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Molecular insights into the bi-clonal presence of inversion 16 and Philadelphia chromosome in relapsed post-treatment acute myeloid leukemia

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 Albany Medical Center, New York. 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 myeloid leukemia (AML) stands out as the most prevalent form of leukemia, constituting 80 percent of cases in adults and 15 to 20 percent in children. It arises from the clonal proliferation of genetically aberrant hematopoietic stem and progenitor cells, impeding normal hematopoiesis.[1] AML is linked to a variable number of cytogenetic abnormalities,[2] and the identification of these abnormalities holds crucial implications, given their association with an elevated risk of inherited AML.[3] Furthermore, cytogenetic abnormalities offer insights into the genetic processes contributing to the histological, morphological, and clinical heterogeneity of AML, serving as vital prognostic predictors.[3]

One of the most prevalent cytogenetic abnormalities in AML is an inversion of chromosome 16(p13q22), associated with a favorable prognosis, longer remission, and improved survival.[3,4] The Philadelphia chromosome (Ph), t(9;22)(q34;q11), is identified in one to two percent of cases of de novo AML and has been added as a new provisional category of AML with BCR::ABL1 fusion.[2] The coexistence of inversion of chromosome 16 and the Philadelphia chromosome is rare, with only a few cases reported in de novo AML.[4] To the best of our knowledge, no case report has been published documenting the coexistence of inversion 16 and the Philadelphia chromosome in post-treatment relapsed AML.

Case. A 68-year-old female, previously diagnosed with AML with inversion of chromosome 16(p13q22), underwent induction chemotherapy followed by consolidation chemotherapy with high-dose cytarabine, concluding a year ago. Since then the patient has been on regular follow-up and surveillance bone marrow biopsies, which showed normal blood counts and no residual acute leukemia. However, the patient presented again after a year with petechiae and bruising. Her complete blood count revealed a low white blood cell count and a decreased platelet count, which prompted an early bone marrow biopsy (**Table 1**). The bone marrow biopsy indicated 25 percent blasts (**Fig. 1**, next page), consistent with relapsed AML.

Chromosomal analysis. Oncology chromosome analysis revealed an interval change in the karyotype compared with the most recent previous study. The analysis showed an abnormal female karyotype with bi-clonal, pericentric inversion of chromosome 16(p13q22) and the presence of the Philadelphia chromosome, t(9;22).

Among the analyzed cells, nine cells exhibited pericentric inversion of chromosome 16 (**Fig. 2**), consistent with the previous identification in this patient, while five cells showed the Philadelphia chromosome t(9;22) as the sole anomaly (**Fig. 3**). The presence of the Philadelphia chromosome was further confirmed by interphase FISH, conducted as an internal quality control maneuver on residual specimens in the original specimen tube from which concurrent cytogenetic studies were initiated.

Table 1

Complete blood count with normal references	Results
WBC 4.1–9.3 10*3/µL	2.93×10^3/µL
Hemoglobin 11.0–14.7 g/dL	12.4 g/dL
HCT 33.0–44.0%	37.8%
MCV 82.3–93.2 fL	101.6 fL
RDW 12.0–15.0%	16.5%
PLT 130–350 10*3/µL	61×10^3/µL

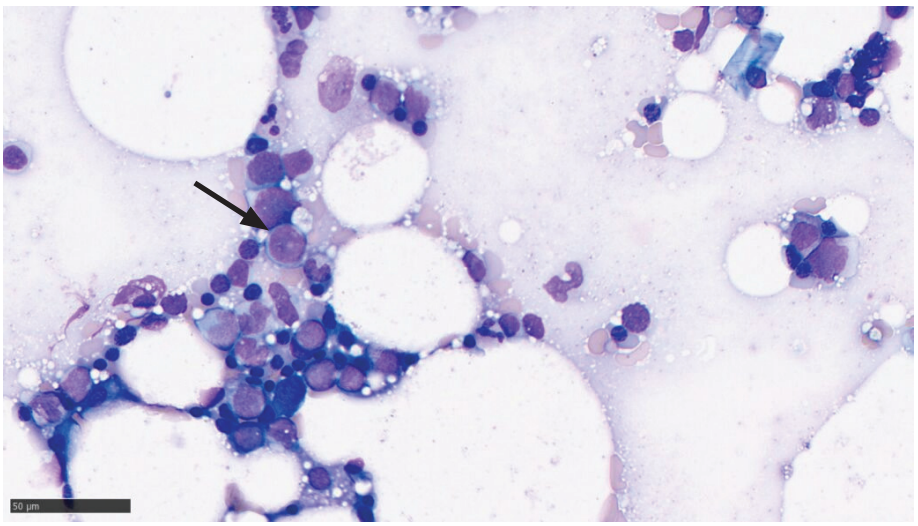

Fig. 1. Bone marrow aspirate showing myeloblast (black arrow) with high nuclear-to-cytoplasmic (N/C) ratio and prominent nucleoli (40× magnification).

Cytogenetic analysis. No mutations or pathogenic alterations were found in the following genes: *FLT3*, *NPM1*, *IDH1*, and *IDH2*.

Treatment and outcomes. The patient underwent salvage chemotherapy followed by consolidation therapy using fludarabine, cytarabine, idarubicin, granulocyte colony-stimulating factor (G-CSF), and venetoclax, which she tolerated well. Subsequently, she experienced count recovery, and a bone marrow biopsy at day 28 revealed bone marrow with only trace blasts, with a negative BCR::ABL fusion on a polymerase chain reaction assay. Additional tyrosine kinase therapy was not administered at that time due to concern about toxicity from the quadruplet combination. She later underwent an allogeneic bone marrow transplant and is now being followed up at an outside hospital.

Discussion. Inversion of chromosome 16 stands out as the most prevalent cytogenetic abnormality in de novo AML,[5] resulting from the fusion of the myosin heavy chain gene 11 (MYH11) at 16p13 and the core-binding factor beta subunit (CBFB) at 16q22.[4] The human core-binding factor (CBF), a heteromeric protein comprising CBF alpha and CBF beta subunits, plays a crucial role in the regulation of hematopoiesis in normal hematolymphoid tissues.[4] The inversion of chromosome 16 abnormality in AML leads to the fusion of the myosin heavy chain 11 gene at 16p13 and the core-binding factor beta subunit at 16q22, disrupting the normal differentiation process by acquiring chromatin-modifying histone deacetylase activities.[4]

The BCR-ABL gene rearrangement, known as the Philadelphia chromosome (Ph), is a characteristic feature of chronic myelogenous leukemia (CML). Approximately 0.5 percent to three percent of acute myeloid leukemia cases also exhibit this gene rearrangement.[6] However, only a few case reports have described the bi-clonal appearance of inversion 16 and the Philadelphia positive (Ph+) gene rearrangement in de novo AML, characterized by the p190 fusion protein. Among these reports, only one case documented bi-clonal appearance in treated and relapsed cases of AML.[4,7-10] In their case report, Kim, et al., suggested that the additional BCR-ABL gene rearrangement with inversion 16 is a part of chemotherapeutic-associated clonal evolution.[9]

Our patient initially presented with inversion 16 AML, underwent treatment with cytarabine, and later relapsed with the appearance of inversion 16 and the Ph+ chromosome in different cell lines, a phenomenon uncommon in reported cases. Neuendorff, et al., explained that BCR-ABL gene rearrangement transiently occurs in a healthy individual.[6] The occurrence of BCR-ABL gene rearrangement during clonal hematopoiesis and its persistence during the maturation of the cell leads to the development of BCR-ABL+ AML, chronic myelogenous leukemia, or a detectable BCR-ABL subclone.[6] This could potentially explain our patient's unique case as well.

Conclusion. In summary, the occurrence of the Ph+ chromosome and inversion 16 in AML is a rare phenomenon primarily associated with de novo AML, although it could also be linked to treatment-related clonal evolution.[9] A comprehensive literature review shows that AML with BCR-ABL gene rearrangement and inversion 16 exhibits a more favorable prognosis than cases with inversion 16 alone. However, this data is primarily based on the coexistence of BCR-ABL gene rearrangement and inversion 16 within the same cell lines.[10,11] Our case is unique as it presented with BCR-ABL gene rearrangement and inversion 16 in different cell lines. To the best of our knowledge, recent literature offers limited insights into the prognosis of such cases. Min, et al., have also proposed favorable outcomes for patients with both BCR-ABL positivity and inversion 16 AML, particularly when treated with tyrosine kinase inhibitors followed by allogeneic hematopoietic cell transplantation.[2]

This case highlights the need for greater insight into the prognosis of such cases and underscores the importance of understanding clonal evolution resulting from treatment. It also emphasizes the critical need for molecular assessments and supports the recommendation of Kim, et al., for regular follow-up cytogenetic evaluations in these patients.[9] □

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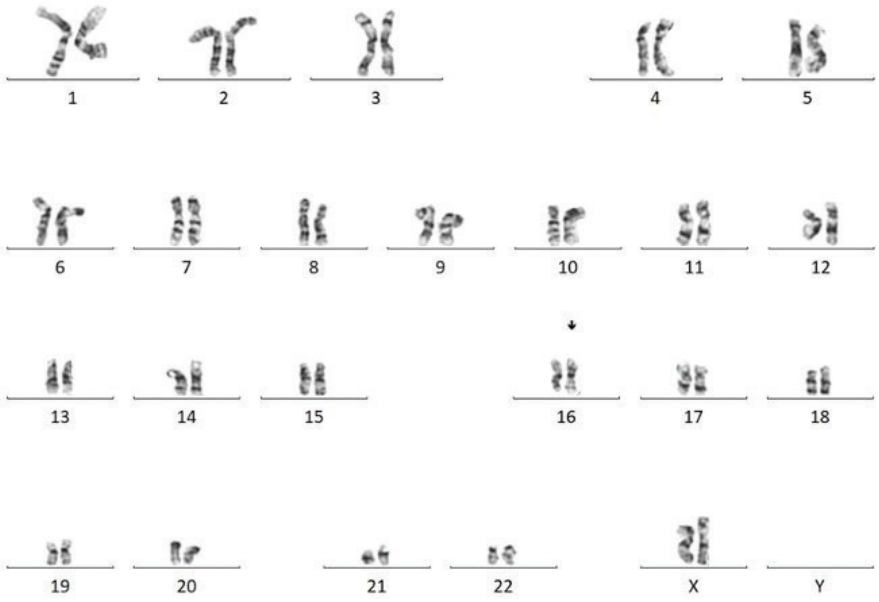

Fig. 2. Oncology chromosome analysis exhibited pericentric inversion of chromosome 16.

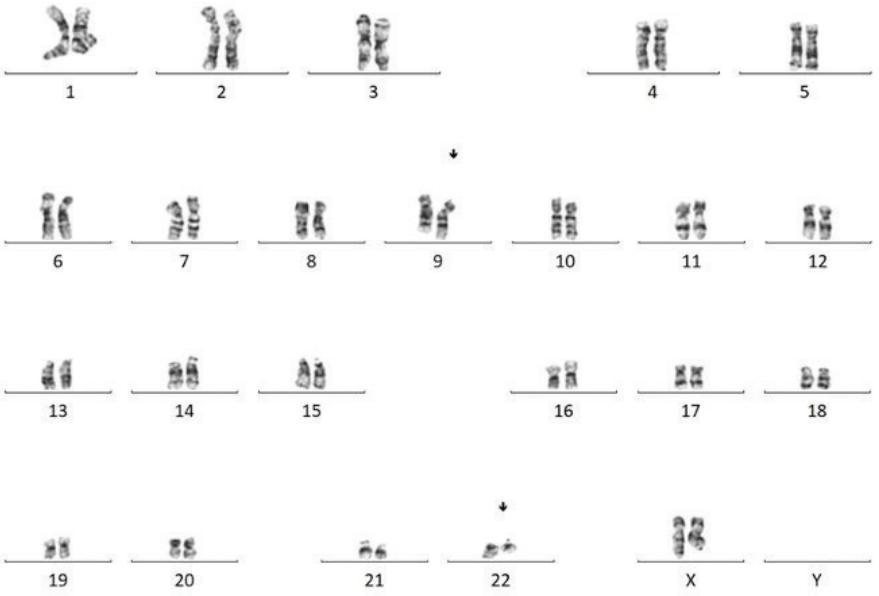

Fig. 3. Oncology chromosome analysis exhibited the presence of the Philadelphia chromosome, t(9;22).

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 cytogenetic abnormality is associated with a favorable prognosis in acute myeloid leukemia?
a. t(9;22)(q34;q11)
b. inv(16)(p13q22)
c. t(15;17)(q22;q21)
d. t (8;21)(q22;q22)

2. What is the frequency of the Philadelphia chromosome (Ph) in de novo acute myeloid leukemia cases?
a. One to two percent
b. Five to 10 percent
c. Ten to 15 percent
d. Twenty to 25 percent

3. Which of the following statements is true about the BCR-ABL gene rearrangement in acute myeloid leukemia?
a. It is a characteristic feature of chronic myelogenous leukemia and occurs in approximately 20 percent of AML cases.
b. It is rarely seen in AML, occurring in approximately 0.5 percent to three percent of cases.
c. It is associated with a favorable prognosis in all cases of AML.
d. It is commonly associated with inversion 16 in AML cases.

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