

**Case study****Diffuse large B-cell lymphoma with *TEL/ETV6* translocation**

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**Summary** Cytogenetic abnormalities of chromosome 12p involving the *TEL/ETV6* gene are observed in a variety of hematopoietic neoplasms including acute leukemias, myelodysplastic syndromes, and myeloproliferative disorders. Karyotypic aberrations, including rearrangements, deletions, and amplifications of chromosome 12p, have been documented in B-cell non-Hodgkin lymphoma; however, rearrangements targeting *TEL* have rarely been reported. Here we describe a diffuse large B-cell lymphoma that had a complex karyotype including t(9;12)(q22;p13), which was confirmed by fluorescence in situ hybridization to represent rearrangement of *TEL*. Additional cytogenetic abnormalities included t(3;14)(q27;q32) involving the variant, alternative breakpoint region of the *BCL6* gene and del(6)(q13q23), resulting in the loss of 1 allele of *BLIMP1*. This case reiterates the importance of correlating morphologic and phenotypic findings with the results of cytogenetic analysis to avoid errors in diagnosing hematologic neoplasms and highlights the rare association of B-cell non-Hodgkin lymphoma with aberrations of *TEL*.

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**1. Introduction**

Translocations and deletions of chromosome 12p13 involving the *TEL* gene occur in a variety of hematologic neoplasms, including acute myeloid and lymphoblastic leukemias, myelodysplastic syndromes (MDSs), and myeloproliferative disorders [1,2]. Aberrations of 12p have also been reported in 15% to 22% of B-cell non-Hodgkin lymphomas (B-NHLs); however, the targeted genes are not yet known in most cases [3,4]. We report a unique case of diffuse large B-cell lymphoma (DLBCL), which occurred in a child with history of classic Hodgkin lymphoma and

presented with multiple lytic bone lesions and pleural effusions at relapse. The lymphoma had a complex karyotype, which included translocation of 12p13 that was confirmed to involve the *TEL* gene; rearrangement involving the variant, alternative breakpoint region (ABR) of the *BCL6* gene; and 6q deletion.

**2. Methods**

The computed tomography-guided biopsy of a lytic lesion of the lumbar vertebral body (L5) and a cell block prepared from the pleural fluid were fixed in formalin (10% neutral buffered). Paraffin-embedded sections of these specimens were stained with hematoxylin and eosin, and a cytospin preparation was stained with Wright-Giemsa for morphologic review.

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## 2.1. Immunohistochemistry, in situ hybridization, and flow cytometry

Immunohistochemical (IHC) stains were performed on the vertebral bone biopsy and pleural fluid cell block with the primary antibodies CD20, PAX5, BCL6, CD10, MUM1, BCL2, CD5, CD3, Ki-67, and p53 after heat-induced antigen retrieval; and Envision plus (DAKO, Carpinteria, CA) and diaminobenzidine (DAB) were used for visualization. IHC staining for *TEL* was also performed on the bone biopsy using an antibody directed against *ETV6* (clone HPA000264, rabbit polyclonal; Sigma, St Louis, MO). In situ hybridization for Epstein-Barr virus–encoded small RNAs (EBER) was performed using the supplied protocol (INFORM EBER; Ventana, Tucson, AZ).

Four-color flow cytometry of the pleural fluid was performed using FACSCalibur (Becton Dickinson, San Diego, CA) and the following primary antibodies: CD45, CD19, CD20, CD10, CD43,  $\kappa$  and  $\lambda$  light chains, CD3, CD16, and CD56 (Becton Dickinson). Data were analyzed using Cellquest software (Becton Dickinson).

## 2.2. Cytogenetic analysis

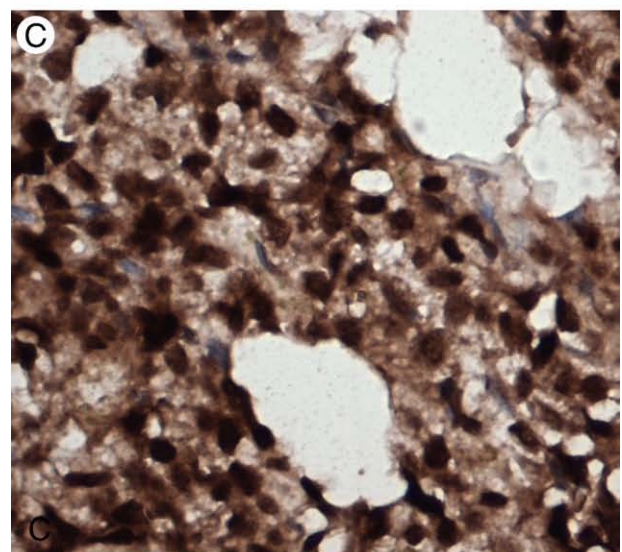
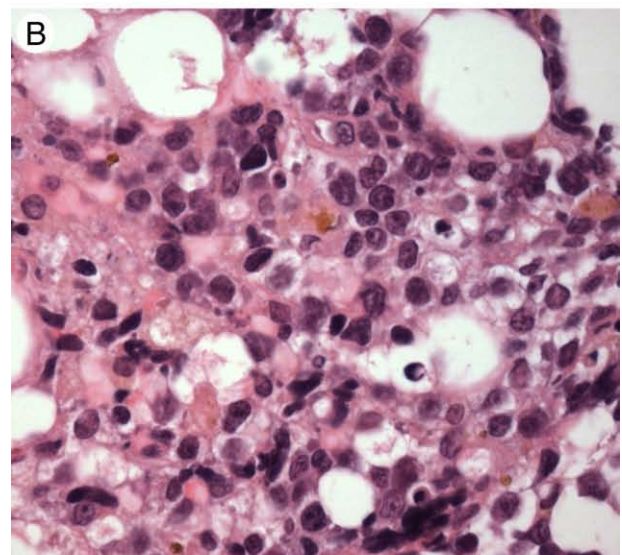
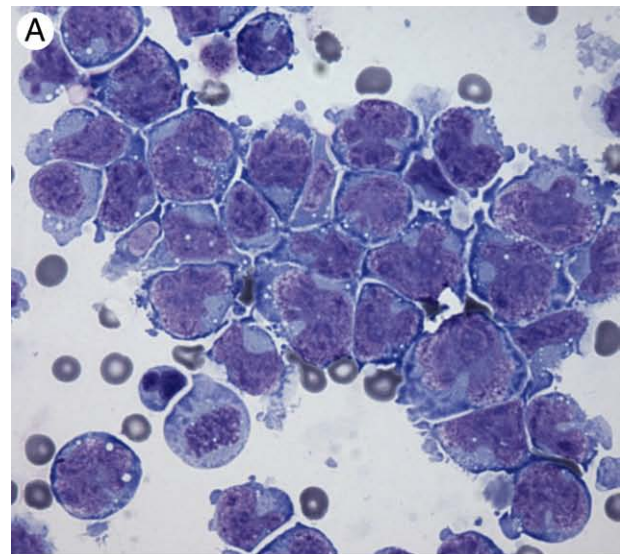
Giemsa banding was performed on metaphase preparations obtained after short-term (12 hours) culture of the pleural fluid using standard methods. Fluorescence in situ hybridization (FISH) analyses were performed on methanol-acetic–fixed cells using *IGH*, *BCL6*(3q27), *TEL/ETV6* (12p13), and *c-MYC*(8q24) dual-color break-apart probes (VYSIS, Downers Grove, IL); a locus specific probe for *BLIMP1*(*PRDMI*)(6q21) [5]; and a CEP6 probe (VYSIS), according to standard protocols; and 200 to 300 cells were analyzed. Fluorescence signals were captured after counterstaining with 4'-6-diamidino-2-phenylindole (DAPI) using the Cytovision Imaging system attached to a Nikon Eclipse 600 microscope (Applied Imaging, Santa Clara, CA).

## 2.3. Immunoglobulin heavy chain gene rearrangement

Polymerase chain reaction analysis to detect rearrangement of the immunoglobulin heavy chain gene variable region was performed on a pleural fluid specimen using the Biomed-2 primers (IVS Gene Clonality Assay Kit; *InVivo*-Scribe Technologies, San Diego, CA).

## 2.4. Case report

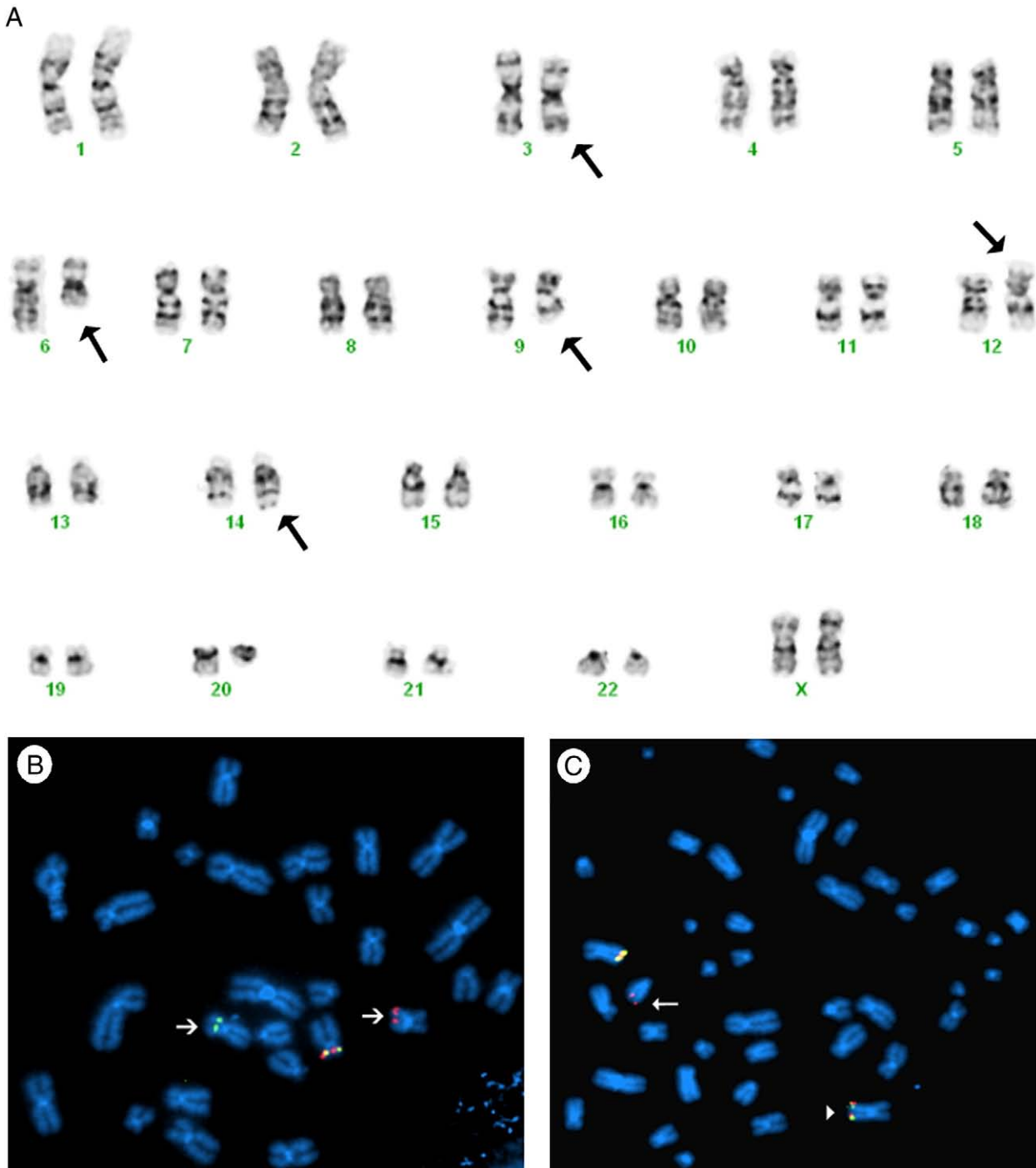
A 22-year-old woman was diagnosed with stage 4B classic Hodgkin lymphoma, nodular sclerosis type, 8 years



**Fig. 1** A, Cytospin of pleural fluid showing large pleomorphic lymphocytes (Wright-Giemsa, 400 $\times$ ). B, Vertebral body biopsy showing an infiltrate of DLBCL (hematoxylin and eosin, 400 $\times$ ), with (C) intense nuclear staining for *TEL* (*ETV6*, 400 $\times$ ).

before her current presentation. She was refractory to a variety of chemotherapeutic regimens, including 8 cycles of COPP-ABVD (cyclophosphamide, prednisone, procarbazine, vincristine, bleomycin, dacarbazine, doxorubicin,

vinblastine) followed by 2 cycles of ICE (carboplatin, etoposide, ifosfamide), high-dose BCNU (carmustine), etoposide, and autologous stem cell rescue, experiencing multiple relapses. Remission was eventually achieved after 10 cycles



**Fig. 2** A, G-band karyotype obtained from culture of pleural fluid. Arrows indicate  $t(9;12)$ ,  $t(3;14)$ , and  $del(6q)(q13q23)$ . B, FISH with *TEL* break-apart probes shows 1 green (split) signal on the short arm of the abnormal chromosome 12 and 1 red (split) signal on the long arm of the abnormal chromosome 9 consistent with *TEL* translocation (arrows). A single yellow (fusion) signal is present on the normal chromosome 12. C, FISH with *BCL6* break-apart probe shows 1 small red signal (arrow) translocated to the long arm of chromosome 14 and 1 green-yellow signal (unequal split, arrowhead) on the long arm of chromosome 3 consistent with ABR translocation; 1 yellow (fusion) signal (normal allele) on the nonrearranged chromosome 3 is also present.

of gemcitabine and vinorelbine with mantle field (chest, neck, and axilla) radiation. Three years before presentation at our institute, she developed pancytopenia; and a bone marrow biopsy showed an infiltrate of DLBCL. She was then treated with CHOP (cyclophosphamide, doxorubicin, prednisone, vincristine) in conjunction with vincristine, cyclophosphamide, prednisone, doxorubicin, and rituximab for 6 months, again relapsing with bone marrow and central nervous system involvement. She was started on the COG 5961 (group C) consolidation-phase regimen with high-dose Ara-C and etoposide with rituximab, intrathecal triple therapy, and conditioning with fludarabine and busulfan in preparation for a bone marrow transplant. She then underwent a reduced-intensity stem cell transplant from her mother 4 months before current presentation. Her most recent presentation was due to development of a large left pleural effusion, and positron emission tomographic scans showed increased uptake in the sternal manubrium and the L5 vertebral body.

Cytologic examination of the pleural fluid was notable for increased numbers of large atypical lymphocytes that had large round to ovoid nuclei, with occasional cells showing irregular nuclear contours, fine chromatin, multiple small nucleoli, and moderate to abundant basophilic cytoplasm (Fig. 1A). Biopsy of the vertebral lytic bone lesion revealed an infiltrate of large lymphocytes with similar morphologic features (Fig. 1B). Flow cytometry of the pleural fluid showed a population of  $\lambda$ -light chain restricted medium to large B-cells exhibiting intermediate to high forward scatter; bright CD45, CD19, CD20, and CD43 expression; and dim, partial CD10 expression. IHC staining of the vertebral bone biopsies and pleural fluid cell block demonstrated the following phenotypes of the neoplastic cells: CD20+, PAX5+, BCL6+, MUM1+, BCL2+, and CD43+. The lymphocytes also showed intense nuclear staining for TEL protein (Fig. 1C). The proliferation index, as assessed with a Ki-67 stain, was markedly elevated (90%). Aberrant nuclear expression of p53 was seen in most cells (80%). Result of in situ hybridization for Epstein-Barr virus (EBER) was negative.

Cytogenetic analysis performed on the pleural fluid sample showed a complex karyotype (Table 1, Fig. 2A). FISH using the *ETV6* (*TEL*)(12p13) break-apart probe confirmed rearrangement of *TEL* (split red and green signals, Fig. 2B). A probe for *BLIMP1*(*PRDM1*)(6q21) indicated loss of 1 copy of *BLIMP1*. *IGH* and *BCL6* break-apart probes confirmed t(3;14). However, the signal pattern (unequal split of signals) was consistent with a break 5' to the *BCL6* gene, involving the ABR (Fig. 2C). No *MYC* translocation was detected by FISH analysis.

A clonal product was detected by polymerase chain reaction for immunoglobulin heavy chain rearrangement in the pleural fluid sample. Together, these findings were diagnostic of a DLBCL involving the lumbar spine and pleural fluid.

The patient was admitted for management of her pleural effusion and consideration of another bone marrow trans-

plant. Her hospital course was complicated by sepsis, renal failure, and disseminated intravascular coagulation; and she died shortly after admission.

### 3. Discussion

We describe a case of DLBCL with a complex karyotype and rearrangement of the *TEL* gene. To the best of our knowledge, this is only the fourth reported case of B-NHL with *TEL* rearrangement (Table 1) [6-8]. The precise morphologic, phenotypic, and clinical details of 2 cases are unknown because they were reported only as B-NHL that occurred in a 4-year-old girl [7] and a 30-year-old man [6]. The third reported case [8] was of a marginal zone lymphoma occurring in a 70-year-old man with involvement of the bone marrow, blood, lymph node, and spleen. That patient was alive 10 months after treatment with chlorambucil.

Rearrangements involving the *TEL* gene (also known as the *E26 transforming specific [ETS] translocation variant gene 6; ETV6*) are frequently detected in a variety of hematopoietic neoplasms, specifically acute leukemias, MDSs, and myeloproliferative disorders [1,2]. However, since the initial characterization of this gene and identification of *TEL* rearrangements and deletions over a decade ago, the function of *TEL* remains unclear. Structural studies have demonstrated that *TEL* contains both a 3' *ETS* DNA binding domain, which also mediates protein-protein interactions, and a 5' helix-loop-helix domain involved in homo- and heterodimerization of transcription factors and signal transducing proteins [9]. *TEL* is involved in chromosomal translocations with a variety of partners forming chimeric fusion transcripts (eg, *TEL-PDGFRB*, *TEL-ABL*, *TEL-AML1*), and the function of *TEL* is thought to differ depending on the fusion partner. Bohlander [1] subdivided the fusion partners into different functional groups, for example, tyrosine kinases, transcription factors, as well as "unproductive fusions," in an attempt to further elucidate the role of *TEL* in hematologic disorders. Within the first category, *TEL* partnering with *JAK2*, a tyrosine kinase fusion protein, leads to constitutive activation of the protein tyrosine kinase domain [10], whereas in cases of *TEL-AML1* rearrangement, it is hypothesized that the chimeric fusion protein causes constitutive repression of *AML1* leading to deregulation of *AML1* target genes [11]. Finally, in addition to rearrangements involving *TEL* and formation of fusion genes or protooncogene activation, recurrent loss of genetic material from 12p13, the region containing the *TEL* gene, is also frequently described, suggesting possible function as a tumor suppressor. In many hematologic disorders with *TEL* rearrangement, deletion of the nonrearranged allele of *TEL* occurs in 60% to 75% of cases [3,7]. Such a concomitant deletion was described in 2 of the previous B-NHLs with *TEL* rearrangement (cases 3 and 4), but was not detected in our case (Table 1). Of note, allelic loss of a microsatellite

**Table 1** B-NHLs with *TEL* rearrangements

Case	Age/Sex	G-band karyotype	Diagnosis	Ref
1	22/F	46, XX,t(3;14)(q27;q32),del(6)(q13q23),t(9;12)(q22;p13),del(20)(q11.2)[11]/46,idem,t(1;16)(q44;p13.1)[9]	DLBCL	Current case
2	30/M	46,XY,t(2;12)(q31;p13),del(11)(q21)	B-NHL	[6]
3	4/F	47,X,der(X)del(X)(q2?6;q2?8)t(X;12)(q2?6;p13);del(12)(p11p13),der(12)t(X;12)(q2?6;p13),+21[13]/47,idem,add(13)(q32)[14]	B-NHL	[7]
4	70/M	48, XY,der(1)t(1;17)(p34;q24)add(1)(q21),der(5)t(5;12;?)(p11;p11p13;?),der(7)t(1;7)(q21;q31),der(12)del(12)(p13p13)t(12;14)(p11;q32),dic(12;17)(p11;p11)del(12)(p13p13),del(14)(q32),der(14)t(12;14)(p11;q32),der(17)t(1;17)(p34;q24),der(17)t(12;17)(p13;q11),-19,-20,-2,+5mar	MZBCL	[8]

Abbreviation: MZBCL, marginal zone B-cell lymphoma.

within the *TEL* gene has been documented on transformation of follicular lymphoma [4], further supporting a tumor-suppressor function in certain contexts. It would be of interest to see if other mechanisms leading to loss of function of *TEL* (eg, mutations) play a role in progression of B-NHL. Our case demonstrated *TEL* rearrangement with the locus 9q22, a region that has previously not been described in B-NHL. However, rearrangement involving this locus has been reported in a case of MDS [12]. In the latter, the fusion partner on 9q22 was identified as tyrosine kinase gene *Syk*; and the *TEL-Syk* fusion was shown to lead to interleukin-3-independent growth of Ba3 cells [12].

The time of acquisition of *TEL* rearrangement in our case is not clear because cytogenetic analysis was not performed at the time of primary presentation of the DLBCL. It is possible that the translocation of *TEL* occurred later during clonal evolution. The patient had received multiple chemotherapeutic regimens for both Hodgkin lymphoma and DLBCL; and thus, a role, if any, of these agents in inducing rearrangement of *TEL* cannot be excluded. A role of *TEL* in mediating resistance to chemotherapy is also unclear. Of note, 3 of the 4 B-NHLs with *TEL* translocations (including our case) had a complex karyotype (Table 1). Thus, as has been previously hypothesized, *TEL* rearrangement in these cases may represent a class II mutation, blocking differentiation and/or interfering with apoptosis, and, although required, is not sufficient for development of malignancy [13].

In the absence of fresh or frozen material, we could not analyze the messenger RNA expression level of *TEL*. However, intense nuclear staining for *TEL* was observed in the neoplastic lymphocytes by IHC analysis. This finding is suggestive of *TEL* overexpression because normal lymph node and tonsillar B-cells did not show any staining for *TEL*. It is unclear at present whether mechanisms other than translocations can also lead to deregulated expression of *TEL* in certain B-NHLs because a survey of *TEL* messenger RNA or protein expression in B-NHLs has not been performed. It should be mentioned that although we observed overexpression of *TEL*, the functional status of *TEL* protein in our case is unknown.

Other genes on chromosome 12p have been previously implicated in B-NHL. These include p27<sup>Kip1</sup>, a cyclin-

dependent kinase inhibitor located centromeric to *TEL*, which is expressed in lower levels in high-grade B-NHL, including DLBCL and mantle cell lymphoma [14-16]. The cyclin-D2 gene (*CCND2*) is located telomeric to *TEL*. Cyclin D2 overexpression via amplification or rearrangement with immunoglobulin heavy or light chains has been described in mantle cell lymphoma and more rarely in other B-NHLs, including Burkitt lymphoma and chronic lymphocytic leukemia (at transformation) [3,17-19]. Rearrangements involving 12p are often accompanied by deletions in the adjacent regions. Because the size of the deletions is often large, at times encompassing the loci of both *TEL* and p27<sup>Kip1</sup>, an impact of a loss of individual genes, or a combination of both, on disease inception or evolution of previously reported cases is unknown [3,6,7]. The findings of G-band karyotype and FISH ruled out structural aberrations of p27<sup>Kip1</sup> and *CCND2* in the current case.

In addition to rearrangement of *TEL*, our DLBCL demonstrated a translocation involving the ABR of the *BCL6* gene. This breakpoint is located between 245 and 285 kilobases 5' to *BCL6*, and translocations involving this region are more commonly seen in follicular lymphomas; however, they are also detected in approximately 12% of DLBCLs with 3q27 translocation and 4% of DLBCLs overall [20]. Karyotype analysis also showed deletion of 6q, and FISH confirmed loss of 1 allele of the *BLIMP1* gene. *BLIMP1* is a transcriptional repressor that is required for terminal B-cell differentiation. Alterations or loss of *BLIMP1* has been described in 25% of activated B-cell like DLBCLs and are considered uncommon in germinal center B-cell like DLBCLs [5]. Because *BCL6* translocations and *BLIMP1* deletions are considered mutually exclusive events [5], it has been hypothesized that alterations of genes leading to a block in plasma cell differentiation might be redundant in the same neoplasm. The significance of both these lesions occurring in our case is not known. It is possible that the deletion of *BLIMP1* may represent a nonspecific or "bystander" deletion due to the large interstitial deletion of 6q.

Lastly, but importantly, the rarity of *TEL* rearrangements in the setting of B-NHL, as described in our case, highlights a potential diagnostic pitfall if results of cytogenetic analysis

are interpreted in the absence of morphologic and phenotypic data. This is especially pertinent for pediatric hematopoietic malignancies because rearrangements of *TEL* are seen in approximately 25% of cases of B-cell acute lymphoblastic leukemia [1].

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