CAP TODAY

Value of targeted NGS in a diagnostically challenging case of CMML

CAPTODAY *and the Association for Molecular Pathology have teamed up to bring molecular case reports to* CAPTODAY *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. Case report No. 9, which begins here, comes from the Divisions of Hematopathology and Hematology and Medical Oncology and the Knight Cancer Institute, Oregon Health & Science University. If you would like to submit a case report, please send email to the AMP at* amp@amp.org. *For more information about the AMP and all previously published case reports, visit* www.amp.org.

Matthew Geller, DO
Elie Traer, MD, PhD
Jennifer Dunlap, MD

The 2008 World Health Organization diagnostic criteria for chronic myelomonocytic leukemia (CMML) integrate peripheral blood and bone marrow findings, including morphologic and chromosomal abnormalities. Notably, there is no single pathognomonic finding specific to CMML,[1,2] and it is important to exclude secondary causes of monocytosis **(Fig. 1)**. Diagnostic difficulty is often encountered in cases in which a technically challenging bone marrow biopsy limits appropriate morphologic evaluation or in cases in which clinical and histologic features do not clearly meet diagnostic criteria.

In CMML, identifying a chromosomal abnormality can make it much easier to reach a diagnosis, particularly in cases where dysplasia is minimal or absent. However, cytogenetic abnormalities detected by metaphase karyotype or fluorescence in situ hybridization are seen in only about 30 percent of cases. Next-generation sequencing technology provides the ability to screen many potentially disease-associated genes for

Fig. 1. WHO diagnostic criteria for CMML

■ Persistent peripheral blood monocytosis $> 1 \times 10^9$/L.

■ No Philadelphia chromosome or *BCR-ABL1* fusion gene.

■ No rearrangements of *PDGFRA, PDGFRB,* or *FGFR1.*

■ Less than 20 percent blasts (includes promonocytes and monoblasts) in blood and bone marrow.

■ Dysplasia in one or more myeloid lineages. If dysplasia is absent or minimal, diagnosis of CMML can be made if other requirements are met and:

■ an acquired clonal cytogenetic or molecular genetic abnormality is present;

■ monocytosis has persisted for at least three months; *and*

■ all other causes of monocytosis have been excluded.

pathogenic mutations and is becoming increasingly important in identifying targeted therapies for patients. Furthermore, depending on the platform, NGS requires relatively little DNA (about 10–250 ng), has a lower limit of detection of about five percent, and can be performed in paraffin-embedded tissue. Here, we discuss the diagnostically challenging case of a man with an ambiguous clinical presentation and bone marrow biopsy whose diagnosis was aided by the use of targeted next-generation sequencing.

Case. The patient is a 65-year-old male with a history of type 2 diabetes mellitus, coronary artery disease, and a monoclonal gammopathy of unknown significance (MGUS) who presented with clinical deterioration including dyspnea, fatigue, abdominal bloating, and lower extremity edema. Over the past year he experienced an 80-pound weight loss with repeated episodes of pleural effusions, ascites, lymphadenopathy, anemia, splenomegaly, and intermittent leukocytosis. These episodes led to several prolonged hospitalizations without a unifying diagnosis. His clinical course was further complicated by a sudden retroperitoneal hemorrhage involving the left renal sack,
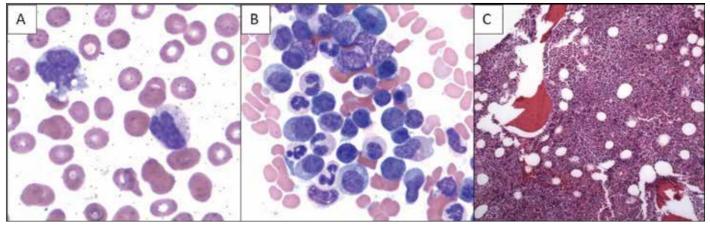
Fig. 2. A) Peripheral blood with circulating myeloid precursor and monocyte. **B)** Bone marrow aspirate with mild multilineage dysplastic changes. **C)** Bone marrow core biopsy with hypercellular marrow. Blasts are not increased and there is no significant reticulin fibrosis.

with subsequent acute renal failure, fever of unknown origin, and failure to thrive.

During his hospitalizations, CBCs revealed anemia, thrombocytopenia, and fluctuating levels of leukocytosis with absolute monocytosis and neutrophilia. Early in his disease course, the monocytosis was not sustained, and at several time points the absolute monocyte count was normal. Multiple tissue biopsies were performed. Biopsy of an abdominal lymph node revealed fibrotic/reactive changes with no evidence of malignancy. A liver biopsy showed an increased sinusoidal inflammatory infiltrate of unclear etiology but raised the possibility of a myeloproliferative neoplasm. Two bone marrow biopsies were non-diagnostic; the aspirate smears lacked marrow particles but did not show overtly dysplastic features. A third bone marrow biopsy revealed a hypercellular marrow with mild dysplastic changes in hematopoietic precursors. The CBC at this time showed a leukocytosis with absolute monocytosis and neutrophilia **(Fig. 2)**. The peripheral blood and bone marrow findings were suspicious for CMML, although the dysplasia was mild and cytogenetic studies including metaphase karyotype and FISH were negative. No $JAK2$ V617F, MPL, or KIT mutations were detected.

A targeted next-generation sequencing assay was performed on the bone marrow aspirate using an ion semiconductor-based sequencing platform (Ion Torrent, Thermo Fisher Scientific). Multiplex PCR covering a custom panel of 42 genes relevant to myeloid neoplasms was used to generate amplicons that were then sequenced on an Ion PGM System. This technology detects pH changes as protons are released into solution with each addition of a complementary base-pair during DNA sequencing. Sequence alignment and variant calling were performed using Torrent Suite software version 2.0. Using this approach, mutations in the $IDH2$ (R140Q) and CBL (C404Y) genes were identified at allele frequencies of 50 percent and 90 percent respectively, providing evidence of a clonal hematopoietic disorder. Both of these mutations have been reported in CMML, among other myeloid neoplasms,[3–5] and both are predicted to be functionally deleterious. The results of the NGS assay corroborated the morphologic and histologic findings of the biopsy and confirmed the diagnosis of CMML.
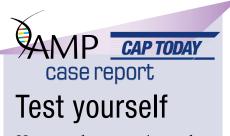
Discussion. Isocitrate dehydrogenase (IDH1/2) enzymes play a key role in intermediate metabolism and cellular energy production and normally catalyze the oxidative decarboxylation of isocitrate to 2-oxoglutarate (2OG). 2OG is a Krebs cycle intermediate and an essential cofactor for a variety of enzymes, including histone demethylases, TET 5-methylcytosine hydroxylases, and others.[6] Mutant $IDH1/2$ acquires a novel enzymatic activity facilitating the reduction of alpha-ketoglutarate to 2-hydroxyglutarate, a putative oncometabolite that may function to inhibit 2OG-dependent enzymes. Initially described in gliomas, mutations in IDH have been identified in acute myeloid leukemia (cumulative frequency of five to 20 percent in de novo AML) as well as myelodysplastic syndromes and CMML (five to 10 percent). In an ongoing phase one clinical trial of AG-221, an IDH2 inhibitor, seven of 10 patients with relapsed or refractory AML or MDS had achieved complete remission as of 2014 with no dose-limiting toxic effects, generating

Table 1. Commonly observed mutations in CMML and MDS

Gene	Frequency	
	CMML	**MDS**
TET2	50–60%	20–25%
ASXL1	40%	14%
SRSF2	46%	12.4%
RUNX1	15%	9–20%
SETBP1	15%	3.8–4.4%
NRAS	11%	3.6%
CBL	10%	2.3%
KRAS	8%	0.9%
ZRSR2	8%	3.1%
IDH1/IDH2	1–6%	2–3.5%
SF3B1	6%	14.5% (80% with RS*)

tremendous enthusiasm for this drug in the clinic.[7]

The *CBL* gene encodes a ubiquitin protein ligase (E3) that down-regulates tyrosine-kinase–mediated signal transduction pathways and functions in hematopoietic stem cell maintenance. The *CBL* C404Y mutation identified in this case is located in the RING finger domain (RFD). Mutations affecting the RING finger domain of *CBL* appear to affect the regulation of cell proliferation. In animal studies, *CBL* knockout mice in which RFD-mutated *CBL* was reintroduced developed a myeloproliferative disorder that eventually evolved into leukemia. Furthermore, in transgenic mice affected by MDS, progression to AML is associated with acquisition of *RAS* and *CBL* mutations.[8] In clinical studies, *CBL* mutations in CMML have been associated with more frequent extramedullary disease and an inferior overall survival in univariate analysis, although further confirmation is needed.[3]

In similar cases with technical limitations and ambiguous clinical and pathologic findings, patients often undergo multiple biopsies before a diagnosis is established, as this patient did. Targeted next-generation sequencing can provide supportive evidence to aid in the diagnosis of myeloid neoplasms earlier in the disease course, particularly in cases of MDS/MPN (CMML) or MDS where reactive etiologies remain in the differential diagnosis. With regard to CMML, reactive causes of monocytosis must be excluded, broadening the differential to include infectious causes (for example, tuberculosis, fungal or viral infections, endocarditis), autoimmune etiologies, or sarcoid. An underlying non-hematopoietic malignancy should also be considered. Hematopoietic malignancies associated with monocytosis include CMML as well as other myeloproliferative neoplasms. Clonal cytogenetic abnormalities are present in only about 30 percent of patients with CMML while almost 90 percent will harbor a gene mutation, frequently in epigenetic modifiers and spliceosome genes. Some of these mutations may be prognostic. For example, *ASXL1* and *SRSF2* have been associated with a worse prognosis, although studies are conflicting.[3,6] Similarly, in MDS, about 50 percent of patients have a cytogenetic abnormality while nearly 70 percent have a detectable gene mutation.

It is important to emphasize that detection of a hematologic-disease–associated mutation in the absence of other supportive clinical and pathologic findings does not, by itself, warrant a diagnosis of a hematopoietic malignancy. Low-level gene mutations have recently been described in persons without overt evidence of a hematologic malignancy.[13,14] These recent studies highlight the emergence of clonal hematopoiesis with aging, and low-level gene mutations in otherwise healthy older individuals should be interpreted cautiously.

Table 1 lists the most commonly observed mutations in CMML and MDS and their corresponding frequencies based on the current literature.[3–5,9–12]

To summarize, this report illustrates the utility of targeted next-generation sequencing in diagnostically challenging cases by establishing a hematopoietic clone and identifying disease-associated gene mutations. While this testing may not be indicated in all cases, a strong argument can be made for the diagnostic utility and benefit in these difficult cases. Furthermore, targeted NGS may provide prognostic information and identify potential therapeutic targets, such as the *IDH* mutation identified in this patient. ☐

AMP CAP TODAY case report

Test yourself

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

1. What are the three most commonly mutated genes in CMML?
a) *TET2, ASXL1, SRSF2*
b) *TET2, SRSF2, RUNX1*

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2. What percentage of patients with CMML harbor a cytogenetic abnormality?
a) 20 percent
b) 25 percent
c) 30 percent

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3. Can a diagnosis of CMML be made in the absence of dysplasia?
a) Yes, if a cytogenetic or molecular abnormality is identified, the monocytosis has persisted for three or more months, and all other reactive causes of monocytosis have been excluded.
b) No, dysplasia must be present.

1. Parikh SA, Tefferi A. Chronic myelomonocytic leukemia: 2012 update on diagnosis, risk stratification, and management. *Am J Hematol*. 2012;87(6):610–619.

2. Swerdlow S, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissue*. 4th ed. Lyon, France: WHO Press; 2008.

3. Itzykson R, Kosmider O, Renneville A, et al. Prognostic score including gene mutations in chronic myelomonocytic leukemia. *J Clin Oncol*. 2013;31(19):2428–2436.

4. Shih AH, Abdel-Wahab O, Patel JP, Levine RL. The role of mutations in epigenetic regulators in myeloid malignancies. *Nat Rev Cancer*. 2012;12(9):599–612.

5. Bejar R, Stevenson K, Abdel-Wahab O, et al. Clinical effect of point mutations in myelodysplastic syndromes. *N Engl J Med*. 2011;364(26):2496–2506.

6. Losman JA, Kaelin WG Jr. What a difference a hydroxyl makes: mutant IDH, (R)-2-hydroxyglutarate, and cancer. *Genes Dev*. 2013;27(8):836–852.

7. Stein E, Tallman M, Pollyea DA, et al. Clinical safety and activity in a phase I trial of AG-221, a first in class, potent inhibitor of the IDH2-mutant protein, in patients with IDH2-mutant positive advanced hematologic malignancies. In: Proceedings from the AACR Annual Meeting; April 5–9, 2014. San Diego. Abstract CT103.

8. Makishima H, Cazzolli H, Szpurka H, et al. Mutations of e3 ubiquitin ligase cbl family members constitute a novel common pathogenic lesion in myeloid malignancies. *J Clin Oncol*. 2009;27(36):6109–6116.

9. Bejar R, Levine R, Ebert BL. Unraveling the molecular pathophysiology of myelodysplastic syndromes. *J Clin Oncol*. 2011;29(5):504–515.

10. Makishima H, Yoshida K, Nguyen N, et al. Somatic SETBP1 mutations in myeloid malignancies. *Nat Genet*. 2013;45(8):942–946.

11. Patnaik MM, Padron E, LaBorde RR, et al. Mayo prognostic model for WHO-defined chronic myelomonocytic leukemia: ASXL1 and spliceosome component mutations and outcomes. *Leukemia*. 2013;27(7):1504–1510.

12. Thol F, Kade S, Schlarmann C, et al. Frequency and prognostic impact of mutations in SRSF2, U2AF1, and ZRSR2 in patients with myelodysplastic syndromes. *Blood*. 2012;119(15):3578–3584.

13. Genovese G, Kähler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med*. 2014;371(26):2477–2487.

14. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med*. 2014;371(26):2488–2498.

Dr. Geller is an attending pathologist, Department of Pathology, Winthrop University Hospital, Mineola, NY. Dr. Dunlap is an assistant professor in the Division of Hematopathology, and Dr. Traer is an assistant professor in the Division of Hematology and Medical Oncology, Oregon Health & Science University, Portland. Drs. Dunlap and Traer are with the OHSU Knight Cancer Institute. Dr. Geller was previously a hematopathology fellow at OHSU.