The (11;14)(q13;q32) Translocation in Multiple Myeloma  
A Morphologic and Immunohistochemical Study  

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Abstract
We identified 24 cases of multiple myeloma with the t(11;14)(q13;q32). In 22 cases, the t(11;14)(q13;q32) was part of a complex karyotype, and in 2 cases it was an isolated abnormality. All patients had clinical and laboratory features consistent with multiple myeloma. The median degree of plasma cell involvement in the bone marrow was 60%, and in 10 cases, the plasma cells had a lymphoplasmacytoid appearance. Of the 24 cases, 21 had intermediate or high proliferative rates based on labeling index studies. Immunohistochemical studies performed on all bone marrow biopsy specimens showed strong cyclin D1 nuclear positivity in 19 cases. There also was strong cyclin D1 nuclear positivity found in 6 of 30 additional cases without the t(11;14)(q13;q32) demonstrated by routine cytogenetics. The t(11;14)(q13;q32) in multiple myeloma results in overexpression of the cyclin D1 protein, which can be demonstrated by immunohistochemical stain. The cyclin D1 stain results in the additional cases of multiple myeloma suggest that the t(11;14)(q13;q32) may be more common than previously thought and may be missed by routine cytogenetics, particularly if the proliferative rate is low.

Cytogenetic studies have a key role in the evaluation of many malignant neoplasms. This is particularly true of hematologic malignant neoplasms in which many disorders are associated with specific cytogenetic abnormalities. The most notable of these is the t(9;22)(q34;q11) characteristic of chronic myelogenous leukemia.1,2 Other important cytogenetic abnormalities include the t(15;17) (q22;q21) associated with acute promyelocytic leukemia,3,4 the t(14;18)(q32;q21) seen with some of the follicular lymphomas,5,6 and translocations involving chromosome 8q24 associated with Burkitt lymphoma.7

The t(11;14)(q13;q32) is also an important cytogenetic abnormality in hematologic disorders and has been recognized as a key finding in mantle cell lymphoma.8-11 This translocation is identified in 40% to 70% of cases, either by routine cytogenetics or by various molecular methods.12-15 In this translocation, the PRAD-1 (CCND1) proto-oncogene at 11q13 is juxtaposed to the immunoglobulin heavy chain gene at 14q32, resulting in overexpression of the protein product, cyclin D1.16-18

Cyclin D1 is known to have a key role in cell cycle regulation, particularly in the progression of cells from G1 to S phase. This is accomplished by cyclin D1 combining with its cyclin-dependent kinase partner (usually CDK4) and phosphorylating the product of the retinoblastoma gene (pRB). This releases a transcription factor (E2F), which is normally bound to the unphosphorylated retinoblastoma protein. E2F can then activate several genes required for DNA synthesis. This pathway increases cellular proliferation primarily by shortening the G1 phase and pushing cells into the S phase of the cell cycle.19-21 Previous studies have shown that the cyclin D1 overexpression in mantle cell lymphoma can be detected by immunohistochemical methods, which has become a key

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component in the workup of possible mantle cell lymphoma cases.

Although commonly associated with mantle cell lymphoma, the t(11;14)(q13;q32) is also the most common translocation found in multiple myeloma. However, it is found in a much lower frequency in multiple myeloma, representing 2% to 4% of all cases studied by routine cytogenetics, but 10% to 25% of cases with abnormal cytogenetics. Recent reports have shown that at the molecular level, there are differences in the translocation breakpoint sites between mantle cell lymphoma and multiple myeloma. Translocation involving the immunoglobulin heavy chain gene in multiple myeloma seems to involve an error in heavy chain switch recombination rather than the heavy chain gene in multiple myeloma. Translocation involving the immunoglobulin locuses in mantle cell lymphoma. This is consistent with plasma cells being at a later stage of B-cell differentiation and having already undergone VDJ recombination. Studies analyzing the breakpoints on chromosome 11 in myeloma specimens also have shown them to be widely scattered and not solely located in the major translocation cluster, where up to 40% of the breakpoints are located in mantle cell lymphoma.

The goals of the present study were to describe the morphologic features of multiple myeloma cases containing t(11;14)(q13;q32) and to determine whether, like mantle cell lymphoma, this translocation results in overexpression of the cyclin D1 protein by immunohistochemical studies. A previous study showed cyclin D1 overexpression in a subset of cases of multiple myeloma; however, these were not correlated with cytogenetic analysis.

Materials and Methods

The cytogenetic and hematopathology files of the Mayo Clinic, Rochester, MN, were searched from January 1988 to January 1999, and we identified 24 cases of documented multiple myeloma with a t(11;14)(q13;q32) by standard cytogenetic analysis. Wright-Giemsa–stained peripheral blood and bone marrow aspirate slides, H&E-stained biopsy slides, and paraffin-embedded biopsy specimens blocks were available for all cases. All bone marrow biopsy specimens were fixed in B-5. Labeling index studies also were performed on all bone marrow aspirate specimens by the method of Greipp et al. This procedure uses fluorescent-labeled bromodeoxyuridine incorporation as a measure of the proliferative rate of the plasma cells. Results are reported as the percentage of plasma cells that are positive for bromodeoxyuridine uptake. Medical record reviews were done in all cases to confirm the clinical impression of multiple myeloma (eg, lytic bone lesions, monoclonal paraprotein, no organomegaly or lymphadenopathy).

Immunoperoxidase stains were performed on the paraffin-embedded decalciﬁed bone marrow biopsy specimens in all cases using an autostainer (Ventana Medical Systems, Tucson, AZ). This instrument used a labeled streptavidin-biotin method with aminoethylcarbazole as the chromogen. A summary of monoclonal antibodies is summarized in Table 1.

Anti–cyclin D1 staining was performed using a mouse monoclonal antibody cocktail (DCS-6/P2D11F11, Novocastra Laboratories, Burlingame, CA) by the method of Vasef et al. and Kurtin et al. The slides were pretreated before staining. Slides were placed in 200 mL of a 1-mmol/L concentration of EDTA buffer, pH 8.0, and microwaved at 700 W for 5 minutes. After addition of 50 mL of distilled water, the slides were microwaved again for 5 minutes and allowed to remain in the EDTA buffer for an additional 15 minutes. They were then transferred to an ultrasonic cleaner (Branson Ultrasonics, Danbury, CT) that had been filled with 1 L of boiling EDTA buffer (1-mmol/L concentration), pH 8.0, sonicated for 30 seconds, and rested for 10 minutes in the sonication buffer.

Following antigen retrieval, the slides were rinsed, endogenous peroxidase activity was blocked in methanolic peroxide, the slides were rinsed again, and the primary antibody cocktail was applied. All slides were stained using a Biokem Techmate 500 (Biokem Solutions, Santa Barbara, CA). Antibody incubations were performed at room temperature, and the avidin-biotin-peroxidase complex method was used. Diaminobenzidine at a concentration of 40 mg/dL was used as the chromogen. After development of the chromogen, all slides were counterstained with hematoxylin using a progressive method.

In addition to cases of multiple myeloma with t(11;14)(q13;q32), 5 cases of known mantle cell lymphoma, 25 normal bone marrow specimens, and 30 cases of multiple myeloma without the t(11;14)(q13;q32) also were stained with cyclin D1 for comparison. The multiple myeloma cases...
had normal cytogenetics (n = 15) or complex cytogenetic abnormalities (n = 15). In 3 cases of multiple myeloma with the t(11;14)(q13;q32), previous bone marrow specimens were available, in which the translocation had not been identified. These biopsy specimens also were stained with anti-cyclin D1 for comparison.

Chromosome analysis was performed on bone marrow aspirates in all cases. Specimens were cultured and procured using a standard direct technique and an in situ unstimulated culture method. Metaphases were stained by Giemsa stain with trypsin pretreatment (GTG banding) or fluorescent staining with quinacrine mustard (QFQ-banding); photomicrographs of representative metaphases were taken in all cases for documentation purposes. The cytogenetic results were recorded in accordance with the standard rules for the International System for Human Cytogenetic Nomenclature.

### Results

A summary of the clinical and laboratory data is given in Table 2. All patients had a clinical spectrum consistent with the diagnosis of multiple myeloma. In all 24 patients, lytic bone lesions were present, either at diagnosis or later in the disease. No patients had lymphadenopathy or organomegaly on examination. In 4 patients, there were sufficient circulating plasma cells (>20% plasma cells or absolute number >2.0 × 10^9/L) that warranted a diagnosis of plasma cell leukemia. A more detailed description of the clinical features, treatment, and survival of the majority of these patients has been published.

In 23 of 24 patients, a monoclonal serum, or urine paraprotein, or both were present, with the subtypes as listed in Table 2. One patient had a nonsecretory myeloma. There were no patients with an IgM paraprotein, and the kappa/lambda distribution was 12:11. Plasma cell labeling index studies performed on the bone marrow aspirate material were available in all cases. In 21 of 24 cases, there was an intermediate or high labeling index, defined as more than 0.3% of the plasma cells in S phase.

The results of the cytogenetic studies show that in 22 (92%) of 24 cases, the t(11;14)(q13;q32) was a part of a complex karyotype. These karyotypes usually showed numerous deletions and additions of other chromosomes in a karyotype typically seen in multiple myeloma. Although there was no consistent abnormality besides the t(11;14)(q13;q32), 10 patients had an additional abnormality of chromosome 13, which previously has been shown to be an adverse prognostic indicator in myeloma. In the majority of cases, the number of abnormal metaphases was less than 50%, and in only 1 case were all metaphases abnormal. In 21 of 22 cases with complex karyotypes, the t(11;14)(q13;q32) was present in all of the abnormal metaphases. However, in 4 of these 22 cases, there was a subgroup of metaphases that contained additional chromosomal abnormalities not found in all cells. The remaining case had only 2 abnormal metaphases. One metaphase contained the t(11;14)(q13;q32) as a part of a complex karyotype; the other abnormal metaphase contained only a del(1)(q21).

In 21 of 24 patients, the t(11;14)(q13;q32) was detected at relapse; the remaining 3 were identified at initial diagnosis. Significantly, 2 of these 3 patients who had the t(11;14)(q13;q32) detected at presentation were the only patients who had this as a single isolated abnormality by cytogenetic karyotyping. In 3 of the 21 patients who experienced relapse, the previous karyotypes obtained at presentation showed normal results. In the other 18 patients with relapse, the cytogenetic status at the time of initial diagnosis was not known, either because cytogenetic studies were not obtained initially, or the patients were referred from another institution for a second opinion later in their disease course.

Of the 24 patients in the present study, complete follow-up was available for 23. One patient was lost to follow-up. Nineteen patients have died of their disease. One patient received a peripheral blood stem cell transplant and is free of disease 27 months after diagnosis. Another patient received...
an autologous bone marrow transplant, but experienced relapse 45 months after transplantation. Two patients were diagnosed more recently, and the time has been insufficient for meaningful follow-up. The median survival since diagnosis of the multiple myeloma was 43.1 months. However, the median survival after detection of the t(11;14)(q13;q32) was only 11.9 months.

A summary of the morphologic and immunohistochemical results is given in Table 3. In 13 cases, typical plasma cell morphologic features (either mature or immature) were seen on the bone marrow aspirate smear. In 10 cases, however, the plasma cells were small with scant cytoplasm, giving a lymphoplasmacytoid appearance (Image 1). In the remaining case, the plasma cells had plasmablastic morphologic features. The degree of infiltration seen on both the bone aspirate smears and biopsy sections was usually quite high. The median degree of marrow involvement was 60% (range, 10%-95%).

The results from the immunohistochemical stains were in keeping with results expected for plasma cells (CD20−; strong cytoplasmic kappa or lambda light chain positivity). In 2 cases, there was weak CD20 positivity. When appropriate, the heavy chain subtype of the monoclonal paraprotein was confirmed by immunohistochemical studies. In 19 of the 24 cases, there was strong nuclear cyclin D1 positivity seen (Image 2). In addition, strong nuclear cyclin D1 positivity was seen in all 5 cases of mantle cell lymphoma, and no positivity was observed in the 25 normal bone marrow specimens. Interestingly, strong nuclear cyclin D1 positivity was observed in 6 of the 30 cases of multiple myeloma without the t(11;14)(q13;q32) identified by karyotypic analysis. In 5 of these 6 cases, a normal karyotype was obtained. The remaining case had a complex karyotype, which included a 14q abnormality.

In 3 of the 24 myeloma cases with a t(11;14)(q13;q32), an earlier bone marrow study had been done that showed normal cytogenetic findings with no translocations. Each of these cases, however, had substantial involvement by multiple myeloma. Immunohistochemical staining for cyclin D1 on the bone marrow biopsy specimens of these 3 cases showed no nuclear staining for cyclin D1 in the plasma cells. In all cases, a nonspecific punctate cytoplasmic staining pattern was observed with the anti–cyclin D1 antibody cocktail, as substitution of the primary antibody with a mouse control IgG abolished this staining. This same phenomenon has been observed in plasma cells seen in lymph nodes stained with cyclin D1 (P.J.K., unpublished observations). This cytoplasmic positivity was not considered true positivity, but it had the fortuitous effect of clearly labeling the plasma cells.

**Table 3**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Data</th>
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</thead>
<tbody>
<tr>
<td>Morphologic</td>
<td></td>
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<tr>
<td>Plasma cell (mature/immature)</td>
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<tr>
<td>Lymphoplasmacytoid</td>
<td>10</td>
</tr>
<tr>
<td>Plasmablastic</td>
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<tr>
<td>Median percentage of plasma cell infiltration (range)</td>
<td>60 (10-95)</td>
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<tr>
<td>Immunohistochemical stains on bone marrow biopsy specimens (No. positive)</td>
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</tr>
<tr>
<td>CD20</td>
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<tr>
<td>kappa</td>
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<td>IgD</td>
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</tr>
<tr>
<td>Cyclin D1</td>
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**Image 1** Multiple myeloma with the t(11;14)(q13;q32) with lymphoplasmacytoid morphologic features. The plasma cells are small with scant cytoplasm (Wright-Giemsa, original magnification ×150).

### Discussion

The t(11;14)(q13;q32) is the most common structural abnormality identified to date in patients with multiple myeloma. However, it occurs in only a small subset of all patients with myeloma and accounts for a much lower frequency than can be identified in mantle cell lymphoma. Although there are differences in the translocation breakpoint at the molecular level, in both diseases, the
Translocation results in dysregulation of the PRAD-1 proto-oncogene and overexpression of the cyclin D1 protein. The results of our study show that this cyclin D1 overexpression can be detected by routine immunohistochemical methods in the majority of cases.

In our study, cases of multiple myeloma with t(11;14)(q13;q32) usually showed evidence of higher plasma cell proliferative activity, such as a high labeling index and extensive bone marrow involvement. There also seems to be an increased propensity for the development of plasma cell leukemia. Although plasma cell leukemia usually accounts for only 1% of all cases of multiple myeloma, it represented 17% (4/24) of our study cases. Previous studies also have shown the t(11;14)(q13;q32) in cases of plasma cell leukemia. Although these features may be due to the influence of cyclin D1 overexpression, they also may be a manifestation of disease progression, as the t(11;14)(q13;q32) was detected at relapse in the majority of cases.

In approximately one half of cases, the plasma cells had a lymphoplasmacytoid appearance. This appearance could result in the misdiagnosis of these cases as lymphoplasmacytic lymphoma, if they were evaluated in morphologic isolation. This underscores the necessity of correlating the morphologic findings with the clinical history, radiologic findings, and laboratory data, including immunophenotypic findings. In equivocal cases, immunohistochemical studies can be very helpful, as plasma cells usually will show strong cytoplasmic immunoglobulin staining and are typically negative with CD20 staining.

It is unclear whether the t(11;14)(q13;q32) in multiple myeloma is a primary or secondary event. The fact that the majority of cases in our study had the translocation detected at relapse would support it as a secondary event. However, in many of our cases, cytogenetic studies were not performed or not available at initial diagnosis. Conversely, the t(11;14)(q13;q32) was present as an isolated abnormality at initial diagnosis in 2 cases. In 4 cases, the t(11;14)(q13;q32) was part of a complex karyotype that contained a subclone with additional karyotypic abnormalities. Both of these findings suggest that the t(11;14)(q13;q32) may be a primary event in some cases of multiple myeloma. Likewise, in 6 of the 30 cases of multiple myeloma without the t(11;14)(q13;q32) by routine cytogenetics, there was strong nuclear cyclin D1 positivity, suggesting that the translocation may have been present in these myelomas but simply not detected by cytogenetics. A likely explanation for this is that multiple myeloma, at least in its initial phases, is usually a slowly proliferative tumor that may not provide representative metaphases for routine karyotyping studies. A normal karyotype from a myeloma specimen, therefore, may simply be derived from the normal bone marrow elements. In addition, in many of our cases, cytogenetic studies were not ordered on the initial presenting bone marrow sample, and, thus, the karyotype of these cases at diagnosis is not known. The cyclin D1 positivity in the cytogenetically normal cases of multiple myeloma indicates that the prevalence of t(11;14)(q13;q32) may be higher than that detected by routine cytogenetic karyotyping methods. Other methods of detecting the t(11;14)(q13;q32), such as fluorescence in situ hybridization techniques, may provide additional answers to these karyotypically normal, cyclin D1–positive cases. A recent study was successful in using fluorescence in situ hybridization to detect the t(11;14)(q13;q32) with a much higher frequency than that detected by routine cytogenetics.

**Image 2** Anti–cyclin D1 immunohistochemical staining in a case of multiple myeloma with t(11;14)(q13;q32). A, An area of the bone marrow biopsy specimen with increased plasma cells (H&E, original magnification ×100). B, Anti–cyclin D1 staining showing strong nuclear positivity (hematoxylin, original magnification ×100).
hybridization techniques to demonstrate the t(11;14)(q13;q32) in 26 of 26 cases of mantle cell lymphoma.  

The survival of patients after identification of the t(11;14)(q13;q32) was usually quite short. Once again, this simply may be due to the majority of cases being identified at relapse. The survival curve for these patients is similar to that of any group of patients with relapsed myeloma.  

Thus, the prognostic significance of t(11;14)(q13;q32), if any, remains unclear.

The t(11;14)(q13;q32) in multiple myeloma results in overexpression of the cyclin D1 protein, which can be detected by immunohistochemical studies in the majority of cases. The clinical and prognostic significance of the t(11;14)(q13;q32) in this group of patients remains to be determined. However, if the presence of t(11;14)(q13;q32) does confer a worse prognosis, cyclin D1 staining by immunohistochemistry may be a useful adjunct in the evaluation of patients with multiple myeloma.

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