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Efficacy of Dasatinib in a Very Elderly CML Patient Expressing a Rare E13a3 *Bcr-Abl1* Fusion Transcript: A Case Report

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Abstract. *We report the case of an 89-year-old male diagnosed with chronic-phase CML and expressing a rare e13a3 BCR-ABL1 fusion transcript. His cytogenetic analysis showed the t(9;22) translocation generating the Philadelphia chromosome (Ph), with a multiplex RT-PCR detecting an atypical fragment. Using two primers complementary to exon 10 of BCR and exon 4 of ABL1, a larger PCR product was observed, where after Sanger sequencing, an e13a3 BCR-ABL1 transcript was revealed. Given the diagnosis, the patient received 100 mg of dasatinib every other day and was then monitored by measuring both hematological and cytogenetic parameters, while his BCR-ABL1 transcripts were examined by PCR and semi-nested-PCR. According to the 2013 European Leukemia Network criteria, after six months of dasatinib the patient's response was classified as warning as he displayed 20% of Philadelphia-positive metaphases. Sequencing of the ABL1 catalytic domain did not detect point mutations. A complete cytogenetic response was achieved after one year of dasatinib. However, semi-nested-PCR confirmed the presence of the e13a3 BCR-ABL1 fusion transcript that has persisted up to the latest follow-up visit.*

The Philadelphia Chromosome (Ph) is generated by a reciprocal translocation t(9;22) (1, 2) leading to the assembly of a chimeric *BCR-ABL1* oncogene that modulates the proliferation, apoptotic rate, cytoskeletal dynamics and microenvironment interaction of the hematopoietic stem cell, thereby giving rise to chronic myeloid leukemia (CML) (3-7). Usually, the *BCR-ABL1* fusion encompasses exon 2 of the *ABL1* gene that is fused in frame with one of three different breakpoints on the *BCR* gene: i) major (M-BCR), ii) minor (m-BCR) and iii) micro (μ-BCR). M-BCR includes the fusion transcripts in proximity of exons 13 or 14 of *BCR*, generating the more common e13a2 or e14a2 *BCR-ABL1* variants. The remaining m-BCR and μ-BCR breakpoint clusters involve less common rearrangements affecting exons 1 or 19 of *BCR* that generate the e1a2 (8) or e19a2 fusions, respectively (9). Moreover, additional uncommon breakpoints have been previously described involving *BCR* exons 6 and 8 or *ABL1* exon 3 (10). Interestingly, *BCR-ABL1* isoforms involving exon 3 of *ABL1* (e13a3, e14a3 and e19a3) have been described both in CML patients (11-13), with contrasting clinical outcomes, and in individuals diagnosed with Ph+ acute lymphoblastic leukemia (14). In the present study we report the case of a very elderly CML patient expressing a rare e13a3 *BCR-ABL1* transcript displaying a partially satisfactory response to dasatinib (DAS) treatment.

Case Report

An 89-year-old male, with a history of neutrophilic leukocytosis was subjected to a bone marrow aspirate in order to perform a karyotype analysis by G-banding (Table I) that showed 45, X, -Y, t (9;22) in 20/20 analyzed metaphases (Ph+100%) (Figure 1A). Subsequently, total RNA was extracted from his peripheral white blood cells (WBCs) and reverse transcribed using Superscript III according to the manufacturer's instructions (ThermoFisher, Waltham, MA, USA). The obtained cDNA was then used to perform multiplex polymerase chain reaction (PCR) in order to detect the expressed *BCR-ABL1* fusion transcript as

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previously described (15). This procedure revealed an atypical band of approximately 160 bp (Figure 1B) that was not amplified in a control Real-Time quantitative PCR carried out as previously described (16, 17). To investigate this amplification product, we performed a new PCR reaction using the platinum Pfx DNA polymerase (ThermoFisher) with forward (*BCR-10*: 5'-TATGACTG CAAATGGTACATTCC-3') and reverse (*ABL1-4*: 5'-TCGTAGTTGGGGGACACACC-3') primers recognizing exon 10 and 4 of the *BCR* and *ABL1* genes, respectively. A band of approximately 909 bp was obtained (Figure 1C) and cloned into the pcr4-TOPO-TA vector according to the manufacturer's protocol (ThermoFisher). After Sanger sequencing, plasmid DNA derived from ten individual bacterial colonies detected an e13a3 fusion transcript generated by a rearrangement involving *BCR* exon 13 and *ABL1* exon 3 (Figure 1D). Hence, the patient was diagnosed with chronic-phase CML expressing an uncommon e13a3 *BCR-ABL1* fusion. His Sokal and Hasford risk scores were 0.97 (intermediate) and 2020 (high), respectively (Table I). Given the patient's age, he initially received 2000 mg/die hydroxyurea (HU), rapidly achieving a complete hematological response. He was then switched to a TKI (October 2013) and, considering his mild renal insufficiency and the previously reported reductions in glomerular filtration rates observed with standard dose imatinib (18), was prescribed dasatinib (DAS). Given his age, the tyrosine kinase inhibitor (TKI) was given at 100 mg every other day. At this time, we began to monitor his molecular response by semi-nested-PCR (SN-PCR) (Figure 2A).

After 6 months of treatment, a cytogenetic analysis showed 20% Philadelphia-positive metaphases. Thus, according to the 2013 European Leukemia Network (ELN) recommendations (19), the patient was included in the warning category. In order to establish if his response to DAS was dependent on point mutations in the drug-binding site, we sequenced the *ABL1* kinase domain (KD). To this end, we amplified cDNA from total RNA extracted from peripheral WBCs using the following primers: forward 5'-CGCAACAAGCCCACTGTCT-3' and reverse 5'-TCCACT TCGTCTGAGATACTGGATT-3' (20). An ~840 bp amplicon was then cloned into the pcr4-TOPO-TA vector and the resulting plasmid DNA was subjected to Sanger sequencing failing to detect any point mutations. Hence, the patient remained on DAS treatment and, in September 2014 (after 12 months of therapy), he achieved a complete cytogenetic response (CCyR; Figure 2B). At this time, a semi-nested-PCR (SN-PCR) performed using the indicated forward *BCR-12* (5'-GTGCAGAGTGGAGGGAGAACA-3') and reverse *ABL1-4* (5'-TCGTAGTTGGGGGACACACC-3') primers recognized a 589 bp cDNA fragment between exon 12 of *BCR* and exon 4 of *ABL1* (Figure 2C). During the following years we repeated both cytogenetic and SN-PCR analyses as indicated in Figure 2A and C, and found that the patient maintained a CCyR with persistence - albeit at progressively lower levels - of the e13a3 *BCR-ABL1* transcript. At the last follow-up in February 2019, the patient - currently 94-years old - is in fair general conditions (Performance Status according to ECOG 2) and continues 100 mg DAS every other day with a remarkable lack of any meaningful toxicity. His CCyR is stable and the e13a3 *BCR-ABL1* fusion is still detectable in SN-PCR (Figure 2).

Table I. *Patient characteristics at diagnosis.*

Male	92-year-old
Peripheral blood cell counts	
Platelets	69.000
WBCs/μl	77.450×10^3
Neutrophils	65%
Eosinophils	4.1%
Basophils	0.3%
Lymphocytes	10.9%
Monocytes	4.7%
Metamyelocytes	3%
Myelocytes	7%
Promyelocytes	3%
Myeloblasts	2%
Cytogenetic	
Karyotype	46, X, -Y, 100% metaphases (9;22) (q34;q11)
Fusion transcript detected	
BCR-ABL	e13a3
Relative risk	
Sokal	0.97 (Intermediate)
Hasford	2020 (High)

WBCs: White blood cells.

Discussion

In the present report we describe the clinical history of a very elderly CML patient with the t(9;22) reciprocal translocation detected by G-banding encoding for a rare e13a3 *BCR-ABL1* transcript. Although individual accounts of single patients exhibiting *BCR-ABL1* transcripts lacking exon 2 of *ABL1* have been previously published (21-23), to the best of our knowledge this is the first case reporting the occurrence of this unusual fusion in a very elderly individual treated with a second-generation TKI. Uncommon *BCR-ABL1* isoforms can go undetected since the classical RT-PCR multiplex employed to diagnose the disease can sometimes generate atypical PCR fragments often interpreted as nonspecific products. In this study, the use of primers recognizing exons distant from the common *BCR-ABL1* breakpoint region allowed the identification of an atypical

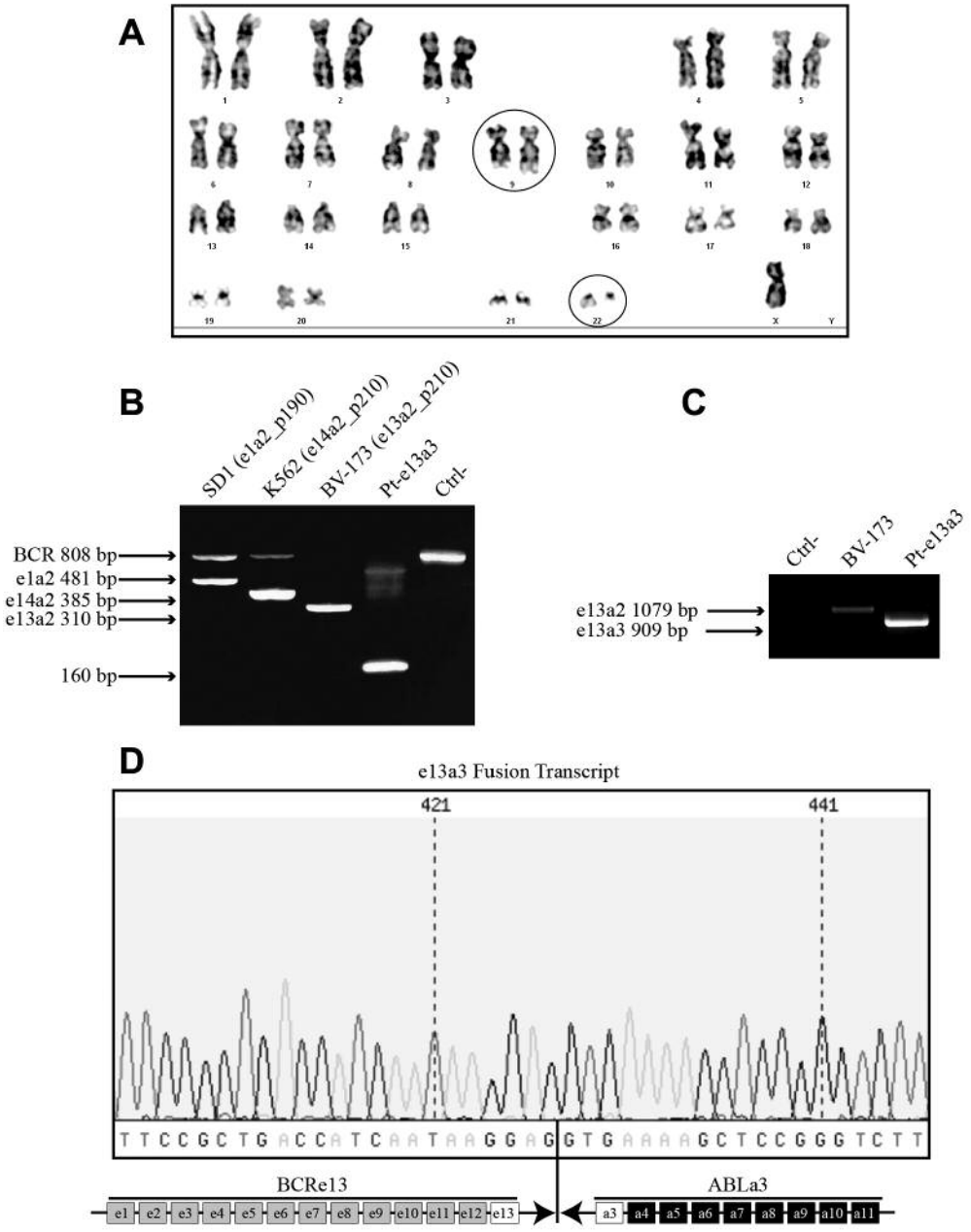

Figure 1. *Cytogenetic, multiplex PCR and Sanger sequencing of the e13a3 BCR-ABL1 fusion transcript. A. Image showing the G-banded karyotype. Circles indicate the t(9;22) translocation. B. Multiplex RT-PCR of different BCR-ABL1 fusion transcripts was performed on total RNA extracted from the indicated immortalized cell lines, used as a positive control. Ctrl- refers to the RNA derived from a healthy donor and Pt-e13a3 shows the atypical e13a3 breakpoint measuring 160 bp. The 808 bp BCR band represents an internal control that is amplified when the sample is negative for BCR-ABL1 expression. C. RT-PCR showing a ~909 bp band obtained using BCR-10 and ABL-4 primers, amplifying the e13a3 fusion. Ctrl- and the immortalized cell line BV-173 were used as negative and positive controls, respectively. D. Schematic representation of the e13a3 BCR-ABL1 transcript and a representative pherogram obtained by Sanger sequencing showing the BCRe13-ABL1a3 exons fusion.*

BCRe13 and *ABL1a3*. Although imatinib often represents the compound of choice for the first-line treatment of chronic-phase CML (24-27), extensive data demonstrate that, in some cases, IM may prove ineffective because of BCR-ABL-dependent or -independent resistance mechanisms requiring alternative therapeutic approaches (28-30). Furthermore, multiple parameters including the size of the BCR portion retained in the oncogenic fusion protein,

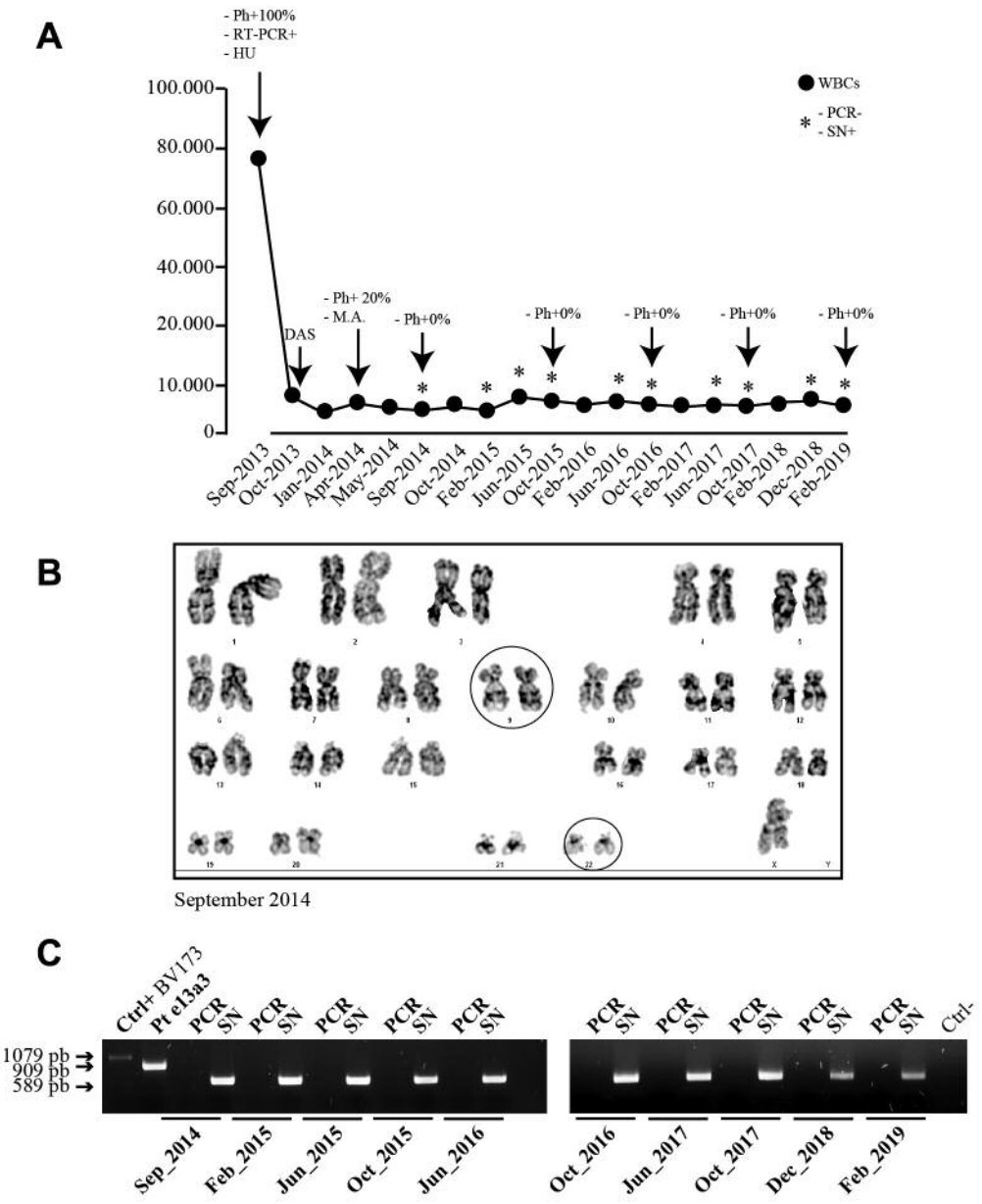

Figure 2. *Hematological, cytogenetic, and molecular responses during DAS treatment. A. The graph indicates the WBC counts from diagnosis to the last follow-up and reports the time points in which both cytogenetic and molecular analyses were performed. B. Representative image of the G-banded karyotype showing achievement and maintenance of CCyR at the indicated time points. Circles indicate normal chromosomes 9 and 22. C. PCR and SN-PCR of the e13a3 BCR-ABL1 transcript at the indicated time points. Ctrl+ and Pte13a3 indicate the cDNA obtained from the BV173 cell line and the WBCs of the patient at diagnosis, respectively. Ctrl- represents the negative control in which cDNA was missing in the PCR reaction mix. Ctrl+: Positive control; WBCs: white blood cells; DAS: dasatinib; Ph+: Philadelphia-positive metaphases; SN: semi-nested-PCR; BA: BCR-ABL1; HU: hydroxyurea; PCR: polymerase chain reaction; CCyR: complete cytogenetic response.*

presence of hyperdiploidy, complex variant translocations as well as intron-derived insertions/truncations in the *BCR-ABL1* kinase domain, have been associated to unfavorable clinical outcomes and poor response to IM (1, 11, 31-35).

The a2 exon of *ABL1* encodes for a Src homology domain 3 (SH3) that plays a critical role both as negative regulator of ABL1 kinase activity and as a modifier of its tertiary structure that affects drug response (14, 22, 36). Based on these data and on the limited indications for IM in patients with renal insufficiency, we prescribed reduced dose DAS to our patient and attained a slow but steady decrease in Ph+ metaphases, achieving a CCyR after one year of treatment.

The SN-PCR method employed to monitor his *BCR-ABL1* mRNA detected a persistent e13a3 transcript even after 5 years of treatment, indicating that - at this dosage - DAS did not eradicate the leukemic clone but was able to maintain a complete cytogenetic remission.

In summary, CML patients expressing e13a3 *BCR-ABL1* fusions may be misdiagnosed because an atypical PCR product can be erroneously interpreted as a non-specific amplicon when the control real-time PCR is negative. In these cases, the cytogenetic analysis is critical, as identification of the Ph chromosome indicates the need to investigate the presence of alternative *BCR-ABL1* transcripts. Moreover, our data suggest that patients diagnosed with chronic-phase CML expressing the e13a3 *BCR-ABL1* may fail to rapidly achieve the expected treatment outcomes, although in our case the lack of a deep molecular response may be partially explained by the reduced dosage of the second-generation TKI prescribed to this very elderly patient.

Ethics Approval and Consent to Participate

The patient provided written informed consent allowing us to anonymously report his clinical case. The study adheres to the declaration of Helsinki and the biological samples were collected following an institutionally approved protocol at the Azienda Ospedaliero Universitaria "Policlinico - Vittorio Emanuele", Catania, Italy.

Conflicts of Interest

The Authors declare that they have no competing interests.

Author's Contributions

MM drafted the manuscript; MM, ET and SS were responsible of study concept, designed and performed the experiments; MM, ET, SS, MP, AP, SRV and CR analyzed and interpreted the data; FS and VZ made a critical revision of the paper and managed the patient; FDR supervised the project; LM conceived the original idea and supervised the project. All Authors have seen and approved the final version of the manuscript.

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