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Rapid decline of Philadelphia-positive metaphases after nilotinib treatment in a CML patient expressing a rare e14a3 *BCR-ABL1* fusion transcript: A case report

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Abstract. We report a case of chronic myeloid leukemia in a 52-year-old male expressing a rare e14a3 *BCR-ABL1* fusion transcript. Cytogenetic analysis showed the t(9;22) translocation and multiplex RT-PCR detected an atypical fragment of approximately 230 base pairs. Using two primers recognizing exon 10 of *BCR* and exon 4 of *ABL1,* a larger PCR product was identified, cloned, sequenced and defined as an e14a3 *BCR-ABL1* rearrangement. The patient was treated with nilotinib and monitored measuring cytogenetic and hematological parameters, while *BCR-ABL1* transcripts were surveyed by conventional and semi-nested PCR. The patient achieved a complete hematologic response after two months of treatment followed by a complete cytogenetic remission two months later. Furthermore, PCR and semi-nested PCR failed to detect the e14a3 *BCR-ABL1* mRNA after 15 and 21 months of nilotinib, respectively.

Introduction

The BCR-ABL oncoprotein is the culprit of chronic myeloid leukemia (CML) as it transforms the hematopoietic stem cell altering its survival, proliferation and interaction with both the cell cytoskeleton and the bone marrow microenvironment (1-6). In the last 15 years, multiple tyrosine kinase inhibitors (TKIs) have been approved for the first line treatment of the disease including imatinib (IM), dasatinib (DAS), nilotinib (NIL) and bosutinib (BOS) (7-12). From a genetic standpoint, the *BCR-ABL1* chimeric oncogene derives from the t(9;22) reciprocal translocation that generates the Philadelphia (Ph) chromosome (13). Most patients express one of three fusion transcripts juxtaposing *BCR* exons 1, 13 or 14 with exon 2 of *ABL1* (14). However, several alternative breakpoints involving different *BCR* and/or *ABL1* exons have been previously described. Specifically, *BCR* exons 1, 6, 8, 13, 14 and 19 can rearrange with *ABL1* exons 2 or 3, generating e6a2, e8a2, e1a3, e13a3, e14a3, e19a3 fusions (15).

BCR-ABL1 fusions involving *ABL1* exon 3 are extremely rare (0.3%) and are usually associated with contrasting clinical outcome (16,17). In the present study we report a CML patient expressing an atypical e14a3 *BCR-ABL1* transcript that was successfully treated with NIL.

Case Report

In October 2016 a 52 year-old male (Table I) with a history of neutrophilia in the absence of thrombocytosis was admitted to the Hematology ward and subjected to a bone marrow aspirate in order to perform a karyotype analysis by G-banding. His cytogenetic profile showed the t(9;22) translocation in 100% of the analyzed metaphases (20/20) (Fig. 1A). At this time, his white blood cells (WBCs) were lysed and used to extract total messenger RNA (mRNA) that was reverse transcribed in cDNA and employed to perform a multiplex RT-PCR in order to determine his *BCR-ABL1* fusion transcript variant (18). An atypical band of approximately 230 base pairs was detected (Fig. 1B) with no amplification of the common *BCR-ABL1* variants in real-time PCR, performed as previously described (19,20). However, the detection of the Ph chromosome by G-banding led to exclude that this 230 base pair band was a nonspecific RT-PCR product. Hence, we employed the cDNA for a new PCR reaction using forward (BCR-10 5'-*TATGAC*

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TGCAAATGGTACATTCC-3') and reverse (ABL-4 5'-*TCG TAGTTGGGGGACACACC*-3') primers recognizing exons 10 and 4 of *BCR* and *ABL1*, respectively. The 977 bp PCR product (Fig. 1C) generated by the Pfx platinum DNA polymerase enzyme was then cloned in the pcr4-TOPO-TA vector according to the manufacture's protocol (all from Thermofisher Scientific). Plasmid DNA obtained from ten individual bacterial colonies was then subjected to Sanger sequencing and each colony showed the e14a3 fusion transcript. One representative pherogram displaying the *BCR*e14 and *ABL1*a3 gene exons rearrangement is depicted in Fig. 1D. Finally, Sokal (0.79, low), Hasford (949, intermediate), EUTOS (11, low) and ELTS (1.39, low) scores were calculated as indicated in Table I. In conclusion, the patient was diagnosed with chronic phase CML expressing a rare e14a3 *BCR-ABL1* variant. Although IM represents an excellent first line treatment for CML, previous data show that not all patients expressing the e14a3 isoform benefit from this drug (16,21). Thus, the patient, with no additional medication, received NIL 300 mg *bis in die* (BID), as this compound is a more potent inhibitor of ABL1 catalytic activity.

Disease evolution was monitored measuring both hematological and cytogenetic parameters. After two months of NIL, the patient achieved a complete hematological response (CHR) and, in February 2017, a new cytogenetic analysis failed to detect Ph+ metaphases suggesting a complete cytogenetic response (CCyR). During the following months, the patient maintained both his CHR and CCRy (Fig. 2A and B).

To molecularly monitor the disease, we evaluated the presence of the e14a3 *BCR-ABL1* gene by PCR and the negative reaction was confirmed by semi nested-PCR (SN-PCR) at the time points indicated in Fig. 2A. A first round of PCR was performed using the primers specified above while for the SN-PCR we employed a Fw primer recognizing exon 12 of the *BCR* gene (BCR-12 5'-*GTGCAGAGTGGAGGGAGA ACA*-3') obtaining a PCR product of approximately 668 bp. In December 2016 the PCR detected the e14a3 fusion transcript. However, in July 2017, *BCR-ABL1* mRNA-reverse transcribed by Superscript III One-Step RT-PCR (Thermofisher Scientific)-was detected only after SN-PCR and by December 2017 and June 2018 neither reaction detected the e14a3 fusion transcript suggesting a strong reduction in the overall number of *BCR-ABL1*-positive cells (Fig. 2B).

In order to evaluate the depth of the response to NIL, in January 2019 we isolated CD34+ cells from peripheral blood performing a colony forming units (CFU) assay as previously described (22). Ten CD34+-derived colonies were collected and total RNA extracted using the Trizol reagent (Life Technologies). We subsequently employed the Superscript III One-Step RT-PCR (Life Technologies) to detect the e14a3 *BCR-ABL1* fusion. Negative colonies for the first *BCR-ABL1* amplification were subjected to a semi-nested PCR as described above. Interestingly, we failed to detect the fusion transcript in any of the analyzed colonies (data not show).

At the last control (January 2019), the patient is still receiving NIL with no clinical, hematological, cytogenetic or molecular signs of disease progression. Furthermore, he has not reported side effects to NIL treatment during the course of his regular outpatient visits.

Table I. Patient characteristics at the time of diagnosis.

Patient characteristic	Values
Complete blood count	
Platelets (μl)	207.000
WBCs (μl)	53910x10^3
Neutrophils	64%
Eosinophils	3%
Basophils	1%
Lymphocytes	10%
Monocytes	1%
Metamyelocytes	5%
Myelocytes	10%
Promyelocytes	4%
Myeloblasts	2%
Haemoglobin (g/dl)	12.5
Cytogenetic analysis	
Karyotype	46, XY,100% (9;22)(q34;q11)
Fusion transcript	
BCR-ABL1	e14a3
Relative risk	
Sokal	0.79 (Low)
Hasford	949 (Intermediate)
EUTOS	11 (Low)
ELTS	1.39 (Low)

Discussion

BCR-ABL1 transcripts displaying breakpoints lacking *ABL1* exon 2 comprise e1a3, e13a3 and e14a3 rearrangements and represent infrequently occurring oncogenic isoforms (21). A methodological issue may have contributed to the uncommon detection of these transcripts as multiplex RT-PCR reactions can generate atypical PCR fragments often interpreted as nonspecific products. In our study, the detection of the t(9;22) translocation by G-banding indicated the presence of a Ph chromosome. We therefore decided to employ primers recognizing more distant exons from the common *BCR-ABL1* breakpoints and successfully identified the atypical *BCR-ABL1* e14a3 rearrangement. To date, different cases of CML patients expressing this isoform have been reported (16,21,23,24). Although IM represents an excellent first line therapy for most CML patients (7), extensive published data suggest that patients receiving this drug may more frequently develop both BCR-ABL-dependent and BCR-ABL-independent resistance to therapy (8,19,25) requiring alternative treatments (26). Furthermore, an inverse correlation has been reported between the size of the BCR portion retained in the oncogenic fusion protein and CML aggressiveness (27). Indeed, complex variant translocations, intron-derived insertions/truncations in the BCR-ABL1 kinase domain or hyperdiploidy suggest that CML patients displaying these genetic alterations often present an unfavorable clinical outcome and inferior IM responses (13,16,21,28,29).

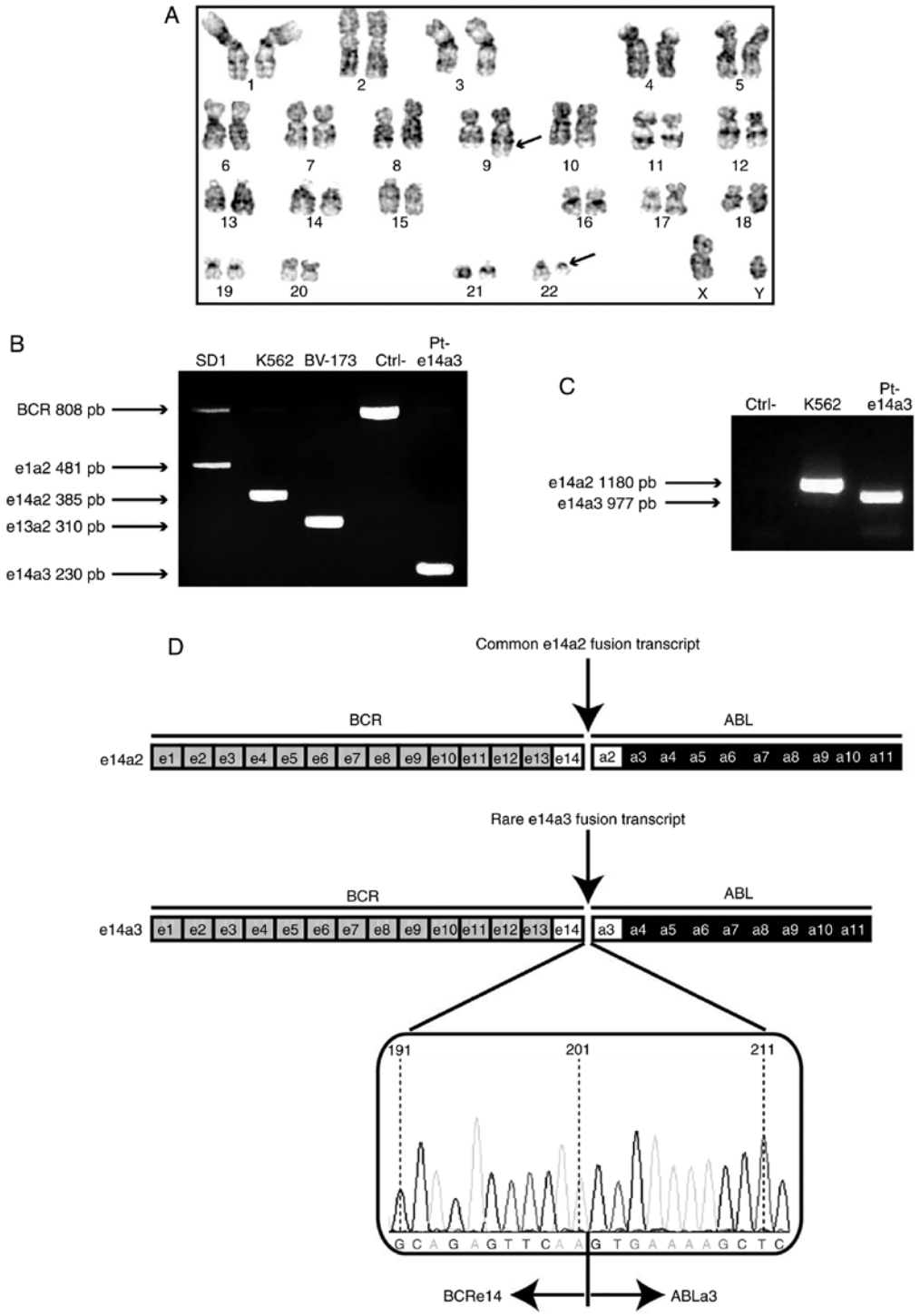

Figure 1. e14a3 *BCR-ABL1* fusion transcript identification. (A) The image shows the G-banded karyotype. Arrows indicate the t(9;22) translocation. (B) Multiplex RT-PCR of different *BCR-ABL1* fusion transcripts performed on total RNA extracted from immortalized cell lines SD1 (BCR-ABL p190-e1a2), K562 (BCR-ABL p210-e14a2) and BV-173 (p210-e13a2). Ctrl- indicates RNA derived from healthy donor and Pt-e14a3 indicates the atypical e14a3 fragment of 230 bps. The 808 bp BCR band represents a PCR internal reaction control amplified when the sample is negative for *BCR-ABL1* expression. Numbers indicate the size of the bands obtained by multiplex PCR (18). (C) The panel shows bands generated with a PCR reaction using BCR-10 and ABL-4 primers. Ctrl-(reaction mix missing cDNA) and K562 were used as negative and positive controls, respectively. (D) Schematic representation of the e14a3 *BCR-ABL1* fusion transcript and one representative pherogram obtained after Sanger sequencing of each bacterial colony showing the *BCRe14* and ABL1a3 exons junction.

The *ABL1* a2 exon encodes for a Src homology domain 3 (SH3) and, although its loss may lead to reduced leukemogenesis with a benign clinical course, its role as a negative regulator of the ABL kinase domain (SH1) may explain the reportedly more aggressive CML phenotype (30,31). Moreover, while the e14a3 *BCR-ABL1* breakpoint preserves the ATP binding domain, the lack of the SH3 domain may modify the SH1 domain tertiary structure thus affecting drug response (32).

On the base of these findings and the controversial data on IM efficacy in subjects displaying the e14a3 transcript (16), we wanted to employ a second-generation TKI (2G-TKI) as first line treatment. Nilotinib was selected for his treatment

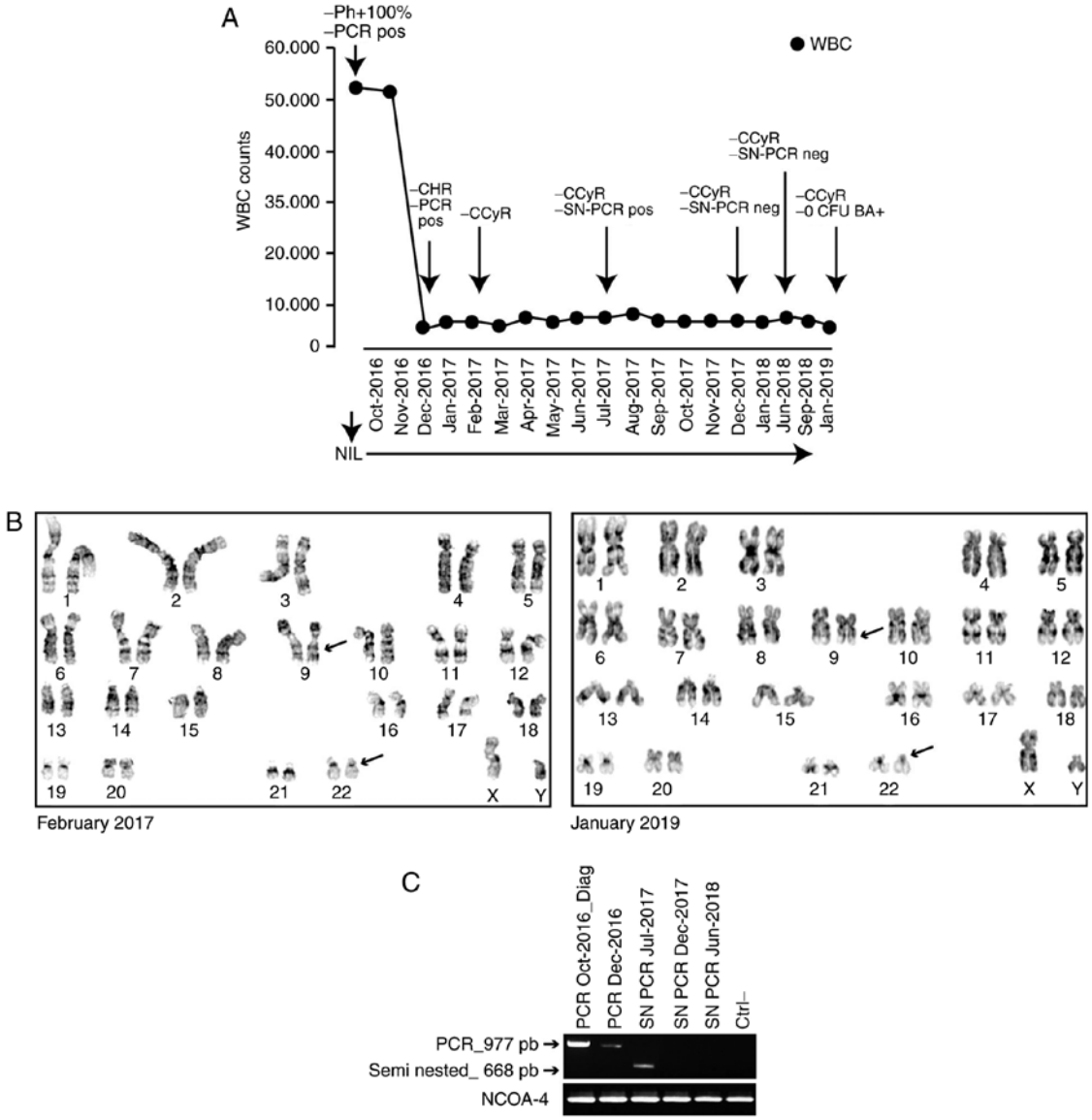

Figure 2. Hematologic, cytogenetic and molecular monitoring of the patient. (A) The graph depicts white blood cells (WBC) counts from the time of diagnosis (October 2016) to the last follow-up (January 2019) and indicates the time points of the cytogenetic and molecular analyses. (B) Follow-up of G-banded karyotype showing achievement and maintenance of CCRy at the indicated time points. Arrows indicate the normal chromosomes 9 and 22. (C) PCR and SN-PCR for e14a3 detection performed at the indicated time points. Ctrl- indicates the reaction mix missing cDNA. Nuclear receptor coactivator 4 (NCO-A4) was used as loading control and amplified by the Fw 5'-*TGGAGAAGAGAGGCTGTATCT*-3' and Rv 5'-*ATTGAAGAAATTGCAGGCTC*-3' primers. WBC, White Blood Cells; NIL, nilotinib; Ph+, Philadelphia-positive metaphases; CHR, Complete Hematological Response; CCyR, Complete Cytogenetic Response; CFU, Colony Forming Units; SN, Semi Nested; BA, BCR-ABL.

because DAS was temporarily unavailable in our pharmacy at the time. Since the patient is an accountant who works from home, he has shown excellent compliance with the administration of the drug 1 h before meals.

Using NIL, a more potent ABL1 inhibitor (9), we observed a rapid decline in Ph metaphases that generated a CCyR after 4 months of NIL. Finally, employing a semi-nested RT-PCR we failed to detect *BCR-ABL1* transcripts both in peripheral WBCs and in CFUs grown in methylcellulose, indicating that the drug induced a rapid decline in the overall number of leukemic cells.

In summary, CML patients expressing the e14a3 *BCR-ABL1* fusion transcript may be undiagnosed because this rearrangement generates an atypical PCR product often misinterpreted as a nonspecific band that is then coupled to a negative real-time PCR result. Hence, performing a cytogenetic analysis is critical to identify these CML patients. Moreover, the difficulty to perform a precise quantitative molecular monitoring and the rare incidence of atypical *BCR-ABL1* fusion transcripts does not allow the design of randomized clinical trials that may compare the efficacy of IM vs. 2G-TKIs.

We conclude that the BCR-ABL1 oncoprotein, derived from *BCRe14* and *ABL1a3* exons rearrangement, is highly sensitive to NIL, suggesting that chronic phase CML patients exhibiting this rare rearrangement may quickly achieve CCyR followed by strong reductions in the size of the leukemic clone.

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Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

Authors' contributions

MM drafted the manuscript; MM, ET and SS were responsible for study conception, and designed and performed the experiments; MM, ET, SS, MSP, AP, SRV and CR analyzed and interpreted the data; MLC performed cytogenetic analysis; FS and VZ made a critical revision of the paper and managed the patient; FDR supervised the project and contributed to study design; LM conceived the original idea and supervised the project. All authors approved the final version of the manuscript to be published.

Ethics approval and consent to participate

The patient provided written informed consent to participate. 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).

Patient consent for publication

Written informed consent obtained from patient for publication of this report.

Competing interest

The authors declare that they have no competing interests.

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