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Case Report
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Acquisition of *TCF3* and *CCND3* Mutations and Transformation to Burkitt Lymphoma in a Case of B-Cell Prolymphocytic Leukemia

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B-cell prolymphocytic leukemia (B-PLL) is a very rare disease; it accounts for <1% of all chronic B-cell leukemias. According to the World Health Organization's definition, B-PLL is diagnosed when peripheral blood (PB) prolymphocytes account for more than 55% of lymphoid cells in a de novo context. B-PLL generally occurs in elderly people presenting B symptoms, a rapidly rise in the lymphocyte count, massive splenomegaly but little or no lymphadenopathy. There are no specific genetic abnormalities; B-PLL has genomic similarities with other chronic B-cell malignancies but displays well-defined combinations of alterations. The karyotype is frequently complex. *MYC* aberrations resulting from mutually exclusive translocations or gains are observed in about 75% of cases. These translocations place the *MYC* gene under the control of an enhancer (usually immunoglobulin genes *IGH*, *IGK*, or *IGL* enhancers) and lead to *MYC* overexpression. Deletions of the short arm of chromosome 17 including the *TP53* gene (del(*TP53*)) are also frequent. *TP53*, *MYD88*, *BCOR*, *MYC*, *SF3B1*, *SETD2*, *CHD2*, *CXCR4*, and *BCLAF1* are the most frequently mutated genes in B-PLL. We recently reported on 3 subgroups in which the prognosis depended on the *MYC* and *TP53* status. Patients with both a *MYC* aberration and del(*TP53*) belong to the high-risk subgroup and have a short mean overall survival time.[1]

Burkitt lymphoma (BL) is an aggressive mature B-cell lymphoma that occurs in adults and children. BL is subdivided into a sporadic subtype (often diagnosed in developed countries, accounting for ~1% of adult lymphomas and ~30% of pediatric lymphomas), the Epstein-Barr-virus-associated endemic subtype, and an HIV-associated subtype. This lymphoma comprises medium-sized monomorphic B-cells with round nuclei, finely clumped chromatin, and deeply basophilic cytoplasm that usually contains lipid vacuoles, numerous mitoses, and tingible body macrophages with a "starry sky" appearance. Although BL characteristic morphology and immunophenotype often enable a rapid diagnosis, testing for genomic aberrations is needed to differentiate BL from other high-grade B-cell neoplasms. Although *MYC* rearrangements are not specific for BL, they are considered as a hallmark feature and are found in almost all cases. The typical t(8;14)(q24;q32) rearrangement (*MYC-IGH*) occurs in 80% of cases. Rearrangements involving the light chain loci *IGL* t(2;8) or *IGK* t(8;22) are less frequent.[2] *MYC* is also the most frequently mutated gene in BL (in 70% of cases). Mutations in the transcription factor 3 (*TCF3*) gene or its negative regulator *ID3* have been reported in about 70% of sporadic subtypes. The other frequently mutated genes are *CCND3*, *TP53*, *RHOA*, *SMARCA4*, and *ARID1A*.[3–5]

Here, we describe a case of concomitant B-PLL and BL. Cytogenetic and molecular analyses revealed a common origin, with the acquisition of additional genetic lesions in the BL clone.

A 46-year-old woman with an unremarkable medical history presented with hyperleukocytosis and thrombocytopenia but no splenomegaly or lymphadenopathy. The white blood cell count was 14.1 × 10^9/L with 79% lymphocytes, the hemoglobin level was 96 g/L, and the platelet count was 25 × 10^9/L. In a blood smear examination prolymphocytes accounted for 72% of lymphoid cells. Flow cytometry of PB cells revealed a $CD5^+CD23^-CD79b^-FMC7^+IgM\lambda^{weak}$ clonal B-lymphocyte population. The karyotype (K) was 46,XX,t(8;22)(q24;q11)[2]/46,XX[34]. fluorescence in situ hybridization (FISH) analyses confirmed the t(8;22) with *MYC* rearrangement in 52% of the nuclei and revealed a cryptic del(*TP53*) in 71% of the nuclei. Cohybridization with *MYC* and *TP53* FISH probes showed that 55% of the cells harbored both abnormalities, and 18% had a del(*TP53*) only; hence, the *MYC* translocation had occurred after the del(*TP53*). Our diagnosis was de novo B-PLL. A bone marrow (BM) aspirate showed a massive infiltration by BL cells (accounting for 87% of the BM cells). The clonal BM B-cells' immunophenotype

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was $CD5^{-}CD10^{+}bcl2^{-}IgM\lambda^{high}$. The K was 46,X,-X,t(8;22)(q24;q11),der(13)t(7;13)(q21;q34),+20[19]/46,XX[1], and a FISH analysis detected a *MYC* rearrangement and a del(*TP53*) in 59% and 84% of the nuclei, respectively. *IGHV* sequencing in PB and BM samples showed that both displayed the same VH3-21/DH3-10/JH6 recombination, with full sequence identity. The sequences contained somatic mutations (96.9% homology with the germline counterparts). The patient was diagnosed with medullar BL clonally related to B-PLL. After treatment with a cyclophosphamide/oncovin/adriamycin/prednisolone/methotrexate regimen, the patient achieved a complete response in the BM but prolymphocytic cells persisted in the PB. She relapsed 5 months later, with massive BL cell invasion of the BM. An allogeneic BM transplant was performed but she died 1 month later, following BL relapse.[6]

To further investigate the clonal relationship between the B-PLL and BL cells, we performed whole exome sequencing (WES) on DNA extracted from sorted CD19+CD5+ PB tumor cells, BM cells, and sorted nontumor CD3+ PB cells (considered to be germinal controls) sampled at the time of diagnosis. Somatic coding mutations were confirmed by polymerase chain reaction-based targeted deep resequencing (See Supplemental Digital Content, http://links.lww.com/HS/A154). In the PB sample, 19 genes were mutated and the variant allele frequency (VAF) ranged from 29.9% to 95.83%; these included *TP53* (c.T824A, p.L275Q, VAF: 95.83%); *CHD2* (c.4160_4178del, p.P1387Rfs*13, VAF: 47.06%), and *SETD2* (c.2628_2629insAG, p.G878Qfs*14, VAF: 34.88%). The same 19 mutations were present in the BM sample, with similar VAFs. Twenty-seven additional mutations were detected in the BM, including *TCF3* (c.G1663C, p.E555Q, VAF: 40.53%) and *CCND3* (c.T875A, p.L292Q, VAF: 37.25%) (Supplemental Digital Content, Table 1, http://links.lww.com/HS/A155). The copy number aberration analysis from WES data confirmed the chromosomal abnormalities observed by K/FISH, and detected cryptic 17q gain and 14q loss in the PB, and 17q gain and 11q loss in the BM (Figure 1; Supplemental Digital Content, Table 2, http://links.lww.com/HS/A154).

This case provided an unusual illustration of a dual B-cell neoplasm, with B-PLL in the PB and BL in the BM. The B-PLL cells harbored a translocation that deregulates *MYC* expression and had biallelic inactivation of *TP53* (by deletion and mutation); these are the 2 most prevalent abnormalities in B-PLL and, when combined, confer a poor prognosis. Mutations in *CHD2* and *SETD2* (involved in chromatin remodeling) are also frequent in B-PLL.[1] The medullar BL cells carried the same somatic mutations, chromosomal abnormalities and *VDJ* recombination (using the *IGHV3-21* gene) as the B-PLL cells but also had other genetic lesions. *IGHV3* is the predominant subgroup in both BL and B-PLL cells.[1,7] Our results demonstrate that the B-PLL and BL cells had the same clonal origin and suggest strongly that the BL developed from the B-PLL (Figure 2).

The transformation of a chronic B lymphoproliferative disease into an aggressive lymphoma is well known in chronic lymphocytic leukemia (CLL, as Richter's syndrome [RS]), follicular lymphoma (FL), and marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue lymphomas (MALT) but has not been described previously in B-PLL. Our case is unique in this respect. In CLL, RS occurs in 2%–8% of patients. Most frequently, clonally related (80%) or unrelated (20%) diffuse large B-cell lymphoma (DLBCL) develops, whereas transformation to classical Hodgkin lymphoma is rare.[8] Transformation to BL is very unusual in CLL; only a few cases have been reported.[9–11] Transformation is linked to the acquisition of additional chromosomal abnormalities and somatic mutations. In DLBCL-type RS, genetic lesions typically affect the *TP53*, *NOTCH1*, *MYC*, and *CDKN2A* genes. The *MYC* network is deregulated in ~70% of samples.[8] *MYC* pathway deregulation is also considered to be a key event in transformation of MALT (40%–80% of cases) and FL (~40%) to DLBCL, and is often associated with *TP53* aberrations.[12,13] In our case, transformation to BL was not due to *MYC* deregulation or *TP53* inactivation alone because these aberrations were already present in the B-PLL cells. Moreover, *MYC* deregulation is known to be insufficient for BL oncogenesis. Additional genetic lesions cooperate with *MYC* to generate human BL.[14] The additional mutations observed in our patient's BM included mutations in the *TCF3* and *CCND3* genes, both of which are frequently mutated in de novo BL. The L292Q *CCND3* missense mutation, novel in BL, affects a

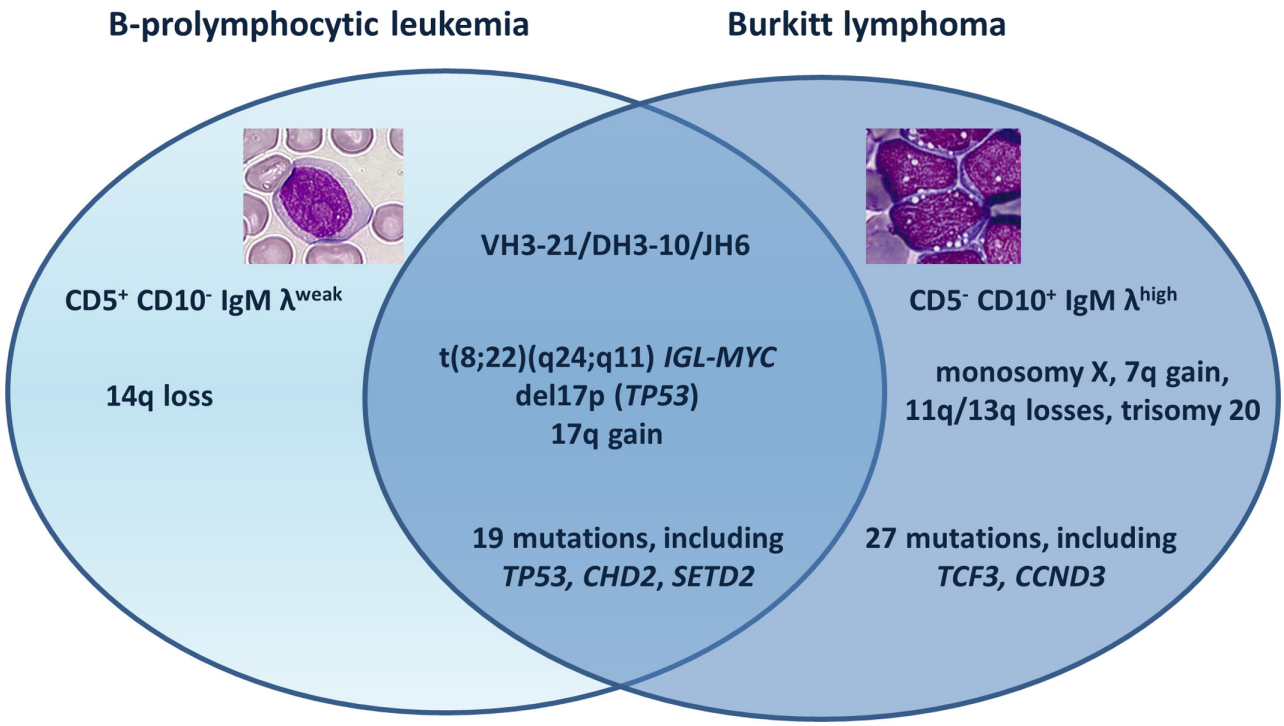

Figure 1. Venn diagram summarizing the biological data.

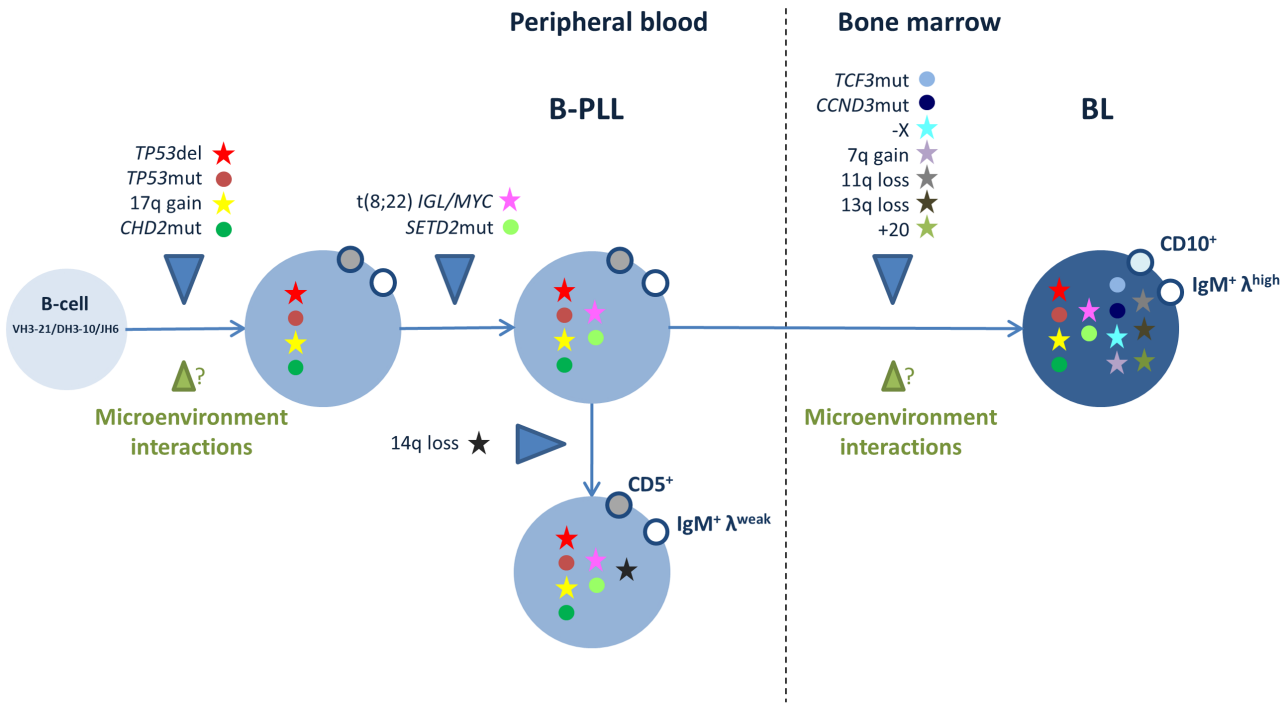

Figure 2. Hypothetical model of the development of B-PLL and BL in the case described here. The illustration depicts the putative sequential acquisition of chromosomal abnormalities and gene mutations, and the possible role of interactions with the microenvironment. B-PLL = B-cell prolymphocytic leukemia; BL = Burkitt lymphoma.

conserved residue in the proline (P), glutamic acid (E), serine (S), and threonine (T) (PEST) domain, which has a role in the protein's degradation. The great majority of the *CCND3* mutations observed in BL and other B-lymphoid neoplasms target the PEST domain and result in the intracellular accumulation of cyclin D3 and deregulation of the cell cycle.[5] The E555Q *TCF3* mutation, already identified in BL,[5] affects the basic helix–loop–helix domain. Gain-of-function monoallelic mutations in *TCF3* and biallelic inactivating mutations in the *ID3* gene (encoding TCF3's inhibitor) activate B-cell receptor signaling, and thus sustain BL cell survival by engaging the phosphoinositide-3-kinase pathway. These mutations are essentially absent in other mature B-cell malignancies, suggesting that the TCF3/ID3 module has a determining role in the pathogenesis of BL. Indeed, it has been shown that TCF3 contributes to the BL phenotype by enforcing a germinal center-derived transcriptional program; it controls a centroblast-restricted gene expression signature that is "inherited" by BL cells and is intensified in cases with *TCF3/ID3* aberrations.[5] Hence, in the present case, the acquisition of the *TCF3* and *CCND3* mutations may have contributed strongly to the development of BL.

We described a unique chemotherapy-refractory case of de novo high-risk B-PLL with concomitant, clonally related BL. Deregulation of *MYC* is a nonspecific, oncogenic event shared by B-PLL and BL (and other lymphoid malignancies). It is necessary for tumor transformation but does not fully explain the phenotype, which is probably dictated by specific combinations of genetic and epigenetic abnormalities. Clonal evolution, cell migration, disease progression, and drug resistance may all be influenced by the tumor microenvironment.[15] This case further confirms the crucial role of *TCF3* and *CCND3* in BL lymphomagenesis.

Disclosures

The authors have no conflicts of interest to disclose.

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