Detection of Chromosome 11q13 Breakpoints by Interphase Fluorescence In Situ Hybridization

A Useful Ancillary Method for the Diagnosis of Mantle Cell Lymphoma

Ruth L. Katz, MD,1 Nancy P. Caraway, MD,1 Jun Gu, MD,1 Feng Jiang, MD,1 Lori A. Pasco-Miller, MT,1 Armand B. Glassman, MD,1 Rajyalakshmi Luthra, PhD,1 Kimberly J. Hayes, CLSP(CG),1 Jorge E. Romaguera, MD,2 Fernando F. Cabanillas, MD,2 and L. Jeffrey Medeiros, MD1

Key Words: Mantle cell lymphoma; Fluorescence in situ hybridization; Chromosome 11q13; Cyclin D1

Abstract

We assessed cytologic specimens from 11 mantle cell lymphomas (MCLs) and 32 other B-cell non-Hodgkin lymphomas (NHLs) for 11q13 breakpoints using a 2-color fluorescence in situ hybridization (FISH) assay that uses an 11q13 probe centered on the CCND1 gene and a centromeric chromosome 11 probe (CEP11). The number of nuclei in 200 cells were counted, and results were expressed as an 11q13/CEP11 ratio. All MCLs showed a high percentage of interphase nuclei with 3 or more 11q13 signals (mean, 74.8%; range 57%-90%). In contrast, in other B-cell NHLs the mean percentage of cells with 3 or more 11q13 signals was 9.2%. All MCLs had an elevated 11q13/CEP11 ratio (mean, 1.38). The mean ratio for other B-cell NHLs was 0.99. Two non-MCL cases, 1 large B-cell and 1 B-cell unclassified NHL, had high 11q13/CEP11 ratios of 1.15 and 1.30, respectively. Conventional cytogenetic analysis performed on the former case revealed a t(5;11)(q31;q13). Interphase FISH analysis using 11q13 and CEP11 probes is a convenient ancillary method for assisting in the diagnosis of MCL. This commercially available assay is simple to use on cytology or imprint specimens, and results can be obtained within 24 hours.

Mantle cell lymphoma (MCL) is a clinically aggressive, generally incurable type of non-Hodgkin lymphoma (NHL) representing up to 6% of all NHLs in the United States.1,2 MCL can be clinically and histologically difficult to distinguish from other types of small B-cell NHL and leukemia, such as follicular small cleaved cell or small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL), which have very different prognoses and treatment.2

One way to support the diagnosis of MCL is to identify the t(11;14)(q13;q32), now widely accepted as the hallmark of MCL, in which the CCND1 (PRAD-1, bcl-1) gene at 11q13 is juxtaposed with the joining region of the immunoglobulin (Ig) heavy chain gene at 14q32.2,3 The CCND1 gene is up-regulated, resulting in overexpression of cyclin D1, which acts at the G1 to S phase checkpoint of the cell cycle to drive cell proliferation.4

Unfortunately, currently available methods to demonstrate the t(11;14) have substantial limitations. Conventional cytogenetics can detect the t(11;14) in up to 75% of MCLs, but karyotyping is a cumbersome procedure that requires fresh tissue, meticulous sample preparation, and adequate metaphases.5 Southern blot analysis can detect bcl-1 locus rearrangements in up to 70% of cases, but this approach requires multiple probes and is laborious and time consuming.6,7 Polymerase chain reaction (PCR) methods are available to detect the t(11;14) involving the major translocation cluster (MTC) region of bcl-1, but only 30% to 40% of MCLs have chromosome 11 breakpoints that occur in the MTC region.8 Other chromosome 11 breakpoints cannot be assessed routinely by PCR methods at this time.
Alternatively, others have assessed cyclin D1 expression in MCL, because the t(11;14) results in cyclin D1 overexpression regardless of breakpoint location. However, methods to assess cyclin D1 expression also have limitations. Northern blot methods are time consuming and laborious and require relatively large samples of fresh or frozen tissue from which high quality RNA can be extracted. Reverse transcriptase–PCR methods require much less RNA but are not well suited for quantitative methods. Immunohistochemical assays for cyclin D1 overexpression are valuable in the assessment of tissue sections. However, these methods are not optimized for assessing cytologic smears or cytocentrifuged slides, and, in our experience, the results using cytologic preparations are less reliable than those obtained using tissue sections.

For these reasons, we became interested in fluorescence in situ hybridization (FISH) methods and, in particular, a recently developed, commercially available FISH assay that simultaneously assesses 11q13 and centromeric chromosome 11 loci in interphase nuclei. Although this assay assesses only chromosome 11, the ease and convenience of this approach is attractive. In a cohort of 11 MCL cases and 32 non-MCL B-cell NHLs, all 11 MCLs had 3 or more 11q13 breakpoints. In contrast, other types of B-cell NHL rarely had a substantial number of cells with more than 2 11q13 signals.

Materials and Methods

Tissue Samples

For most samples tested, cells were obtained by fine-needle aspiration of nodal-based cases of MCL, follicular lymphoma, large B-cell lymphoma, and B-cell SLL/CLL.

Table 1. Following Ficoll-Hypaque gradient separation of aspirated lymphoid cells collected in RPMI, mononuclear cells were prepared by cytocentrifugation on silane-coated slides at a concentration of 2 × 10⁶ per mL. A minimum of 12 cytocentrifuged preparations were prepared and submitted for FISH or immunohistochemical studies. In a few cases, imprint preparations were prepared from unfixed excisional biopsy specimens of lymphoid neoplasms. The slides were obtained by touching fresh tissues against silane-coated slides and fixing the slides in Carnoy solution.

In all cases, cells were analyzed by 3-color flow cytometry immunophenotypic methods using a battery of monoclonal antibodies specific for lymphoid antigens including Ig kappa and lambda light chains, CD3, CD5, CD10, CD11c, CD19, CD20, CD23, and FMC7 (Becton Dickinson, San Jose, CA).

The diagnosis of each case was based on a combination of morphologic and immunophenotypic findings. Thirty-one of 32 patients in the study also underwent tissue biopsy, in most cases within 1 year of the time the fine-needle aspiration specimen for the present study was obtained, and the diagnosis of NHL was confirmed. For a subset of patients with follicular NHL, in some cases the excisional biopsy specimen was involved by a tumor of different grade than that observed in the fine-needle aspiration specimen. The 1 patient without tissue biopsy had a primary effusion lymphoma.

DNA Probes

The locus-specific 11q13 probe is labeled with spectrum orange. This probe is 300 kilobases (kb) long and is centered on the CCND1 gene (Figure 1). The alpha-satellite centromeric chromosome 11 probe (CEP11) is labeled with spectrum green. This probe hybridizes to band region 11p11.1-q11. Both probes are premixed and commercially available (Vysis, Downers Grove, IL).

FISH Method

Slides were pretreated in a series of solutions: 0.1N hydrochloric acid–0.2% Triton-X 100 in 2x sodium chloride citrate (SSC); 2x SSC; 1x phosphate-buffered saline (PBS); 1% formaldehyde–PBS; and PBS in 2x SSC. The slides then were denatured in 70% formamide at 74°C for 5 minutes and dehydrated in a series of cold alcohol solutions. The slides were dried on a 45°C slide warmer for 5 minutes. A mix of 0.6 µL of probe, 1.2 µL of sterile water, and 4.2 µL of hybridization buffer was denatured for 5 minutes at 74°C and placed on ice for 15 minutes. The probes then were applied to slides, covered, and sealed with rubber cement. Washings after overnight hybridization at 37°C were as follows: 3 rinses in 50% formamide–2x SSC at 45°C for 10 minutes each; 1 rinse in 2x SSC at 45°C for 10 minutes; and 1 rinse in 2x SCC–0.1% Nonidet P-40 at 45°C for 5 minutes. Interphase nuclei were counterstained with propidium iodide.
with 1 µg/mL 4′,6-diamidino-2-phenylindole (DAPI)-containing antifade solution.

**Visualization and Scoring of FISH Signals**

Hybridization sites were analyzed using a Labophot-2 microscope (Nikon, Tokyo, Japan) equipped with appropriate filter sets for visualizing spectrum green or orange and DAPI fluorescence signals. At least 200 nuclei from each slide were scored. Slides were analyzed only if 80% of the nuclei in the field of view were interpretable. Each nucleus was scored individually for the number of 11q13 and CEP11 signals, simultaneously. To avoid misinterpretation owing to false monosomies or deletions due to insufficient hybridization efficiency, nuclei were counted only if at least 1 bright CEP11 signal (green) and 1 bright 11q13 (orange) signal were present. Only nonoverlapping intact nuclei were scored. Split centromere signals were counted as 1, and minor centromere signals were disregarded. We used normal lymphocyte nuclei as an external control to assess hybridization efficiency **Image 1**. Two 11q13 signals were counted as 1 signal if they were near each other (0.5 µm or less), to avoid misinterpretation owing to sister chromatids of cells in S or G2 phase.

As a convenient measure of the 11q13 and CEP11 signals, a 11q13/CEP11 ratio was calculated. The 11q13/CEP11 ratio allows one to distinguish cases with extra copies of chromosome 11 (polysomy), in which the 11q13/CEP11 ratio remains near 1, from cases with translocations or amplifications specifically involving 11q13, in which the cyclin 11q13/CEP11 ratio is elevated **Figure 2**. **Table 2** illustrates 1 case of MCL in which the percentage of cells with 3 or more 11q13 signals was 87%, with 0.5% of cells with 3 or more CEP11 signals. The 11q13/CEP11 ratio in this case was 1.63.

**Figure 1** Map of the chromosome 11q13 locus. The 300-kilobase locus-specific 11q13 probe (Vysis, Downers Grove, IL) is shown in orange. The **CCND1** gene is shown in green. Other nearby loci also are indicated.

**Image 1** Fluorescence in situ hybridization results in normal lymphocytes (control). Two 11q13 (orange) and 2 CEP11 (centromeric chromosome 11 probe; green) signals are present (cytocentrifuged preparation, ×32). The 11q13 and CEP11 probes are from Vysis, Downers Grove, IL.

**Table 2**

<table>
<thead>
<tr>
<th>Probe/Color</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6 or More</th>
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</thead>
<tbody>
<tr>
<td>11q13/orange</td>
<td>0</td>
<td>13</td>
<td>54</td>
<td>31</td>
<td>1.5</td>
<td>0.5</td>
</tr>
<tr>
<td>CEP11/green</td>
<td>3</td>
<td>96.5</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The ratio calculation was as follows:

Total 11q13: (0 × 1) + (13 × 2) + (54 × 3) + (31 × 4) + (0.5 × 5) + (0.5 × 6) = 322.5

Total CEP11: (3 × 1) + (96.5 × 2) + (0.5 × 3) + (0 × 4) + (0 × 5) + (0 × 6) = 197.5

11q13/CEP11 = 322.5/197.5 = 1.63

* Data given are percentages; 200 cells were counted. The probes were an 11q13 probe centered on the **CCND1** gene and a centromeric chromosome 11 probe (CEP11) (Vysis, Downers Grove, IL).
We also have noted rare cases in which an individual cell nucleus had a cluster of 11q13 signals, probably representing amplification. Within an individual nucleus, probable amplification of 11q13 was considered to be present when 11q13 signals were 3 times greater than CEP11 signals.

Serial Dilution Experiments

Serial dilutions were performed to determine the minimum percentage of abnormal cells that can be detected using this FISH technique. Two cell lines, Z-138 and Z-181, were used (donated by Zvi Estrov, MD, Houston, TX). Z-138 was established from a patient with CLL whose disease underwent transformation to an aggressive mature B-cell acute lymphoblastic leukemia.13 This cell line exhibited multiple cytogenetic abnormalities, including the t(8;14), t(14;18), and t(11;14), resulting in marked overexpression of cyclin D1 messenger RNA. More than 95% of the interphase nuclei have 3 11q13 and 2 CEP11 signals. Z-181 was established from bone marrow cells obtained from a patient with acute myeloid leukemia (French-American-British classification M1).14 This cell line has normal chromosome 11 number and structure, and interphase nuclei have 2 11q13 and 2 CEP11 signals. Z-138 cells were diluted with the Z-181 cells in order that the Z-138 cells represented the following percentages of all cells: 50%, 12.5%, 6.3%, 3.2%, 1.6%, and 0.8%. Cytocentrifuged slides were prepared from these dilutions, which were used for FISH. After hybridization and washing, the percentage of nuclei with abnormal numbers of 11q13 signals was determined and compared with projected values.

Cytogenetic Analysis

Conventional G-band karyotype analysis was performed on 13 specimens. Cells from lymph nodes, bone marrow, and effusions were placed in 10 mL of Ham F10 medium with 20% fetal calf serum at a concentration of 2 to 4 × 10^6 nucleated cells per milliliter. Cultures were incubated overnight at 37°C. Standard harvesting procedures were used. Colcemid (0.1 mL/10 mL) was added to the culture for 30 minutes. For the hypotonic treatment, a 0.075-mol/L concentration of potassium chloride was used for 30 minutes at room temperature. The fixation procedure consisted of 3 changes of methanol/glacial acetic acid (3:1) with a 10-minute waiting period between each change. The Thermotron drying chamber (Thermotron Industries, Holland, MI) was used for slide preparation. Slides were placed in a 60°C oven overnight in preparation for GTG banding. For cytogenetic analysis, a maximum of 20 metaphases was analyzed. The karyotype formulas were written using the International System for Human Cytogenetic Nomenclature.15

![Figure 2](https://example.com/figure2.png)

**Figure 2** Idiogram depicting t(11;14)(q13;q32). A, Normal chromosomes 11 and 14. B, Chromosomes 11 and 14 after t(11;14). C, Normal interphase cell with 2 11q13 and 2 CEP11 (centromeric chromosome 11 probe) signals. D, Interphase cell with t(11;14) with 3 11q13 and 2 CEP11 signals. 11q13 is indicated in orange, and CEP11 is indicated in green. The 11q13 and CEP11 probes are from Vysis, Downers Grove, IL.

![Image 2](https://example.com/image2.png)

**Image 2** Fine-needle aspiration specimen showing a case of mantle cell lymphoma. The neoplastic cells are small with irregular nuclear contours. Large cells are absent. Flow cytometry immunophenotypic studies showed that the neoplastic cells expressed monotypic immunoglobulin, B-cell antigens, and CD5 and were negative for CD10 and CD23 (rapid Romanowsky, x50).
Results

The study group included 11 cases of MCL: 6 classic small cell and 5 blastoid (Table 1). All MCLs expressed monotypic Ig light chain (6 Ig kappa and 5 Ig lambda) and the CD5, CD19, CD20, and FMC7 antigens and were negative for CD10, CD11c, CD23, and T-cell antigens. The mean age of the patients with MCL was 66.8 years (range, 47-73 years); there were 9 men and 2 women.

The study group also included 32 other types of B-cell NHL: 18 follicular lymphomas of all grades, 10 large B-cell lymphomas, 3 cases of B-cell SLL/CLL, and 1 unclassified B-cell lymphoma. Each case expressed pan-B-cell antigens, and 30 (94%) of 32 expressed monotypic Ig light chain (15 Ig kappa, 15 Ig lambda); 2 cases were Ig-negative. Of 14 follicular lymphomas tested, 14 expressed the CD10 antigen. All 3 cases of SLL/CLL were positive for CD5. The mean age of the patients with other types of B-cell NHL was 59.2 years (range, 33-75 years), and the male/female ratio was 0.88.

A comparison between the distribution of 11q13 and CEP11 signals for the non-MCL and MCL cases is shown in Figure 3 and Figure 4. Two 11q13 signals were noted in a mean of 87.3% of nuclei from the non-MCL group compared with 26.1% of nuclei in the MCL group (P = 0.00; Student t test). In contrast, 3 11q13 signals were observed in a mean of 56.1% of nuclei in the MCL group compared with a mean of 5.8% of nuclei in the non-MCL group (P = 0.00; Student t test).

The MCL group also demonstrated significantly increased percentages of nuclei with more than 4 11q13 signals compared with the non-MCL group (Figure 4). In the MCLs, the mean percentage of nuclei with 4 11q13 signals was 10.9%. Five and 6 or more 11q13 signals were found in 5.5% and 3.2% of nuclei, respectively. In contrast, in 32 other B-cell NHLs the mean percentage of nuclei with 4 11q13 signals was 3%. Five and 6 or more 11q13 signals were found in 0.1% and 0% of nuclei, respectively.

The presence of 4, 5, or 6 or more 11q13 signals also correlated with blastoid cytologic features in the MCL group. In blastoid neoplasms, the mean percentages of nuclei with 4, 5, and 6 or more 11q13 signals were 20.2%, 12%, and 7%, respectively. In contrast, in classic small cell MCL, the mean percentages of nuclei with 4, 5, or 6 or more 11q13 signals were 1.7%, 0%, and 0%, respectively.

The 11q13/CEP11 ratio was significantly higher in the MCL group than in other B-cell NHLs (Table 3 and Figure 5) (P = 0.00; Fisher exact test). In the MCLs, the mean was 1.38, the SD was 0.11, and the 95% confidence levels ranged from 1.31 to 1.46. The mean 11q13/CEP11 ratio was 1.43 for blastoid and 1.35 for classic small cell MCL; this difference was not significant (P = 0.25). For other B-cell NHLs, the mean 11q13/CEP11 ratio was 0.99, the SD was 0.09, and the 95% confidence interval ranged from 0.96 and 1.03 (Table 3).

Of the non-MCL cases, 4 tumors had an increased percentage of nuclei with 3 or 4 11q13 signals. In 2 cases...
with 3 or 4 11q13 signals, 1 follicular mixed and 1 large B-cell NHL, the number of CEP11 signals also was increased, and these cases had normal 11q13/CEP11 ratios of 1.02 and 0.89, respectively. In 2 cases, 1 B-cell unclassified and 1 large B-cell NHL, 3 11q13 signals were identified without an increase in CEP11 signals, resulting in 11q13/CEP11 ratios of 1.30 and 1.15, respectively. Conventional cytogenetics done on the large B-cell NHL revealed the t(5;11)(q31;q13), explaining the increased number of 11q13 signals in this neoplasm. Conventional cytogenetic studies were not performed on the unclassified B-cell NHL.

We compared these FISH results with the results of conventional cytogenetics, molecular studies, and immunohistochemical studies for cyclin D1 overexpression in MCL

Discussion

Using traditional cytologic or histologic methods, it can be difficult to differentiate MCL from other small B-cell NHL and leukemias. Nevertheless, this distinction is vital, as MCL has a poorer prognosis compared with that of other small B-cell NHL and leukemias. Presently, patients with MCL at our institution receive intensive chemotherapy regimens followed by autologous or allogeneic stem cell transplant, which clearly is not appropriate for less aggressive small B-cell neoplasms.

MCLs carry a characteristic cytogenetic abnormality, the t(11;14)(q13;q32), and conventional cytogenetics or other methods to detect this translocation are useful for distinguishing MCL from other small B-cell NHL. However, currently used techniques have substantial limitations. Conventional cytogenetics is a cumbersome procedure and is successful in only 75% of cases. Negative results may be attributed to inadequate metaphase spreads resulting from poor morphologic quality of chromosomes or lack of mitoses. Southern blot analysis can be used, but is also laborious and time consuming, and detects bcl-1 locus rearrangements in
approximately 70% of MCLs if multiple probes are used. The widely scattered chromosome 11 breakpoints are a substantial obstacle to PCR assays. Currently, PCR assays are established that detect translocations affecting the \( bcl-1 \) MTC region, present in 30% to 40% of MCLs.8

As an alternative to \( t(11;14) \) detection, immunohistochemical assays that detect cyclin D1 overexpression can be used.10 However, these assays require heat-induced microwave retrieval and are optimized for paraffin-embedded tissue sections. In our hands, demonstration of cyclin D1 overexpression in cytologic smears or cytocentrifuged preparations is relatively less reliable. Moreover, even with ideal assay conditions and materials, a subset of MCLs are immunohistochemically negative for cyclin D1.16

The most sensitive assays reported for detecting the \( t(11;14) \) are those that use interphase FISH methods, most of which have been used in a research setting.17-23 Some assays have demonstrated 11q13 translocations, using either a single-color yeast artificial chromosome probe spanning the 11q13 locus or a painting probe for chromosome 11. The latter assay, however, is nonspecific because trisomy 11 will result in 3 signals that mimic the presence of the \( t(11;14) \). Colocalization double-color FISH assays using probes immediately flanking the 11q13 or 14q32 breakpoints have a reported sensitivity of 87% to 100%.22,23 Recently Li and coworkers23 described a FISH method using probes for \( CCND1 \) and the Ig heavy chain gene that can be used on both fresh and paraffin-embedded tumor samples. They report 100% sensitivity for diagnosing MCL with this technique, which uses the same commercial probe for 11q13 as used in the present study, combined with a 14q32 probe derived from a bacterial artificial chromosome clone. DNA fiber FISH, using a barcode series of cosmid and P1 probes spanning 11q13 breakpoints over a 250-kb distance, is also sensitive and was reported to detect the \( t(11;14) \) in 95% of MCLs in 1 study.24 These FISH techniques are powerful and elegant but are not routinely available in many laboratories.

The FISH assay used in the present study is commercially available and combines 11q13 and CEP11 DNA probes, each labeled with a different fluorochrome (Figures 1 and 2). The 11q13 probe, directly labeled with spectrum-orange, is approximately 300 kb long and is centered on the \( CCND1 \) locus (Figure 1). The CEP11 probe, labeled with spectrum green, hybridizes with band region 11p11.1-q11. In normal peripheral blood lymphocyte nuclei, the 2 probes are seen as closely apposed green and red signals (Image 1). Following 11q13 disruption, the red signal is split, resulting in an extra red signal relative to the 2 green signals representing CEP11 (Image 2). This unique combination of the 11q13 and CEP11 probes also allows expression of the results as an 11q13/CEP11 ratio.

By using this FISH assay, an elevated percentage of nuclei with 3 or more 11q13 signals was demonstrated in all 11 MCL cases. The percentage of cells with 3 or more 11q13 signals ranged from 57% to 90%, with a mean of 74.8%. Conventional cytogenetic studies were performed on 4 MCLs, and all carried the \( t(11;14) \). In contrast, in 28 of 32 other B-cell NHLs, no case showed more than 7% of nuclei

![Figure 6](https://academic.oup.com/ajcp/article-abstract/114/2/248/1757910)

**Table 3**

<table>
<thead>
<tr>
<th>Histologic Diagnosis</th>
<th>No. of Cases</th>
<th>11q13/CEP11 (mean ± SD)</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mantle cell lymphoma</td>
<td>11</td>
<td>1.38 ± 0.11</td>
<td>1.31-1.46</td>
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<tr>
<td>Blastoïd</td>
<td>5</td>
<td>1.43 ± 0.16</td>
<td>1.23-1.63</td>
</tr>
<tr>
<td>Nonblastoïd</td>
<td>6</td>
<td>1.35 ± 0.05</td>
<td>1.30-1.39</td>
</tr>
<tr>
<td>Other B-cell NHL</td>
<td>32</td>
<td>0.99 ± 0.09</td>
<td>0.96-1.03</td>
</tr>
</tbody>
</table>

CEP11, centromeric chromosome 11 probe.

* The 11q13/CEP11 ratio was significantly higher in mantle cell lymphoma compared with other types of B-cell NHLs (\( P < .001 \); Fisher exact test). The 11q13 and CEP11 probes are from Vysis, Downers Grove, IL.
with 3 or more 11q13 signals. These 28 cases included 3 SLL/CLLs, 17 follicular lymphomas, and 8 large B-cell lymphomas. Conventional cytogenetics performed on 9 of these cases did not show the t(11;14).

The 11q13/CEP11 ratio was not significantly higher in the blastoid variant compared with the classic small cell variant of MCL. The lack of a significant difference is likely due to a relatively equal increase in 11q13 and CEP11 signals. Blastoid MCLs are often tetraploid as shown by DNA image analysis25 and were tetraploid for chromosome 11 in the present study (Table 4).

Four non-MCL cases in the present study are of interest because a high percentage of nuclei with 3 or 4 11q13 signals were identified, 21% to 89%. In 2 cases, 1 follicular mixed and 1 large B-cell, the presence of 3 or 4 11q13 signals is explained by extra chromosome 11 copy number, proven by an increased number of CEP11 signals and a normal 11q13/CEP11 ratio. These 2 cases highlight the value of simultaneously using the 11q13 and CEP11 probes.

Table 4

<table>
<thead>
<tr>
<th>Case No.</th>
<th>MCL Variant</th>
<th>Cells With &gt;3 11q13 Signals (%)</th>
<th>11q13/ CEP11</th>
<th>Cyclin D1 Immunostain</th>
<th>bcl-1 Rearrangements</th>
<th>t(11;14) PCR</th>
<th>Cytogenetics</th>
</tr>
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<tbody>
<tr>
<td>1 B B</td>
<td>66</td>
<td>1.19                           +</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td></td>
<td></td>
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<tr>
<td>2 B B</td>
<td>72</td>
<td>1.38                           ND</td>
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<td>3 B B</td>
<td>91</td>
<td>1.46                           +</td>
<td>–</td>
<td>–</td>
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<tr>
<td>4 B B</td>
<td>88</td>
<td>1.63                           +</td>
<td>–</td>
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<tr>
<td>5 B B</td>
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<tr>
<td>6 C C</td>
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<td>7 C C</td>
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<tr>
<td>8 C C</td>
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<tr>
<td>9 C C</td>
<td>70</td>
<td>1.37                           +</td>
<td>+</td>
<td>+</td>
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<tr>
<td>10 C C</td>
<td>65</td>
<td>1.36                           +</td>
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<tr>
<td>11 C C</td>
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B, blastoid; C, classic small cell; CEP11, centromeric chromosome 11 probe; MCL, mantle cell lymphoma; ND, not done; PCR, polymerase chain reaction; U, uninterpretable; +, positive; –, negative.

* The 11q13 and CEP11 probes are from Vysis, Downers Grove, IL.

Table 5

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<tr>
<th>Case No.</th>
<th>Diagnosis</th>
<th>Chromosome Analysis*</th>
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<tbody>
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<td>51,X,–Y,del(1)(q1q25),–2,add(3)(q27),add(4)(p11),del(5)(p51)(q13),del(6)(q23),add(7)(q36),add(8)(q24.3),XZ,add(13)(q22),add(14)(p11),del(15)(q21),del(16)(q21),+16,add(18)(q23),add(19)(p13.3),+der(19) (t1;19)(q22;p13.3),add(20)(p13),add(21)(q22),der(21)(q11.2),+5mar[17][53],idem,+5,+17[13]</td>
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<tr>
<td>2 FL-L</td>
<td>46,XX[22]</td>
<td></td>
</tr>
<tr>
<td>3 FL-S</td>
<td>47:50, X,del(Xp22),add(1)[1q12],–2,–6,del(11)[q12],add(18)[q23],add(19)[q13.4],+5-6mar[cp18]</td>
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</tr>
<tr>
<td>4 LCL</td>
<td>49:52,XX,inv(3)[p24q29],+5,–7,–8,–9,add(9)[p24],+12,13,t(14;18)[q32;21],+18,+18,der(18)t(14;18),+21, +5-7mar[cp12][46,XX[8]]</td>
<td></td>
</tr>
<tr>
<td>5 LCL</td>
<td>46,XX,t(1;9)[q32;p24][cp2][46,XX[18]</td>
<td></td>
</tr>
<tr>
<td>6 MCL</td>
<td>43:46,XX,t(1;2)[p31.1;p23],+3,del(6)[q21]–10,t(11;14)[q13;32],+1-2mar[cp24]+6,XX[1]</td>
<td></td>
</tr>
<tr>
<td>7 FL-L</td>
<td>46,XY[20]</td>
<td></td>
</tr>
<tr>
<td>8 LCL</td>
<td>46,XY[19]</td>
<td></td>
</tr>
<tr>
<td>9 MCL</td>
<td>43:46,XY,del(6)[q14],del(9)[q13],–9,t(11;14)[q13;32],–15,–2,3mar[cp6]46,XY[7]</td>
<td></td>
</tr>
<tr>
<td>10 MCL</td>
<td>45,XX,t(1;6)[p22;p21],–6,–10,t(11;14)[q13;32],–12,add(13)[p12],der(14)t(11;14)[q13;32],add(14)[p11], +18,der(20)[q13],+mar[18]46,XX[2]</td>
<td></td>
</tr>
<tr>
<td>11 SLL/CLL</td>
<td>46,XY[del(20)[q11][20]</td>
<td></td>
</tr>
<tr>
<td>12 MCL</td>
<td>37:45,t(11;14)[q13;32][cp5]</td>
<td></td>
</tr>
<tr>
<td>13 LCL</td>
<td>48,XX,del(4)[q12],+6,del(6)[q21]–8,+10x2,t(14;18)[q32;q21],add(18)[q32],–22[2][46,XX[23]</td>
<td></td>
</tr>
</tbody>
</table>

FL-L, follicular lymphoma, large cell; FL-S, follicular lymphoma, small cleaved cell; LCL, large B-cell lymphoma; MCL, mantle cell lymphoma; SLL/CLL, small lymphocytic lymphoma/chronic lymphocytic leukemia.

* The t(11;14) translocations found only in the MCL cases are shown in bold type.
tumor originally was classified as follicular and diffuse large B-cell lymphoma, as the neoplasm was positive for CD10. However, the neoplastic cells are intermediate in size, and PCR studies showed no evidence of the t(14;18) involving either the major or minor breakpoint cluster regions of the bcl-2 gene. We also retrospectively considered the possibility that this neoplasm was blastoid MCL. However, the neoplasm is negative for t(11;14) by PCR and is immunohistochemically cyclin D1 negative. Thus, we cannot classify this neoplasm, and it is possible that a translocation involving the 11q13 locus other than the t(11;14) could explain the FISH results. Conventional cytogenetic analysis was done on the large B-cell lymphoma with 21% nuclei with 3 or more 11q13 signals, which revealed a complex karyotype including the t(5;11)(q31;q13) in 17 of 20 cells.

As these 2 cases illustrate, a drawback to the FISH assay used in the present study is that the presence of increased 11q13 signals is not direct proof of the t(11;14). The 11q13 locus may be translocated with sites other than 14q32, resulting in a fusion product that is not the t(11;14). However, this is not a practical problem. In most instances in which this assay is needed, the differential diagnosis includes MCL and other small B-cell NHLs or leukemias, and translocations involving the 11q13 locus other than the t(11;14) are rare in these neoplasms.

Amplification of the 11q13 locus, with or without translocation, also may result in detection of extra 11q13 signals. In the MCL cases, we observed a statistically increased incidence of neoplasms in which a subset of cells had 5 or more 11q13 signals, suggestive of amplification. Amplification of 11q13 has been reported in a variety of carcinomas, including those arising in the breast and head and neck. However, amplification has not received much emphasis in MCL. Correlation of 11q13 locus amplification with cyclin D1 protein levels or prognosis may be of interest. Genetic instability of MCL may, at least in part, be involved in 11q13 locus amplification. We did not find evidence of 11q13 amplification in the 32 small B-cell NHLs.

The FISH assay we used in the present study is rapid, simple, and sensitive and can be performed on a minimal number of cells. Most of the cases we assessed were fine-needle aspiration specimens from which cytocentrifuged preparations were prepared. As this method seems to be simpler, cheaper, and as sensitive as other well-established molecular methods, we believe it may prove to be diagnostically useful and convenient if tested in a larger cohort.

From the Departments of 1Pathology and 2Hematology, The University of Texas M.D. Anderson Cancer Center, Houston, TX.

References


Address reprint requests to Dr Katz: The University of Texas M.D. Anderson Cancer Center, Dept of Cytopathology, Box 53, 1515 Holcombe Blvd, Houston, TX 77030.

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