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Evaluation of the genetic findings in B-cell lymphoma in the context of clinicopathological data

CAP TODAY and the Association for Molecular Pathology have teamed up to bring molecular case reports to CAP TODAY readers. AMP members write the reports using clinical cases from their own practices that show molecular testing's important role in diagnosis, prognosis, and treatment. The following report comes from Vanderbilt University Medical Center. If you would like to submit a case report, please send an email to the AMP at amp@amp.org. For more information about the AMP and all previously published case reports, visit www.amp.org.

Alexander Gross, MD; Hamama Bushra, MD
Emily Mason, MD, PhD; Nico Lopez-Hisijos, DO
Rebecca B. Smith, PhD; Laura A. Lee, MD, PhD
Barbara Nelson, PhD; Ashwini Yenamandra, PhD

Case. A 73-year-old male with a clinical history of benign prostatic hypertrophy and pituitary macroadenoma status post-resection presented with lymphocytosis. This incidental lymphocytosis was noted within a preoperative CBC for a prostate procedure. At the time he was asymptomatic; medications included hydrocortisone, testosterone, and levothyroxine. Lymphadenopathy and splenomegaly were absent on physical examination. Complete blood counts showed WBC 25.8×10^9/L, hemoglobin 13.9 g/dL, hematocrit 42 percent, and platelets 134×10^9/L. Peripheral blood was remarkable for leukocytosis with 56 percent atypical lymphocytes, morphologically consistent with prolymphocytes, displaying dispersed chromatin and prominent nucleoli (**Fig. 1**). Flow cytometry of peripheral blood identified a CD5-positive, lambda-restricted B-cell population (63 percent of total cells) also positive for CD19 (bright), CD20 (dim), CD38 (moderate-bright), and CD45 (bright) and negative for CD10, CD23, CD34, and CD200. Forward scatter was variably increased.

The bone marrow core biopsy showed 20 percent CD20-positive B cells, which were negative for cyclin D1 by immunohistochemistry. A PET-CT demonstrated splenomegaly and avidity in right iliac, right inguinal, retroperitoneal, right axillary, and left cervical lymph nodes.

Lymph node biopsy revealed intermediate to large B cells with open chromatin, prominent nucleoli, and abundant amphophilic cytoplasm (**Fig. 2**, next page). Lesional cells were positive for CD20, BCL6, BCL2, and MYC (80–90 percent) and negative for CD10, MUM1, and EBER. SOX11 was negative and LEF-1 was positive in a subset of lesional cells. Ki-67 showed a low proliferation index (five to 10 percent).

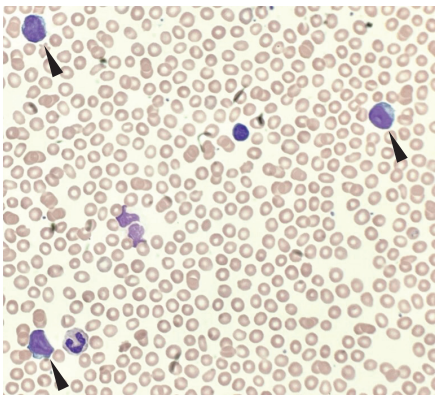

Fig. 1. Peripheral blood with atypical lymphocytes (arrowheads) with dispersed chromatin and prominent nucleoli (Wright-Giemsa, 10×).

Next-generation sequencing of a peripheral blood sample identified pathogenic alterations in *BCOR* (p.Y1384*, 57.9 percent), *BIRC3* (p.R549fs, 7.2 percent), and *NSD2* (p.E1099K, 1.5 percent) and variants of uncertain significance in *IDH2* (p.V335I, 48.9 percent), *MAP3K7* (p.K589E, 29.4 percent), *NSD1* (p.E1978K, 21 percent), *NUDT15* (p.I95T, 26.1 percent), and *PPP1R15A* (p.IT596VA, 47.1 percent). Transcriptome RNA sequencing detected no gene fusions.

Fluorescence in situ hybridization analysis of the bone marrow was negative for all the tested CLL-associated genetic abnormalities, including *IGH::CCND1* rearrangement, trisomy 12, deletion 13q, and *TP53* abnormalities. Subsequent cytogenetic analysis of the bone marrow detected an abnormal karyotype: 46,XY,t(8;22)(q24.1;q11.2),-20,+mar[15]/46,XY[5] (**Fig. 3**). Metaphase FISH analysis of the bone marrow culture with *MYC* break-apart probe (Abbott Molecular) revealed a *MYC* rearrangement, with *MYC* translocated to chromosome 22, consistent with t(8;22) observed in the karyotype (**Fig. 4**). This is suggestive of an *IGL::MYC* rearrangement.

This case was diagnosed as CD5-positive large B-cell lymphoma with

a differential diagnosis including B-cell prolymphocytic leukemia (or splenic B-cell lymphoma/leukemia with prominent nucleoli) and CD5-positive DLBCL NOS with leukemic progression.

Discussion. This case illustrates a B-cell lymphoma with large cell morphology and a *MYC* translocation but with a low proliferative index and indolent clinical course; accurate diagnosis relied on complementary ancillary genetic testing. The *IGL::MYC* fusion is reported in approximately 15 percent of Burkitt lymphoma cases[1] and in 12 percent of aggressive B-cell lymphomas overall.[2] This specific translocation places the proto-oncogene *c-MYC* at 8q24 region under constitutive activation of the immunoglobulin lambda promoter, resulting in unregulated *MYC* expression and increased B-cell proliferation.[3] Of note, this rearrangement does not yield an mRNA fusion, since the promoter region of lambda is not transcribed, and thus underscores the crucial role of FISH.[4] Yet this translocation is not diagnostic of aggressive malignancy, and the low Ki-67 and absence of rapid clinical progression in this case argue against a diagnosis of aggressive high-grade B-cell lymphoma. This case highlights that the genetic findings in B-cell lymphoma must be interpreted carefully in the context of other clinicopathologic data.

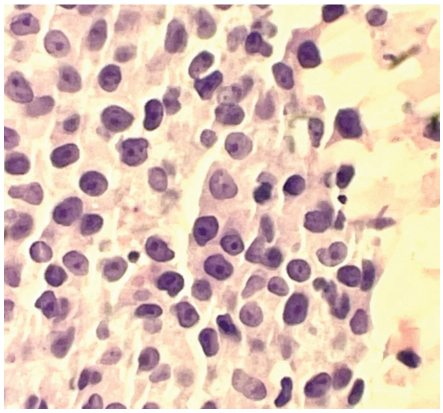

Fig. 2. Inguinal lymph node excisional biopsy involved by discohesive intermediate to large B cells with open chromatin, prominent nucleoli, and abundant amphophilic cytoplasm (H&E, 40×).

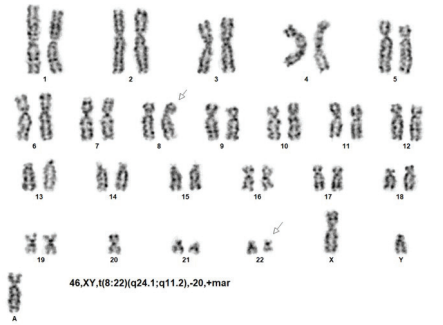

Fig. 3. Karyotype with t(8;22)(q24.;q11.2),-20 and a marker chromosome.

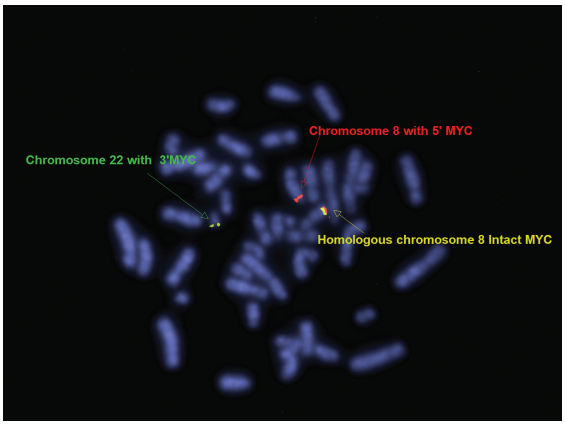

Fig. 4. Metaphase FISH with *MYC* break-apart probe confirming the t(8;22) rearrangement.

In the fifth edition of the World Health Organization Classification of Haematolymphoid Tumours, B-prolymphocytic leukemia (B-PLL) has been deleted as an entity and instead is being regarded as a heterogeneous category including cases of hairy cell leukemia variant (HCLv), leukemic mantle cell lymphoma (MCL), and CLL/SLL progressed to B-PLL. Thus, now it has been in part absorbed in the new entity named "splenic B-cell leukemia with prominent nucleoli" (SBLPN) that also includes HCLv. Conversely, the International Consensus Classification still regards B-PLL as an entity but recommends its diagnosis only in cases without previous history of B-CLL (to exclude CLL progressing to B-PLL), negative for cyclin D1 and SOX11 (to exclude MCL), and lacking hairy surface projections and intrasinusoidal bone marrow infiltration (to exclude HCLv and splenic marginal zone lymphoma). B-PLL usually carries a complex karyotype with rearrangement and/or increased copy number of *MYC* (62 percent), del17p (38 percent), and trisomy 18 (30 percent). B-PLL patients are treated according to B-CLL guidelines.

The patient was managed as if he had large cell lymphoma and treated accordingly. There is not much clinical follow-up; he was initiated on treatment essentially without symptoms. □

1. Janz S. *Myc* translocations in B cell and plasma cell neoplasms. *DNA Repair (Amst)*. 2006; 5(9–10):1213–1224.

2. Gagnon MF, Pearce KE, Greipp PT, et al. *MYC* break-apart FISH probe set reveals frequent unbalanced patterns of uncertain significance when evaluating aggressive B-cell lymphoma. *Blood Cancer J*. 2021;11(11):184.

3. Zeidler R, Lipp M, Joos S, et al. Breakpoints of burkitt's lymphoma t(8;22) translocations map within a distance of 300 kb downstream of MYC. *Genes Chromosomes Cancer*. 1994;9(4):282–287.

4. Szankasi P, Bolia A, Liew M, et al. Comprehensive detection of chromosomal translocations in lymphoproliferative disorders by massively parallel sequencing. *J Hematopathol*. 2019;12(3):121–133.

Dr. Gross is a clinical fellow, molecular genetics; Dr. Bushra is a clinical fellow, hematopathology; Dr. Mason is associate professor; Dr. Lopez-Hisijos is assistant professor; Dr. Smith is assistant professor; Dr. Lee is associate professor; Dr. Nelson is a clinical fellow, laboratory genetics and genomics; and Dr. Yenamandra is associate professor—all in the Department of Pathology, Microbiology, and Immunology, Vanderbilt School of Medicine and Vanderbilt University Medical Center, Nashville.

See 'Test yourself' questions, next page.

Test yourself

Here are three questions taken from the case report. Answers are online now at www.amp.org/casereports *and will be published next month in* CAP TODAY.

1. Approximately what percentage of Burkitt lymphoma cases bear the t(8;22) *IGL*::*MYC* translocation?
a. 90 percent
b. 75 percent
c. 50 percent
d. 15 percent

2. What gene rearrangements lead to fusions that are undetectable by RNA transcriptome sequencing methods?
a. Coding sequence to coding sequence
b. Coding sequence to intron
c. Regulatory elements (i.e. promoter) to coding sequence
d. Coding sequence to short tandem repeat

3. What FISH probe design works best to detect a gene with an unknown fusion partner?
a. Dual color, dual fusion
b. Break-apart
c. Enumeration
d. Centromere