Identifying the signal in the signal: incidental detection of B-cell lymphoproliferative disorder-related variants in the molecular profiling of a spindle cell sarcoma

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 Massachusetts General Hospital and Harvard Medical School. 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.

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We report the case of a 75-year-old female who initially presented with a 10-cm left pretibial mass on MRI. A biopsy revealed a high-grade spindle cell sarcoma with myofibroblastic differentiation. PET CT showed intense uptake in osseous lesions at the scapula, vertebral bodies, iliac bones, sacrum, and femoral head. A sacrum biopsy confirmed metastatic spindle cell sarcoma. The patient underwent chemotherapy and radiotherapy. A follow-up CT scan several months later revealed new liver and lung metastases, prompting molecular analysis of the sacrum specimen.

The patient's sacrum biopsy was analyzed using the PGDx Elio assay (Personal Genome Diagnostics, Baltimore), which targets more than 500 genes using a hybrid-capture-based next-generation sequencing system. NGS showed sequence variants in the following genes (codon, variant allele frequency): *MYD88* (p.L265P, 17.8 percent; **Fig. 1A**, next page), *CXCR4* (p.G340Wfs*8, 21.3 percent; **Fig. 1B**), *BARD1* (p.H506Q, 18.6 percent), *H3F3A* (p.G35V, 15.1 percent), and *ERBB4* (p.P637T, 13.9 percent) (**Table 1**). The tumor mutational burden was low (4.6 mutations/Mb).

The *MYD88* p.L265P variant is a recurrent driver mutation in both Waldenström's macroglobulinemia (WM) and IgM monoclonal gammopathy of undetermined significance (IgM MGUS). WM is defined by an IgM monoclonal component in the blood associated with elevated serum viscosity, in the setting of lymphoplasmacytic lymphoma (LPL). The *MYD88* mutation is detected in more than 90 percent of patients with LPL/WM and in up to 80 percent of those with IgM MGUS.[1-3] It may also be present in a subset of other hematologic malignancies, such as diffuse large B-cell lymphoma and chronic lymphocytic leukemia.[3] Additionally, nonsense and frameshift mutations in the C-terminus of *CXCR4* are observed in approximately 40 percent of LPL/WM cases, often occurring alongside *MYD88* mutations.[4] *CXCR4* mutations have also been observed in patients with IgM MGUS.[5] The *H3F3A* p.G35V variant, which is equivalent to p.G34V depending on which initial methionine is used for numbering, is thought to likely be related to the sarcoma given it has been reported in mesenchymal tumors, specifically a subset of giant cell tumors of bone, and other variants at the *H3F3A* p.G34 locus have been described in osteosarcoma.[6-8] In addition, *H3F3A* p.G35V has not been reported in lymphoproliferative disorders. *BARD1* p.H506Q and *ERBB4* p.P637T are considered variants of uncertain significance, without previous reports in the literature.

Table 1. Variants identified on next-generation sequencing

Genes	Variant annotation (coding, amino acid)	Variant allele frequency
MYD88	c.794T>C, p.Leu265Pro	17.80%
CXCR4	c.1016dupG, p.Gly340Trpfs*8	21.30%
BARD1	c.1518T>G, p.His506Gln	18.60%
H3F3A	c.104G>T, p.Gly35Val[1]	15.10%
ERBB4	c.1909C>A, p.Pro637Thr	13.90%

[1]Equivalent to p.Gly34Val, depending on which of two adjacent methionines is included in the codon numbering.
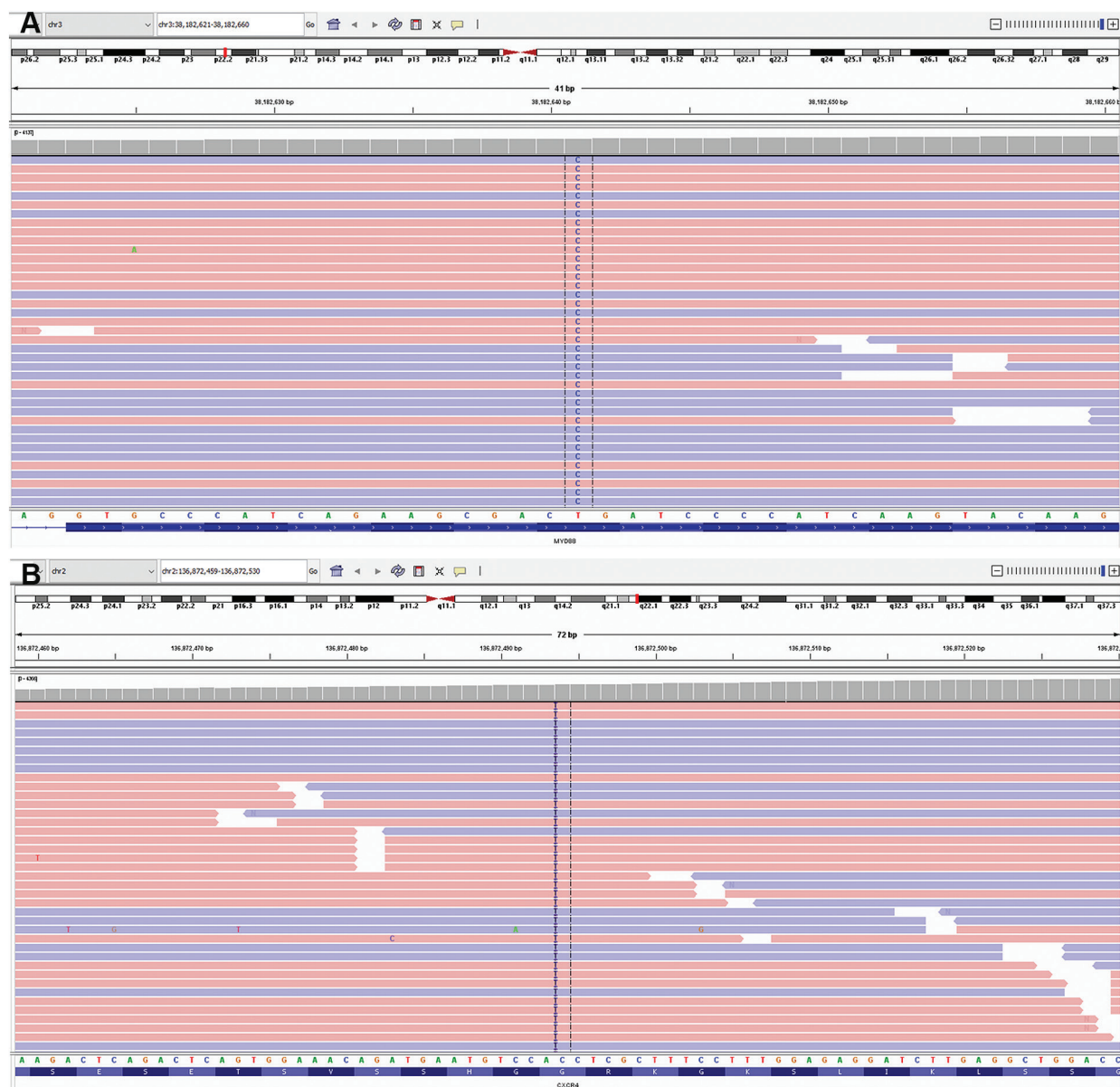
Fig. 1. Integrative Genomics Viewer (Broad Institute, Cambridge, Mass.) showing exon 5 of the *MYD88* gene with sequencing reads showing a confident signal for *MYD88* c.794T>C (p.L265P) hotspot variant (**A**), as well as exon 2 of the *CXCR4* gene with a confident signal for *CXCR4* c.1016Gdup, resulting in a p.G340Wfs*8 truncating frameshift alteration (**B**).

Following the molecular results, re-review of the sacrum biopsy revealed, in addition to the metastatic sarcoma, patchy infiltrates of small lymphocytes with slightly irregular nuclei, mature chromatin, and occasional intermixed plasma cells, present within the bone marrow (**Fig. 2A**, next page). Subsequent immunohistochemistry showed that most of the lymphocytes were CD20+ PAX5+ B cells (**Fig. 2B**), which were CD5 negative. CD3 highlighted fewer interspersed small T cells (**Fig. 2C**). MUM1 was positive in a few intermixed plasma cells, which appeared polytypic for kappa and lambda light chains by chromogenic in situ hybridization; only rare plasma cells were IgM positive. Overall, the CD20+ B-cell infiltrates occupied 10 to 15 percent of the intertrabecular marrow space.

This B-cell-rich infiltrate raised concern for a concurrent small B-cell lymphoma, specifically LPL. A conclusive lymphoma diagnosis on the biopsy was precluded by the limited lymphoid infiltrate and lack of definitive light chain clonality in the plasma cell component. Further evaluation showed a trace band of monoclonal IgM kappa light chain on serum protein electrophoresis (SPEP), and increased serum free kappa light chain at 35.7 mg/dL (ref: 3.3–19.4) as well as elevated serum kappa/lambda free light chain ratio at 2.08 (ref: 0.3–1.7). Peripheral blood flow cytometry did not detect clonal B cells, and the complete blood count was normal aside from a slight increase in neutrophils and a mild decrease in lymphocytes. Taken together, the histo-
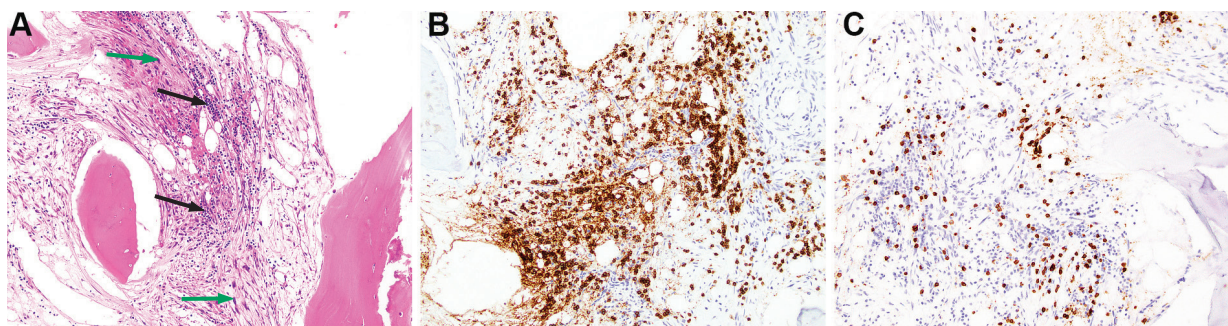
Fig. 2. Hematoxylin and eosin stain of the sacrum biopsy showing patchy infiltrates of small lymphocytes with mature chromatin (black arrows), intermixed with metastatic spindle cell sarcoma cells (green arrows) in a hypocellular marrow (**A**, 200×). Immunohistochemistry demonstrated that the lymphocytic infiltrates were predominantly CD20+ B cells (**B**, 200×), with a few scattered small CD3+ T cells (**C**, 200×).

logical and laboratory findings in the setting of the molecular results strongly suggested a B-cell lymphoproliferative disorder. A bone marrow biopsy was recommended for further evaluation, which the patient elected to defer during the treatment of her metastatic sarcoma. Following additional chemotherapy and radiotherapy for sarcoma, the patient experienced disease progression and was subsequently placed on home hospice care.

IgM MGUS is defined by the presence of a circulating monoclonal IgM at a concentration of less than 3 g/dL and, if assessed, a bone marrow containing less than 10 percent monoclonal plasma cells in asymptomatic patients. Most of the bone marrow B cells and plasma cells are polyclonal in IgM MGUS.[9] In this case, a trace band of monoclonal IgM kappa light chain was identified on SPEP without definitive histological evidence of clonality on the sacrum biopsy, despite a patchy B-cell-rich lymphoid infiltrate. These findings, along with the *MYD88* p.L265P and *CXCR4* truncating alterations, may be indicative of IgM MGUS. Moreover, recent studies have shown that *MYD88* p.L265P may be present in B-cell precursors and normal nonclonal B cells in patients with LPL/WM.[9,10] Given the limited sampling from the sa-

crum biopsy, an overt LPL cannot be entirely excluded without a more comprehensive bone marrow biopsy for better characterization.

In conclusion, we report a case of metastatic sarcoma with incidental detection of molecular variants associated with B-cell lymphomas. These findings, cross-referenced with histological and laboratory results, suggested the presence of an underlying B-cell lymphoproliferative disorder, most likely IgM MGUS. Subtle findings of neoplastic lymphoid infiltrates may be difficult to detect based on morphology alone. However, molecular findings, sometimes incidental, may enable early detection and facilitate close monitoring of disease progression, emphasizing the importance of clear and timely communication by pathologists with patient-facing clinical colleagues. □

1. Landgren O, Staudt L. MYD88 L265P somatic mutation in IgM MGUS. *N Engl J Med.* 2012; 367(23):2255–2257.

2. Jiménez C, Sebastián E, Chillón MC, et al. MYD88 L265P is a marker highly characteristic of, but not restricted to, Waldenström's macroglobulinemia. *Leukemia.* 2013;27:1722–1728.

3. Alcoceba M, García-Álvarez M, Medina A, et al. *MYD88* mutations: transforming the landscape of IgM monoclonal gammopathies. *Int J Mol Sci.* 2022;23(10):5570.

4. Kaiser LM, Hunter ZR, Treon SP, Buske C. CXCR4 in Waldenström's macroglobulinema: chances and challenges. *Leukemia.* 2021; 35:333–345.

5. Moreno DF, López-Guerra M, Paz S, et al. Prognostic impact of *MYD88* and *CXCR4* mutations assessed by droplet digital polymerase chain reaction in IgM monoclonal gammopathy of undetermined significance and smouldering Waldenström macroglobulinaemia. *Br J Haematol.* 2023;200(2):187–196.

6. Leske H, Dalgleish R, Lazar AJ, Reifenberger G, Cree IA. A common classification framework for histone sequence alterations in tumours: an expert consensus proposal. *J Pathol.* 2021;254(2):109–120.

7. Yamamoto H, Iwasaki T, Yamada Y, et al. Diagnostic utility of histone H3.3 G34W, G34R, and G34V mutant-specific antibodies for giant cell tumors of bone. *Hum Pathol.* 2018;73:41–50.

8. Koelsche C, Schrimpf D, Tharun L, et al. Histone 3.3 hotspot mutations in conventional osteosarcomas: a comprehensive clinical and molecular characterization of six H3F3A mutated cases. *Clin Sarcoma Res.* 2017;7:9.

9. Rodriguez S, Celay J, Goicoechea I, et al. Preneoplastic somatic mutations including *MYD88*[L265P] in lymphoplasmacytic lymphoma. *Sci Adv.* 2022;8(3):eabl4644.

10. Kaushal A, Nooka AK, Carr AR, et al. Aberrant extrafollicular B cells, immune dysfunction, myeloid inflammation, and MyD88-mutant progenitors precede Waldenstrom macroglobulinemia. *Blood Cancer Discov.* 2021;2(6):600–615.

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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. Which of the following is a recurrent driver mutation in lymphoplasmacytic lymphoma (LPL), Waldenström's macroglobulinemia (WM), and IgM monoclonal gammopathy of undetermined significance (IgM MGUS)?
a. *TP53* p.R175H
b. *MYD88* p.L265P
c. *BRAF* p.V600E
d. *KRAS* p.G12D

2. An inactivating mutation of which additional gene is most commonly observed in LPL/WM and IgM MGUS?
a. *BCL6*
b. *TP53*
c. *CXCR4*
d. *NOTCH1*

3. What are some of the considerations when unexpected hotspot variants are detected by next-generation sequencing in a tumor?
a. The alteration may represent a less common or previously unreported variant for the tumor type being tested, typically recognized on further literature review.
b. A mixup in sample labeling or processing could result in variants being attributed to the wrong patient or tissue, prompting review of samples tested around the same time.
c. External DNA contamination from another sample could introduce unexpected alterations, prompting review of samples tested around the same time.
d. The presence of a separate clonal/neoplastic process, prompting review of the submitted specimen's histology and the patient's clinical history.
e. All the above.