Human Pathology (2015) **46**, 260–271

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Human PATHOLOGY

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Original contribution

# Lymphoblastic transformation of follicular lymphoma: a clinicopathologic and molecular analysis of 7 patients☆

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Received 4 August 2014; revised 23 October 2014; accepted 29 October 2014

**Keywords:**
Follicular lymphoma;
Lymphoblastic transformation;
*MYC* rearrangement;
Double hit lymphoma;
TdT staining

**Summary** Approximately 30% of patients with follicular lymphoma (FL) transform to a more aggressive malignancy, most commonly diffuse large B cell lymphoma. Rarely, FL transformation results in clinical findings, histology, and immunophenotype reminiscent of B-lymphoblastic leukemia/lymphoma. We report the largest series to date with detailed analysis of 7 such patients. Lymphoblastic transformation occurred on average 2 years after initial diagnosis of FL. Five patients had prior intensive chemotherapy. Two patients developed mature high-grade lymphoma, followed by the lymphoblastic transformation. FL had *BCL2* gene rearrangement in 4 of 5 cases. High-grade transformation was accompanied by *MYC* gene rearrangement (5 of 5). Transformation was characterized by expression of TdT, loss of Bcl6, variable loss of immunoglobulin light chain, and persistence of Pax-5, Bcl2, and CD10. Whole-exome sequencing in 1 case revealed presence of several actionable mutations (*CD79B*, *CCND3*, *CDK12*). FL, aggressive mature B cell lymphoma, and lymphoblastic transformation were clonally related in 6 evaluable cases. After transformation, survival ranged from 1 to 14 months. Four patients died of disease, 2 were in remission after stem cell transplant, and 1 was alive with disease.


## 1. Introduction

Follicular lymphoma (FL) is the most common lymphoma in the United States and in western Europe [1]. It is a tumor of mature germinal center B cells that most frequently present in

☆ The authors declare no conflict of interest.

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http://dx.doi.org/10.1016/j.humpath.2014.10.021
0046-8177/

middle-aged individuals and the elderly. Approximately 85% of patients with FL have t(14;18), which results in the overexpression of the antiapoptotic *BCL2* oncogene. FL is typically an indolent lymphoma with cumulative risk of high-grade transformation of approximately 3% per year [2]. The most common and well-described type of high-grade lymphoma occurring in this setting is diffuse large B cell lymphoma (DLBCL). FL also rarely transforms to other lymphomas, including B cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma (B cell lymphoma, unclassifiable [BCLU]) [3], double-hit lymphoma [4–7], plasmablastic lymphoma [8,9], and rarely, B-lymphoblastic leukemia/lymphoma (B-ALL) [4,10–15].

The most recent edition of World Health Organization classification recognized an overlap category between DLBCL and Burkitt lymphoma, provisionally entitled BCLU. It is a heterogeneous and incompletely characterized category that requires further study [1]. The term may be used for cases that have features of both DLBCL and Burkitt lymphoma but do not fulfill diagnostic criteria for either entity. BCLU occur predominantly in older adults who present with advanced disease with generalized lymphadenopathy and/or extranodal involvement [5,6,16–18]. BCLU are very aggressive lymphomas with poor prognosis and limited response to intensive chemotherapy [5,6,16,17,19]. Many lymphomas in this group have evidence of concurrent *MYC* and *BCL2* gene rearrangement (double-hit lymphoma) [1]. Of note, not all double-hit lymphomas correspond to BCLU, as cases of FL, lymphoblastic lymphoma, plasmablastic lymphoma, and plasma cell myeloma have been described to contain both *MYC* and *BCL2* gene rearrangements [6,9,18,19]. In particular, a subset of double-hit lymphoma arises from a preexisting or concurrent FL, presumably by acquisition of *MYC* rearrangement [5,6,10,16]. These cases may have unusual morphologic features, such as diffuse growth, absence of centrocyte-like cells, very high proliferation index, and a starry-sky pattern or focal necrosis [6]. A small subset of the double-hit lymphoma cases express TdT and have blastoid morphology [6]. Such cases are currently classified as B-ALL [6,7].

We have encountered several cases of FL transforming into aggressive high-grade B cell lymphoma/leukemia with morphologic and immunophenotypic features of B-ALL and acquired *MYC* gene rearrangement. Thus, the goal of the study was to bring attention to a poorly understood form of lymphoblastic progression in FL, expand the spectrum of double-hit lymphoma, and clarify the relationship of these neoplasms to B-ALL and BCLU.

## 2. Materials and methods

### 2.1. Case selection

A computer-assisted search of the Departments of Pathology of Weill Cornell Medical College (WCMC), the University Hospital in Lausanne, Switzerland, and the Cleveland Clinic, USA, disclosed 3, 2, and 1 patients with lymphoblastic transformation of FL, respectively. One additional case was identified in the consultation files of one of the authors (J.A.F.). The present research was approved by the internal review boards of the respective institutions, where applicable.

### 2.2. Immunohistochemistry, flow cytometry, cytogenetics, and polymerase chain reaction

At WCMC, immunohistochemical studies were performed using a Bond-Max autostainer (Vision BioSystems, Hingham, MA). Monoclonal antibodies were used as follows: CD45, Pax-5, CD20, CD79a, CD3, Ki-67, Bcl2, Bcl6, TdT, CD10, Mum-1, CD34, CD99, cyclin D1. In situ hybridization was used for EBV-encoded RNA (EBER). Four-color flow cytometric analysis was performed. Fluorescence in situ hybridization (FISH) was performed using the LSI *IGH@-BCL2* dual-color dual-fusion probes and the LSI *C-MYC* dual-color break-apart rearrangement probes (Vysis/Abbott Molecular Inc, Des Plaines, IL) to rule out a t(14;18) translocation and *MYC* gene rearrangement, respectively. To assess the presence of immunoglobulin heavy chain gene rearrangements, a polymerase chain reaction (PCR) was performed using *FR3*, *JH* and *IGK* primers. The BIOMED-2 assays were used to detect the common breakpoints in the *BCL2* gene. PCR products were analyzed by gel electrophoresis for size fractionation.

### 2.3. Exome sequencing

Bone marrow (BM) aspirate material from patient 1 was analyzed as follows. DNA was extracted from frozen tissue sections. Samples were first digested overnight with 0.5 mg/mL Proteinase K and 0.625% SDS in 4 mL of nucleic lysis buffer at 37°C. After digestion, 1 mL of saturated NaCl was added, and samples were shaken vigorously for 15 seconds before spun at 2500 rpm for 15 min. Supernatant was transferred to a new tube and mixed with 2 volume of room temperature (RT) 100% ethanol. DNA was precipitated by centrifugation at maximum speed for 30 min, washed twice with 70% ethanol, and finally dissolved in Tris EDTA (TE) or nuclease-free water overnight at RT. Exome sequencing samples were prepared using the Agilent SureSelect Human All Exon 50MB Target Enrichment System for Illumina Paired-End Sequencing Library kit. PE75 sequencing was performed on Illumina HiSeq 2000.

### 2.4. Single-nucleotide variant discovery

Short sequencing reads were aligned to human genome assembly GRCh37/hg19 using the BWA aligner. Duplicated paired reads were filtered, and variant detection was performed as previously described [20]. Coding region single-nucleotide variants (not present in SNP132) were