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

By Glenn H. Segal, Aneal Masih, Allyson Canavan Fox, Trevor Jorgensen, Marda Scott, and Raul C. Braylan

Mantle cell lymphomas (MCLs) are typically CD5-expressing B-cell non-Hodgkin’s lymphomas (NHLs) that frequently harbor the chromosomal translocation t(11;14) or 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, and in order to avoid using somewhat arbitrary and subjective morphologic definitions, 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 a 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 higher 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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CD5+ B-CELL LYMPHOMA WITH bcl-1 REARRANGEMENT

Chain expression,\textsuperscript{7,12,17} and (3) typical absence of CD23 antigen.\textsuperscript{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 Hematology 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.\textsuperscript{17,19} Antibody selection and data interpretation were similar to that of a recent report from our group\textsuperscript{17}; 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 (FFIT 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.\textsuperscript{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 3'3' 3' complementarity site" was used in hybridization experiments with the Ig heavy chain gene, respectively. Arrows denote primers (MCL-1 or JH) and their respective orientation in the reaction. The solid line denotes the internal oligonucleotide probe (MCL-2). Asterisk designates the bcl-1/JH junction, a consequence of t(11;14).

**Fig 1.** Schematic representation of a t(11;14) chromosomal translocation showing the orientation and relative locations of primers and probe. JH and CH represent joining and constant regions on the Ig heavy chain gene, respectively. Arrows denote primers (MCL-1 or JH) and their respective orientation in the reaction. The solid line denotes the internal oligonucleotide probe (MCL-2). Asterisk designates the bcl-1/JH junction, a consequence of t(11;14).

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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.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)16 on ethidium bromide screening were further analyzed for specificity verification through SBH and nonisotopic probe detection. Briefly, PCR products were Southern-transferred to positively-charged nylon membranes (Boehringer Mannheim) by semidry blotting (BioRad, Hercules, CA). Following a 1-hour prehybridization, membranes were hybridized with 10 pmol/mL labeled probe. The hybridization procedure was verified through SBH and nonisotopic probe detection. Briefly, four separate 10-μL 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 fragments were separated by electrophoresis in 0.7% agarose gels, evaluated by ethidium bromide/UV transillumination for adequacy of fractionation, and transferred to nylon membranes by the Southern method. Probes, labeled with 32P by random hexamer priming, were serially hybridized with the prepared blots. Finally, hybridized blots were exposed to SAR-2 film (Eastman Kodak, Rochester, NY) for 2 to 5 days at −70°C. Three different genomic probes were used for hybridization experiments, including (1) bcl-1 at MTC (2.1-kb SacI fragment, probe designated b216 and provided by Dr Y. Tsujimoto, Wistar Institute, Philadelphia, PA); (2) bcl-1 at an alternate breakpoint site approximately 24 kb telomeric to MTC (460-bp PstI-SmaI fragment, probe designated p9421 and provided by Dr T. Meeker, University of Kentucky, Lexington, KY); and (3) Ig heavy chain gene joining region (5.4-kb fragment; Oncogene Science Inc, Manhasset, NY). Although additional genomic bcl-1 probes are reported in the literature,22 they were not available to us at the time of this study. A germline placent DNA control was run with each gel, along with lambda DNA/HindIII fragments (GIBCO/BRL) as a DNA marker.

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 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-
nodular component was remarkably different. Patients whose tumors had any nodularity present had a better survival rate (57% to 99% 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.

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.9,11-13 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 cytologic 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.

Table 3. Morphologic and DNA Content Data

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Architectural Features</th>
<th>Predominant Cytologic Component</th>
<th>DNA Content</th>
<th>S-Fraction (%)</th>
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<tr>
<td>1</td>
<td>Diffuse only</td>
<td>Small cell</td>
<td>Diploid</td>
<td>8</td>
</tr>
<tr>
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<td>Medium cell</td>
<td>Aneuploid (D1 = 1.95)</td>
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<tr>
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<td>Small cell</td>
<td>Diploid</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>Diffuse and nodular*</td>
<td>Small cell</td>
<td>Diploid</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>Diffuse and nodular*</td>
<td>Small cell</td>
<td>Aneuploid (D1 = 1.9)</td>
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</tr>
<tr>
<td>6</td>
<td>Nodular</td>
<td>Small cell</td>
<td>Diploid</td>
<td>7</td>
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<td>7</td>
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<td>Diploid</td>
<td>3</td>
</tr>
<tr>
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<td>Diploid</td>
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</tr>
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<td>Large cell</td>
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<td>Medium cell</td>
<td>Diploid</td>
<td>11</td>
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</tbody>
</table>

Abbreviation: D1, DNA index.
* Predominantly diffuse pattern with only focal nodular areas.
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; HLADr, 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 two temporally distinct samples from the same patient (case 8a/b) are considered separately in this section. 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).
sequence of additional nucleotides (N nucleotides) at the translocation joining site were unique to each sample and ranged from 2 to 38 bp in length. Ig heavy chain gene joining region family usage was as follows: JH1, two patients; JH4, three patients; JH5, two patients; and JH6, five patients.

Using the SBH technique, 10 cases showed nongermline, rearranged bcl-1 fragments. Eight were identified with the MTC probe, and two were identified with the alternative breakpoint site probe (ie, p94). A direct comparison of PCR and SBH in this setting was somewhat limited, as only 8 of the 13 PCR-positive samples were evaluated by SBH. However, all eight had detectable bcl-1 MTC gene rearrangements with both techniques. The two samples detected with the alternative non-MTC breakpoint probe could not be amplified with the bcl-1 (MTC)/JH PCR assay. All cases with bcl-1 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-1 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 detectable bcl-1 gene rearrangements. This suggests that a detectable bcl-1 gene rearrangement in CD5-expressing B-cell NHL may define, or may be one of, a spectrum of lymphomas with certain clinical characteristics and molecular genetic features of this entity. Although MCLs without detectable bcl-1 gene rearrangements or CD-5 expression undoubtedly exist, only CD5-expressing B-cell neoplasms with evidence of bcl-1 gene rearrangement were included in the present study.

In our series, we have shown that CD5-expressing B-cell NHLs with bcl-1 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-1 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-1 gene rearrangement in CD5-expressing B-cell NHL may define, or may be one of, a spectrum of lymphomas with certain clinical characteristics and molecular genetic features of this entity. Although MCLs without detectable bcl-1 gene rearrangements or CD-5 expression undoubtedly exist, only CD5-expressing B-cell neoplasms with evidence of bcl-1 gene rearrangement were included in the present study.
ing the clinical behavior of well-characterized and uniformly treated MCLs will be 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 data from our series suggest that a mantle zone pattern may be infrequent in MCLs harboring a bcl-1 gene rearrangement; however, a larger study is required to determine if this is a valid conclusion.

The most significant ancillary morphologic features noted were the consistent absence of proliferation centers characteristic of SLL/CLL and the invariably 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 other previous works 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 bi- 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. This contrasts with low-grade NHLs, such as SLL/CLL, and most follicular lymphomas that almost invariably have low 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. 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 shown that up to 73% of MCLs have detectable bcl-1 gene rearrangements when multiple breakpoint site probes are used. More recently, we and other investigators have shown that PCR can be used for direct amplification of the bcl-1 (MTC)/JH junction. 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. Rikmoh et al have reported similar findings in 15 of 16 MCL samples. In contrast, the MTC-based bcl-1 PCR assay does 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.

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.

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, and as illustrated through a literature comparison (Fig 3), several specimens from different reports have had the same MTC breakpoint. Given the limited region in which re-arrangement 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, supporting the notion that a strong genetic relationship exists among these morphologically heterogeneous but immunophenotypically uniform neoplasms.

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