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Efficacy of Nilotinib in a CML Patient Expressing the Three-way Complex Variant Translocation t(2;9;22)

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Abstract. *Background/Aim: Chronic myelogenous leukemia (CML) is characterized by the presence of the Philadelphia chromosome, resulting from the reciprocal translocation involving chromosomes 9 and 22. About 5-10% of newly diagnosed patients in chronic-phase (CP) CML show complex additional chromosomal aberrations (ACA), that may involve one or more chromosomes in addition to 9 and 22. Data concerning the prognostic significance of ACA in CP-CML subjects at diagnosis are controversial. Furthermore, there is no evidence showing that selection of imatinib (IM) or second-generation tyrosine kinase inhibitors (2G-TKI) would be of benefit for these patients. Case Report: We report the three-way complex variant translocation t(2;9;22) in a CP-CML patient. Conventional cytogenetic analysis was employed to identify the ACA. Multiplex reverse transcription-PCR was used to identify the BCR-ABL1 transcript and its levels were measured using quantitative real-time-PCR. This rare ACA t(2;9;22) in our young patient displayed primary resistance to IM, but was responsive to second-line treatment with nilotinib. Conclusion: CP-CML patients exhibiting this rare aberration at diagnosis may benefit from a 2G-TKI therapy compared to IM.*

Chronic myelogenous leukemia (CML) is a clonal myeloproliferative disorder deriving from the transformation of hematopoietic stem cells (1). It is characterized by the presence of the Philadelphia (Ph) chromosome (2), resulting from a reciprocal translocation involving the long arms of chromosomes 9 and 22. This cytogenetic alteration t(9;22)(q34;q11) causes a genomic recombination between the breakpoint cluster region (*BCR*) gene on chromosome 22 and the abelson (*ABL1*) gene on chromosome 9, resulting in their juxtaposition, which generates the *BCR-ABL1* fusion gene (3). This chimeric oncogene encodes for a constitutively active tyrosine kinase conferring growth advantages to leukemic cells by deregulating cell proliferation, favouring cytokine-independent growth, inhibiting apoptosis and altering several cell-adhesion pathways (4-8). The essential role of *BCR-ABL1* for the pathogenesis of CML has been confirmed by the therapeutic success of selective first, second or third-generation tyrosine kinase inhibitors (TKIs) (9-14).

About 5-10% of newly diagnosed chronic-phase (CP) CML patients show complex additional chromosomal aberrations (ACA), that may involve one or more chromosomes in addition to 9 and 22 (15, 16). Although ACA have low incidence in these patients, their occurrence is high in CML patients in accelerated phase (AP) (30-40%) or blast crisis (BC) (50-80%) (17, 18). Their higher incidence in the last phases of the disease is certainly related to increasing genomic instability and linked to unfavourable prognosis (19).

Data regarding the prognostic significance of ACA at the time of diagnosis are controversial. Indeed, ACA are heterogeneous collections of karyotypic abnormalities with a different prognostic impact on the outcome of CML patients (20, 21). In general, ACAs are classified into "major" or "minor" route changes. The negative impact on CML prognosis is usually attributed to "major" route abnormalities, including trisomy 8, an additional Ph chromosome, isochromosome 17 [i(17)(q10)] and trisomy 19 (22). European Leukemia Network (ELN) recommendations defined these "major" ACA as a

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warning sign even if in daily practice they did not require different initial treatments (23). However, "major" ACAs occurring during TKI therapy are considered as treatment failure as they are usually linked to a clonal evolution of the disease from CP to AP or BC (24). On the contrary, "minor" ACAs (such as trisomy 21, loss of Y chromosome, numerical aberrations, hypodiploidy, hyperdiploidy, and polyploidy) are considered as sporadic and infrequent aberrations (25). Generally, these "minor" route ACAs do not have an adverse effect on prognosis or TKI treatment response (21, 26, 27).

Herein, we describe the rare three-way complex variant translocation t(2;9;22) in a young patient diagnosed with CP-CML that exhibited primary resistance to imatinib (IM), but benefited from second-line treatment with nilotinib (NIL).

Case Report

In March 2011, a 20 year-old male was referred to the Division of Haematology of the Azienda Ospedaliero Universitaria "Policlinico-Vittorio Emanuele" in Catania because of high white blood cell (WBC) and platelet (PLT) counts. Specifically, WBCs were 222×10^9/L (37% neutrophils, 1% lymphocytes, 3% eosinophils, 1% basophils, 4% myeloblasts, 4% promyeloblasts, 30% myelocytes and 20% metamyelocytes) while PLTs were 982×10^9/L with haemoglobin (Hgb) levels at 8.6 g/dL. At this time a peripheral blood smear was compatible with a diagnosis of CML. The patient underwent bone marrow aspiration and conventional cytogenetic analysis by G-banding detected a complex three-way Philadelphia translocation variant identified as t(2;9;22) (q23-31; q34; q11) in 20 out of 20 analysed metaphase cells (Figure 1A). Multiplex reverse transcriptase - polymerase chain reaction (RT-PCR) revealed the presence of the e14a2 *BCR-ABL1* transcript (Figure 1B), while a real-time RT-PCR (Q-PCR) showed a *BCR-ABL1/GUS*^{IS} ratio of 9.06% (28) and a *BCR-ABL1/ABL1*^{IS} of 50.89% (Figure 1C). Calculation of four risk parameters at diagnosis showed a high Sokal score, intermediate Euro and low EUTOS (European Treatment and Outcome Study) and ELTS (EUTOS Long Term Survival) scores, respectively (29-32). The patient began hydroxyurea (3 g/day) and – after one week – was switched to first-line IM 400 mg/day. In the following 90 days he achieved a complete hematologic response (CHR) but, after 6 months, he developed resistance to the drug. At that time, his *BCR-ABL1/ABL1*^{IS} levels were >10% with a concomitant mutational analysis failing to detect alterations in the *BCR-ABL1* kinase domain. He was then switched to NIL at 300 mg twice daily displaying a progressive decrease in BCR-ABL1 transcripts and attaining a molecular response (MR<10%) after 2 months of treatment (*BCR-ABL1/ABL1*^{IS} 9.4%). After further 3 months his *BCR-ABL1/ABL1*^{IS} levels were 1.3% (Figure 1, panel C). An additional cytogenetic analysis performed after one year of NIL treatment failed to detect the three-way complex variant Ph+ metaphase, suggesting a complete cytogenetic response (CCyR). Currently, the patient is still receiving NIL with no clinical, haematological and molecular signs of disease progression. In fact, the patient shows a stable major molecular response (MR$^{3.0}$) with *BCR-ABL1* ratio of 0.088% (Figure 1, panel C).

The patient signed an informed consent releasing anonymously his sample for research purposes in accordance with the Declaration of Helsinki (33).

Banding cytogenetics. Conventional cytogenetic analysis was performed as previously described (34). Cells were then incubated using standard protocols, processed by conventional methods, and chromosomes were stained with Giemsa. G-banding was performed by tripsin treatment stained with Giemsa (GTG-banding technique) (35). A total of 20 metaphases were analysed. The karyotype was described according to the International System for Human Cytogenetic Nomenclature (36).

RNA isolation, qualitative and quantitative polymerase chain reaction. For polymorphonuclear RNA extraction, peripheral blood was subjected to red cell lysis. Subsequently, 10×10^6 cells were resuspended in 600 μL of lysis buffer (RLT buffer) provided with the Qiagen RNeasy mini kit (Qiagen KJ Venlo, The Netherlands) and RNA was extracted according to manufacture's protocol. The RNA was then quantified using an Eppendorf BioSpectrometer (Eppendorf-AG Hamburg Germany) and reverse transcribed employing the Moloney Murine Leukemia Virus (M-MLV) Reverse Transcriptase (Invitrogen Paisley, UK) and random hexamer primers, starting from 1μg of total RNA. To determine the *BCR-ABL1* fusion transcript variants, a multiplex RT-PCR was performed as previously described (37). *BCR-ABL1* copy numbers were quantified using Q-PCR. cDNA was amplified by 50 cycles using Taqman Universal Master Mix (Applied Biosystems Foster City, CA, USA) according to the manufacturer's instructions and the ABI 7500 sequence detection system (Applied Biosystems), as previously reported (38). *BCR-ABL1/GUS*^{IS} and *BCR-ABL1/ABL1*^{IS} levels were calculated according to the current EUTOS criteria (28).

Discussion

The t(9;22) translocation is present in more than 90% of CML patients at diagnosis. However, approximately 5% of these individuals show one or more ACAs (15, 16, 39). The role of each individual chromosomal aberration in CML evolution and outcome is controversial as it is greatly influenced by the time of occurrence, the type of aberration ("major" or "minor"), TKI choice and treatment timing.

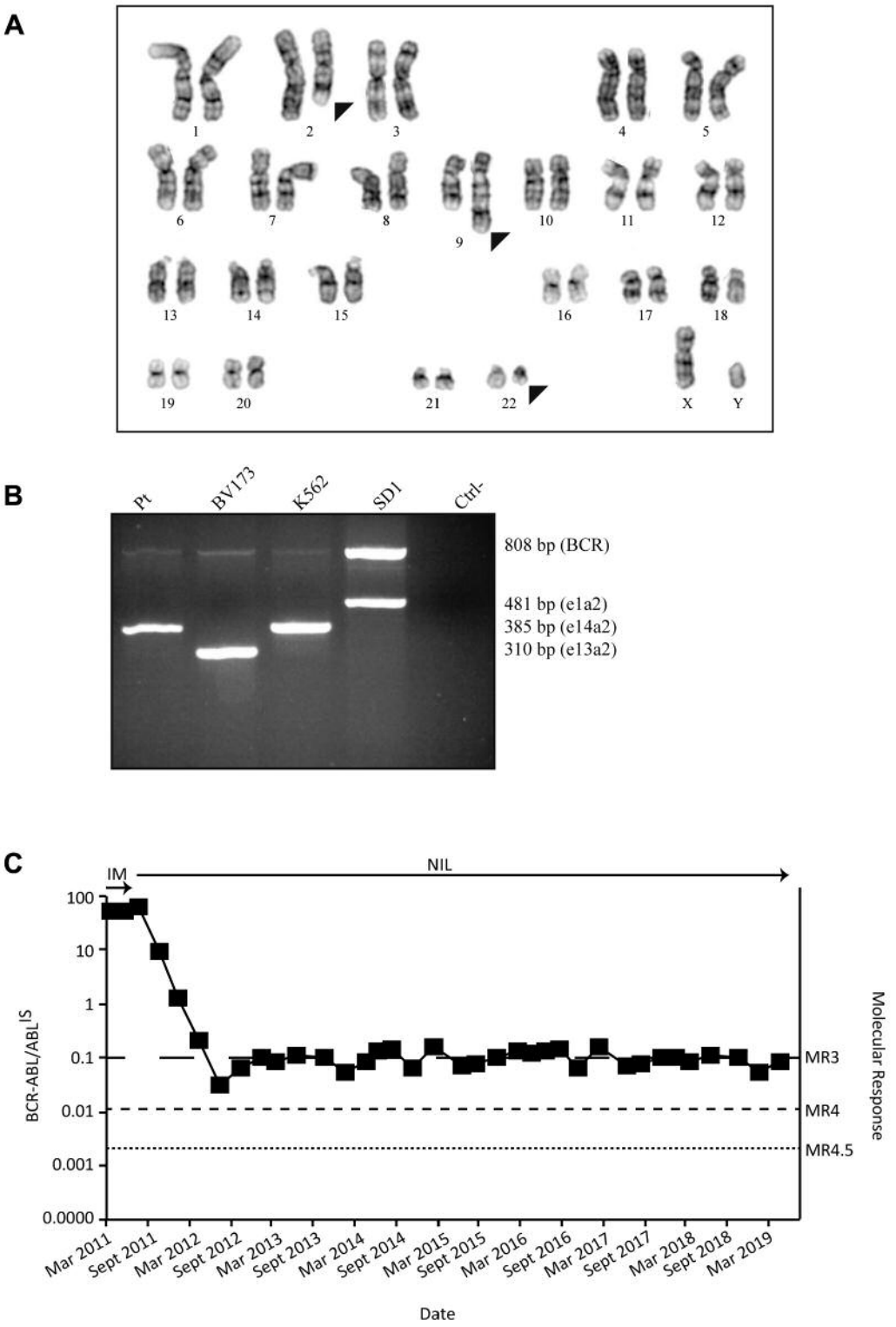

Figure 1. *(A) G-banded karyotype showing the t(2;9;22) translocation. Arrowheads indicate the chromosomes involved in the three-way translocation. (B) Multiplex RT-PCR analysis of different BCR-ABL1 fusion transcripts performed on total RNA extracted from the patient (lane 1) and the indicated immortalized cell lines (lanes 2-4). Lane 5: negative control (Ctrl-). (C) BCR-ABL/ABL^{IS} ratio over the depicted time period in relation to the specified tyrosine kinase inhibitor treatment. Dotted lines represent the indicated molecular responses (MR$^{3.0}$, MR$^{4.0}$, MR$^{4.5}$). IM: Imatinib; NIL: nilotinib; Pt: patient; Ctrl-: negative control.*

In the present study, we described the case of a young patient diagnosed with CP-CML, who presented the three-way t(2;9;22) chromosome translocation. He presented primary resistance to IM but was responsive to second-line treatment with NIL.

Several articles have previously described this three-way complex variant translocation (26, 40, 41), classified as a "minor" route aberration, suggesting that it may be generated by two different mechanisms. Indeed, the occurrence of this atypical translocation can occur because of multiple concomitant breaks (one-step mechanism) or serial genetic events in close succession (two-step mechanism) (42). In each scenario, increasing genomic instability is likely involved in these events, favouring the development of chromosomal aberrations, increasing the risk of secondary neoplasm and resistance in CML patients (43-45). Unfortunately, we were unable to identify the exact mechanism, leading to the development of the t(2;9;22) complex translocation, as the available biological material was inadequate for the required FISH analysis.

While "major" route aberrations are associated with an inferior outcome, "minor" route alterations usually do not impact prognosis. In addition, the best treatment strategy for CML patients displaying these aberrations is still uncertain. The patient described in this case report received standard dose IM as first-line therapy but failed the prescribed TKI after 6 months, as he did not achieve cytogenetic or molecular responses. Thankfully, subsequent treatment with NIL was associated with a rapid decrease in *BCR-ABL1*/*ABL1*^{IS} transcripts, leading to a CCyR and an MR3.0 that the patient has maintained for the least eight years. The prognostic relevance of ACAs in patients receiving IM is highly debated, as several studies have shown inferior overall survival with IM (46), while multiple reports have shown that presence of ACAs at diagnosis does not confer an inferior prognosis (47, 48). These contrasting findings are likely attributable to the great heterogeneity of chromosomal abnormalities reported to date (3, 21, 26, 49). Thus, while the presence of ACAs at diagnosis should not alter the treatment strategy selected for these patients (20), the presence of these alterations may represent a "warning" sign, as suggested by the latest ELN recommendations, thus requiring careful disease monitoring (50).

In conclusion, we report a rare case of CP-CML with a complex three-way Philadelphia translocation t(2;9;22) responsive to NIL treatment. Furthermore, our data suggest that CP-CML patients exhibiting this rare aberration at diagnosis may benefit from this second-generation TKI compared to IM.

Conflicts of Interest

FDR and FS received an honoraria from BMS, Incyte, Novartis, Pfizer. No potential conflicts of interest were disclosed by the other Authors.

Authors' Contribution

ET, MLC, MM and SS designed and performed the experiments. ET, SS, MM, CR, MSP, SRV, SDG and AP analyzed and interpreted the data. ET wrote the paper, while MCP, EM, FS and VZ made a critical revision of paper and managed the patient. FDR helped supervise the project. LM and FS conceived the original idea and supervised the project.

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