Residual Monotypic Plasma Cells in Patients With Waldenström Macroglobulinemia After Therapy

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Abstract

Waldenström macroglobulinemia (WM) is currently defined as lymphoplasmacytic lymphoma involving bone marrow (BM) associated with a serum IgM paraprotein.1 WM is typically composed of small lymphocytes, plasmacytoid lymphocytes, and plasma cells in variable proportions, which can change after therapy. In this study, we assessed 41 WM cases that required chemotherapy, 39 showing persistent disease in restaging BM specimens. In 10 cases, there was persistent monotypic plasmacytosis in BM in the absence of demonstrable monotypic B cells. The monotypic plasma cells represented 0.5% to 46% of the cellularity and persisted 1 to 50 months after the last course of chemotherapy. The plasma cells were best quantified by immunohistochemical analysis on paraffin sections. We conclude that WM can persist as a pure plasma cell population after therapy. This finding has implications for the immunophenotypic assessment of WM after therapy and may explain persistent IgM paraproteinemia in patients with WM with no evidence of a clonal B-lymphocyte population.

Waldenström macroglobulinemia (WM) is currently defined as lymphoplasmacytic lymphoma (LPL) involving bone marrow (BM) associated with a serum IgM paraprotein of any level.1 A subset of patients with WM also can have lymphadenopathy and splenomegaly or, less commonly, hyperviscosity syndrome if serum IgM paraprotein levels are high. Pathologically, WM most commonly involves the BM in an interstitial pattern and is composed of small lymphocytes, plasmacytoid lymphocytes, and plasma cells in variable percentages. However, WM can involve the BM in a number of other patterns, and plasmacytoid differentiation, including Marschalko-type (mature-appearing) plasma cells, can be minimal or marked.2 Immunophenotypically, the B cells in WM are typically surface IgM+, CD5−, CD10−, CD19+, CD20+, CD22+, CD23−/dim+, CD25+, CD27+, FMC7+, and CD103−.2,3 By flow cytometry, one can often identify 2 distinct subsets of cells in WM: monotypic B lymphocytes that are surface IgM+, CD20+, and CD138− and monotypic plasma cells that are cytoplasmic IgM+, CD138+, and CD20−/+.4

Because patients with WM often have a clinically indolent course, therapy is reserved for patients with symptoms. The major therapeutic options for patients with WM include alkylating agents, purine analogues, and monoclonal antibodies such as rituximab. The latter is a chimeric antibody that targets CD20. Rituximab induces antibody-dependent cellular cytotoxicity, complement-mediated lysis, and apoptosis and has been used in combination with conventional chemotherapy for many types of B-cell lymphoma, including LPL/WM.5

The presence of monotypic IgM in serum, also known as IgM paraprotein, is a tumor-specific marker that can be easily assessed in a noninvasive manner. As a result, assays for IgM paraprotein levels have been used to monitor patients with WM after therapy. Patients with WM commonly show a good...
response to therapy, as assessed by a drop in serum IgM paraprotein level, but it is known that maximal response can be delayed for a number of months after all types of chemotherapy, including single-agent rituximab. Pathologic examination of BM in WM patients after therapy often shows a substantial decrease or complete absence of B lymphocytes, even when the patients have persistent IgM paraproteinemia.6

In this study, we assessed 41 WM cases that required chemotherapy, in most cases including rituximab, and that had morphologic and flow cytometric immunophenotypic assessment of follow-up BM samples. We show that WM can persist in the BM as monotypic plasmacytosis, without B lymphocytes, after therapy in a subset of patients. This finding has implications for the flow cytometric immunophenotypic analysis of residual disease and may explain the persistence of IgM paraproteinemia in some WM cases without evidence of lymphoid disease in the BM.

Materials and Methods

We began this study by focusing on a series of 75 WM cases previously reported by Konoplev et al,7 with approval from the institutional review board. This study group was obtained by a search of the files of the Department of Hematopathology, The University of Texas M.D. Anderson Cancer Center, Houston. For this study, we focused on WM cases treated with rituximab, alone or as a part of a combined chemotherapy regimen, and in which follow-up BM samples were obtained for histologic and flow cytometric immunophenotypic analysis. The original diagnosis of WM for each case was verified by review of BM aspirate smears and biopsy specimens and the flow cytometric immunophenotypic data. The medical records were searched for demographic data, clinical symptoms, type of therapy, and the results of serum protein electrophoresis and immunofixation at time of initial diagnosis and after therapy. Conventional cytogenetic and molecular results were reviewed, if performed. For this study, we used the definition of WM initially proposed by Owen et al8 as part of the 2002 International Workshop on WM, which was essentially adopted in the 2008 World Health Organization classification. Thus, we included cases with IgM monoclonal gammapathy of any concentration and BM infiltration by small lymphocytes showing plasmacladoyt or plasma cell differentiation, composed of clonal B cells with a surface IgM+/CD5+/CD10+/CD19+/CD20+ immunophenotype.

Immunohistochemical Methods

Immunohistochemical analysis was performed using 4-μm thick, formalin-fixed, paraffin-embedded tissue sections of BM biopsy or aspirate clot specimens. The slides were deparaffinized in xylene, rehydrated with graded alcohols, and heated in EDTA buffer at pH 8.0 in a steamer (Black and Decker, Towson, MD). After blocking endogenous peroxidase activity with hydrogen peroxide, the slides were washed with buffer and then incubated with primary antibody for 30 to 60 minutes at 23°C. The DAKO Autostainer (DakoCytomation, Carpinteria, CA) was used for staining. The washing buffer was 0.05 mol/L tris(hydroxymethyl)aminomethane-buffered saline with 0.05% polysorbate. Diaminobenzidine was used as the chromogen. Appropriate positive and negative control samples were included for each run. The antibodies used in various cases included CD3 (polyclonal, dilution 1:80; DAKO), CD20 (dilution 1:700; DAKO), immunoglobulin κ and λ light chains (dilution 1:10,000; DAKO), BCL-6 (dilution 1:10; DAKO); CD138 (dilution 1:20; DAKO); CD5 (dilution 1:20; Lab Vision, Fremont, CA), cyclin D1 (SP4, dilution 1:40; Lab Vision); CD10 (dilution 1:75; Novoceastra-Leica Microsystems, Newcastle upon Tyne, England), CD23 (dilution 1:15; Novocastra), and PAX5 (dilution 1:35; BD Biosciences, San Jose, CA).

Flow Cytometric Immunophenotypic Analysis

Flow cytometric immunophenotyping was performed on whole BM aspirate specimens using a standard stain-lyse-wash procedure, using Ortho Lyse buffer (BD Biosciences). For the study, 1 × 10⁶ nucleated BM cells per tube were stained. For assessment of cytoplasmic immunoglobulin light chain expression, cells were stained using a 0.25% saponin permeabilization and 4% formaldehyde fixation procedure. The following antibodies were assessed using the following monoclonal antibodies: CD3, CD5, CD19, CD20, CD22, CD23, CD38, CD56, CD79b, CD138, surface and cytoplasmic immunoglobulin κ and λ light chains (BD Biosciences), and FMC7 and CD10 (Beckman Coulter, Fullerton, CA). Cells were stained with 3- or 4-color combinations of antibodies conjugated to fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein–cyanine (Cy) 5.5, and allophycocyanin. Isotype-matched negative control antibodies were used to set thresholds for positive staining, with a cutoff of 20% of cells above background used to score positive expression. Data were acquired on FACSCalibur cytometers using CellQuest software (BD Biosciences). In most cases, 3 × 10⁴ cells were acquired. Analysis of flow cytometric data was performed using CellQuest and Paint-A-Gate (BD Biosciences). Lymphocytes were identified as CD45-bright cells with low side-scatter, and surface antigen expression levels were characterized. Plasma cells were gated on CD38 vs side scatter (SSC) or CD138 vs SSC plots; the preferred antigen varied from case to case depending on brightness for CD38. The surface antigen and cytoplasmic immunoglobulin light chain expression levels of plasma cells were characterized.

Cytogenetic Analysis

Conventional G-band karyotype analysis was performed on 13 cases at time of initial diagnosis of WM using methods...
described previously. The karyotype formula was written using the International System for Human Cytogenetic Nomenclature (1994).

**Molecular Analysis**

DNA extracted from BM aspirate material was analyzed for immunoglobulin heavy chain gene rearrangements using polymerase chain reaction–based methods and 3 primer sets that included variable region primers derived from framework 1, framework 2, and framework 3 regions in combination with a mixture of fluorescently labeled joining region primers as previously described. The control sequences were amplified indicating that the quality of DNA was adequate.

**Statistical Analysis**

The 2-tailed Student t test was used for numeric comparison between 2 groups. Differences between 2 groups were considered statistically significant if P values were less than .05 in a 2-tailed test.

**Results**

**Study Group**

From the initial study group of 75 cases reported previously, 41 patients with WM required treatment for symptoms and received chemotherapy, usually with a combination of agents including rituximab. Initial restaging BM samples of 2 patients showed no evidence of persistent WM. The remaining 39 patients had persistent disease, including 10 patients who had only persistent monotypic plasmacytosis in the absence of monotypic B lymphocytes. This subgroup of 10 patients is the focus of this study.

As shown in Table I, there were 9 men and 1 woman. The age at the initial diagnosis ranged from 56 to 77 years (median, 65 years). The signs and symptoms of the patients were variable; however, most patients had fatigue, night sweats, and/or weight loss. Most patients were initially observed without treatment. Serum IgM paraprotein levels at initiation of chemotherapy ranged from 2,200 to 8,000 mg/dL (median, 6,500 mg/dL). For comparison, the pretreatment IgM levels of the remaining treated patients not included in this study (available for 26/31 cases) ranged from 400 to 8,700 mg/dL (median, 2,800 mg/dL). The study group had a significantly higher range of pretreatment serum IgM levels (P = .016).

The therapies used for the patients with residual plasma cells in the absence of clonal B lymphocytes are summarized in Table 1. All patients received 1 or more combinations of chemotherapy agents in all but 1 case (case 1) including rituximab. For all but 1 case, the results in Table 1 reflect the initial chemotherapeutic course(s), before the first restaging BM biopsy (including aspiration). The remaining patient (case 4) received initial chemotherapy (3 courses of cladribine) 4 years before the set of BM biopsies described in Table 1. The other 31 treated patients received a similar range of chemotherapeutic regimens.

**Table I**

<table>
<thead>
<tr>
<th>Case No./ Sex/Age (y)</th>
<th>BM Lymphocytes/ Plasma Cells (%)</th>
<th>Pattern of Infiltration</th>
<th>Serum IgM (mg/dL)</th>
<th>Treatment Before Restaging Bone Marrow Aspiration and Biopsy</th>
<th>BM Plasma Cells (%)</th>
<th>Serum IgM (mg/dL)</th>
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</thead>
<tbody>
<tr>
<td>1/M/62</td>
<td>46/12</td>
<td>Interstitial and diffuse</td>
<td>N/A</td>
<td>Fludarabine, mitoxantrone, dexamethasone × 8</td>
<td>0.5</td>
<td>N/A</td>
</tr>
<tr>
<td>2/M/56</td>
<td>20/6</td>
<td>Paratrabecular and interstitial</td>
<td>3,500</td>
<td>Bortezomib, rituximab × 3</td>
<td>8</td>
<td>1,600</td>
</tr>
<tr>
<td>3/M/72</td>
<td>22/4</td>
<td>Paratrabecular and interstitial</td>
<td>6,500</td>
<td>Rituximab-CHOP × 2</td>
<td>3</td>
<td>4,000</td>
</tr>
<tr>
<td>4/F/64</td>
<td>54/4</td>
<td>Interstitial</td>
<td>8,000</td>
<td>Rituximab × 8, cladribine; rituximab-CHOP × 4</td>
<td>4</td>
<td>1,200</td>
</tr>
<tr>
<td>5/M/76</td>
<td>40/4</td>
<td>Interstitial</td>
<td>3,800</td>
<td>Fludarabine, rituximab × 4</td>
<td>18</td>
<td>N/A</td>
</tr>
<tr>
<td>6/M/65</td>
<td>68/0.1</td>
<td>Interstitial</td>
<td>7,400</td>
<td>Cladribine, cyclophosphamide, rituximab × 2</td>
<td>7</td>
<td>3,200</td>
</tr>
<tr>
<td>7/M/59</td>
<td>40/5</td>
<td>Interstitial</td>
<td>9,600</td>
<td>Cladribine, cyclophosphamide, rituximab × 2</td>
<td>20</td>
<td>5,700</td>
</tr>
<tr>
<td>8/M/77</td>
<td>91/0.4</td>
<td>Diffuse</td>
<td>4,600</td>
<td>Rituximab × 8, cladribine, cyclophosphamide, rituximab × 1</td>
<td>5</td>
<td>1,000</td>
</tr>
<tr>
<td>9/M/64</td>
<td>34/40</td>
<td>Interstitial</td>
<td>7,600</td>
<td>Cladribine, cyclophosphamide, rituximab × 2</td>
<td>46</td>
<td>7,100</td>
</tr>
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<td>10/M/71</td>
<td>86/2</td>
<td>Diffuse</td>
<td>2,200</td>
<td>Cladribine, cyclophosphamide, rituximab × 1</td>
<td>8</td>
<td>500</td>
</tr>
</tbody>
</table>

BM, bone marrow; N/A, not available (consult cases); CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone.

* Percentage of total marrow cellularity. All values >1% taken from the greater of the aspirate smear differential count or estimation from immunoperoxidase stains; values <1% from flow cytometric analysis.

† Posttreatment, only clonal plasma cells were detected, and the pattern of involvement was interstitial in all cases.
Following therapy, serum IgM levels decreased in 8 of 8 tested patients in the study group, by from 500 to 6,800 mg/dL. The percentage decrease in serum IgM ranged from 6% to 77% (median, 55%). In comparison, in the nonstudy group of treated patients with available IgM data (25/31), the posttherapy serum IgM levels ranged from 900 mg/dL higher to 4,300 mg/dL lower. The percentage change ranged from an increase of 41% to a decrease of 90% (median, 38% decrease). Compared with the nonstudy group of 31 patients, the patients in the study group showed significantly greater decreases in absolute IgM levels ($P = .019$) and percentage change in IgM ($P = .049$).

**Morphologic and Immunohistochemical Findings**

Before therapy, numerous small lymphocytes and plasmacytoid lymphocytes and, usually, fewer plasma cells were identified in BM aspirate smears. The extent of replacement of the BM in biopsy specimens ranged from approximately 20% to 90% [Image 1].

After the conclusion of chemotherapy, a monocytic plasma cell population was identified in BM biopsy specimens from 0.25 to 10 months later (median, 2.5 months). In all 10 cases, the plasma cells were cytologically unremarkable and were not enlarged or immature-appearing. In BM aspirate smears, plasma cells represented from fewer than 1% to 46% of all cells, with a median of 7.5%. In most cases, the percentage of plasma cells in the BM actually increased after therapy. However, in most cases, this was due in part to decreased overall BM cellularity, so that residual plasma cells were proportionally increased. In BM aspirate clot or biopsy specimens, the plasma cells were present singly or in small clusters and did not form nodules or large sheets. In all cases assessed by immunohistochemical analysis, the plasma cells were positive for CD138 and monotypic cytoplasmic immunoglobulin light chain of the same type as detected in plasma cells and B lymphocytes before therapy [Image 2]. The plasma cells were negative for CD3, CD10, CD20, CD23, PAX5, cyclin D1, and BCL-6 in all cases assessed.

**Flow Cytometric Immunophenotyping Results**

Before therapy, in most cases of WM, the monotypic B lymphocytes outnumbered plasma cells [Image 3]. B lymphocytes were positive for CD19 and CD20 in all cases and were positive for surface immunoglobulin $\kappa$ light chain in 9 cases and immunoglobulin $\lambda$ light chain in 1 case. Of 8 cases analyzed, 1 had B lymphocytes positive for CD5. The plasma cells in each case expressed the same cytoplasmic immunoglobulin light chain as the lymphocytes and were positive for CD38 and CD138. The plasma cells were also positive for CD19 in 4 (44%) of 9 and CD56 in 2 (22%) of 9 cases analyzed. In 1 case, plasma cells expressed CD19 and CD56.

In 7 of 7 posttherapy specimens tested, clonal plasma cells were identified by flow cytometry [Image 4]. The level of involvement ranged from 0.05% to 0.5% in 6 cases and was 12% in the seventh case. The plasma cells expressed CD38, CD138, and the same immunoglobulin light chain as expressed by plasma cells and B lymphocytes before therapy. B lymphocytes were rare or absent, and no evidence of monotypic lymphocytes was identified. In the 5 cases examined by immunohistochemical analysis and flow cytometry, the number of plasma cells identified by immunohistochemical analysis was 10- to 400-fold higher than by flow cytometry.

**Clinical Follow-up**

Additional clinical follow-up data for the 10 cases in the study group [Table 2] ranged from 12 to 70 months (median, 31 months) after the prechemotherapy BM staging biopsies detailed in Table 1. Of 10 patients, 9 received additional therapy, ranging from only rituximab (1 patient) to combination chemotherapy (7 patients) to autologous stem cell transplantation (SCT, 1 patient). Of 8 evaluable cases, 7 had persistent clonal BM plasma cells, with no detectable clonal B-cell population. Case 2, the SCT recipient, showed pathologic complete remission (CR), with no residual clonal plasma cells or B cells in the BM after transplantation. Case 10 received no additional chemotherapy after a single course of cladribine, cyclophosphamide, and rituximab (C/C/R). Subsequent BM biopsies in this case, up to 50 months later, showed small clonal plasma cell populations, all in the absence of a detectable clonal B-cell population. All 10 cases in the study group had a persistent serum paraprotein at last follow-up. Of the 10, 8 were alive with disease, and 2 died of disease.

As noted, among the remaining 31 treated nonstudy group cases, 2 showed a pathologic CR (with adequate assessment of plasma cells, by immunohistochemical analysis or flow cytometry) after initial chemotherapy. They were treated with cladribine alone or C/C/R, respectively. In 1 additional case, there was subsequent pathologic CR after a second course of C/C/R. In 2 of the 3 cases, the disease eventually relapsed, with reappearance of clonal plasma cells and clonal B cells. In 2 additional cases in which autologous SCT was done, there was subsequent pathologic CR.

**Cytogenetic and Molecular Results**

In 6 cases in the study group, conventional cytogenetic analysis was done on a BM specimen showing a significant level of disease involvement (>30%). In 1 case there was a normal karyotype, and 1 man showed an extra X chromosome in 20 of 20 metaphases, suggesting constitutional Klinefelter syndrome. Another man showed deletion of chromosome Y in 4 of 20 metaphases, a finding of uncertain significance in men in this age group. Of 6 cases, 3 showed a chromosomal abnormality that appeared to be clearly associated with disease: trisomy 8 (5/20 metaphases); del(13)(q12;q14) (6/20), confirmed to involve the RB1 locus by fluorescence in situ.
Image 1 (Case 7) Waldenström macroglobulinemia before treatment with rituximab. The bone marrow biopsy (A, H&E, ×400) showed hypercellular (70%) bone marrow with increased interstitial plasma cells and poorly formed lymphoid aggregates. Bone marrow aspirate smears (B, Wright-Giemsa, ×1,000) showed increased lymphocytes, plasmacytoid lymphocytes, and mature plasma cells. CD20 was positive by immunohistochemical analysis (C, ×200) in most lymphocytes, while staining for CD138 (D, ×200) showed only scattered clusters of plasma cells. Most of the plasma cells and lymphocytes were positive for immunoglobulin κ light chain (E, ×200) and negative for immunoglobulin λ (F, ×200).
hybridization (FISH) analysis; and a complex karyotype, 47,XY,+4,del(6)(q22;27),add(8)(p11.2) (2/20), respectively.

Three cases with monotypic plasma cells were tested by polymerase chain reaction for immunoglobulin heavy chain (IgH) rearrangements; 2 had demonstrable monoclonal IgH gene rearrangements.

**Discussion**

WM typically involves BM and is composed of a mixture of small lymphocytes, plasmacytoid lymphocytes, and plasma cells. The lymphocytes are of B-cell lineage and usually have a relatively nonspecific immunophenotype, positive for monotypic surface immunoglobulin light chain surface IgM and pan-B-cell antigens, and negative for CD5 and CD10. In most cases of WM, plasma cells are a minority component, positive for cytoplasmic immunoglobulin light chain and IgM, surface CD38, and CD138, and mostly negative for B-cell antigens. Because these 2 populations have a distinctive immunophenotype, care must be taken to account for lymphocytes and plasma cells in the morphologic and immunophenotypic assessment of WM.

The major finding of this study is the detection of monotypic plasma cells in WM cases in the absence of clonal B lymphocytes after therapy. The plasma cells had the same
immunophenotype as shown before therapy and, therefore, represent persistence of WM. Although the plasma cell population ranged from fewer than 1% to 46% of BM cellular elements, in most cases, the plasmacytosis was present at a low level with a median of 7.5%. This phenomenon occurred as early as 1 month after the initiation of chemotherapy and, in 1 case, persisted for 50 months after the last dose of chemotherapy. Although our study was not designed to assess the frequency of this occurrence, 10 (24%) of 41 treated cases of WM had persistent monotypic plasmacytosis, and, therefore, this phenomenon does not seem to be rare.

Others have made similar observations. Varghese et al\textsuperscript{6} recently reported the persistence of low-level plasma cell clones in 5 of 6 patients with WM after chemotherapy using fludarabine alone or in combination with cyclophosphamide. Goteri et al\textsuperscript{10} described 2 patients with LPL who had clusters of monoclonal plasma cells in BM after rituximab therapy. Robak et al\textsuperscript{11} also described a case of a 54-year-old man with
massive BM and spleen infiltration by LPL associated with IgA gammopathy and crystal-storing histiocytosis. Following therapy with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone, he underwent splenectomy, and only mature plasma cells were present in the spleen and BM.

Although the pathogenesis of this finding is unknown, one hypothesis is that rituximab and/or other chemotherapeutic agents successfully eliminated the B lymphocytes of WM but were unable to eliminate a plasma cell component of WM, which is more resistant to chemotherapy. It is known that normal plasma cells are very hardy and resistant to many forms of chemotherapy. For example, in patients with acute myeloid leukemia or lymphoma who receive high-dose chemotherapy, it is not surprising to observe viable plasma cells around blood vessels in posttherapy BM specimens. Another more theoretical possibility is that WM arises from a progenitor cell that undergoes maturation arrest at multiple stages of development, with therapy preventing the lymphocyte component from expanding.

Although the mechanism is not entirely clear, the finding of monotypic plasma cells in the BM of WM cases after therapy has practical considerations. First, strategies to detect minimal residual disease in patients with WM must account for the 2 components of WM: B lymphocytes and plasma cells. Although B lymphocytes are easily assessed using standard flow cytometric immunophenotyping, the analysis of plasma cells using flow cytometry requires careful gating, and demonstration of cytoplasmic immunoglobulin light chain restriction requires cell permeabilization. The use of cell permeabilization can result in some cell loss and may also lead to higher nonspecific background staining. These and other technical factors often lead to loss of sensitivity of flow cytometry to detect small numbers of plasma cells.12

If immunohistochemical analysis is used to assess BM in WM cases after therapy, one must be aware that lymphoid aggregates can be absent. In some cases, the only evidence of persistent disease may be scattered small plasma cells that will only be recognized reliably if immunostaining with antibodies specific for immunoglobulin light chains is performed.

We note that immunostaining usually highlights a greater percentage of plasma cells than are identified by flow cytometry, and in the 6 evaluable cases in our study group, 10- to 400-fold more plasma cells were seen by immunohistochemical analysis. Specific high-sensitivity flow cytometric assays have been developed to detect minimal residual neoplastic plasma cells (as recently reviewed by Paiva et al13). However, unless such an assay is available, we recommend immunohistochemical analysis (or in situ hybridization studies) for immunoglobulin light chains to assess plasma cell clonality in follow-up BM specimens in WM cases. Staining for a plasma cell marker (eg, CD138) by immunohistochemical methods may help to quantitate residual plasma cells.

Second, the persistence of monotypic plasma cells in BM of WM cases after therapy may have implications for non-invasive monitoring of the therapeutic response. A common approach for following up patients with WM after therapy is to monitor the serum IgM paraprotein level. By using this approach, it has been observed that the maximum response to therapy, in other words, the lowest serum IgM paraprotein levels, occurs 5 to 21 months after various regimens are used, including single-agent rituximab, fludarabine, cladribine, chlorambucil, or alemtuzumab (anti-CD52) or a combination of agents.5 This delayed response to therapy may be explained by the persistence of monotypic plasma cells in the BM and suggests that BM examination after therapy may complement serum paraprotein studies in monitoring patients with WM.

In the cases in our study group, all but 1 showed decreased serum IgM levels after chemotherapy, with a reduction ranging from 15% to 77% (the last case showed apparently stable disease with 6% lower levels). One likely explanation is that the monotypic B lymphocytes secrete IgM and contribute to the overall serum paraprotein level. Their elimination by therapy would, therefore, cause serum IgM paraprotein levels to drop. Alternatively, if the bulk of the serum paraprotein is accounted for by the plasma cell component, the therapy must be eliminating a portion of the neoplastic plasma cells, with only a resistant or possibly more primitive/stem cell–like population of plasma cells persisting.

In our study, the plasma cells were positive for CD138 and CD38 in all cases but were positive for CD19 and CD56 in 44% and 22% of cases, respectively. Our results are somewhat in contrast with those of Morice et al14 who reported that plasma cells in 90% of WM are positive for CD19. This may be due to technical differences in instrumentation and/or fluorochrome use (CD19-FITC vs CD19-PE-Cy7). Our

### Table 2

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Follow-up (mo)</th>
<th>BM Plasma Cells (%)</th>
<th>Serum IgM (g/dL)</th>
<th>Last Known Clinical Status</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>36/36</td>
<td>14</td>
<td>900</td>
<td>AWD</td>
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<tr>
<td>2</td>
<td>38/42</td>
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<td>AWD</td>
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<tr>
<td>4</td>
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<td>51/51</td>
<td>3</td>
<td>300</td>
<td>AWD</td>
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</tbody>
</table>

AWD, alive with disease; BM, bone marrow; DOD, died of disease.

* Duration from initial biopsy to last BM biopsy at M.D. Anderson Cancer Center, Houston, TX; last serum IgM assay.

† Bone marrow plasma cells quantitated as in Table 1.

‡ Plasma cells and B cells did not show demonstrable light chain restriction, but a residual paraprotein was still detectable. In cases 6 and 8, there were no additional bone marrow biopsies after the posttherapy specimens described in Table 1.

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results are generally in agreement with those of Seegmiller et al., who found that monotypic plasma cells in patients with non-Hodgkin lymphomas tend to be positive for CD19, CD45, and surface immunoglobulin and negative for CD56, unlike plasma cell myeloma, in which plasma cells are usually positive for CD56 and negative for CD19. Only 1 (11%) of 9 WM cases in our study group showed a myeloma-like pattern.

Only 3 of 6 evaluable cases in our study group had unequivocally abnormal karyotypes, including trisomy 8, a del(13q) including the RB1 locus, and a complex karyotype unequivocally abnormal karyotypes, including trisomy 8, a del(13q) including the RB1 locus, and a complex karyotypeunequivocally abnormal karyotypes, including trisomy 8, a del(13q) including the RB1 locus, and a complex karyotype. The del(6q) is the most common genetic abnormality in WM, ranging from 40% to 70%. A previous study showed that the frequency of del(6q) by conventional karyotyping was 21%; however, a higher frequency has been shown by FISH in other studies. FISH data for del(6q) were not available for the cases in our series. As reported previously, the (t;14)(p13;q32) is rare or absent in WM, in accord with the results of this study.

In this study, we showed that WM can persist in the BM after therapy as monotypic plasma cells in the absence of clonal B lymphocytes. This finding has practical implications for laboratory diagnosis and patient management. For laboratory diagnosis, the B-lymphocyte and plasma cell populations must be assessed in WM specimens after therapy, and flow cytometric and immunohistochemical panels should be designed to assess both cell populations. For clinical management, the persistence of monotypic plasma cells may explain the delay in the maximum decrease of serum IgM paraprotein levels in patients with WM after therapy. This finding also suggests that BM examination at regular intervals may complement serum protein electrophoresis studies in assessing persistent disease.

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References