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Identification of rare *PML::RARA* variant missed by traditional molecular testing

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 City of Hope Comprehensive Cancer Center, Duarte, Calif., and Cedars-Sinai Medical Center, Los Angeles. 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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Acute promyelocytic leukemia with *PML::RARA* is a unique subtype of acute myeloid leukemia with specific biology and clinical characteristics. APL makes up five to eight percent of AML cases in younger patients but is less common in the elderly.[1] It is characterized by predominance of abnormal promyelocytes and presence of a hallmark translocation t(15;17)(q24;q21), resulting in fusion of the promyelocytic leukemia (*PML*) gene with the retinoic acid receptor alpha (*RARA*) gene.[2,3]

Clinically, this fusion provides an increased sensitivity to specific therapeutic agents, such as all-trans-retinoic acid (ATRA) in combination with chemotherapy or arsenic trioxide (ATO). This treatment has changed APL from a fatal to a highly curable leukemia (approximately 90 percent cure rate), but rapid and accurate diagnosis is crucial to start life-saving therapy promptly.[4] Although *PML::RARA* is found in up to 95 percent of APL cases with typical morphology, it is worth noting that atypical isoforms and variant *RARA* translocations also have been described.[4,5] The various functional domains of the PML protein retained within the *PML::RARA* atypical isoforms or different *RARA* partners could potentially result in different clinical outcomes and response to therapy.[4,6]

Here we describe a *PML::RARA* rearrangement with an atypical breakpoint in *PML*. This case highlights the significance of multiple testing techniques and inability to use common measurable residual disease testing platforms to follow in remission, owing to potentially false-negative results.

Case. A 21-year-old male with a reported history of APL in remission for three years after receiving chemotherapy with ATRA plus ATO presented to the clinic with progressive shortness of breath and pallor. Laboratory evaluation revealed severe pancytopenia. His blood smear showed rare circulating promyelocytes. Bone marrow biopsy revealed predominantly granulocytic precursors consisting almost entirely of abnormal promyelocytes, with virtually no mature forms. Erythroid and megakaryocytic precursors were suppressed by

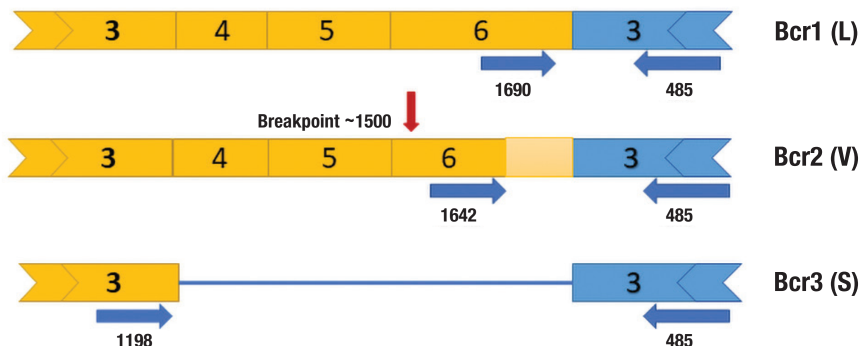

Fig. 1. Schematic of *PML::RARA* transcript isoforms. Exons of *PML* are shown in yellow and exons of *RARA* in blue. Standard qRT-PCR primers and their approximate binding locations are indicated by blue arrows. The patient's novel breakpoint within *PML* exon 6 is marked by the red arrow, demonstrating why the bcr1 and bcr2 primer sets failed to amplify the fusion transcript.

leukemia but showed no overt dysplasia. A differential count showed 85 percent promyelocytes. Flow cytometry demonstrated 82 percent blast equivalents with expression of CD13, CD33, CD38, CD64, CD117, and MPO, and lacking CD34 and HLA-DR. A preliminary diagnosis of AML suggestive of APL was rendered, and the patient was started on ATRA pending confirmation of diagnosis. He achieved morphologic remission with a combination of ATO and ATRA.

A blood sample was sent for *PML::RARA* quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) because of concern for the relapsed leukemia. The result was very low-level positive for short isoform (bcr3) at 0.02 percent. qRT-PCR was repeated on the bone marrow and showed only low-level positivity for *PML::RARA* fusion short form (bcr3) at 0.09 percent. A rapid AML next-generation sequencing panel by amplicon capture, which covers *RARA* fusions with 17 different partners including *PML*, detected fusion reads at the limit of detection. Fluorescent in situ hybridization using a dual-color dual-fusion probe set was positive for *PML::RARA* rearrangement in 179/200 (89.5 percent) of the cells scored on bone marrow aspirate. Due to this discrepancy, Anchored Multiplex PCR (AMP) next-generation sequencing was performed, which enables the detection of novel fusion partners and breakpoints. The results confirmed the presence of an atypical bcr2 transcript involving a novel breakpoint within exon 6 of *PML* and a breakpoint in intron 2 of the *RARA* gene (**Fig. 1**).

Discussion. Based on current guidelines, both morphologic and immunophenotypical studies and confirmatory genetic analysis are part of the diagnostic workup of APL. The confirmatory tests may include cytogenetic analysis for t(15;17) by karyotype and/or FISH and molecular testing by qRT-PCR methods for *PML::RARA*.[2-4,7] Although *PML::RARA* is found in up to 98 percent of APL cases with typical morphology, rare instances may evade detection by standard qRT-PCR.[5,8]

Three distinct typical *PML::RARA* transcript isoforms can be discerned based on specific breakpoints within the *PML* gene: 1) the long isoform (intron 6, known as bcr1); 2) the variant isoform (exon 6, known as bcr2); and 3) the short isoform (intron 3, known as bcr3).[5,9] Only one breakpoint in intron 2 has been identified in the *RARA* gene in these cases.[5,10]

Rare atypical *PML::RARA* transcripts have been reported, with the most common being atypical bcr2 (V-forms). These arise from mis-splicing or breaks involving a novel donor site in *PML* exon 6 and are typically unique.[11,12] These splicing events in *PML* can be linked to concomitant insertions of nucleotides from *RARA* intron 2.[12,13] Moreover, partial deletions of *RARA* exon 3 and alternative isoforms of *RARA* have been observed in atypical transcripts.[14-16]

The majority of reported APL cases with atypical isoforms can be detected using routine qRT-PCR methods; however, in a few cases, as in our case, this assay may not be able to be used to establish a diagnosis.[12] Specifically, the primer for bcr2 is located downstream of the patient's breakpoint (Fig. 1), and the bcr3 primer was able to detect trace signals due to the immense amount of product at diagnosis. However, bcr3 primer would not be expected to identify product during treatment and clinical remission. Therefore, knowledge of these atypical cases is essential for monitoring measurable residual disease.[17] False-negative results would have serious implications for patient therapy, as early treatment intervention in patients displaying MRD positivity leads to more favorable outcomes.[4] FISH can be used as a disease surveillance tool in this case; however, this assay is not sensitive enough for effective MRD monitoring. Similar to qRT-PCR, amplicon-based NGS platforms may fail to detect variant isoforms due to the primer design. Although AMP NGS is a potential alternative NGS method for detecting atypical isoforms, this technology is not capable of detecting transcripts at MRD. A patient-specific qRT-PCR assay could be designed based on the sequence derived from AMP NGS for personalized MRD monitoring.

Patients with classical *PML::RARA* fusions typically respond well to retinoid therapy and have a favorable prognosis. However, this may not hold true for atypical or variant isoforms, which can be associated with variable outcomes and treatment responses. Therefore, beyond confirming the presence of a *PML::RARA* fusion, precise identification of the transcript is crucial for appropriate clinical management and for accurately interpreting outcomes in retinoid-based clinical trials.[4,9]

In summary, we report a case in which routine qRT-PCR and amplicon-based sequencing failed to detect the atypical *PML::RARA* fusion isoform, which was later identified by AMP NGS. Although the patient achieved hematologic remission, the lack of a suitable method for molecular monitoring limits effective follow-up. This highlights the need for agnostic or universal qRT-PCR primers capable of detecting *PML::RARA* fusions regardless of breakpoint location. □

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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 tests is typically used to follow a patient with acute promyelocytic leukemia in clinical remission?

a. Karyotype
b. FISH
c. qRT-PCR
d. Transcriptome sequencing

2. A patient presents with cytopenias and bone marrow aspirate showing abnormal promyelocytes with Auer rods, but dual-color dual-fusion FISH for *PML::RARA* is negative. Which of the following best explains the negative FISH result?

a. The patient has classic APL with t(11;17).
b. The *PML::RARA* fusion is due to a cryptic insertion of *RARA* into *PML.*
c. The patient likely has therapy-related acute myeloid leukemia with *KMT2A* rearrangement.
d. There is no true translocation involving *RARA.*

3. Which of the following findings would most strongly support a diagnosis of acute promyelocytic leukemia in an atypical case?

a. Aberrant coexpression of CD7 and CD56.
b. Myeloperoxidase-negative blasts.
c. Presence of Auer rods and strong MPO positivity.
d. High TdT expression in blasts.