Cyclin D1–Positive Diffuse Large B-Cell Lymphoma With IGH-CCND1 Translocation and BCL6 Rearrangement

A Report of Two Cases

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ABSTRACT

Objectives: To demonstrate and confirm the existence of cyclin D1–positive diffuse large B-cell lymphoma (DLBCL) with IGH-CCND1 rearrangement and discuss the rationale of differentiating this entity from blastoid and pleomorphic variants of mantle cell lymphoma (MCL).

Methods: Two cyclin D1–positive lymphomas with morphologic features of DLBCL and IGH-CCND1 translocations were characterized with respect to clinical features, as well as morphologic, immunophenotypic, cytogenetic, and molecular findings.

Results: The large tumor cells were CD20+, CD5–, CD10–, BCL6+, MUM1+, and cyclin D1+ in both cases. SOX11 was negative. Epstein-Barr virus–encoded RNA in situ hybridization demonstrated diffuse positivity in case 1. BCL6 and IGH-CCND1 rearrangements were identified by fluorescence in situ hybridization in both cases. Specifically, the diagnosis of a relapsed DLBCL with acquisition of IGH-CCND1 was rendered for case 1, molecularly confirmed by the detection of identical monoclonal IGH rearrangements between the initial diagnostic DLBCL and relapse lymphoma.

Conclusions: Our study demonstrates convincingly that IGH-CCND1 rearrangement leading to cyclin D1 overexpression can occur in DLBCL and pose a potential diagnostic pitfall, requiring thorough knowledge of the clinicopathologic findings to allow accurate discrimination from a blastoid or pleomorphic MCL. The coexistence of IGH-CCND1 and IGH-BCL6 rearrangements suggest that BCL6 and cyclin D1 may cooperate in the pathogenesis of DLBCL.

Cyclin D1 is a cell cycle regulator with a critical role in G1-S transition. It is expressed at very low levels in normal B cells but is overexpressed in almost all mantle cell lymphomas (MCLs) by virtue of rearrangement of the CCND1 (BCL1) locus with the immunoglobulin heavy chain (IGH) gene and rarely with the immunoglobulin light chain genes. Aberrant cyclin D1 expression as a result of CCND1 rearrangement is a critical primary pathogenetic event in MCL, promoting transition from G1 to S phase by phosphorylating retinoblastoma protein and releasing the transcription factor E1 and by increasing degradation or blocking of p27, a cell cycle inhibitor. Although cyclin D1 expression is highly specific for MCL, it is not 100% specific. Cyclin D1 is also expressed at variable intensities in around 40% of plasma cell myelomas associated with IGH-CCND1 rearrangement, CCND1 amplification, or no structural alterations of the CCND1 locus. It is also weakly expressed in hairy cell leukemia and is not associated with CCND1 rearrangement.

Cyclin D1 expression has been systematically evaluated in diffuse large B-cell lymphoma (DLBCL) in several studies, which indicate that cyclin D1 expression is infrequent (0%-15%) in DLBCL. The two earlier studies by Zukerberg et al and Bai et al did not detect cyclin D1 expression in 33 and 79 DLBCLs, respectively. Subsequently, a study of 231 DLBCLs by Ehinger et al revealed 10 cyclin D1–positive cases. Rodriguez-Justo et al identified one cyclin D1–positive case in a series of 114 DLBCLs. Cyclin D1 positivity was observed in 10 (15%) of 66 de novo DLBCLs in a study by Vela-Chavez et al. In a recent study of 304 DLBCLs, cyclin D1 positivity was seen in only four (1.3%) cases. Most cyclin D1–positive DLBCLs possess centroblastic morphology.
and a postterminal center immunophenotype, with BCL6 and MUM1 expression and absence of CD10. They are almost always CD5 negative. A rare case of CD5-positive, cyclin D1–positive DLBCL has been reported. Cyclin D1 positivity in a lymphoma with large cell morphology often raises the differential diagnosis of a pleomorphic/blastoid variant of MCL, which needs to be distinguished from cyclin D1–positive DLBCL. CCND1 rearrangement has been considered superior to the immunohistochemical detection of cyclin D1 alone in discriminating between blastoid/pleomorphic MCL and DLBCL. To date, informative fluorescence in situ hybridization (FISH) results have been available for 30 cases of cyclin D1–positive DLBCL, IGH-CCND1 or CCND1 rearrangements were identified in four cases, including two that were very recently published when this article was in preparation. The two older cases described by Ehinger et al included one with IGH-CCND1 fusion in most tumor cells and one with IGH-CCND1 detected in about 10% of the tumor cells, while the rest had partial deletion of the CCND1 locus. Although this finding suggests the existence of a small subset of DLBCLs with cyclin D1 positivity and IGH-CCND1 rearrangement, in neither of these cases could the diagnosis of DLBCL be firmly established and the diagnosis of pleomorphic/blastoid MCL be entirely excluded based on other immunophenotypic and cytogenetic features. These two cases may in fact represent blastoid or pleomorphic MCL misdiagnosed as DLBCL. While the overall features of one of the two cases (case 1) described by Juskevicius et al may be consistent with DLBCL, the other case was probably a blastoid MCL. BCL6 rearrangement, which is frequently detected in DLBCL, has not been reported in any of the cyclin D1–positive DLBCLs. In the current study, we present for the first time two cases of cyclin D1–positive DLBCL with CCND1 and BCL6 rearrangements, whereby the diagnosis of DLBCL can be convincingly established based on integration of clinical, histopathologic, cytogenetic, and molecular data.

Cases: Clinical History

Case 1

A 48-year-old man with a medical history of human immunodeficiency virus/AIDS sought treatment for axillary, inguinal, and cervical lymphadenopathy. Laboratory values were as follows: hemoglobin, 10.4 g/dL; WBCs, 10.6 × 10^3/μL; and platelets, 269 × 10^3/μL. A subsequent biopsy of a left groin lymph node was performed, which was interpreted as diffuse large B-cell lymphoma, not otherwise specified (NOS). There was no evidence of bone marrow involvement. The patient underwent six cycles of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone). In July 2009, a positron emission tomography (PET) scan revealed no evidence of residual disease. In 2012, he noticed swelling in his right neck, and a biopsy of a right cervical lymph node was performed in July 2012. At the time, his laboratory values were as follows: hemoglobin, 9.3 g/dL; WBCs, 2.2 × 10^3/μL; and platelets, 395 × 10^3/μL. The biopsy specimen was read as cyclin D1–positive, Epstein-Barr virus (EBV)–positive DLBCL. IGH-CCND1 rearrangement was identified by FISH analysis. A staging bone marrow specimen was negative for lymphoma involvement. The patient received three cycles of R-DICE (rituximab, dexamethasone, etoposide, ifosfamide, and cisplatin) and underwent an autologous stem cell transplant on October 25, 2012. The latest PET scan in April 2013 showed no enlarged or hypermetabolic lymph nodes in the cervical area.

Case 2

A 60-year-old man with B-symptoms had left testicular swelling with inguinal, cervical, and axillary lymphadenopathy in September 2011. A right axillary excisional lymph node biopsy specimen revealed cyclin D1–positive DLBCL, NOS. FISH detected IGH-CCND1 rearrangement. A staging bone marrow specimen showed involvement by DLBCL. There was also involvement of the central nervous system. Laboratory values were as follows: hemoglobin, 12.8 g/dL; WBCs, 10.6 × 10^3/μL; and platelets, 242 × 10^3/μL. The patient received R-CHOP and liposomal cytarabine for six cycles and four cycles of high-dose methotrexate. A PET scan in May 2012 revealed no evidence of disease. The patient was admitted to the emergency department with neurologic symptoms in March 2013. He had a hypermetabolic lesion in the left posterior parietal lobe with right pelvic lymphadenopathy by PET computed tomography. Cerebrospinal fluid was negative for malignancy. A brain biopsy was canceled after an acute episode of seizures. The patient underwent therapy with corticosteroids, rituximab, and methotrexate. He subsequently had no evidence of lesions in the central nervous system.

Materials and Methods

Case Selection and Histologic Analysis

Both cyclin D1–positive DLBCL cases (cases 1 and 2) were encountered in clinical practice in the Division of Hematopathology, Department of Pathology and Laboratory Medicine, Weill Cornell Medical College, New York, NY, among 1,460 DLBCLs viewed during routine diagnostic
work for the past 10 years. A total of 203 DLBLs and 35
MCLs (30 classic and five blastoid/pleomorphic) were also
identified from the archives of the Department of Pathology
and Laboratory Medicine at Weill Cornell for comparison.
Cases were diagnosed according to the diagnostic criteria
of the World Health Organization (2008) by integration
of results of morphologic evaluation; immunophenotypic
analysis performed on formalin-fixed, paraffin-embedded
tissues; and cytogenetic and molecular analysis. All
the DLBL cases have previous available data on BCL6,
MUM1, and CD10 expressions, and all MCL cases are
cyclin D1 positive. This study was approved by the Institu-
tional Review Board of Weill Cornell Medical College.

Tissue Microarray Construction

A tissue microarray (TMA) block was constructed from
35 MCLs, using a Manual Tissue Microarrayer (Beecher
Instruments, Sun Prairie, WI). For each individual case, two
representative cores of 0.6 mm were taken from the original
paraffin blocks. Serial sections of 4.5 μm were cut from the
tissue array blocks and used for immunohistochemical and
FISH analysis.

Immunohistochemical Analysis

For immunohistochemistry, paraffin-embedded tissue
sections were stained using a Leica (Buffalo Grove, IL)
autostainer according to the company’s protocols, with
minor modifications. The antibodies and the protocols used
are listed in Table 1 and Table 2. BCL6, MUM1, and
SOX11 were considered positive when 30% or more neo-
plastic cell nuclei stained for each marker. Statistical signifi-
cance of the difference in the proportions of BCL6+MUM1+
cases between DLBL and MCL was determined using the
two-proportion z test.

Conventional Cytogenetic Analysis

Cells were obtained from lymph nodes and were cul-
tured for 24 hours without stimulation and then harvested
per standard protocols. G-band metaphases were examined
and reported per the International System for Human Cyto-
genetic Nomenclature.

FISH

FISH was performed on paraffin sections for cases 1
and 2, as well as the MCL TMA. Interphase nuclei cells
were examined. The following probes were used: LSI BCL6
dual-color break-apart rearrangement probe (Vysis/Abbott
Molecular, Des Plaines, IL) to look for BCL6 rearrange-
ment and LSI CCND1 (BCL1)–IGH dual-color dual-fusion
probe (Vysis/Abbott Molecular) to look for IGH-CCND1
translocation. Statistical significance of the difference in the
proportions of BCL6 rearranged cases between DLBL and
MCL was determined using the two-proportion z test.

Immunoglobulin Heavy Chain Gene Rearrangement
Analysis by Polymerase Chain Reaction and Sequencing

Genomic DNA extracted from formalin-fixed, paraf-
fin embedded tissue sections was subjected to seminested
polymerase chain reaction (PCR) analysis for immuno-
oglobulin heavy chain (IGH) gene rearrangement using IGVH
framework region 3 (FR3) primer and JH consensus primers
as described previously. The products were analyzed by
polyacrylamide gel electrophoresis. The dominant rear-
anged bands were extracted from gel and cloned. They
were then sequenced using an ABI Automated 3730 DNA
Analyzer (Applied Biosystems, Foster City, CA). The DNA
sequences were compared with online database IMGT/V-
QUEST (http://imgt.cines.fr) and IGBlast (http://www.ncbi.
nlm.nih.gov/igblast/).
Results

Histologic and Immunophenotypic Findings

Case 1

The initial diagnostic specimen from 2009 was a 3 × 2.3 × 1-cm left groin lymph node. Microscopically, the lymph node architecture was largely effaced by a diffuse atypical large cell proliferation with oval to irregular nuclei, vesicular chromatin, and conspicuous nucleoli (centroblastic morphology). Mitotic activity was brisk. These cells were positive for CD20, BCL-6, and MUM-1 but negative for CD5, CD10, and cyclin D1. In situ hybridization for EBV-encoded RNA (EBER) was negative. The diagnosis of DLBCL, NOS was made.

The second specimen from 2012 was a right cervical lymph node biopsy specimen. Microscopically, there were fragments of lymph node with effacement of normal architecture by a diffuse proliferation of large atypical lymphoid cells with similar morphologic features as those seen in the previous biopsy specimen. Mitotic activity was increased, and there was focal necrosis. The atypical lymphoid cells were positive for CD20, BCL-6, and MUM-1 but negative for CD5 and CD10. The tumor cells also showed strong diffuse positivity for cyclin D1 but were negative for SOX11. Ki-67 staining showed a proliferation index of 90%, and EBER in situ hybridization for RNA was diffusely positive (Image 1). This case was interpreted as a cyclin D1–positive, EBV-positive DLBCL.

Case 2

The specimen, from a right axillary lymph node, measured 4.5 × 3 × 1.5 cm. Microscopically, the nodal architecture was completely effaced by a diffuse proliferation of large atypical lymphoid cells with a centroblastic morphology. The neoplastic cells were positive for CD20, BCL-6, and MUM-1 but negative for CD5 and CD10. The tumor cells also exhibited partial positivity for cyclin D1; SOX11 was negative (Image 2). The Ki-67 proliferation index was 60%. EBER was negative. This case was diagnosed as cyclin D1–positive, DLBCL.

The staging bone marrow specimen had a cellularity of 60%. Aggregates of large lymphocytes were seen (50% of marrow cells) along with trilineage maturing hematopoiesis. Flow cytometry showed monotypic B cells comprising 12% of the analyzed population. These cells did not express CD5 or CD10. The findings were consistent with bone marrow involvement by DLBCL.

To provide further support that these two cases are indeed DLBCL and not blastoid/pleomorphic DLBCL, we compared the BCL6, MUM1, and SOX11 expression status of these two cases with a cohort of DLBCLs and MCLs. BCL6 and MUM1 expressions in the DLBCL cohort were determined during routine clinical diagnostic evaluation. SOX11 expression in DLBCL has been extensively studied and is very infrequent (<1%).18-22 BCL6, MUM1, and SOX11 expressions in MCL were analyzed by immunohistochemistry performed on TMA. Among 203 DLBCLs with available data on BCL6 and MUM1, 116 (57.1%) were positive for both. Eighty-five (73.3%) of these 116 DLBCLs were negative for CD10. In contrast, six (17.1%) of 35 MCLs (P < .0001 vs DLBCL), including one of five blastoid/pleomorphic variants, were positive for BCL6 and MUM1. BCL6 expressions in all positive cases were weak. Twenty-seven of 30 classic MCLs and three of five blastoid/pleomorphic MCLs were positive for SOX11. Among the 35 MCL cases, only one classic MCL had a BCL6+MUM1+SOX11– phenotype. The overall immunophenotypic features of our two cases share greater resemblance to DLBCL than to MCL.

Cytogenetics Findings

Case 1

Interphase FISH was performed on paraffin sections from both the first and second lymph node biopsy specimens. While the first biopsy specimen was positive for BCL6 rearrangement and negative for CCND1 rearrangement, the second biopsy specimen was positive for both IGH-CCND1 and BCL6 gene rearrangements (Image 3).

Case 2

Twenty-one metaphase cells were analyzed. Multiple numerical and structural abnormalities were observed in 19 cells, with the following karyotype: 47, XY, t(3;22)(q27;q11.2), t(4;12)(q12;p11.2), der(8)t(8;8)(p12;q21), ...
Image II Morphologic and immunophenotypic findings of the initial diagnostic and relapsed lymphomas in case 1. A, Initial diagnostic diffuse large B-cell lymphoma (DLBCL), not otherwise specified (H&E; ×400 [inset, ×600]). The tumor cells are positive for CD20 (B), BCL6 (C), MUM1 (D), and cyclin D1 (E). Cyclin D1 is positive in some endothelial cells and histiocytes. F, Relapsed Epstein-Barr virus–positive DLBCL (H&E; ×400 [inset, ×600]). The tumor cells are positive for CD20.
Image II (cont) (G), BCL6 (H), MUM1 (I), and cyclin D1 (J) but negative for SOX11 (K). In situ hybridization for Epstein-Barr virus–encoded RNA (L) demonstrates diffuse nuclear positivity in the lymphoma cells (×400).
Image 2 Morphologic and immunophenotypic findings in case 2. A, Diffuse large B-cell lymphoma, not otherwise specified (H&E; ×400 [inset, ×600]). The tumor cells are positive for CD20 (B), BCL6 (C), MUM1 (D), and cyclin D1 (focal) (E) but negative (F) for SOX11 (×400).
tumors upon gel electrophoresis, implying that these two similar monoclonal rearrangements were seen in the two initial diagnostic and relapse lymphomas, we performed case 1 molecular findings.  

Conventional karyotyping on the staging bone marrow sample identified two abnormal clones in 19 metaphase cells. The first clone (eight cells) had loss of the Y chromosome. The second clone (five cells) had multiple numerical and structural abnormalities. These abnormalities included the following: t(3; 22), t(4; 12), t(8; 13), and t(11; 14) translocations. Interphase FISH performed on the paraffin sections identified BCL6 gene rearrangement in 57.5% of cells analyzed and CCND1 rearrangement in 41% (5.5% double fusions and 35.5% single fusion) of cells. Conventional karyotyping on the staging bone marrow sample identified two abnormal clones in 19 metaphase cells. The first clone (eight cells) had loss of the Y chromosome. The second clone (five cells) had multiple numerical and structural abnormalities. These abnormalities included the following: t(3; 22), t(4; 12), t(8; 13), and t(11; 14) translocations. Interphase FISH performed on the paraffin sections identified BCL6 gene rearrangement in 57.5% of cells analyzed and CCND1 rearrangement in 41% (5.5% double fusions and 35.5% single fusion) of cells. Image 4. Conventional karyotyping on the staging bone marrow sample identified two abnormal clones in 19 metaphase cells. The first clone (eight cells) had loss of the Y chromosome. The second clone (five cells) had multiple numerical and structural abnormalities. These abnormalities included the following: t(3; 22), t(4; 12), t(8; 13), and t(11; 14), which were also seen in the lymph node biopsy specimen. FISH analysis detected IGH-CCND1 gene rearrangements in 4% of 200 interphase nuclei.

The presence of BCL6 rearrangement in these two cases is supportive of the diagnosis of DLBCL despite the concurrent CCND1 rearrangement. Among our cohort of DLBCLs, 44 have available FISH data on BCL6 determined during routine diagnostic workup. Five (22.7%) of 22 of the germinal center B-cell (GCB) subgroup and 16 (72.7%) of 22 of the non-GCB subgroup demonstrated BCL6 rearrangement. FISH for BCL6 rearrangement was performed on paraffin sections of the MCL TMA. Twenty-three of 35 cases yielded successful probe hybridization. BCL6 rearrangement was not identified in any of the MCLs (P = 6 × 10^-5 vs DLBCL), including the classic MCL with a BCL6+/MUM1+/SOX11− phenotype.

Discussion

Herein we report two cases of cyclin D1–positive DLBCL with CCND1 and BCL6 rearrangements. We believe that these two cases represent authentic DLBCLs rather than aggressive variants of MCL. Previously in a study of 231 DLBCLs by Ehinger et al, 10 (4.3%) cases were positive for cyclin D1 by immunohistochemistry. One of these 10 cases was positive for IGH-CCND1 rearrangement by FISH, and another case showed loss of part of the CCND1 locus in most tumor cells and IGH-CCND1 rearrangement in a minority of the tumor cells. However, the possibility that these two cases represent aggressive variants of MCL has not been fully investigated and excluded. The former case was negative for both BCL6 and MUM1, an unusual immunophenotype for DLBCL but a common phenotype for MCL, and therefore could represent an aggressive variant of MCL misdiagnosed as DLBCL. The other case was MUM1+ and BCL6−, which per se is not sufficient to exclude the diagnosis of MCL and justify the diagnosis of DLBCL. About 35% of MCLs are MUM+. Our cohort of MCLs exhibited even higher MUM1 positivity (65.7%) (Table 3).

Several morphologic, immunophenotypic, cytogentic, and molecular features in these two cases support the diagnosis of DLBCL. The neoplastic cells in both cases resemble centroblasts. Aggressive MCL variants predominantly have blastoid or pleomorphic morphology. Both of our cases are positive for BCL6 and MUM1. BCL6+/MUM1+ was seen in most (75%) cyclin D1–positive DLBCLs but observed only in a minority (~8%) of MCLs. In our cohort, BCL6+/MUM+ was present more frequently in DLBCL compared with MCL (57.1% vs 17.1%, P < .05). However, statistical significance cannot be reached vs blastoid/pleomorphic MCL (20%, P < 0.1) because of the small sample size of the latter. Thus, the BCL6+/MUM+ phenotype favors but cannot conclude a diagnosis of DLBCL in cases 1 and 2. The lack of SOX11 expression in these two cases is also in line with DLBCL, although it is by no means diagnostic. SOX11 is a characteristic of SOX11 expression in these two cases is also in line with DLBCL, although it is by no means diagnostic. SOX11 is a characteristic of lymphomas were clonally related Image 5. To confirm that these two rearrangements are identical, we purified, cloned, and subjected the amplicons to Sanger sequencing. Both rearrangements demonstrated the usage of IGHV4-34*05 and IGHJ4*01, with 85.4% overall identity and a similar somatic hypermutation pattern in the V and J genes Figure 1. The exact D gene used could not be delineated due to heavy somatic hypermutations in the complementarity determining region 3. The results of this gene rearrangement analysis indicate that the two lymphomas are indeed derived from the same B-cell clone.
marker highly specific for MCL, and most (~90%) MCLs express SOX11.18-22,25 The sensitivity of nuclear SOX11 staining for MCL, however, may be lower for the blastoid/pleomorphic variant. Before our study, 10 blastoid/pleomorphic variants of MCL, including four cyclin D1 negative, had been analyzed for SOX11 expression by immunohistochemistry.18,21,22 Seven (70%) of 10 demonstrated nuclear staining. The results of SOX11 immunohistochemistry findings in our cohort are consistent with the published literature. In contrast, only two of the nearly 300 DLBCLs analyzed to date, including eight CD5-positive DLBCLs and four cyclin D1-positive DLBCLs, exhibited nuclear staining.18-22 The two positive cases showed only weak to moderate levels of expression.22 Thus, while SOX11 positivity could almost certainly have ruled out a DLBCL in cases 1 and 2, their lack of SOX11 per se cannot discriminate definitively between DLBCL and blastoid/pleomorphic variant MCL. Taking together the expressions of BCL6, MUM1, and SOX11 appears to further increase the specificity for diagnosing DLBCL. The BCL6+MUM1+SOX11– combination, seen in cases 1 and 2, can probably be seen in about half of the DLBCLs based on our current findings and previously published results but was seen in only one classic MCL among 35 MCLs (<3%) in our cohort. None of the blastoid/pleomorphic variant had

Image 3: Fluorescence in situ hybridization findings in the initial diagnostic (A, C) and relapsed (B, D) lymphomas in case 1. BCL6 rearrangement was detected using a break-apart probe. Intact BCL6 genes were seen as yellow fusion signals (A, B). The arrows indicate some of the cells harboring split red and green signals, which represent rearranged BCL6 genes. Both the initial diagnostic and relapsed lymphomas were positive for BCL6 rearrangement. IGH-CCND1 rearrangement was detected using a dual-color, dual-fusion rearrangement probe (C, D). No yellow fusion signals were seen in the initial diagnostic tumor, but they could be seen in many tumor nuclei (some highlighted by arrows) of the relapsed lymphoma (×100).
the BCL6+MUM1+SOX11– phenotype. Thus, the immunophenotypic features of cases 1 and 2 are more supportive of DLBCL than MCL. In addition, EBV positivity has not been reported in MCL, and its presence in case 1 can be taken as solid evidence against the diagnosis of blastoid/pleomorphic MCL.

Molecular studies and FISH results provide the strongest evidence for classifying our cases as DLBCL. For case 1, the second lymphoma was clonally related to the initial DLBCL by IGH gene rearrangement analysis, supporting the interpretation that the second lymphoma represented a relapsed DLBCL rather than an independent blastoid/pleomorphic MCL. The presence of BCL6 rearrangement, which is a frequent genetic event in DLBCL15 and identified in most non-GCB subtype DLBCLs in our comparison cohort, further strengthens the diagnosis of DLBCL in our two cases. FISH performed on TMA did not identify BCL6 rearrangement in any of the MCLs in our cohort, including the classic MCL with BCL6+MUM1+SOX11– immunophenotype. That said, it is important to note that MCL cannot be entirely excluded based on BCL6 rearrangement per se. To date, six cases of MCL, including four conventional type and two blastoid type, with BCL6 expression have been reported to harbor BCL6 rearrangement.26-28 However, no MUM1 or SOX11 expression data were available for any of these cases. It is conceivable that the two blastoid MCLs with BCL6 rearrangement may in fact represent DLBCL with cyclin D1 rearrangement.

Both cases represent a diagnostic pitfall and may potentially be misdiagnosed as aggressive MCL based on the presence of both IGH-CCND1 and cyclin D1 immunohistochemical positivity. For case 1, had the second lymphoma been interpreted in the absence of clinical information (ie, history of DLBCL) and the results of BCL6 FISH, EBER in situ hybridization, and molecular studies, it might have been misdiagnosed as an aggressive variant of MCL, albeit with an unusual BCL6 and MUM1 immunohistochemical positivity. For case 2, a positive BCL6 FISH...
result in conjunction with negative SOX11 expression was crucial in supporting the diagnosis as DLBCL. Our study demonstrates that IGH-CCND1 rearrangement can rarely be seen in DLBCL, and the identification of IGH-CCND1 with cyclin D1 overexpression in lymphomas with large cell/blastoid morphology does not necessarily rule out a DLBCL. To avoid this diagnostic pitfall, IGH-CCND1 and cyclin D1 positivity need to be interpreted in the context of other clinical, immunophenotypic (in particular, SOX11 expression along with BCL6 and MUM1), cytogenetic, and molecular findings.

Very recently, two cases of cyclin 1–positive DLBCL with CCND1 rearrangements have been reported. While one of the cases (case 1) is compatible with DLBCL based on morphologic, immunophenotypic, and cytogenetic features, the other case is likely to be a blastoid MCL. Our cases are unique in that they both harbor BCL6 rearrangement in addition to IGH-CCND1, providing additional evidence for the existence of cyclin D1–positive DLBCL with IGH-CCND1. To our knowledge, this is the first time cyclin D1–positive DLBCL with both CCND1 and BCL6 rearrangements has been reported in the literature. We have not performed a systematic study to determine the exact frequency of this rare type of DLBCL because of the large number of samples required for screening.

Increased CCND1 copy number due to gene amplification or extra chromosome 11 has been demonstrated previously in a small subset of cyclin D1–positive DLBCLs. This alteration in CCND1 locus may result in increased cyclin D1 expression. Our study demonstrates that IGH-CCND1 can also be another genetic mechanism to deregulate cyclin D1 in DLBCL. In most cyclin D1–positive DLBCLs, however, no alterations in the CCND1 locus were identified, implying that alternative mechanisms are responsible for cyclin D1 overexpression in those cases. Regardless of the underlying mechanisms, the detection of cyclin D1 overexpression in DLBCL implies a role for cyclin D1 in its pathogenesis. Interestingly, we have deregulated BCL6 and cyclin D1, suggesting that these two oncogenes may cooperate in the development of DLBCL. It is likely that cyclin D1 rearrangements in these two cases occur during tumor progression rather than as an early initiating event. This is supported in case 1 by the presence of IGH-CCND1 in the relapsed lymphoma, but not in the original diagnostic lymphoma, and in case 2 by the subclonal pattern of IGH-CCND1 and cyclin D1 expression. Further studies are necessary to directly demonstrate cooperation between these two oncogenes.

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