establish the distinction of ALCL from HD or lymphomatoid papulosis. Further studies are needed to determine the prognostic significance of NPM-ALK rearrangement, to resolve whether its detection can aid in the differentiation of ALCL from HD or lymphomatoid papulosis, and to establish the distinction of ALCL from HD or lymphomatoid papulosis.

ACKNOWLEDGMENT

We thank Jackie Creeks for assistance with the manuscript, Michael Cook for assistance with illustrations, and Dr Maureen Goode for scientific editing. We also express our gratitude to the physicians, nurses, social workers, and fellows of the Lymphoma Section who over the years cared for the patients included in this study.

REFERENCES


Table 4. Frequency of NPM-ALK Rearrangement in HD

<table>
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<th>Author</th>
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</tr>
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</table>

Abbreviations: RT, reverse transcriptase; ISH, in situ hybridization; IM, immunocytochemistry; C, cytogenetic analysis.


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Amplification of genomic DNA demonstrates the presence of the t(2;5) (p23;q35) in anaplastic large cell lymphoma, but not in other non-Hodgkin’s lymphomas, Hodgkin’s disease, or lymphomatoid papulosis

AH Sarris, R Luthra, V Papadimitracopoulou, M Waasdorp, MA Dimopoulos, JA McBride, F Cabanillas, M Duvic, A Deisseroth, SW Morris and WC Pugh