Mantle cell lymphomas (MCLs) are typically CD5-expressing B-cell non-Hodgkin’s lymphomas (NHLs) with detectable bcl-1 gene rearrangements. Insufficient data are available on the biologic features and clinical behavior of rigorously characterized MCL. As these NHLs have been reported to exhibit various histologic and cytologic expressions, we chose to study cases of MCL selected on more objective grounds. Specifically, 15 samples (from 14 patients) of CD5-expressing B-cell NHLs with detectable bcl-1 gene rearrangement were included. Overall, these patients had relatively uniform clinical manifestations. Most were older men (mean age, 67 years) who presented with lymphadenopathy, high-stage disease, and bone marrow involvement. All but two patients relapsed, demonstrated residual tumor, or had disease progression after an initial response to various therapies. Nine patients have died; these patients had a median survival of only 19 months. All cases could be classified within the broad morphologic spectrum previously described for MCL, and no predominant histologic subtype was observed. However, cases could be segregated into two major groups according to tissue architecture: one with a purely diffuse pattern and the other with at least a focal nodular component. Patients with purely diffuse tumors had a lower survival rate (0%) than those with tumors having a nodular component (62% survival rate). In contrast to the morphologic variability, these NHL exhibited a rather homogeneous immunophenotypic pattern. All cases demonstrated intense CD20 expression, with typically intense IgM and light chain expression, and relatively weak IgD expression. In no case was CD10 detected on the neoplastic cells. DNA content analysis showed aneuploidy only in three instances, and two groups of cases could be arbitrarily defined on the basis of their S-phase fraction. A relationship between a purely diffuse growth pattern and a high S-phase fraction (greater than 5%) was observed. As expected from this association, patients with tumors having high S-phase fractions fared worse (14% survival rate) than those patients with tumors showing lower S-phase fractions (57% survival rate). Thirteen NHLs from 12 patients had amplifiable bcl-1 gene rearrangements at the major translocation cluster (MTC). The bcl-1 breakpoints aggregated within a 63-bp region of the MTC, and the amplified tumor DNA from each patient had unique N-nucleotide junctional sequences and Ig joining region breakpoint sites. Two additional NHLs had bcl-1 gene rearrangements detected only with Southern blot hybridization using a genomic probe directed at a breakpoint site distant from the MTC. We conclude that CD5-expressing B-cell NHLs with bcl-1 gene rearrangement can be morphologically classified as MCL, have relatively uniform immunophenotypic characteristics, and are associated with an overall poor prognosis. Architectural growth pattern and kinetic information, such as S-phase fraction, may help separate a more aggressive group of these NHL from a relatively less aggressive subset.

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chain expression,7,12,17 and (3) typical absence of CD23 antigen.18 These studies support the notion that despite their morphologic heterogeneity, MCLs may be uniformly defined by their immunophenotypic characteristics. With this information in mind, we chose to examine our pool of CD5-positive B-cell neoplasms for t(11;14) using polymerase chain reaction (PCR) and restriction enzyme digest-Southern blot hybridization (SBH) analyses. This report documents the clinical, morphologic, relevant immunophenotypic, DNA content, and molecular genetic features of 15 of these lymphomas with bcl-1/JH gene rearrangements.

**MATERIALS AND METHODS**

**Case Selection Strategy and Source Material**

The files of the University of Florida/Shands Hospital Hematopathology Laboratory (Gainesville, FL) were reviewed for cases of neoplastic, CD5-expressing, B-cell lymphoproliferative disorders, and 123 cases were identified. Ethanol-fixed or frozen material from these specimens was tested for bcl-1 gene rearrangement by PCR analysis (all cases) and/or restriction enzyme digestion coupled with SBH (only performed in 68 cases due to limited DNA quantity and cost considerations). This study is based on 15 tissue samples (from 14 patients) that harbored bcl-1 gene rearrangements as identified by one or both methodologic techniques. Fourteen samples were lymph node biopsy specimens, and one was from a salivary gland-based mass.

**Clinical and Laboratory Data**

Clinical status and results of laboratory studies at initial diagnosis along with clinical follow-up information were sought on each case through review of referring physician's correspondence or patient charts and/or communication with the patient's physician(s).

**Morphologic Evaluation**

Morphologic characteristics were assessed on routine hematoxylin- and eosin-stained sections from either formalin- or B5-fixed tissues. The architectural features recorded were (1) pattern of involvement (diffuse, nodular, mantle-zone type), (2) presence or absence of residual germinal centers, (3) presence or absence of vascular sclerosis, (4) presence or absence of a histiocytic infiltrate, and (5) presence or absence of proliferation centers. The cytologic features recorded were relative size of neoplastic cells (eg, small, medium, large, or combinations of these cell types) and qualitative degree of nuclear irregularity.

**Flow Cytometric Analysis**

Preparation of single-cell suspensions from fresh tissue, cellular immunophenotyping, and DNA content/kinetic studies were all performed using methods previously published from this laboratory.17,19 Antibody selection and data interpretation were similar to that of a recent report from our group17; specifically, antigen expression and its intensity were both assessed. Intensity was assessed as weak (within log 1 fluorescence intensity), moderate (within log 2 fluorescence intensity), or strong (within log 3 fluorescence intensity). A moderate or strong rating was considered as intense expression of that particular antigen. Monoclonal antibody specificities included CD45, CD19, CD20, HLA-Dr, CD10, CD5, CD3, CD2, CD7, CD4, CD8, CD11c, CD25, CD23 (in two cases), and CD38 (in two cases) [Becton Dickinson Immunocytometry Systems (BDIS), San Jose, CA]. Polyclonal antibodies against Ig heavy chains (IgM, D, G, A) and Ig light chains (kappa, lambda) were also used (CalTag Laboratories, South San Francisco, CA; Kallestad Laboratories, Austin, TX). DNA content analysis was performed using CellFIT software in diploid cases (FITF model, BDIS), and MultiCycle software (Phoenix Flow Systems, San Diego, CA) for aneuploid cases. S-phase fractions were calculated for all cells in each sample. In aneuploid cases, the S-phase fractions reported represent the average value for the diploid and aneuploid cells.

**DNA Extraction**

DNA was extracted by a nonorganic method described by Miller.20 DNA quantity and quality were assessed by spectrophotometric analysis (Beckman DU64, Palo Alto, CA). A 100-ng aliquot (working concentration) of DNA from each sample was boiled for 2 minutes for optimal denaturation and then rapidly cooled on ice before storage (at 4°C) or amplification.

**DNA Amplification (bcl-1/JH Assay)**

*Primer/probe information.* All oligonucleotides were prepared on a DNA synthesizer (Applied Biosystems, Foster City, CA) through phosphoramidite chemistry at a dedicated facility at the University of Florida (Interdisciplinary Center for Biotechnology Research, Gainesville, FL). Each amplification included a JH consensus primer (5'ACC TGA GGA GAC GGT GAC and a primary primer (MCL-1; 5' GAT GGG CTT CTC TCA CCT ACT A) directed to the major translocation cluster (MTC) of bcl-1 complementary to a sequence 412 bp upstream from the SstI/Sac I restriction site on chromosome 11.17 An oligonucleotide probe (MCL-2; 5'TCA GGC CTT GAT AGC TCG) with complementary sequence internal to the MCL-1 primer annealing site and located 362 bp upstream of the SstI/Sac I site17 was used in hybridization experiments (see below). A schematic representation of the relative relationship of the primers and probe is shown in Fig. 1.

**General PCR amplification methods.** DNA amplification was performed in an automated thermocycler of the recirculated air type (Idaho Technology, Idaho Falls, ID) using capillary tubes as the individual reaction vessel. A 100-ng aliquot of genomic DNA was amplified in a 10-µL reaction volume containing 1X PCR buffer (50 mmol/L Tris pH 8.5, 3 mmol/L MgCl2, 500 µg/ml bovine serum albumin), 200 µmol/L each deoxyribonucleotide triphosphate (dNTP), 0.5 µmol/L each primer, and 0.4 U of Taq polymerase (Boehringer Mannheim, Indianapolis, IN). DNA amplification was performed by an initial precycling denaturation hold at 94°C for 30 seconds, followed by 45 cycles of denaturation at 94°C for 1 second, annealing at 60°C for 0 seconds, and extension at 72°C for 26 seconds. Optimal

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conditions were empirically determined in our laboratory (data not shown). This was partly done through reliance on and/or modification of previously published data with the use of an appropriate positive control sample. Rapid temperature cycling techniques that allow minimal denaturation and annealing times have been previously described.17,21,22

**Standard product analysis.** PCR products were subjected to electrophoresis in 2% agarose minigels and subsequently visualized by ethidium bromide staining and ultraviolet (UV) transillumination. A DNA marker (1 KB ladder; GIBCO/BRL, Gaithersburg, MD) was used to provide a size estimate in base pairs. Appropriate positive and negative (ie, no template DNA added) controls were run with each gel. In addition, amplification of a beta globin gene segment was performed on all fully negative samples to assure PCR amplification quality of genomic DNA. Samples with a visible PCR-amplified product within the expected size range (400 to 500 bp) were subsequently placed in a 500-μL microfuge tube, followed by DNA precipitation with 2.5 volumes of ethanol for 5 hours at -20°C. Optimal sample drying was attained through a 15-minute centrifugation in a high-speed centrifuge apparatus (DNA SpeedVac System; Savant, Farmingdale, NY). Reprecipitation was performed in 0.3 Molar sodium acetate/Tris EDTA buffer. Actual DNA sequence analysis was performed by standard methods on a DNA sequence analyzer (Applied Biosystems) in a specialized laboratory dedicated to these analyses (University of Florida, Interdisciplinary Center for Biotechnology Research). Interpretation of sequence data was performed manually through direct comparisons with previously reported bcl-1 MTC sequences23 and JH sequences.24-26

**Restriction Enzyme SBH for bcl-1 and Ig Gene Rearrangements**

SBH was performed according to standard procedures as previously published.25-29 Briefly, four separate 10-μg aliquots of DNA from each specimen were subjected to digestion with the following restriction enzymes: EcoRI (GIBCO/BRL), Pst I (GIBCO/BRL), BamHI (GIBCO/BRL), and HindIII (Boehringer Mannheim). DNA was denatured, and immobilized by a rapid UV crosslinking procedure (Stratagene, La Jolla, CA). Subsequently, membranes were hybridized with a digoxigenin-labeled internal probe (ie, MCL-2 oligonucleotide) and detected with an anti-digoxigenin alkaline phosphatase-based system according to the manufacturer’s instructions (Genius System; Boehringer Mannheim). Specifically, the probe was tailed at its 3' end with digoxigenin-dUTP (Genius System; Boehringer Mannheim), and after a 2-hour prehybridization incubation, membranes were hybridized with 10 pmol/μL labeled probe. A positive reaction was visualized as a blue/black color at the site of complementary sequence binding on the membrane.

**Direct sequence analysis of PCR-amplified products.** Amplified DNA was recovered through band interception in agarose gels using 8 to 10 separate reaction volumes (ie, 80 to 100 μL) for each specimen. Diethyl aminoethyl (DEAE) membranes (NA45; Schleicher and Schuell, Keene, NH) were prepared as directed by the manufacturer by washing for 10 minutes in 10 mM EDTA pH 7.6 and 5 minutes in 0.5 Molar NaOH, followed by three brief washes in distilled water. Briefly, according to manufacturer’s instructions (Schleicher and Schuell) and after routine electrophoretic separation, a strip of NA45 was placed in an incision directly upstream of the band. Electrophoresis was reinitiated until, as judged by ethidium bromide/UV assessment, binding to the inserted strip was complete. The wet strip was subsequently placed in a 500-μL microfuge tube, covered with 250 μL of high salt NET buffer (5 mMolar NaCl, 10 mMolar EDTA, 1 mMolar Tris at pH 8.0), centrifuged for 5 seconds, and then incubated at 55°C to 60°C for approximately 30 minutes. An extraction with 3 volumes of water-saturated n-butanol was performed, followed by DNA precipitation with 2.5 vol of ethanol for 5 hours at -20°C. Optimal sample drying was attained through a 15-minute centrifugation in a high speed centrifuge apparatus (DNA SpeedVac System; Savant, Farmingdale, NY). Reprecipitation was performed in 0.3 Molar sodium acetate/Tris EDTA buffer. Actual DNA sequence analysis was performed by standard methods on a DNA sequence analyzer (Applied Biosystems) in a specialized laboratory dedicated to these analyses (University of Florida, Interdisciplinary Center for Biotechnology Research). Interpretation of sequence data was performed manually through direct comparisons with previously reported bcl-1 MTC sequences23 and JH sequences.24-26

**DNA Amplification (Ig Heavy Chain Gene Clonality Assays)**

The assessment of B-cell clonality by PCR analysis was performed in a similar fashion to that described above and, specifically, according to procedures and protocols recently reported from this laboratory.26

**RESULTS**

**Clinical Data**

Clinical and laboratory features at initial diagnosis. Pertinent clinical and laboratory data are detailed in Table 1. One patient had two separate biopsy samples (case 8a/b); these are considered together unless otherwise noted. There were 12 men and two women, ranging in age from 47 to 89 years (mean, 67 years). Most patients initially presented with lymph node enlargement (13 of 14 patients; 93%) and had high-stage (ie, Ann Arbor Stage III or IV) disease (12 of 13 patients evaluated; 92%). Bone marrow was involved in seven of nine patients (78%) evaluated. A significant proportion had splenic involvement (7 of 13 patients evaluated; 54%), but clinically apparent liver involvement was not observed. Involvement of extranodal sites including cerebrospinal fluid, salivary gland, base of tongue, eyelid, and colon was noted in some patients, as specified in Table 1. Occasional patients had a peripheral blood lymphocytosis (2 of 14) or an elevated serum lactate dehydrogenase level (3 of 14) at the time of diagnosis.

**Initial treatment, response, and survival data (Table 2).** Most tissue specimens used for this study were received for diagnostic purposes from different institutions. Therefore, the individual patients were not treated or followed in a uniform manner. All patients but one were given some form of initial therapy, including local radiotherapy alone (one patient), single-agent chemotherapy (four patients), mild multiagent chemotherapy [ie, cyclophosphamide, vincristine, prednisone (CVP); two patients], or standard multiagent chemotherapy with/without local radiotherapy (six patients).
A single patient was initially observed for approximately 1 year without treatment but was subsequently given single-agent chemotherapy because of progressive disease.

The mean patient follow-up was 24 months (range, 4 months to 52 months; Table 2). All but one patient had a partial (PR) or complete (CR) clinical response. Four of six patients with a CR eventually relapsed from treatment. Of the two additional patients attaining a CR, one died with no apparent clinical recurrence (no autopsy was performed), and the other is without clinical evidence of tumor at 11 months after completion of treatment. A total of nine patients have died, with a median survival of 19 months (range, 5 months to 52 months). Four of the five living patients still have detectable tumor at 4 months to 42 months from diagnosis. The overall median survival was 20 months. Notably, three patients de-

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Treatment (duration)</th>
<th>Initial Response/Subsequent Status</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Clb (9 mos)</td>
<td>No response/disease progression</td>
<td>DWD (28 mos)</td>
</tr>
<tr>
<td>2</td>
<td>Local XRT (1 mo)*</td>
<td>CR/no clinical recurrence</td>
<td>Dead, UGI bleed (19 mos)</td>
</tr>
<tr>
<td>3</td>
<td>COP-BLAM (7 cycles)</td>
<td>PR/persistent residual disease</td>
<td>Dead, AML (14 mos)</td>
</tr>
<tr>
<td>4</td>
<td>Cyclophosphamide (3 mos)</td>
<td>PR/stable off TX (at 1 mo)</td>
<td>AWD (4 mos)</td>
</tr>
<tr>
<td>5</td>
<td>ProMACE-CytobOM (6 cycles)</td>
<td>CR/stable off TX (at 11 mos)</td>
<td>AWOD (16 mos)</td>
</tr>
<tr>
<td>6</td>
<td>CVP (6 cycles)/Clb (24 mos)</td>
<td>PR/progressed off TX</td>
<td>AWD (42 mos)</td>
</tr>
<tr>
<td>7</td>
<td>CVP (6 cycles)</td>
<td>PR/progressed off TX</td>
<td>DWD (12 mos)</td>
</tr>
<tr>
<td>8a/b</td>
<td>Clb, prednisone (6 mos)</td>
<td>PR/progressed off TX</td>
<td>DWD (40 mos)</td>
</tr>
<tr>
<td>9</td>
<td>CHOP (3 cycles)/mBACOD (1 cycle)</td>
<td>CR/relapse (at 4 mos)</td>
<td>DWD (17 mos)</td>
</tr>
<tr>
<td>10</td>
<td>CHOP (4 cycles)</td>
<td>PR/progressed off TX</td>
<td>DWD (5 mos)</td>
</tr>
<tr>
<td>11</td>
<td>Clb, prednisone (6 mos)†</td>
<td>PR/stable off TX (at 3 mos)</td>
<td>AWD (21 mos)</td>
</tr>
<tr>
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<td>High-dose pulse Clb (5 cycles)</td>
<td>CR/relapse (at 2 mos)</td>
<td>DWD (27 mos)</td>
</tr>
<tr>
<td>13</td>
<td>ProMACE-CytobOM (7 cycles)</td>
<td>CR/relapse (at 15 mos)</td>
<td>AWD (38 mos)</td>
</tr>
<tr>
<td>14</td>
<td>CHOP and XRT (unknown)</td>
<td>CR/relapse (at 42 mos)</td>
<td>DWD (52 mos)</td>
</tr>
</tbody>
</table>

Abbreviations: TX, treatment; UGI, upper gastrointestinal; AML, acute myelogenous leukemia; DWD, dead with disease; AWOD, alive without clinical evidence of disease; AWD, alive with disease; Clb, chlorambucil; XRT, X-ray therapy; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; COP-BLAM, CHOP with procarbazine and bleomycin; ProMACE-CytobOM, cyclophosphamide, doxorubicin, etoposide, cytarabine, bleomycin, vincristine, methotrexate, prednisone; CVP, cyclophosphamide, vincristine, prednisone; mBACOD, cyclophosphamide, doxorubicin, vincristine, bleomycin, methotrexate, and dexamethasone.

* XRT involved neck and central nervous system.
† This patient was observed for 1 year after diagnosis, treatment was initiated due to progressive nodal enlargement and associated symptoms.
developed an end-stage leukemic phase (range of white blood cell counts, \(60 \times 10^9/L\) to \(450 \times 10^9/L\)) composed of neoplastic lymphocytes.

**Morphologic Data**

General comments on morphologic evaluation. The architectural and cytologic features of all specimens appeared to fall within the diagnostic boundaries previously described for MCL. Specific morphologic findings on individual cases are shown in Table 3.

Architectural features. The architectural features showed variability. Six cases exhibited an entirely diffuse pattern, four were predominantly diffuse with focal areas of nodularity, and four specimens were predominantly or entirely nodular. Many of the cases with a nodular component had ill-defined or vague nodularity, with the exceptions being two specimens with a mantle zone pattern (cases 5 and 6) and another that had many well-defined nodules (case 11).

Residual germinal centers were present in three cases, two of which had a mantle zone pattern (wide mantles surrounding benign-appearing germinal centers). Vascular sclerosis was observed in five cases, with only two having prominent, generalized changes. Proliferation centers were not apparent in any specimen. Interestingly, all of the lymphomas had a benign-appearing histiocytic infiltrate. In nine cases the infiltrate was marked, with some specimens exhibiting a "starry sky" appearance. The other five cases had a slight to moderate number of histiocytes intermixed with the neoplastic lymphocytes.

The patient survival rate for cases with and without a nodular component was remarkably different. Patients whose tumors had any nodularity present had a better survival rate (62% survival rate, mean follow-up of 20.4 months) than those whose tumors exhibited only a diffuse architectural pattern (0% survival rate, mean follow-up of 24.7 months).

Cytologic features. Similar to the architectural heterogeneity, the cytologic features of the neoplastic cells from different tumors showed variability. Most lymphomas had a predominantly small cell infiltrate (11 specimens) composed of lymphocytes with minimal cytoplasm, variable nuclear border irregularity, and partially condensed chromatin with inconspicuous nucleoli. Each of these 11 cases had a minor component of larger lymphocytes intermixed with the many small cells. The other three lymphomas had a different cyto-logic composition: one (case 9) had mostly large cells, and the other two (cases 2 and 14) were primarily composed of medium size cells. However, all three also contained intermixed, small, atypical lymphocytes. A dispersed chromatin pattern (possibly of blastic type) was observed in one of the lymphomas with cells of medium size. The three cytologically variant cases had a diffuse architectural pattern. Although admittedly subjective and potentially biased by fixation and tissue section thickness, we documented slight nuclear irregularity in one case, moderate nuclear irregularity in 10 cases, and marked nuclear irregularity in three cases. A cleaved nuclear morphology was not a typical finding in any specimen; rather, the irregularity was characterized by variably prominent nuclear indentations, or an undulated appearance.

**Flow Cytometry**

Immunophenotypic features. By study definition, all the cases contained neoplastic cells that coexpressed B-cell–associated and CD5 antigens. The fraction of neoplastic cells in the samples ranged from 57% to 99% (median, 80%) as assessed by immunophenotypic data. The surface phenotype of the neoplastic cells was remarkably uniform. CD20 was intensely expressed (ie, at a minimum, log 2 fluorescence intensity) in all specimens. In addition, CD20 was consistently expressed at a greater intensity relative to CD19 antigen expression. CD10 was not detected on the neoplastic B cells in any specimen. In contrast, surface Ig was identified on the neoplastic B cells in each tumor. Specifically, IgM and IgD were consistently expressed, while IgG and IgA were not found at any significant level of expression in any case. Ig light chain restriction was consistently observed.
with five cases expressing kappa and nine cases expressing lambda epitopes. Ig intensity was typically moderate to strong with respect to IgM and light chains, but only weak to moderate for IgD. The only exception was observed in the patient with two temporally distinct samples (case 8), in which IgM, IgD, and Ig lambda were all weakly expressed.

The expression of remaining antigens was as follows: CD45, 14 of 14 cases; HLA-DR, 13 of 14 cases; non-CD5 T-cell–associated markers, 0 of 14 cases; CD25, 2 of 14 cases; CD11c, 2 of 14 cases; CD23, none of two cases; and CD38, two of two cases.

**DNA content.** DNA data are detailed in Table 3. The majority of tumors were diploid; however, there were three cases with an aneuploid DNA content. The S-phase fractions ranged from 1% to 16% (mean, 6%). A strong correlation was observed between the architectural pattern and the S-phase fraction (ie, all cases with a purely diffuse pattern had an S-phase fraction greater than 5%). When cases were then arbitrarily segregated according to S-phase fraction, patients with tumors exhibiting less than a 5% S-phase fraction had a higher survival rate (57%, mean follow-up of 26.1 months) than those whose tumors had higher S-phase fractions (14% survival rate, mean follow-up of 21.7 months).

**Molecular Genetic Features**

Products of bcl-1/JH were amplified in 13 specimens from 12 patients. The amplified bands were within the expected size range, exhibited appropriate molecular size heterogeneity, and demonstrated a positive hybridization signal with the internal MCL-2 probe (Fig 2).

In addition, direct sequence analysis of these PCR products definitively established them as bcl-1 (MTC)/JH rearranged segments, with each tumor having a distinctive sequence. However, samples 8a and 8b, which were from one patient, had the same breakpoints and general nucleotide sequence. Overall, the bcl-1 breakpoints fell within a 63-bp region of the MTC site on chromosome 11 (Fig 3). Although most had distinct breakpoints, several samples from different patients apparently had the same MTC breakpoint (Fig 3). Each of these tumors, however, had unique DNA sequences at the bcl-1/JH junction and JH breakpoints (Fig 4). The
However, all eight had detectable bcl-l MTC gene rearrangement sites (ie, p94). A direct comparison of PCR over the last several years, the majority of recent reports have focused on the identification of bcl-l rearrangements by SBH and/or PCR (data not shown). The two samples detected with the alternative non-MTC breakpoint probe could not be amplified with the bcl-l (MTC)/JH PCR assay. All cases with bcl-l rearrangements also had detectable IgH gene rearrangements by SBH and/or PCR (data not shown).

**DISCUSSION**

Conceptual views on MCL are currently evolving. Although abundant information on MCL has become available over the last several years, the majority of recent reports have focused on the identification of bcl-l gene rearrangements in examples of this entity classified primarily on morphologic grounds. It is clear that a strong relationship exists, but it is also apparent that not all NHLs designated as MCLs harbor t(11;14). Insufficient data are available on the biologic characteristics and clinical behavior of MCLs that have been characterized through the detection of this genetic abnormality. Furthermore, a recent proposal aimed at defining a unifying construct for MCL documents the difficulty that has existed in interpreting and comparing published series on this subject and openly invites additional studies based on well-characterized examples to assist in gaining a better understanding of their nature. The purpose of our investigation was to provide such additional information on MCL through the detailed and comprehensive analysis of objectively characterized specimens. In contrast to the typical use of relative subjective, non-universally accepted, and evolving morphologic criteria to select potential study cases, we focused our attention on the relatively homogeneous immunophenotypic characteristics and molecular genetic features of this entity. Although MCLs without detectable bcl-l gene rearrangements or CD-5 expression undoubtedly exist, only CD5-expressing B-cell neoplasms with evidence of bcl-l gene rearrangement were included in the present study.

In our series, we have shown that CD5-expressing B-cell NHLs with bcl-l gene rearrangement have relatively uniform clinical and biologic characteristics, and that these generally overlap with the features previously documented for MCL. Clinically affected individuals were mostly men (86%) with a mean age of 67 years. The majority had lymphadenopathy and high-stage disease with frequent bone marrow involvement, and slightly more than half had clinical evidence of splenic enlargement at diagnosis. Other sites of extranodal disease, including peripheral blood involvement, were also observed in some patients.

Despite the fact that most patients in our series had documented therapeutic responses to various regimens, the majority had evidence of disease progression or relapse. Although the clinical presentation and high relapse rate are reminiscent of low-grade NHL in the Working Formulation, the poor survival rate of our patient population (ie, nine patients dead and an overall median survival of 20 months) seems to indicate that CD5-expressing B-cell NHLs with bcl-l gene rearrangement are not typical indolent malignancies. Our findings are in agreement with several prior series of similar patient size and scope that have documented an overall short median survival (20 to 30 months) and surprisingly aggressive course for patients with t(11;14)-harboring B-cell NHLs. In contrast, most prior studies on patients with MCLs in general have reported median survival data that varied more widely, ranging from 24 to 88 months. This suggests that a detectable bcl-l gene rearrangement in CD5-expressing B-cell NHL may define, or may be one important factor in defining, MCL with more aggressive biological potential. This hypothesis cannot be directly substantiated or refuted with available information, as most clinical studies have primarily used morphologic criteria to classify MCL, without considering their bcl-l gene rearrangement status. One exception is a series claiming that no significant clinical differences were apparent between MCL with and without bcl-l gene rearrangements; however, the median clinical follow-up in this study was relatively short (ie, 13 months). Interestingly, almost 50% of their patients with detectable bcl-l gene rearrangements by SBH analysis were already dead in contrast to only 30% of those without a bcl-l rearrangement. Prospective, randomized studies examin-
ing the clinical behavior of well-characterized and uniformly treated MCL is necessary to draw any definitive conclusion. We cannot comment on the clinical impact of the presence of bcl-1 rearrangements in other lymphoproliferative processes, particularly those that do not express CD5.

On morphologic grounds, all of the samples in our series could be classified within the MCL category. We found no predominant histologic subtype, but a rather heterogeneous collection of patterns that have been previously described as lying within this category. In general, we could identify two major architectural subgroups: one with purely diffuse growth and the other with at least focal nodularity. The majority of tumors were primarily comprised of small neoplastic lymphocytes. It is also important to note that neoplasms with a mantle zone pattern were uncommon in our series. This is consistent with the short median survival of our patient population, as the mantle zone variant has been associated with an overall longer survival.

The most significant ancillary morphologic features noted were the consistent absence of proliferation centers characteristic of SLL/CLL and the variable presence of an intermixed benign histiocytic proliferation. This latter observation has not been emphasized in the literature on MCL; however, Lardelli et al clearly document that a histiocytic infiltrate is a common finding in this type of NHL.

In contrast to the morphologic variability of our CD5-expressing B-cell lymphomas with bcl-1 gene rearrangement, we found extremely uniform immunophenotypic features, including intense CD20, IgM, and Ig light chain expression, weak to moderate IgD expression, no CD10 reactivity, and in two cases tested, no reactivity with CD23 antibody. The above CD20 and Ig intensity patterns were observed in all but one case in which the neoplastic B cells expressed weak IgM, IgD, and Ig lambda, while maintaining intense CD20 expression (case 8). There was also an excess of lambda-restricted neoplasms (nine cases) compared with kappa-restricted ones (five cases). These immunophenotypic characteristics have all been previously documented, to some extent and with varied emphasis, in studies focused on MCL and/or t(11;14)-harboring B-cell NHL. Our findings, along with prior data, provide strong evidence that immunophenotyping is an important tool in the critical analysis of MCL, as it assists in accurate categorization of a morphologically heterogeneous group of neoplasms. These data and that of our earlier series clearly document the importance of assessing CD20 intensity, as MCLs with bcl-1 gene rearrangements appear to invariably express this antigen at moderate to strong levels.

Information on DNA content and kinetic features of MCL is scanty. This is a significant deficiency, as S-phase fraction has been shown to correlate well with histologic grade in NHL. This is relevant to MCL, as the grading of this entity is controversial due primarily to (1) a lack of grade assignment within the Working Formulation, (2) the unusual blend of low- and intermediate-grade clinical characteristics, and (3) the inconsistent portrayal of grade in the literature (ie, MCL is typically included with low-grade NHL in some studies, while others suggest that it should be classified within the intermediate-grade category of diffuse small cleaved cell lymphoma). Thus, kinetic data could provide a useful, adjunct biologic parameter for predicting the clinical behavior of MCL.

A previous abstract reported S-phase fractions at 5% or greater in seven of nine t(11;14)-containing NHLs, suggesting that many of these neoplasms are not in a low proliferative, indolent state. In addition, the few analyzed centrocytic lymphomas documented in the literature have also had relatively high S-phase fractions.

We have observed low S-phase fractions, all less than 3%, in MCLs without detectable bcl-1 gene rearrangements. In contrast, in this study we found that MCLs with bcl-1 rearrangements fell into two separate groups with respect to the fraction of cells in S-phase. A correlation was observed between survival rate and S-phase fraction; patients with tumors having low S-phase fractions (less than 5%) had a better survival rate than patients whose tumors exhibited higher S-phase fractions at a comparable length of follow-up evaluation. Similarly, patients whose tumors exhibited at least a focal nodular architectural component had a better survival rate than those with purely diffuse tumors. Not surprisingly, an association between diffuse architecture and high S-phase fraction (greater than 5%) was evident. The relatively small number of individuals in this series, however, precluded a meaningful statistical analysis. Additional studies are needed to optimally determine the clinical utility of kinetic and growth pattern information in this setting. Prior series, however, have suggested that a nodular mantle zone pattern portends a better prognosis in MCL.

A strong association has been established between the cytogenetic identification of t(11;14) and MCL. More recent studies have determined that bcl-1 loci rearrangement with JH gene segments is the molecular genetic equivalent of this karyotypic abnormality. Several different groups have determined that bcl-1 rearrangements are detectable by direct amplification of the bcl-1 (MTC)/JH junction in MCL. In the present investigation, we have extended our molecular genetic findings. First, we have shown that PCR has a comparable bcl-1 gene rearrangement detection rate to SBH at the MTC breakpoint site, as MTC rearrangements were detected in the same eight specimens evaluated by both techniques. Rikos et al have reported similar findings in 15 of 16 MCL samples. In contrast, the MTC-based bcl-1 PCR assay did not appear to detect rearrangements at alternative breakpoints of the bcl-1 locus [ie, SBH analysis with p94 identified rearrangements in two cases that showed no amplification with the bcl-1 (MTC)/JH assay]. Second, through Southern hybridization experiments performed with an inter-
nal bcl-1 (MTC) probe, we have shown that a rapid, nonisotopic detection system is capable of confirming the specificity of a bcl-1/JH amplification product. As direct sequence analysis may not be practical for diagnostic purposes, the probe hybridization procedure can be used subsequent to ethidium bromide screening and size estimation to assist in product verification. The rationale is similar to the internal probe verification step used for bcl-2/JH assays previously reported by our group.11

Finally, we directly sequenced the amplified DNA segments from all 13 samples (12 patients) with MTC breakpoints. Each amplified bcl-1/JH fragment had both common and unique nucleotide sequences. The amplified products of two temporally distinct specimens from one patient (case 8), however, were identical. Both of these observations document the tumor specificity of the amplified product. The bcl-1 breakpoints were tightly clustered within a 63-bp region of the MTC similar to previous reports.23,50 Notably, several MCLs from different patients had identical MTC breakpoints; however, each neoplasm could be differentiated from the other at the DNA level by the N-nucleotide sequence at the bcl-1/JH junction and the JH breakpoint site. Identical bcl-1 breakpoints in different MCLs have been previously documented in a single series,50 and as illustrated through a literature comparison (Fig 3), several specimens from different reports have had the same MTC breakpoint.30,52 Given the limited region in which rearrangement occurs at the MTC, this is not a surprising occurrence. General nucleotide sequence, bcl-1 breakpoint and JH breakpoint sites, and N-region length and heterogeneity were all similar to previously reported bcl-1 (MTC)/JH junctions of MCL DNA,23,30 supporting the notion that a strong genetic relationship exists among these morphologically heterogeneous but immunophenotypically uniform neoplasms.

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CD5-expressing B-cell non-Hodgkin's lymphomas with bcl-1 gene rearrangement have a relatively homogeneous immunophenotype and are associated with an overall poor prognosis

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