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LETTER TO THE EDITOR

CML with e6a2 *BCR-ABL1* transcript: an aggressive entity?

Karolien Anne Beel · Jan Lemmens · Hilde Vranckx · Johan Maertens · Peter Vandenberghe

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Dear Editor,

The *BCR-ABL1* fusion gene is characteristic of CML. Four different breakpoint cluster regions (bcr) have been described in *BCR*. The most common *BCR* breakpoints, in 95% of CML cases, occur in M-BCR, i.e., introns 13 or 14, resulting in an e13a2 (b2a2) or an e14a2 (b3a2) *BCR-ABL1* transcript. "Atypical" transcripts, e8a2, e19a2, e13a3, e14a3, e1a3, and e6a2 *BCR-ABL1*, account for less than 1% of CML cases [1, 2]. Only 11 CML patients have been reported with the e6a2 variant. A male predominance and a worse outcome have been suggested for this variant [3].

Here, we describe an additional CML case with an e6a2 fusion transcript. This 57-year-old male patient presented with hepatosplenomegaly, weight loss, cough, and fever. Laboratory examination showed a hemoglobin level of 12.1 g/dL, a white blood cell count of 43.5×10*9/L with 5% myeloblasts and 10% eosinophils, a platelet count of 129×10*9/L, and an LDH of 3,363 U/L (normal value, 313–618 U/L). An abdominal CT scan confirmed the hepatomegaly (craniocaudal diameter of 24 cm) and splenomegaly (bipolar diameter of 20 cm). Sokal score was 2.11, and Hasford score, 2,211.6 (both high risk). Bone marrow aspirate showed a hypocellular marrow with 10% myeloblasts and marked eosinophilia. On trephine biopsy, a myeloproliferative disease with striking fibrosis was seen. Taqman RQ-PCR was negative for e13a2/e14a2 transcripts and weakly positive for the e1a2 transcript in peripheral blood. A subsequent cytogenetic analysis showed the t(9;22)(q34;q11) translocation in all metaphases and trisomy of chromosome 8 in 6 out of 10 metaphases, indicating clonal evolution. Fluorescent in situ hybridization (FISH), using the Vysis LSI BCR/ABL1 extra signal dual color translocation probe, revealed, in addition to the expected fusion signal on der(22), a small fusion on der(9), further suggesting the presence of a variant breakpoint, located centromeric to M-BCR (Fig. 1). Qualitative reverse transcription polymerase chain reaction (RT-PCR), using forward primers in BCR exon 1 and reverse primers in ABL1 exon 2, confirmed the presence of a variant transcript of 502 bp. This was identified as e6a2 after Sanger sequencing. Based on these findings, a diagnosis of CML in an accelerated phase was made, and the patient was started on imatinib 600 mg daily.

Three months later, while still on imatinib, he was readmitted with CML in blast crisis. He had cutaneous chloromas, severe abdominal pain, a productive cough, and life-threatening dyspnea, necessitating intubation and mechanical ventilation. Peripheral leukocyte count was 465×10*9/L with 29% myeloblasts; he had severe anemia (Hb, 6.5 g/L), thrombocytopenia (75×10*9/L), and an LDH of 10,612 U/L. Chest CT scan revealed multiple pathologically enlarged mediastinal and hilar lymph nodes. Fundoscopic examination showed features of hyperviscosity. Peripheral blood karyotype and FISH now showed an extra derivative der(22)t(9;22) in ~50% of cells. Following leukapheresis and

PV is a senior clinical investigator of the Fund for Scientific Research-Flanders (Belgium) (FWO Vlaanderen).

K. A. Beel · J. Maertens
Department of Hematology, Internal Medicine,
University Hospital Leuven,
Leuven, Belgium

J. Lemmens
Department of Hematology, GZA-ziekenhuizen,
Wilrijk, Belgium

H. Vranckx · P. Vandenberghe (✉)
Center for Human Genetics, University Hospitals Leuven,
Leuven, Belgium
e-mail: peter.vandenberghe@uzleuven.be

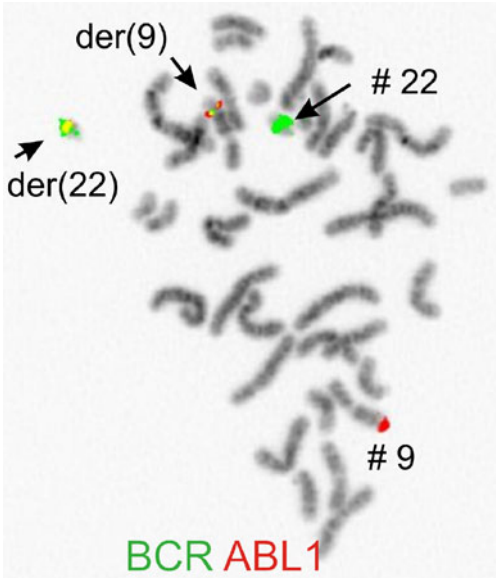

Fig. 1 Metaphase FISH on peripheral blood. The Vysis LSI BCR/ABL extra signal dual color translocation probe was applied on a cytogenetic specimen from peripheral blood. *Text* and *arrows* indicate the der(9), der(22), and the normal chromosomes 9 and 22 without fusion. There is a fusion signal on der(22), but also on der(9). The latter fusion is absent in BCR-ABL1 fusions with a M-BCR breakpoint

while on mechanical ventilation, AML-type chemotherapy with cytosine-arabinoside (200 mg/m2/d×7 in continuous infusion) was started. After a prolonged and complicated recovery, he eventually obtained a complete morphological remission and was discharged on dasatinib 140 mg daily. However, 2 months later, the patient was readmitted in hematological relapse (leukocytosis, 112×10*9/L). After cytosine-arabinoside (1,000 mg/m2, twice daily, 6d) and amsacrine (120 mg/m2/d, 3d), he again achieved a hematological remission, but only a minimal cytogenetic response. Ten months after the initial diagnosis, the patient was admitted in good condition (ECOG 1) for a reduced intensity stem cell transplantation with a sibling donor. He, however, died rapidly due to multi-resistant gram-negative sepsis and subsequent multi-organ failure. Autopsy showed disseminated fungal disease.

The clinical evolution in this case is consistent with the notion that shorter *BCR-ABL1* transcripts are associated with more aggressive disease. Ten out of 11 reported cases with e6a2 *BCR-ABL1* CML (excluding this case) were male. Of these, six had died, and five were in remission. Three of the latter were on imatinib alone; the remaining two were in remission after allogeneic HPCT [1–11].

The broadly used protocols for quantitative RQ-PCR are optimized for detecting and quantifying the typical b2/b3a2 and e1a2 transcripts, in particular, during follow-up. However, as reported earlier, rare *BCR-ABL1* transcript variants can escape detection or yield at most weakly positive reactions in these protocols, as also seen here [12]. In order to avoid missed diagnoses and consequent delays, qualitative RT-PCR protocols for the typical b2/b3a2 and e1a2 transcripts have to be used in combination with cytogenetics/FISH [13]. Also, specific qualitative multiplex RT-PCR protocols have been developed for the diagnostic setting, which detect typical as well as rare *BCR-ABL1* transcripts [14, 15].

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