

# Optimal Response in a Patient With CML Expressing *BCR-ABL1* E6A2 Fusion Transcript With Nilotinib Therapy: A Case Report

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**Abstract.** *Background/Aim:* The Philadelphia chromosome is considered the hallmark of chronic myeloid leukemia (CML). However, although most patients with CML are diagnosed with the e13a2 or e14a2 breakpoint cluster region (BCR)-Abelson 1 (ABL1) fusion transcripts, about 5% of them carry rare BCR-ABL1 fusion transcripts, such as e19a2, e8a2, e13a3, e14a3, e1a3 and e6a2. In particular, the e6a2 fusion transcript has been associated with clinically aggressive disease frequently presenting in accelerated or blast crisis phases; there is limited evidence on the efficacy of front-line second-generation tyrosine kinase inhibitors for this genotype. *Case Report:* We describe a case of atypical BCR-ABL1 e6a2 fusion transcript in a 46-year-old woman with CML. *Results:* The use of primers recognizing more distant exons from the common BCR-ABL1 breakpoint region correctly identified the atypical BCR-ABL1 e16a2 fusion transcript. Treatment with second-generation tyrosine kinase inhibitor nilotinib was effective in this patient expressing the atypical e6a2 BCR-ABL1 fusion transcript.

Chronic myeloid leukemia (CML), is a myeloproliferative disorder characterized by the presence of the Philadelphia

chromosome (Ph+) that results by the reciprocal translocation t(9;22) (q34;q11), leading to a breakpoint cluster region (BCR)-Abelson 1 (ABL1) fusion transcript. This encodes BCR-ABL onco-protein, which has a constitutive tyrosine kinase activity and plays an essential role in the pathogenesis of the disease, as it transforms hematopoietic stem cells, determining survival and proliferation, and interaction with both the cell cytoskeleton and the bone marrow microenvironment (1-8). The introduction of imatinib mesylate dramatically improved the outcome of patients with CML in the chronic phase (8-13). Nevertheless, clinical evidence suggests that patients treated with imatinib mesylate may develop BCR-ABL-dependent or BCR-ABL-independent resistance to therapy (14-20).

To overcome resistance to imatinib mesylate therapy, in the past 10 years, both second- and third-generation tyrosine kinase inhibitors (TKIs) have been developed (dasatinib, nilotinib, bosutinib and ponatinib) (21-23).

Most variants result from chromosomal breaks in BCR introns 1, 13 or 14, and ABL1 exon 2 and are known as e1a2, e13a2, and e14a2 fusion transcript, respectively; and the vast majority of patients with CML have either e13a2 or e14a2 BCR-ABL1 fusion transcripts (24-26). However, several alternative transcripts have been reported, largely resulting from either BCR or ABL1 alternative exon splicing. These uncommon variant transcripts can result in phenotypic variability and affect response to TKI therapy (27). They are generated by rearrangement between BCR exons 1, 6, 8, 13, 14 and 19 and ABL1 exons 2 and 3, accounting for fewer than 1% and their clinical significance is still under investigation (28-31). The atypical e6a2 BCR-ABL1 transcript produces a rare fusion protein of 185 kDa, which confers a poor prognosis in CML due to its association with aggressive phenotype and early transformation, perhaps due

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*Key Words:* BCR-ABL1, e6a2, CML, nilotinib, TKI therapy.

Table I. Patient characteristics at diagnosis.

Complete blood count	
Platelets	276×10 <sup>3</sup>
WBCs (μl)	73.9×10 <sup>3</sup>
Red cells (μl)	3040×10 <sup>3</sup>
Hemoglobin (g/dl)	8.9
Cytogenetic analysis	
Karyotype	46, XX, 95% (9;22)(q34;q11)
Fusion transcripts	
<i>BCR-ABL1</i>	e6a2
Relative risk	
Sokal	Low
Hasford	Low
EUTOS	Low
ELTS	Low

*BCR-ABL1*: Breakpoint cluster region–Abelson 1.

to the lack of an important regulatory *BCR* sequence within the fusion proteins (30). Here we report a case of rare CML presenting with an e6a2 fusion variant and treated with nilotinib.

### Case Report

In October 2018, a 46-year-old female was admitted to the Hematology Section, because of leukocytosis and anemia (Table I). The differential white blood cell count showed the presence of immature myeloid circulating cells, while bone marrow evaluation indicated the presence of the Philadelphia-positive chromosome (32) in 95% of the analyzed metaphases (33) with no further cytogenetic abnormalities. Sokal (34), Eutos (35), Hasford (36) and ELTS (37) risk scores were categorized as low (Table I).

In order to detect *BCR-ABL* fusion transcripts, total RNA extracted from white blood cells derived from bone marrow was reverse transcribed by Superscript III (Invitrogen, Carlsbad, CA, USA) and the cDNA obtained used to employed reverse transcriptase polymerase chain reaction (RT-PCR) multiplex (38, 39).

Molecular analysis showed no amplification of specific products with primers for the detection of the *BCR-ABL1* canonical fusion transcripts e13a2, e14a2 and e1a2. Instead, we found an atypical band at approximately 1,350 bp (Figure 1).

To better characterize this PCR product, a new PCR reaction was performed using forward primer BCR-3 (5'-TGGGTCCTGTGCGGAATCCT-3') and reverse primer for ABL-2 (5'-GTTCCAACGAGCGGCTTCACT-3') recognizing exon 3 and exon 2 of *BCR* and *ABL* genes, respectively. Using platinum SuperFiDNA polymerase enzyme (Thermo Fisher, Carlsbad, CA, USA), we obtained a band of approximately 480 bp (Figure 2). After agarose gel

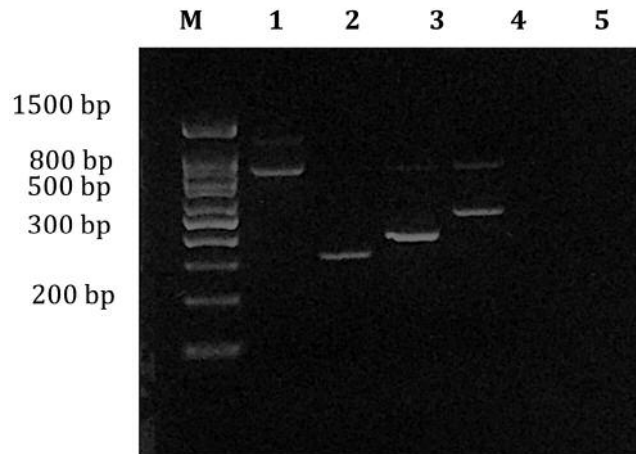


Figure 1. Multiplex reverse transcriptase polymerase chain reaction analysis of different breakpoint cluster region (*BCR*)–Abelson 1 (*ABL1*) fusion transcripts. Lane M: Molecular size marker (100-bp ladder); lane 1: e6a2 (1,350 bp) from the patient; lane 2: e13a2 (310 bp) positive control; lane 3: e14a2 (385 bp) positive control; lane 4: e1a2 (481 bp) positive control; lane 5: negative control.

purification, this DNA fragment was cloned into pcr4-TOPO-TA vector according to the manufacturer’s protocol (Invitrogen) Plasmid DNA derived from 10 individual bacterial colonies was sequenced by Sanger analysis, which detected e6a2 fusion transcript (Figure 2).

Based on clinical and laboratory findings, the patient was diagnosed as having chronic-phase CML expressing an uncommon *BCR-ABL* e6a2 fusion transcript. After informed consent, the patient was treated frontline with nilotinib at conventional dose (300 mg *b.i.d.*).

### Discussion

The concept of precision medicine is based on the identification of specific therapeutic strategies targeting genes responsible for transformation of normal cells into tumor cells (32, 40-44). Hence, the development of small molecules able to target these intracellular molecules represents a useful therapeutic approach to cancer treatment (45-53).

CML is characterized in 95% of patients by the expression of *BCR-ABL1* fusion transcript. Three breakpoint cluster regions have been reported to date: Major, minor and micro, which result in *BCR-ABL* proteins that differ in size and transforming potential, namely p210, in more than 90% of cases, p190 and p230, respectively. Different atypical breakpoints outside these cluster regions have been described. They arise from splicing between whole exons, insertion of small sequences, or genomic breakpoints within exons and often produce proteins with oncogenic potential.

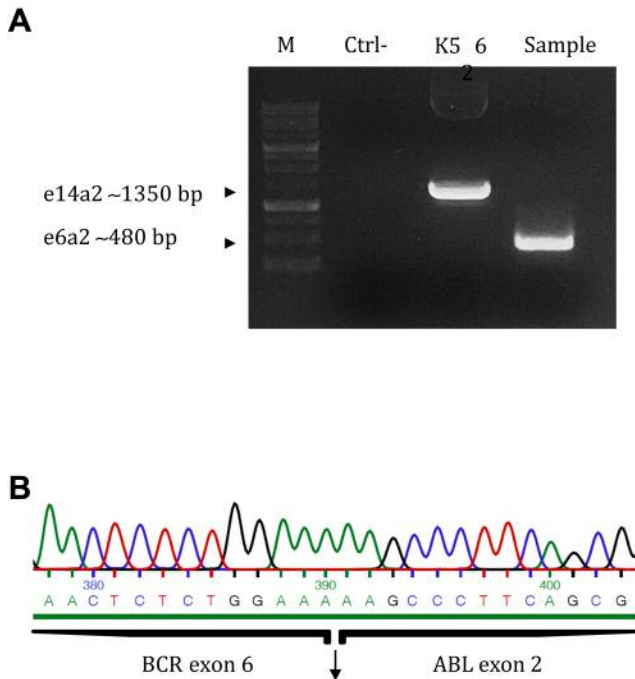


Figure 2. Breakpoint cluster region (*BCR*)–*Abelson 1* (*ABL1*) *e6a2* fusion transcript detection. A: Reverse transcriptase polymerase chain reaction performed on total RNA extracted from immortalized cell lines (K562) used as positive control. Ctrl- indicates the negative control (reaction mix lacking cDNA) and 'Sample' indicates the atypical *BCR-ABL1 e6a2* fusion transcript from patient. B: One representative pherogram obtained after Sanger sequencing of each bacterial colony showing the *BCRe6* and *ABL1a2* exon junctions.

In this regard, the *BCR-ABL1 e6a2* fusion transcript usually occurs in the middle of the guanine nucleotide exchange factor (GEF)/DBL-like domain, which is therefore only partially contained in the resulting *BCR-ABL* protein. Since this region is a GEF-related domain, it mediates the interaction with guanine nucleotide-binding proteins which are involved in cell growth and signaling. Hence, it is possible that its truncation has transforming effects, enhancing the oncogenic potential of *BCR-ABL1* (30).

In this report, we describe the case of a female patient with CML carrying the *BCR-ABL1 e6a2* fusion transcript, which presents diagnostic and therapeutics challenges.

In fact, the use of conventional multiplex RT-PCR usually fails to detect uncommon *BCR-ABL1* rearrangements due to the generation of atypical PCR products, which are often interpreted as nonspecific and this failure to recognize these may lead to a misdiagnosis of acute myeloid leukemia, which excludes the patient from targeted therapy. Therefore, we employed primers recognizing more distant exons from the common *BCR-ABL1* breakpoint region, allowing the identification of the atypical *BCR-ABL1 e6a2* fusion transcript.

Patients carrying atypical *BCR-ABL1 e6a2* fusion transcript are depicted as having an aggressive clinical course; in fact it is reported that this transcript is associated with a poor prognosis, frequently with patients presenting in accelerated or blast crisis phases (54-56). TKI therapy outcomes have not yet been well established. Therefore as previous reports suggested a poor prognosis in patients harboring atypical *BCR-ABL1 e6a2* isoform (24, 57) and because of the young age of patient, we employed nilotinib therapy from the beginning.

The patient soon achieved complete hematological response and complete cytogenetic response within 6 months of treatment, and after 14 months of nilotinib therapy she is in complete cytogenetic response and her clinical outcome is good.

In conclusion, in order to define the best treatment choice for these patients with CML, it would be mandatory to investigate the molecular and hematological characterization of more patients with *BCR-ABL1 e6a2*-bearing CML to verify the true correlation of this transcript with the aggressiveness of the disease.

## Conflicts of Interest

FDR and FS declare honoraria from Bristol Mayers-Squibb, Incyte, Novartis, Pfizer. All the others Authors have no competing interests.

## Authors' Contributions

LM wrote the article, designed and performed the experiments; ET, SRV, AP, MSP, SDG and CR analyzed and interpreted the data; MLC and LT performed cytogenetics; FS and FDR undertook the clinical care; FS, FDR, LM critically revised the article; LM conceived the original idea and supervised the project.

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Received February 7, 2020

Revised March 10, 2020

Accepted March 11, 2020