A Subset of CD5– Diffuse Large B-Cell Lymphomas Expresses Nuclear Cyclin D1 With Aberrations at the CCND1 Locus

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Abstract

In 231 diffuse large B-cell lymphomas, the expression of cyclin D1 and CD5 was evaluated. All cases were CD5–. Ten (4.3%) were positive for cyclin D1 and were subjected to fluorescence in situ hybridization at the CCND1 locus. One case showed the t(11;14). In another case, the telomeric probe signal for cyclin D1 was lost in most tumor cells, and in a small proportion of the cells, there were fluorescence signals indicative of the t(11;14). Two other cases displayed additional cyclin D1 signals in the absence of the t(11;14). All cases but 1 were positive for bcl-6 or MUM1, disfavoring the possibility of misdiagnosed blastoid variants of CD5– mantle cell lymphomas. Thus, contrary to the current view, there seems to exist a certain number of cyclin D1+ and CD5– diffuse large B-cell lymphomas, some of which have structural aberrations at the CCND1 locus, including the t(11;14).

The nuclear protein cyclin D1 is of considerable interest in lymphomagenesis because of its well-defined molecular role as a permissive factor for the progression of the G1 phase of the cell cycle.1,2 Cyclin D1 is expressed at very low levels in normal B cells,3 and its deregulation probably contributes to the pathogenesis of certain lymphomas by promoting the G1–S transition.4-6 The overexpression of cyclin D1 is, with some exceptions,4,7-10 restricted to 3 lymphoma subtypes, ie, mantle cell lymphoma,4,11,12 plasma cell myeloma,5,13,14 and hairy cell leukemia.6,15 In about 90% of mantle cell lymphomas, there is overexpression of cyclin D1.16 The inability to detect cyclin D1 in the remaining 10% is apparently not due to insensitive assays because gene expression profiling experiments can demonstrate that approximately 10% of lymphomas with typical morphologic features and the characteristic gene expression signature of mantle cell lymphoma lack cyclin D1 messenger RNA.17 In the vast majority of mantle cell lymphomas with immunohistochemical evidence of cyclin D1 expression, the t(11;14) between the immunoglobulin heavy chain (IGH) and cyclin D1 (CCND1) genes can be found with fluorescence in situ hybridization (FISH).18 The expression of CD5 is also highly characteristic, present in at least 90% of mantle cell lymphomas,19 although the existence of CD5– cases is well recognized.20,21 Plasma cell myelomas express cyclin D1 in about 40% of the cases, some of which contain the t(11;14).13,14 In hairy cell leukemia, deregulated expression of cyclin D1 is very common.6,15 In 1 study, all cases examined were positive for cyclin D1 on immunohistochemical analysis, apparently in the absence of the t(11;14).15

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of all lymphomas, representing about 30%
of all primary diagnosed lymphomas. It is a heterogeneous group of lymphomas with respect to morphologic features, protein expression pattern, gene expression profile, and clinical behavior. DLBCLs are generally believed to be cyclin D1+. However, a study of 70 DLBCLs and Burkitt lymphomas reported 7 cases with weak positivity of scattered tumor cells. In our routine practice, we have seen a few convincing cyclin D1+ cases with weak but distinct reactivity. For this reason, we examined a large number of DLBCLs for cyclin D1 expression. Cases with overexpression of cyclin D1 were further subjected to extended immunohistochemical phenotyping and FISH to determine the status of the CCND1 locus on chromosome 11.

**Materials and Methods**

**Patient Samples**

We selected 251 consecutive cases of DLBCL with available paraffin blocks from 3 university hospitals in Sweden (Lund, Uppsala, and Umeå). Tissue samples from each patient were obtained and reevaluated according to the World Health Organization classification of tumors of hematopoietic and lymphoid tissues. Of the cases, 20 were excluded from the study because of inadequate material (13 cases) or an unclear or altered diagnosis (7 cases). Of the remaining 231 samples of DLBCLs, 210 were the centroblastic subtype, 11 the anaplastic subtype, 6 the T-cell/histiocyte rich subtype, and 4 the immunoblastic subtype.

**Morphologic and Immunohistochemical Analyses**

The paraffin blocks were cut in 4- to 6-µm thin sections, dried overnight at 60°C, and deparaffinized in xylene. Subsequently, the sections were rehydrated through graded alcohol in water. Some of the sections were stained with H&E for routine microscopic evaluation.

For immunohistochemical analysis, heat-epitope retrieval was achieved by boiling the sections in EDTA buffer, pH 8.9, in a microwave oven at 800 W for 7 minutes and 300 W for 15 minutes. After boiling, the sections were allowed to cool at room temperature for 20 minutes, rinsed thoroughly with water, and placed in a tris(hydroxymethyl) aminomethane (Tris)-buffered saline for 5 minutes. The primary antibodies were incubated for 25 minutes at room temperature. Endogenous peroxidase was blocked with peroxidase block solution for 25 minutes, and the slides were rinsed or washed with Tris-buffered saline. Immunostaining was performed using the Tech-Mate instrument (DAKO, Glostrup, Denmark) according to the manufacturer’s instructions. The following antibodies were used: CD20 (clone L26, DAKO), CD10 (clone 56C6, Novocastra Laboratories, Newcastle upon Tyne, England), CD5 (clone CD5/54/F6, DAKO), MUM-1 (clone MUM1p, DAKO), bcl-6 (clone PG-B6p, DAKO), bcl-2 (clone 124, DAKO), cyclin D1 (NCL-CYCLIN D1-GM [clone P2D11F11], Novocastra, or cyclin D1 [clone SP4], NeoMarkers, Fremont, CA), p53 (clone D07, Novocastra), and Ki-67 (clone MIB1, DAKO).

For cyclin D1, immunostaining was judged as focal if fewer than 30% of the tumor cells were positive. Fewer than 10% positive tumor cells was considered as negative. For Ki-67, the percentage of positive tumor cells was estimated.

**DNA FISH**

Of the 10 cyclin D1+ cases, 7 paraffin blocks were available for further molecular studies. DNA FISH with IGH/cyclin D1 fusion probes was performed according to the manufacturer’s instructions (Abbott, Abbott Park, IL). Briefly, the IGH/cyclin D1 fusion translocation probe set is a mixture of an IGH probe set labeled with spectrum green and a cyclin D1 probe labeled with spectrum orange. The IGH probe set contains 2 spectrum green–labeled probes designed to hybridize with sequences on either side of the IGH J breakpoint region on 14q32 associated with the t(11;14). The cyclin D1 probe is designed to bind to and span the common breakpoint region that lies centromeric to CCND1. IGH/cyclin D1 probes hybridized to a cell harboring the t(11;14) with breakpoints at the common breakpoint region on 11q13 and at the IGH J region on 14q32 will result in 2 orange/green (yellow) fusion signals, 1 on each of the abnormal chromosomes 11 and 14, and single green and orange signals from the remaining intact chromosomes.

DNA FISH with cyclin D1 split probes was performed according to the manufacturer’s instructions (Abbott). Briefly, hybridization with the Abbott cyclin D1 break-apart rearrangement (split) probe set (consisting of 2 probes) will identify rearrangements of the CCND1 gene 11q13 region independently of the translocation partner. The first probe, labeled with spectrum green, is designed to bind to sequences centromeric to the CCND1 gene. The second probe, labeled with spectrum orange, extends telomerically from the CCND1 gene. When hybridized with the split probe, the common breakpoint region has 2 spectrum green–labeled probes designed to hybridize with sequences on either side of the IGH J breakpoint region. The first probe, labeled with spectrum green, extends telomeric from the IGH J breakpoint region and a fluorescein isothiocyanate–labeled DNA probe (CCND1-upstream) covering 163 kb centromeric to the CCND1 common breakpoint region with a fluorescein isothiocyanate–labeled DNA probe (CCND1-downstream) covering 644 kb telomeric to the IGH J breakpoint region. The second probe, labeled with spectrum orange, extends centromeric to the IGH J breakpoint region with a Texas Red–labeled DNA probe (CCND1-upstream) covering 644 kb telomeric to the IGH J breakpoint region and a fluorescein isothiocyanate–labeled DNA probe (CCND1-downstream) covering 163 kb centromeric to the CCND1 common breakpoint region.
**Results**

**Cyclin D1 Is Expressed in a Minority of DLBCLs**

We stained 231 cases of DLBCLs with antibodies against CD20, cyclin D1, and CD5. The cyclin D1 antibody was from Novocastra (clone P2D11F11). Ten cases were positive for cyclin D1 as judged by the staining of the tumor nuclei. Of these 10, 9 were of the centroblastic subtype and 1 of the anaplastic subtype. None of the 10 cases showed plasmacytic differentiation. Of the 10 cases, 7 displayed focal staining, and the remaining 3 cases were diffusely positive (Table 1). The intensity of cyclin D1 staining was weak in all cases except 1 (Table 1 and Images 1-3). Surprisingly, none of the 231 cases, including the cyclin D1+ cases, was positive for CD5 despite adequate internal controls (T lymphocytes) in every case (Table 1 and Image 1).

All cyclin D1+ lymphomas were subjected to further immunohistochemical studies to determine their phenotype in more detail. The results of the immunostaining for CD20, CD10, CD5, cyclin D1, MUM1, bcl-2, bcl-6, p53, and Ki-67 are summarized in Table 1. In addition, 7 of the 10 cyclin D1+ cases were available for further immunohistochemical studies with a rabbit monoclonal cyclin D1 antibody (clone SP4, NeoMarkers). All positive cases could be confirmed with very similar staining patterns compared with the cyclin D1 antibody from Novocastra (not shown). Every case except one (case 4) expressed bcl-6 or MUM1. This pattern of expression argues against the possibility of them being CD5− blastoid variants of mantle cell lymphoma. Thus, some DLBCLs are cyclin D1+ in the absence of expression of CD5. The clinical features of the patients are provided in Table 2.

**Some Cyclin D1+ DLBCLs Have Aberrations at the CCND1 Locus**

Of the 10 cyclin D1+ DLBCLs, 7 were available for further molecular studies. These were subjected to FISH with fusion or split probes to determine the status of the CCND1 and IGH genes as described in the “Materials and Methods” section. In 1 case (case 4) with centroblastic morphologic features (Image 1A) and strong expression of cyclin D1 (Image 1C), a t(11;14) could be demonstrated as judged by the fluorescence pattern with fusion and split probes (Table 1 and Images 1D and 1E). In another case (case 1) with centroblastic morphologic features (Image 2A), a minority of the tumor cells (approximately 10%) contained a fusion signal as detected by the t(11;14) fusion probe (Table 1 and Image 2C). In addition, the telomeric cyclin D1 probe was lost in many tumor cells, resulting in 1 fusion signal and 1 green signal (owing to the loss of the orange telomeric cyclin D1 probe) when assessed by the Abbott split probe (Table 1 and Image 2D). The results were confirmed by the cyclin D1 split probe from DAKO (Table 1 and Image 2E), which showed 1 fusion signal and 1 orange signal (owing to the loss of the green telomeric cyclin D1 probe) in many of the tumor nuclei. These results indicate a break in the CCND1 gene in the majority of the tumor cells and a translocation to the IGH locus in a subclone of the lymphoma. In 2 other cases (cases 2 and 3) with anaplastic (Image 3A) and centroblastic (not shown) morphologic features, respectively, the expression of cyclin D1 (Image 3B) was associated with additional CCND1 signals by FISH with fusion and split probes (Table 1 and Images 3C and 3D). In the remaining 3 available cases (cases 5-7), the pattern of FISH was normal (Table 1), suggesting that the overexpression of cyclin D1 was related to posttranslational mechanisms.

### Table 1
**Characterization of 10 Cases of Cyclin D1+ Diffuse Large B-Cell Lymphoma by Immunohistochemical Analysis and FISH Using Fusion or Split Probes to Detect the t(11;14) Translocation, Additional Cyclin D1 Gene Signals, and Deleted Chromosome 11**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>CD10</th>
<th>CD5</th>
<th>Cyclin D1</th>
<th>MUM1</th>
<th>bcl-2</th>
<th>bcl-6</th>
<th>p53</th>
<th>Ki-67 (%)</th>
<th>FISH</th>
</tr>
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<tr>
<td>1</td>
<td>−</td>
<td>−</td>
<td>+ (weak, diffuse)</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+ (focal)</td>
<td>90</td>
<td>Abnormal cyclin D1 gene</td>
</tr>
<tr>
<td>2</td>
<td>−</td>
<td>+</td>
<td>+ (weak, focal)</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>80</td>
<td>Additional cyclin D1 signals</td>
</tr>
<tr>
<td>3</td>
<td>−</td>
<td>+</td>
<td>+ (weak, focal)</td>
<td>+ (focal)</td>
<td>+</td>
<td>+ (focal)</td>
<td>+ (focal)</td>
<td>90</td>
<td>Additional cyclin D1 signals</td>
</tr>
<tr>
<td>4</td>
<td>−</td>
<td>+</td>
<td>+ (strong, diffuse)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>50</td>
<td>t(11;14)</td>
</tr>
<tr>
<td>5</td>
<td>−</td>
<td>+</td>
<td>+ (weak, focal)</td>
<td>+ (focal)</td>
<td>+</td>
<td>+ (focal)</td>
<td>+</td>
<td>90</td>
<td>Normal</td>
</tr>
<tr>
<td>6</td>
<td>−</td>
<td>+</td>
<td>+ (weak, focal)</td>
<td>+</td>
<td>+</td>
<td>+ (focal)</td>
<td>+</td>
<td>90</td>
<td>Normal</td>
</tr>
<tr>
<td>7</td>
<td>−</td>
<td>+</td>
<td>+ (weak, focal)</td>
<td>+ (focal)</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>50</td>
<td>Normal</td>
</tr>
<tr>
<td>8</td>
<td>−</td>
<td>+</td>
<td>+ (weak, focal)</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>9</td>
<td>−</td>
<td>+</td>
<td>+ (weak, focal)</td>
<td>+</td>
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<td>ND</td>
<td>ND</td>
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<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>−</td>
<td>+</td>
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<td>ND</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
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FISH, fluorescence in situ hybridization; ND, not determined; +, positive immunohistochemical result; −, negative immunohistochemical result.

*Immunostaining was judged as focal if fewer than 30% of the tumor cells were positive. Fewer than 10% positive tumor cells was considered as negative. For Ki-67, the percentage of positive tumor cells was estimated. All cases were CD20+ (not shown).
Image 1 (Case 4) B-cell lymphoma with classic centroblastic morphologic features (A, H&E, ×40); CD20+ (not shown), CD5− (B, ×40), and diffuse strong nuclear staining with cyclin D1 (C, ×40). D, When hybridized with a fusion probe to detect the t(11;14), yellow (orange/green) fusion signals can be seen in many of the tumor nuclei indicating the t(11;14) translocation. Some of these yellow (orange/green) signals are highlighted with arrows. The native chromosomes 11 and 14 are seen as orange and green signals, respectively (×100). E, When hybridized with a split probe, intact \textit{CCND1} genes are seen as yellow (orange/green) fusion signals. The split red and green signals seen in many of the tumor nuclei (some of which are highlighted with circles) represent rearranged \textit{CCND1} genes (×100).
Image 2A (Case 1) Diffuse large B-cell lymphoma with centroblastic morphologic features (A, H&E, ×40); CD20+ (not shown), CD5− (not shown), and diffuse weak nuclear staining with cyclin D1 (B, ×40). C, When hybridized with a fluorescent fusion probe to detect the t(11;14), most cells had normal orange and green signals, but in approximately 10% of the cells, yellow (orange/green) fusion signals (highlighted with arrows) were detected (×100). D, When hybridized with a fluorescent split probe (Abbott, Abbott Park, IL), many cells contained 1 normal yellow (orange/green) fusion signal (representing an intact \textit{CCND1} allele) and 1 green signal (representing loss of the orange telomeric cyclin D1 probe). Some of the green signals are highlighted with arrows (×100). E, The results were confirmed using another fluorescent split probe (DAKO, Glostrup, Denmark). Many cells contain 1 normal yellow (orange/green) signal and 1 red-orange signal (representing loss of the green telomeric cyclin D1 probe). Some of the red-orange signals are highlighted with arrows (×100). These results indicate the t(11;14) translocation in a minority of the tumor cells and loss of part of the \textit{CCND1} gene locus in many tumor cells.
Discussion

This study demonstrates unequivocal overexpression of cyclin D1 in 4.3% (10/231) of DLBCLs in the absence of expression of CD5. Several previous investigations have failed to demonstrate cyclin D1 positivity in DLBCLs, possibly because of problems in finding a sufficiently sensitive method. The only exception that we are aware of is a series of 70 DLBCLs and Burkitt lymphomas in which 10% of the cases displayed scattered weakly cyclin D1+ tumor cells, stained with the DCS-6 monoclonal antibody from Novocastra. The expression of CD5 was not studied in this series. The cyclin D1+ and CD5+ DLBCLs reported by Zhang et al possibly represent blastoid variants of mantle cell lymphomas.

Most of the cyclin D1+ cases in the present study are unlikely to represent CD5– blastoid variants of mantle cell lymphoma because all but 1 of 10 cases were positive for bcl-6 or MUM1. This immunohistochemical pattern is typical of some DLBCLs but argues against a diagnosis of mantle cell lymphoma.
cell lymphoma, even though rare cases of mantle cell lymphoma seem to be able to express bcl-6. Hence, in a large study of 315 mantle cell lymphomas, Camacho et al found 5 bcl-6+ cases (1.6%).

The remaining case in the present study, associated with evidence of the t(11;14), was negative for CD10, bcl-6, and MUM1 and could represent a misdiagnosed mantle cell lymphoma. This case showed strong cyclin D1 positivity, whereas in the other 9 positive cases, cyclin D1 staining was weak yet distinct. We used a cyclin D1 antibody from Novoceastra that proved to work well with adequate staining of internal controls such as endothelial cells. The available positive cases were confirmed with another cyclin D1 antibody from NeoMarkers. The introduction of new antibodies during the last years has increased the sensitivity and reliability of immunohistochemical analysis of cyclin D1, which could explain the discrepancy between our results and earlier studies. The rabbit monoclonal from NeoMarkers is probably more sensitive than previously available mouse monoclonal antibodies and may have the potential to detect more cyclin D1+ cases.

Cyclin D1 is part of a molecular complex that promotes progression through the G1 phase of the cell cycle and, hence, cell proliferation. By binding to certain cyclin-dependent kinases, cyclin D1 triggers a conformational change that activates the kinase with subsequent phosphorylation of critical substrates for the G1-S transition such as the retinoblastoma protein. Hyperphosphorylation of the retinoblastoma protein in turn allows G1-S transition by the release of the E2F family of transcription factors, which control the expression of genes required for the initiation or propagation of DNA-synthesis and cell cycle progression. This mechanism is obviously not restricted to mantle cells or even lymphoid cells. Indeed, deregulated expression of cyclin D1 probably contributes to carcinogenesis in many epithelial malignancies such as, eg, breast cancer and head and neck squamous carcinoma. It is, therefore, not surprising that overexpression of cyclin D1 can be seen in several types of lymphomas, reflecting the oncogenic pathway in the particular lymphoma examined. Rather, given the general molecular role of cyclin D1 in cell cycle regulation, it is remarkable that cyclin D1 overexpression is largely limited to a few lymphoma subtypes. It is still not understood why deregulation of cyclin D1 is associated with a few specific malignant lymphoid phenotypes.

In the present study, 3 (cases 5-7) of 7 cyclin D1+ cases showed no structural or numeric aberrations of the CCND1 gene by FISH, suggesting that overexpression of cyclin D1 was related to posttranslational mechanisms (cases 8-10 not studied because paraffin blocks were no longer available). Of 7 available cyclin D1+ cases, 2 (cases 2 and 3) displayed additional CCND1 signals by FISH. A possible explanation for the overexpression of cyclin D1 in these cases would be CCND1 gene amplification, although it is difficult to rule out extra copies of chromosome 11 as a result of hyperdiploidy. These cases should not pose diagnostic problems because, in the absence of the t(11;14) and CD5 expression but with positivity for bcl-6 or MUM1, they do not fall within the boundaries for the criteria of mantle cell lymphoma. The expression of cyclin D1 likely represents one of the oncogenic hits that has led to unrestrained proliferation and survival of the tumor cells. These cases should, therefore, be classified as DLBCLs with aberrant expression of cyclin D1. However, the finding of cyclin D1+ cases should encourage the search for the t(11;14) to exclude the possibility of a CD5−blastoid mantle cell lymphoma.

In another cyclin D1+ case (case 1), a subpopulation (approximately 30% of the cells) was cyclin D1+ by immunohistochemical analysis. In this case, many tumor cells had evidence of structural abnormalities in 1 of the CCND1 alleles. Two cyclin D1 split probes had similar hybridization results with loss of signals from the telomeric cyclin D1 probe in

### Table 2

<table>
<thead>
<tr>
<th>Case No./Sex/Age (y)</th>
<th>Elevated LD Level</th>
<th>Extranaodl Involvement</th>
<th>IPI*</th>
<th>Response to Primary Treatment</th>
<th>Survival Data (mo)</th>
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<tr>
<td>1/M/56</td>
<td>No</td>
<td>IV</td>
<td>BM</td>
<td>Low risk</td>
<td>Dead with disease (44)</td>
</tr>
<tr>
<td>2/M/79†</td>
<td>Yes</td>
<td>IV</td>
<td>BM</td>
<td>High risk</td>
<td>Dead with disease (1)</td>
</tr>
<tr>
<td>3/F/69</td>
<td>Yes</td>
<td>IV</td>
<td>BM and CNS</td>
<td>High risk</td>
<td>Dead with disease (1)</td>
</tr>
<tr>
<td>4/F/71</td>
<td>ND</td>
<td>IV</td>
<td>BM</td>
<td>ND</td>
<td>Dead with disease (29)</td>
</tr>
<tr>
<td>5/F/58†</td>
<td>Yes</td>
<td>I</td>
<td>None</td>
<td>Low risk</td>
<td>Alive without disease (50)</td>
</tr>
<tr>
<td>6/F/71</td>
<td>Yes</td>
<td>I</td>
<td>None</td>
<td>Low risk</td>
<td>Dead with disease (12)</td>
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<tr>
<td>7/M/84</td>
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<td>I</td>
<td>None</td>
<td>Low risk</td>
<td>Dead without disease (39)</td>
</tr>
<tr>
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<td>III</td>
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<td>High risk</td>
<td>Dead without disease (55)</td>
</tr>
<tr>
<td>9/M/68</td>
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<td>BM</td>
<td>High risk</td>
<td>Dead with disease (97)</td>
</tr>
<tr>
<td>10/M/66</td>
<td>No</td>
<td>I</td>
<td>None</td>
<td>Low risk</td>
<td>Dead with disease (9)</td>
</tr>
</tbody>
</table>

BM, bone marrow; CNS, central nervous system; CR, complete remission; IPI, international prognostic index; LD, lactate dehydrogenase; ND, not determined; PD, progressive disease; PR, partial remission.

* Low risk indicates low risk and low-intermediate risk equivalent to an IPI score of 1 or 2; high risk indicates high-intermediate risk and high risk equivalent to an IPI score of 3, 4, or 5.

† Also had rheumatoid arthritis.
many of the tumor cells together with CCND1/IgH fusion signals in a subclone. This finding most likely reflects molecular heterogeneity within the tumor and suggests that structural abnormalities of the CCND1 gene could be one of the pathogenetic events of this particular DLBCL. This lymphoma could be classified as a DLBCL with aberrant expression of cyclin D1 despite the finding of a subclone with the t(11;14).

From a clinical perspective, none of these 9 cyclin D1+ cases (cases 1-3 and 5-10) would favor a diagnosis of mantle cell lymphoma over DLBCL. Rather, the involvement of the central nervous system in 1 case and the association with rheumatoid arthritis in 2 other cases are features more frequently associated with DLBCL. In addition, 4 (44%) of these 9 patients had stage I disease (Table 2), which is more often seen in patients with DLBCL than in patients with mantle cell lymphoma.39

In the remaining case (case 4) associated with strong, diffuse expression of cyclin D1, evidence of the t(11;14) translocation was found by FISH. Staining for bcl-2 was positive. In view of the t(11;14) and the absence of CD10, bcl-6, and MUM1, it is likely that this case actually represents a misdiagnosed blastoid variant of mantle cell lymphoma. However, it would be difficult to completely rule out the possibility of DLBCL based on the classic centroblastic morphologic features and the absence of CD5 despite the t(11;14) and the strong expression of cyclin D1.

Indeed, translocations between chromosomes 11 and 14 are not entirely specific for mantle cell lymphoma because they can be found in, eg, plasma cell myeloma along with overexpression of cyclin D1.13 Certainly no pathologist would classify a plasma cell myeloma with overexpression of cyclin D1 and a t(11;14) translocation as a mantle cell lymphoma, simply because of its discriminating morphologic features and clinical picture. The morphologic features and clinical behavior of lymphomas (or any malignancy) are the sum of all molecular events leading to the transformed phenotype. Only a minority of these events are understood or can be visualized. Hence, molecular and genetic characteristics should not be used alone to classify any lymphoma. Whenever the morphologic or clinical setting is uncharacteristic, a definitive diagnosis should be made with caution. In such cases, gene expression profiling may serve as a useful future tool in the differential diagnosis. In current clinical practice, we would advocate immunostaining with CD5 and cyclin D1 in all cases of DLBCLs. All equivocal cases of large B-cell lymphomas with nuclear staining for cyclin D1 (including putative mantle cell lymphomas with other than classic mantle cell morphologic features) should be subjected to FISH and immunostaining with CD10, bcl-6, and MUM1.

The present study demonstrates that some DLBCLs are cyclin D1+ in the absence of expression of CD5. In most of these cases, there is little doubt as to the correct diagnosis. However, in exceptional cases, the distinction between DLBCLs and mantle cell lymphomas may be difficult, even if immunohistochemical and cytogenetic methods are used.

References


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