**Chronic Lymphocytic Leukemia FISH Panel**

**Impact on Diagnosis**

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**Key Words:** Chronic lymphocytic leukemia; Fluorescence in situ hybridization; FISH; 14q32 Translocation

**Abstract**

Interphase fluorescence in situ hybridization (FISH) is an alternative to conventional chromosome analysis of chronic lymphocytic leukemia (CLL) cells. We analyzed 172 samples from 136 possible CLL cases using a FISH panel. Reflex testing with probes to CCND1, BCL2, BCL3, BCL11A, c-MYC, MALT1, and a break-apart immunoglobulin heavy chain (IGH) probe was done if more than 2 signals for 14q32 occurred. For 111 cases, there were sufficient data for analysis. Of 111 cases, 81 (72.9%) had 1 or more genetic abnormalities. The most frequent abnormality was 13q–, followed by trisomy 12, 11q–, and 17p–. In 13 cases, there were IGH abnormalities. Two cases with CCND1/IGH fusion were reclassified as mantle cell lymphoma. Four CLL cases had IGH fusion with BCL2, BCL3 (2 cases), and BCL11A; no fusion partner was detected in 7 cases. Morphologic features were atypical for CLL in 2 cases with IGH fusion (BCL11A and BCL3). The FISH CLL panel is useful to identify prognostic aberrations and to clarify diagnosis in cases with unusual morphologic features.

B-cell chronic lymphocytic leukemia (CLL) is the most common leukemia in adults. The morphologic features and immunophenotype of CLL cells are well characterized. A unique, recurring genetic alteration has not been identified in CLL, but chromosomal aberrations occurring in CLL have been linked to prognosis.1-3 For example, deletions of chromosomes 17p and 11q are associated with an adverse clinical outcome, with overall survival of 3 years and 6 to 7 years, respectively. Patients with isolated 13q deletions have a favorable clinical course and best overall survival, 11 years. The clinical course of patients with CLL with trisomy 12q is intermediate between the groups with inferior and favorable outcomes.

Early genetic studies of CLL using conventional chromosome banding analysis detected chromosomal aberrations in 40% to 60% of cases.4,5 However, only dividing cells are evaluated by chromosome banding techniques. Because it has been difficult to stimulate CLL cells to divide, novel stimulation techniques have been reported to improve the detection of chromosomal aberrations, particularly translocations, in CLL lymphocytes.1 Nevertheless, fluorescence in situ hybridization (FISH), which allows analysis of dividing and nondividing cells, is increasingly being offered as an alternative to conventional chromosome banding. With FISH, up to 80% of CLL cases demonstrate genetic alterations.2

In this report, we review the Northwestern Memorial Hospital (NMH; Chicago, IL) experience with a FISH panel for CLL after it was implemented as part of the routine procedure to assess prognosis. Morphologic and flow cytometric evaluation of peripheral blood and/or bone marrow samples is routinely performed at our institution in all new cases in which CLL is considered a possible diagnosis. When FISH is performed, the results are correlated with the initial diagnostic impression.
In this study, the FISH results not only provided prognostic information, but also added data that in some cases supported CLL when the morphologic features were not classic for CLL and in others changed the initial diagnostic impression.

Materials and Methods

Between June 2002 and November 2005, samples of peripheral blood, bone marrow, or lymph nodes from 136 patients with a working or established diagnosis of CLL were analyzed with a FISH panel at Mayo Medical Laboratories, Rochester, MN, using the following probes: c-MYB (6q23), D6Z1 (6cen), ATM (11q22.3), CCND1 (11q13), D11Z1 (11cen), D12Z3 (12cen), MDM2 (12q15), D13S319 (13q14), LAMP1 (13q34), p53 (17p13.1), D17Z1 (17cen), and immunoglobulin heavy chain (IGH; 14q32). When more than 2 signals for chromosome 14q32 were identified, reflex testing using additional FISH probes was performed to detect fusion of IGH with CCND1 (11q13), BCL2 (18q21), BCL3 (19q13), BCL11A (2p13), c-MYC (8q24), or MALT1 (18q21). A break-apart probe for IGH was also used to determine if the additional IGH signal was due to trisomy 14 or IGH translocations involving loci other than those tested. Normal values, sensitivity, and specificity for each probe and set of probes were established at Mayo Medical Laboratories and have been previously published.6,7 Commercial probes for cyclin D1, bcl-2, bcl-6, c-myc, and MALT-1 were purchased from Vysis, Des Plaines, IL. “Homebrew” probes for bcl-3 and bcl-11a were made at Mayo Medical Laboratories. Cases were stratified into 3 risk groups as follows: good, normal or 13q− only; intermediate, +12 only; or poor, 6q−,11q−, or 17p−. CLL cases with more than 1 genetic abnormality but with 17p− were placed in the 17p− group, and those with 11q− but not 17p− were placed in the 11q− group.

Immunophenotyping using flow cytometry was performed on blood samples, bone marrow aspirates, or lymph node biopsy specimens in all cases using previously published procedures.8 Briefly, 4-color immunophenotyping was performed evaluating surface antigens using antibodies in the following combinations: CD45/CD19/CD56/CD3; CD2/CD3/CD7/CD5; CD8/CD3/CD4/CD25; CD10/CD19/κ; CD19/CD20/CD11c/CD103; CD38/CD19/CD5/CD79b; CD25/CD19/CD23/FMC7; CD34/CD45/CD10/CD19; and CD33/CD45/CD13/CD14. In addition, after the cells were incubated with antibodies directed against surface antigens, cells were permeabilized using the Intra-Prep kit (Beckman Coulter, Miami, FL) to evaluate for intracellular antigens terminal deoxynucleotidyl transferase (TdT), myeloperoxidase, and CD79a in the following combinations: CD45/CD19/CD3/TdT and CD45/CD3/CD79a/myeloperoxidase. The antibodies were directly labeled with fluorescein isothiocyanate, phycoerythrin, phycoerythrin–cyanin 5, or phycoerythrin–Texas red. CD79b was purchased from DAKO, Fort Collins, CO, and CD52 from Caltag (Invitrogen), Carlsbad, CA; the remainder of the antibodies were purchased from Beckman Coulter, Miami, FL.

The following immunophenotype was interpreted as typical for CLL: CD5+, dim to moderate CD20+, and CD23+ monotypic B cells. Brightly positive staining for CD20, FMC7, and/or CD79b or negative staining for CD23 was regarded as an atypical immunophenotype for CLL. CD38 was regarded as positive if a distinct population of the lymphocytes displayed greater staining intensity than the granulocytes in the sample and as dim if the staining intensity overlapped with the granulocytes and was consistent even following blocking with 100% normal mouse serum. The ζ-associated protein (ZAP-70) staining pattern was evaluated in selected cases using previously published procedures.9

CBC count and leukocyte differentials were performed on peripheral blood samples. Peripheral blood samples and/or bone marrow aspirate smears were stained with Wright-Giemsa for morphologic evaluation. B-5–fixed, decalcified bone marrow trephine biopsy specimens were stained with H&E before histologic evaluation. The morphologic features of lymphocytes in blood and/or bone marrow were reviewed in all cases without knowledge of immunophenotype or FISH results.

Lymphocyte morphologic features were considered typical for CLL if the lymphocytes were small with round nuclei, condensed chromatin, and scant cytoplasm and prolymphocytes were fewer than 10%; morphologic features were considered atypical if lymphocytes displayed irregularly shaped nuclei or dispersed chromatin or prolymphocytes were greater than 10%. The morphologic features were correlated with the immunophenotype to arrive at an initial diagnostic impression. When the FISH results became available, they were correlated with the initial diagnostic impression.

Results

Summary of Cases

A total of 136 cases of presumed CLL were referred for the FISH panel. Three cases were excluded from analysis because neither immunophenotypic nor morphologic data were available to correlate with the FISH results, and 22 were not CLL based on review of the morphologic features and immunophenotype. The remaining 111 cases included 71 men and 40 women ranging in age from 25 to 84 years (median, 61 years). Specimens used for FISH analysis were peripheral blood (61), bone marrow (49), and lymph node (1).
FISH Results

Cases With CCND1/IGH Fusion Classified as Mantle Cell Lymphoma

Two cases initially regarded as CLL based on clinical manifestations demonstrated CCND1/IGH fusion, indicating bcl-1 gene rearrangement. One patient, a 41-year-old man, had marked lymphocytosis in the peripheral blood (WBC count, 155,400/µL [155.4 × 10⁹/L]; hemoglobin, 10.6 g/dL [106 g/L]; mean corpuscular volume [MCV], 90 µm³ [90 fL]; platelet count, 343 × 10³/µL [343 × 10⁹/L]; lymphocytes, 96.5% [0.97]; and neutrophils, 3.5% [0.04]). The lymphocytes varied from small cells with condensed chromatin and round nuclei to medium-sized cells with slightly irregular nuclei. In addition to CCND1/IGH fusion, FISH also showed deletion of chromosomes 11q and 13q.

The second patient, a 76-year-old man, had absolute lymphocytosis (WBC count, 11,500/µL [11.5 × 10⁹/L]; hemoglobin, 10.4 g/dL [104 g/L]; MCV, 76 µm³ [76 fL]; platelet count, 155 × 10³/µL [155 × 10⁹/L]; lymphocytes, 50.2% [0.50]; neutrophils 35.4% [0.35]; monocytes, 10.3% [0.10]; eosinophils, 3.1% [0.03]; and basophils, 1.0% [0.01]). Lymphocyte morphologic features were unusual for CLL; the cells were primarily small with condensed chromatin and scant cytoplasm but included occasional larger cells with visible nucleoli. Flow cytometric immunophenotyping of the blood showed monotypic B cells that were CD5+, CD10–, CD20+, dim CD23+, bright FMC7+, and bright CD79b+. In addition to CCND1/IGH fusion, 13q and 17p deletions were also identified.

Although both cases were clinically regarded as CLL, their morphologic features and immunophenotype were atypical for CLL. Flow cytometric immunophenotyping of the blood showed monotypic B cells that were CD19+, CD20+, CD5+, bright CD23+, bright FMC7+, and bright CD79b+. In addition to CCND1/IGH fusion, FISH also showed deletion of chromosomes 11q and 13q.

CLL Cases

Of 109 CLL cases, 79 (72.5%) had 1 or more genetic abnormalities; the remaining 30 cases (27.5%) had normal FISH results. The majority of the patients with genetic alterations, 67% (53/79), had a single abnormality. Two alterations were present in 25% (20/79), and 8% (6/79) had 3 or more genetic alterations.

Chromosome 13q deletion (13q–) was the most common finding. Abnormalities of chromosomes 17p and 14q32 occurred with similar frequencies. The least common abnormality was deletion of chromosome 6q.

An abnormal signal pattern for the IGH gene was present in 11 CLL cases. Translocations were identified in 4 of these cases. One or more extra IGH signals without an identifiable fusion partner were present in 6 cases, and the 3' end of the IGH variable region (IGHv) signal was absent in 1 case.

FISH Results Correlated With Phenotypic, Morphologic, and Other Pathologic Findings

Immunophenotypic and Morphologic Findings

A total of 109 cases displayed typical phenotypes, and 2 were atypical with bright CD20+ results. The 2 CLL cases...
Nelson et al / CLL, FISH, AND 14q32 TRANSLOCATIONS

**Table 2**

Chronic Lymphocytic Leukemia Cases With Translocations Involving Chromosome 14q32

<table>
<thead>
<tr>
<th>Case No./Sex/Age (y)</th>
<th>Tissue Source</th>
<th>6q–</th>
<th>11q–</th>
<th>+12</th>
<th>13q–</th>
<th>17p–</th>
<th>CD38</th>
<th>ZAP-70</th>
<th>Gene</th>
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<tr>
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<td>NA</td>
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<tr>
<td>2/M/52</td>
<td>PB</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>NA</td>
<td>bcl-3</td>
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<tr>
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<td>PB</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NA</td>
<td>bcl-3</td>
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<tr>
<td>4/M/52</td>
<td>PB</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>bcl-2</td>
</tr>
</tbody>
</table>

BM: bone marrow; NA: not available; PB: peripheral blood; ZAP, ζ-associated protein; +, positive result; –, negative result.

**Table 3**

Chronic Lymphocytic Leukemia Cases With IgH Abnormalities and No Translocations Identified

<table>
<thead>
<tr>
<th>Case No./Sex/ Age (y)</th>
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<th>IgH Abnormality</th>
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<th>11q–</th>
<th>+12</th>
<th>13q–</th>
<th>17p–</th>
<th>CD38</th>
<th>ZAP-70</th>
<th>Rai Stage</th>
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<td>–</td>
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<td>0</td>
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<td>2/N/62</td>
<td>PB</td>
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<td>–</td>
<td>–</td>
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<td>–</td>
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<td>IV</td>
</tr>
<tr>
<td>3/M/53</td>
<td>BM</td>
<td>3 signals</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>NA</td>
<td>IV</td>
</tr>
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<td>–</td>
<td>–</td>
<td>+</td>
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<td>–</td>
<td>–</td>
<td>NA</td>
<td>IV</td>
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<td>LN</td>
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<td>–</td>
<td>+</td>
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<td>–</td>
<td>NA</td>
<td>I</td>
</tr>
<tr>
<td>7/N/29</td>
<td>BM</td>
<td>+ signal deletion</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>III</td>
</tr>
</tbody>
</table>

BM: bone marrow; IgH, immunoglobulin heavy chain gene; LN, lymph node; NA, not available; PB, peripheral blood; ZAP, ζ-associated protein; +, positive result; –, negative result.

with bright CD20 staining had isolated trisomy 12. Nine CLL cases displayed atypical morphologic features for CLL; they included 2 with translocations involving chromosome 14q32 (bcl-3 and bcl-11a; discussed in the next section), 3 with isolated trisomy 12, 2 with isolated 13q deletion, and 2 with normal FISH results.

**CLL Cases With Chromosome 14q32 Translocations**

Eleven CLL cases demonstrated abnormalities involving the IgH gene located at chromosome 14q32. Of these, 4 had translocations (Table 2). These cases are discussed in more detail because the FISH results were informative for the diagnoses.

The first case, a 46-year-old man, had absolute lymphocytosis in the blood (WBC count, 32,100/µL [32.1 × 10⁹/L]); hemoglobin, 14.3 g/dL [143 g/L]; hematocrit, 43.8% [0.44]; MCV, 84 µm³ [84 fl]; platelet count, 286 × 10⁹/µL [286 × 10⁹/L]; lymphocytes, 86% [0.86]; neutrophils, 10% [0.10]; bands, 2% [0.02]; monocytes, 1% [0.01]; and eosinophils, 1% [0.01]) associated with lymphadenopathy and splenomegaly. A bone marrow biopsy showed a lymphoid infiltrate with a diffuse growth pattern involving approximately 80% of the section. The lymphocytes were primarily small with condensed chromatin but included many cells with angulated or cleaved nuclei Image 2A. The morphologic features of the lymphocytes were not typical for CLL and raised the possibility of another type of non-Hodgkin lymphoma.

Flow cytometric immunophenotyping findings, however, were characteristic of CLL: κ surface immunoglobulin (sIg) light chain–restricted B cells that were CD19+/CD20+/CD5+/CD10–/CD23+/CD79b–/FMC7–. Small lymphocytic lymphoma/CLL was confirmed with a lymph node biopsy that showed effacement of the normal architecture by a proliferation of small lymphocytes with a diffuse growth pattern that included proliferation centers Image 2B. FISH analysis revealed fusion of IgH and bcl-11a, indicating t(2;14)(p13;q32), which rarely occurs in CLL. ¹⁰,¹¹ Deletion of the long arm of chromosome 11 was also identified.

His illness was characterized by development of massive generalized lymphadenopathy that encased major blood vessels, including the inferior vena cava, aorta, common iliac arteries, portal vein, splenic vein, and superior mesenteric vein. Multiple bilateral pulmonary nodules, splenomegaly that required radiation therapy for symptom management, severe thrombocytopenia (platelet count, 4 × 10⁹/µL [4 × 10⁹/L]), and anemia (hemoglobin, 7.4 g/dL [74 g/L]) also developed. He was treated with several different chemotherapeutic regimens throughout the disease course. The therapies included fludarabine; fludarabine/cyclophosphamide; cyclophosphamide, doxorubicin, vincristine, and prednisone; and pentostatin/rituximab. He died of progressive disease 51 months after initial diagnosis.

The second case was a 52-year-old man who had absolute lymphocytosis in the blood (WBC count, 85,000/µL [85.0 ×
10^9/L; hemoglobin, 14.3 g/dL [143 g/L]; MCV, 90 µm^3 [90 fL]; platelet count, 170 × 10^3/µL [170 × 10^9/L]; lymphocytes, 87% [0.87]; neutrophils, 11% [0.11]; and monocytes, 2% [0.02]) and lacked lymphadenopathy. The lymphocytes were morphologically atypical for CLL and included small cells with condensed chromatin and round nuclei Image 3A and many cells with irregular nuclear contours and larger lymphocytes with more abundant cytoplasm and visible nucleoli. The bone marrow trephine biopsy section was hypercellular with an extensive lymphoid infiltrate that displayed interstitial Image 3B and diffuse growth patterns. Flow cytometric immunophenotyping showed κ slg light chain–restricted B cells that were CD19+, CD20+, CD5+, CD10–, dim CD23+, and dim FMC7+.

**Image 2** A, Chronic lymphocytic leukemia (CLL) with bcl-11a translocation involving the bone marrow aspirate. The lymphocytes are primarily small with condensed chromatin. However, many display angulated nuclei and do not resemble typical CLL cells (Wright-Giemsa, ×600). B, CLL with bcl-11a translocation involving the lymph node. The infiltrate is composed of small lymphocytes with a diffuse growth pattern and includes proliferation centers that are typical of CLL (H&E, ×100).

**Image 3** A, Chronic lymphocytic leukemia (CLL) with bcl-3 translocation involving the blood. The lymphocytes are morphologically heterogeneous and include small cells with condensed chromatin and round nuclei, many cells with irregularly shaped nuclei, and also larger cells with visible nucleoli (Wright-Giemsa, ×600). B, CLL with bcl-3 translocation involving the bone marrow core biopsy section. The infiltrate includes increased numbers of lymphocytes with visible nuclei and more dispersed chromatin (H&E, ×200).
The phenotype was compatible with CLL, but the unusual morphologic features and dim CD23 staining raised the possibility of MCL. Although conventional chromosome banding of the bone marrow demonstrated a normal male karyotype, FISH showed trisomy 12 and fusion of the bcl-3 gene located at 19q13 to the IGH gene, resulting in t(14;19)(q32;q13), a translocation reported in rare CLL cases. Absence of CCND1/IGH fusion in this case excluded MCL. CLL with atypical morphologic features was diagnosed. After almost 2 years with a stable clinical course, he was treated with fludarabine, cyclophosphamide, and rituximab for progressive disease that responded well to therapy. He was alive with disease at 38 months.

The third patient with CLL with IGH fusion was a 47-year-old woman who also had a bcl-3 translocation. She was first given a diagnosis of Rai stage 0 CLL in May 1997 when a CBC count showed absolute lymphocytosis, anemia, and a normal platelet count (WBC count, 50,200/µL [50.2 × 10⁹/L]; hemoglobin, 10.8 g/dL [108 g/L]; platelet count, 236 × 10³/µL [236 × 10⁹/L]; lymphocytes, 85% [0.85]; neutrophils, 12% [0.12]; and monocytes, 3% [0.03]). Flow cytometry performed on the blood in 2001 showed that the lymphocytes were dim κ sIg light chain restricted, dim CD20+, CD5+, CD10–, CD23+, CD38+, CD52+, FMC7–, and dim CD79b+. A bone marrow biopsy specimen was hypercellular (80%) with a dense lymphoid infiltrate composed of small, mature-appearing cells.

Fludarabine was given for 6 months beginning in August 1997. She achieved and remained in remission until March 2000, when lymphocytosis recurred. Additional combination chemotherapy was given, resulting in a partial response. Autologous stem cell transplantation was performed in June 2002 because of progressive disease that was no longer responsive to chemotherapy; she achieved a complete morphologic and molecular remission with negative IGH gene rearrangement shown by polymerase chain reaction of blood and bone marrow specimens.

In June 2003, she was referred to NMH for recurrent disease. At this time, the blood smear showed many large lymphocytes with visible nucleoli in addition to small, mature-appearing lymphocytes consistent with prolymphocytoid transformation. The immunophenotype was similar to that in the initial study except that CD23 was now negative. The initial history of CLL was not immediately available, and non-Hodgkin lymphoma, in particular MCL, was considered. However, FISH analysis of the blood (performed at the time of prolymphocytoid transformation) showed a hyperdiploid clone including 3 or 4 copies of chromosomes 6, 11, 12, 13, and 14 with 13q–, 17p–, and fusion of IgH and bcl-3, indicative of t(14;19)(q32;q13). Based on these data, the history of CLL, and review of the prior blood and bone marrow specimens, the patient was given a diagnosis of prolymphocytoid transformation of CLL and treated with more aggressive chemotherapy—cyclophosphamide, etoposide, methotrexate, bleomycin, and vincristine—without significant response, and she died 83 months after initial diagnosis.

The last CLL case with a translocation involving chromosome 14q32 was a 52-year-old man with absolute lymphocytosis in the blood (WBC count, 30,300/µL [30.3 × 10⁹/L]; hemoglobin, 16.7 g/dL [167 g/L]; hematocrit, 47.9% [0.48];
MCV, 92 µm³ [92 fL]; platelet count, 300 x 10^3/µL [300 x 10^9/L]; lymphocytes, 84% [0.84]; neutrophils, 15% [0.15]; and monocytes, 1% [0.01]) that was first identified during an annual physical examination. Left axillary lymph nodes were also enlarged, but neither a bone marrow nor a lymph node biopsy was performed. The lymphocytes in the blood were small with condensed chromatin and scant cytoplasm. Flow cytometric analysis of the blood showed CD19+, CD20+, CD5+, CD10−, CD23+, CD79b−, FMC7−, and κ slg light chain–restricted B cells. CLL was diagnosed.

FISH analysis demonstrated fusion of the bcl-2 and IGH genes, resulting in t(14;18)(q32;q21), as well as deletion of the long arm of chromosome 13. Although an unusual presentation of follicular lymphoma with leukemic phase could not be completely excluded, the immunophenotype and morphologic features of the lymphocytes were considered most consistent with CLL. His disease was stable without treatment, and he was alive 44 months after initial diagnosis.

**CLL Cases With Chromosome 14q32 Aberrations With No Translocation Partner Identified**

Seven CLL cases without translocations had an abnormality involving the IGH gene. Six CLL cases had at least 1 extra signal for chromosome 14q32 without fusion of IGH with CCND1, bcl-2, bcl-3, bcl-11A, c-myc, or MALT1. They included 3 men and 3 women ranging in age from 53 to 74 years (Table 3). All had typical lymphocyte morphologic features and immunophenotype for CLL. Four had Rai stage IV CLL, 1 had stage I, and 1 had stage 0. Deletion of chromosome 13q was detected in 3 cases, trisomy 12 in 2 cases, and 17p deletion in 1 case; no additional aberration was present in 2 cases.

The final CLL case with a chromosome 14q32 aberration and no identified translocation had loss of the 3’ IGHv signal and trisomy 12. The results of chromosome banding analysis were similar; in 12 cells, they showed an interstitial deletion in chromosome 14 with breakpoints at 14q24 and 14q32, as well as trisomy 12. This patient, a 29-year-old man, had typical lymphocyte morphologic features and immunophenotype for CLL. Four had Rai stage IV CLL, 1 had stage I, and 1 had stage 0. Deletion of chromosome 13q was detected in 3 cases, trisomy 12 in 2 cases, and 17p deletion in 1 case; no additional aberration was present in 2 cases.

**Correlation of FISH Prognostic Groups With CD38/ZAP-70 Status**

The majority of the 109 CLL cases (63 [57.8%]) were in the favorable prognostic group with isolated 13q− or normal FISH results. Thirteen cases (11.9%) were in the intermediate prognostic group with isolated trisomy 12; 25 (22.9%) were in the least favorable prognostic group, with 17p− or 11q−; this group included the 4 patients with 6q− because all 4 also had 17p−. Of the 90 CLL cases analyzed for CD38, 81 were placed in prognostic groups. Nineteen (23%) of the 81 were CD38+. A similar percentage of CD38+ cases was present in cases with 17p (2/6 [33%]) and 11q deletion (4/11 [36%]) and cases with normal FISH results (9/27 [33%]) (Table 4). CLL cases with trisomy 12 and isolated 13q− had the lowest percentage of CD38+ cases; 15% (2/13) and 8% (2/24), respectively. ZAP-70 was tested in 36 cases; 10 were positive. In this limited number of cases tested for ZAP-70, the ZAP-70 staining pattern did not correlate with different genetic prognostic groups and was negative in all 3 cases with IGH anomalies that were tested (Table 3).

The majority of cases in the poor prognostic FISH groups of isolated 17p− (10/12 [83%]) and isolated 11q− (8/13 [62%]) had advanced disease with Rai stage III or IV. More patients in the intermediate and good prognostic groups had disease with a low Rai stage, 0 to II: trisomy 12 (7/11[64%]),

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normal FISH results (16/25 [64%]), and isolated 13q– (20/32 [62%]) Table 5. Interestingly, the majority (5/7) of CLL cases with an IGH anomaly but without an identifiable translocation partner had Rai stage III or IV disease.

**Discussion**

We evaluated our experience with a FISH CLL panel after it had been implemented by clinicians at NMH to assess prognosis. We found that FISH results were also useful to clarify the diagnosis in cases in which the phenotype and/or morphologic features were atypical. This was especially true for cases with chromosome 14q32 aberrations.

Two cases were classified as MCL after FISH analysis showed CCND1/IGH fusion, indicating t(11;14)(q13;q32). Both patients had blood lymphocytosis and some immunophenotypic features that resembled CLL. Both cases were CD20+, CD5+, and CD23+ (one bright and one dim). However, both cases had atypical morphologic features for CLL and were also FMC7+ and CD79b+, features that are unusual for CLL. The FISH results confirmed the diagnosis of MCL.

Although aberrations of chromosome 14q32 are reported to be rare in CLL, we found translocations of chromosome 14q32 in about 15% of CLL cases. In 4 of these cases, translocations involving chromosome 14q32 that have only rarely been encountered in CLL were found; some of these cases had morphologic features that suggested another non-Hodgkin lymphoma. One of these cases was a CLL with a bcl-11a translocation.

The bcl-11a gene codes for a transcription factor that is expressed in many hematopoietic tissues, including bone marrow cells and germinal center B cells, and is required for normal lymphoid development. The bcl-11a translocation is extremely rare in CLL. Prior reported cases include the initial description of 2 children with CLL. Both children died 1 to 4 years after receiving bone marrow transplants for progressive CLL. Two adults with CLL and bcl-11a translocations have also been described. Both had an aggressive clinical course and died with progressive disease. Our patient with a bcl-11a translocation also had a relatively aggressive course characterized by massive generalized lymphadenopathy, splenomegaly, and extensive bone marrow involvement associated with severe thrombocytopenia and anemia. His disease showed no response to chemotherapy, and he died 51 months after diagnosis.

Deletion of chromosome 11q was also present in the patient with a bcl-11a translocation. Chromosome 11q deletion is associated with extensive lymphadenopathy and a more advanced stage of CLL. The extent to which chromosome 11q deletion contributed to the aggressive clinical behavior is unclear. The prior reported CLL cases with bcl-11a translocations also had complex karyotypes that included chromosome 11q deletion in at least 1 adult.

Recognition of a bcl-11a translocation occurring in CLL cases is important for at least 2 reasons. First, as in our case, the morphologic features of the lymphocytes may suggest non-Hodgkin lymphoma, making it difficult to establish a definitive diagnosis of CLL based on analysis of only peripheral blood samples and bone marrow trephine biopsy sections. This is particularly true when proliferation centers are not present in the trephine biopsy section. Although the immunophenotype of the lymphocytes may be typical for CLL, a lymph node or other extramedullary tissue biopsy may be necessary to confirm the diagnosis. Second, because CLL cases with the bcl-11a translocation seem to be clinically aggressive, their recognition may allow for initiation of early and possibly innovative therapy.

Two CLL cases with a chromosome 14q32 translocation had t(14;19)(q32;q13), resulting in fusion of the bcl-3 and IGH genes; trisomy 12 was also present in both cases, and 13q– and 17p– were also present in 1 case. Prior studies have suggested that atypical morphologic features, trisomy 12, and an aberrant immunophenotype are features of CLL cases with a bcl-3 translocation. The findings in our cases are consistent with the prior observations. Both cases had relatively aggressive courses requiring therapy within 2 years, similar to

**Table 5**

FISH Prognostic Group Correlated With Rai Clinical Stage

<table>
<thead>
<tr>
<th>Chromosomal Status</th>
<th>17p– (n = 12)</th>
<th>11q– (n = 13)</th>
<th>Trisomy 12q Only (n = 13)</th>
<th>Normal (n = 30)</th>
<th>13q– Only (n = 33)</th>
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<td>8</td>
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<td>3</td>
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<td>5</td>
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<td>0</td>
<td>2</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

FISH, fluorescence in situ hybridization.

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what has been observed in some CLL cases with bcl-3 translocations.\(^3,16\) The bcl-3 translocation has also been described in other non-Hodgkin lymphomas.\(^1\)

In the fourth CLL case with a translocation, FISH analysis demonstrated fusion of bcl-2 and IGH, indicating t(14;18)(q32;q21); deletion of the long arm of chromosome 13 was also present. The MALT1 gene was not involved because it showed a normal signal pattern. The lymphocytes had the typical morphologic features and immunophenotype of CLL, and lymphocytosis was incidentally found during a routine physical examination. Therefore, this case was thought to be more consistent with CLL than follicular lymphoma, even though bcl-2 gene rearrangement occurs much more frequently in follicular lymphoma.

Although it rarely occurs, CLL with bcl-2 gene rearrangement has been documented in several cases.\(^1,17,18\) Similar to our patient, all 7 CLL cases with t(14;18)(q32;q21) reported in 1 series had low-stage disease and the typical immunophenotype and morphologic features for CLL.\(^17\) Our case had an indolent course.

The bcl-2 translocations occurring in CLL may be different from those that typically occur in follicular lymphoma in that the translocation partner may be an immunoglobulin light chain rather than the heavy chain gene, and the fusion site within the bcl-2 gene may occur at the 5\(^{\prime}\) instead of the 3\(^{\prime}\) regions.\(^17,19\) In addition, breakpoints within the bcl-2 gene may also occur between the major breakpoint region and minor cluster region instead of within these sites.\(^17,20\) The bcl-2 translocation in our patient was conventional in that it involved the IGH gene. However, more detailed molecular analysis was not performed to determine the precise breakpoint within the bcl-2 gene.

Three or more IGH signals were present in 6 CLL cases using the CCND1/IGH probe set in the initial phase of FISH analysis. Multiple dual fusion probe sets were used to look for specific translocation partners (bcl-2, bcl-3, bcl-11a, c-myc, or MALT1) in these cases. A break-apart IGH probe for chromosome 14q32 was also used to distinguish between multiple copies of chromosome 14 and separation of the IGH locus due to an IGH translocation. Although the break-apart IGH probe demonstrated a separation of the IGH locus in 5 cases, a partner chromosome was not identified. This finding indicates that IGH may be involved in translocations with more loci than just the ones used in this investigation. One case seemed to represent trisomy 14 because all abnormal nuclei had 3 intact IGH signals with the break-apart probes.

One of the 7 CLL cases with an aberration of chromosome 14q32 had deletion of the 3\(^{\prime}\) IGH signal involving the IGHv; no translocation was identified. This patient had stage III disease at initial diagnosis and required therapy. Others have described deletion of the IGHv in CLL cases, with progressive or stable CLL, but IGH deletion more commonly occurred in the group with progressive disease.\(^6\)

Our experience supports the use of a FISH panel with inclusion of probes directed at 14q32 in the evaluation of CLL. Reflex testing using probes directed at previously identified IGH fusion partners is particularly useful to exclude MCL that shows an immunophenotype and/or morphologic features similar to those of CLL and to identify CLL cases with atypical morphologic features that may otherwise be regarded as other types of non-Hodgkin lymphomas.

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References


