ANTICANCER RESEARCH *39*: 6965-6971 (2019)
doi:10.21873/anticanres.13918

Detection and Clinical Implications of a Novel *BCR-ABL1* E12A2 Insertion/Deletion in a CML Patient Expressing the E13A2 Isoform

STEFANIA STELLA[1,2*], MICHELE MASSIMINO[1,2*], ELENA TIRRÒ[1,2*], SILVIA RITA VITALE[1,2], VINCENZO ACCURSO[3], ADRIANA PUMA[1,2], MARIA STELLA PENNISI[1,2], SANDRA DI GREGORIO[1,2], CHIARA ROMANO[1,2], FRANCESCO DI RAIMONDO[4,5], SERGIO SIRAGUSA[3] and LIVIA MANZELLA[1,2]

[1]*Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy;*
[2]*Center of Experimental Oncology and Hematology, A.O.U. Policlinico-Vittorio Emanuele, Catania, Italy;*
[3]*Division of Hematology, A.O.U. Policlinico "P. Giaccone", University of Palermo, Palermo, Italy;*
[4]*Division of Hematology and Bone Marrow Transplant, A.O.U. Policlinico-Vittorio Emanuele, Catania, Italy;*
[5]*Department of Surgery, Medical and Surgical Specialities, University of Catania, Catania, Italy*

Abstract. *Background/Aim: The Philadelphia chromosome is the most frequent cytogenetic abnormality in chronic myelogenous (CML). More than 95% of CML patients are diagnosed with the e13a2 or e14a2 BCR-ABL1 fusion transcripts while, in about 1% of these individuals, the break generates the e1a2 rearrangement. Furthermore, about 5% of CML patients are diagnosed with rare BCR-ABL1 fusion transcripts, such as e19a2, e8a2, e13a3, e14a3, e1a3 and e6a2. However, there is limited evidence concerning the clinical and prognostic implications of these infrequent oncogenic variants for CML patients receiving tyrosine kinase inhibitors (TKIs). Case Report: We describe a novel atypical e12a2 insertion/deletion (Ins/Del) BCR-ABL1 fusion identified in a CML 59-year-old man diagnosed with a common e13a2 BCR-ABL1 isoform. The use of primers recognizing more distant exons from the common BCR-ABL1 breakpoint region correctly identified and monitored in time the atypical e12a2 Ins/Del BCR-ABL1 fusion. Conclusion: Treatment with second- (nilotinib) and third-generation (ponatinib) TKIs was effective in suppressing leukemic clones exhibiting the atypical e12a2 Ins/Del BCR-ABL1.*

*These Authors contributed equally to this study.

Correspondence to: Stefania Stella, Department of Clinical and Experimental Medicine, Center of Experimental Oncology and Hematology, University of Catania, Building 8D/2 - Via Santa Sofia, 78, 95123 Catania, Italy. Tel: +39 0953781946, Fax: +39 0953781949, e-mail: stefania.stel@gmail.com

Key Words: e13a2, CML, *BCR-ABL1*, nilotinib, TKIs, ponatinib, e12a2.

The Philadelphia (Ph-) chromosome, generated by the reciprocal translocation of the *ABL1* and *BCR* genes, is the most frequent cytogenetic abnormality in both chronic myelogenous (CML) and adult acute lymphoblastic (ALL) leukemia (1-5). At the molecular level, the t(9,22) translocation originates from the *BCR-ABL1* chimeric oncogene encoding for an oncoprotein with constitutive tyrosine kinase (TK) activity that alters the proliferation rates, survival signaling, cytoskeleton dynamics and microenvironment interactions of the hematopoietic stem cell (6-10).

More than 95% of CML patients are diagnosed with the e13a2 or e14a2 *BCR-ABL1* fusion transcripts while, in about 1% of these individuals, the break in the *BCR* gene occurs between exons 1 and 2, generating the e1a2 rearrangement. Approximately 5% of CML patients present atypical *BCR-ABL1* mRNAs created by fusions involving alternative exons, gene insertions or unusual breakpoints. These infrequent isoforms include e19a2, e8a2, e13a3, e14a3, e1a3, e6a2 and e12a2 and may escape detection when using standard methods optimized for typical variants (11-14).

Over the past 20 years, the development of *BCR-ABL1* tyrosine kinase inhibitors (TKIs) has significantly improved the outcomes of most CML patients, generating unprecedented rates of complete hematological (CHR), cytogenetic (CCyR) and molecular (MR) responses (15, 16). In the current therapeutic scenario, several TKIs may be chosen as first-line treatment for CML (17, 18), although extensive data have shown that the efficacy of these compounds may be compromised by both *BCR-ABL1*-dependent or -independent mechanisms of resistance often requiring alternative therapeutic approaches (19). Currently, very limited evidence is available on the efficacy of different TKIs in CML patients expressing atypical *BCR-ABL1*

rearrangements, let alone those displaying the coexistence of two (one typical, the other atypical) *BCR-ABL1* transcripts (14, 20, 21).

In the present case report, we describe the case of a patient successfully treated with nilotinib (NIL) and ponatinib (PON) for a disease initially characterized by the common e13a2 isoform that subsequently developed an atypical e12a2 insertion/deletion (Ins/Del).

Case Report

In June 2007, a 59-year-old man presented with a 2-week history of fever, fatigue and abnormal blood cell counts. At the time, his hemoglobin (Hgb) was 13.4 g/dl with 76,530 white blood cells (WBC) and 224,000 platelets (Table I). The spleen was palpable 2 cm below the left costal margin while liver size was normal. Conventional cytogenetics, performed by G-banding (22, 23), detected the Philadelphia chromosome in all examined metaphases with no additional cytogenetic abnormalities [karyotype 46,XY, t(9;22)(q34;q11)] (Table I). Multiplex reverse transcriptase (RT)-PCR revealed the presence of the e13a2 *BCR-ABL1* transcript with *BCR-ABL1/ABL1* levels of 198.82% measured by real-time (Q-PCR) (24) (Figure 1A and B).

Based on these clinical findings the patient was diagnosed with chronic-phase CML with low Sokal (25), low Hasford (26) and intermediate ELTS (27) scores (Table I). Soon thereafter, he began imatinib (IM) 400 mg/day achieving a complete hematological response with persistence of the Philadelphia chromosome in 4/20 (20%) metaphases after 3 months. At this time, Q-PCR detected *BCR-ABL1/ABL1* transcript levels of 85.15% (Figure 1B) and the WBC count was 8120 (Figure 2A). In November 2007, the patient had to discontinue IM because of grade II toxicity (squamous erythema) and after 20 days commenced dasatinib (DAS) 100 mg/day. After six months, he was still in CHR, and displayed a partial cytogenetic response (PCyR) with $BCR\text{-}ABL1/ABL1^{IS}$ transcripts of 1.71% (Figure 1B). In November 2008, the patient eventually attained a complete cytogenetic response (CCyR) with a corresponding decrease in his molecular response ($BCR\text{-}ABL1/ABL1^{IS}$ 1.04%), but after three months he exhibited a rise in his oncogenic transcripts ($BCR\text{-}ABL1/ABL1^{IS}$ 1.96%). Soon thereafter he presented with an increase of his WBCs although a mutation analysis of the *ABL1* kinase domain by clonal sequencing (28, 29) failed to detect any sequence alterations (Figure 2A). As the patient admitted occasional discontinuations of the drug, in the absence of a kinase domain mutation he continued his treatment with DAS. However, after nine months, his WBCs were 15,000, with 35% of Ph-positive metaphases, while a quantitative RT-PCR unexplainably detected a $BCR\text{-}ABL1/ABL1^{IS}$ value of 0.19% (Figures 1B and 2A). To investigate the presence of a possible new fusion transcript, we employed the same total RNA to perform a different qualitative RT-PCR using previously described (30) forward (*BCR*-10: 5'-TATGACTGCAAATGGTACATTCC-3') and reverse (*ABL1*-4: 5'-TCGTAGTTGGGGGACACACC-3') primers. The resulting amplicon was smaller than the expected e13a2 *BCR-ABL1* transcript and Sanger sequencing identified the lack of exons 13 and 14 in *BCR* fused with a partial deletion of *ABL1* exon 2 with a 39bp insertion that matched the genomic region from 29534693 to 29534732 (GRCh38) located on chromosome 20. This Ins/Del generated a breakpoint between exons 12 of *BCR* and 2 of *ABL1* giving rise to an e12a2 Ins/Del *BCR-ABL1* fusion (Figure 2B and C). The patient was then put on nilotinib (NIL) 800 mg/day and after 6 months achieved a CHR, CCyR and a major molecular response (MR^3) ($BCR\text{-}ABL1/ABL1^{IS}$ 0.06%; Figure 1B). Molecular follow-up of the e13a2 isoform continued every 3 months with Q-PCR, while the RT-PCR with *BCR-10* and *ABL1-4* primers was employed to detect the e12a2 Ins/Del transcript with failure to amplify this isoform after 6 months of NIL (data not shown).

Over the next 6 years, the patient maintained his CHR and CCyR and exhibited molecular responses varying between an MR^3 and a deeper MR^4. However, in December 2015 he

Table I. *Patient characteristics at the time of diagnosis.*

Complete blood count	
Platelets	224,000
WBCs (μl)	$76{,}530 \times 10^3$
Neutrophils	75%
Eosinophils	2.5%
Basophils	1%
Lymphocytes	11%
Monocytes	1%
Metamyelocytes	5%
Myelocytes	10%
Promyelocytes	5%
Myeloblasts	2.5%
Haemoglobin (g/dl)	13.4
Cytogenetic analysis	
Karyotype	46, XY, 100% (9;22)(q34;q11)
Fusion transcripts	
BCR-ABL1	e13a2 and e12a2 ins/del
Relative risk	
Sokal	0.76 (Low)
Hasford	770.54 (Low)
EUTOS	15 (Low)
ELTS	1.61 (Intermediate)

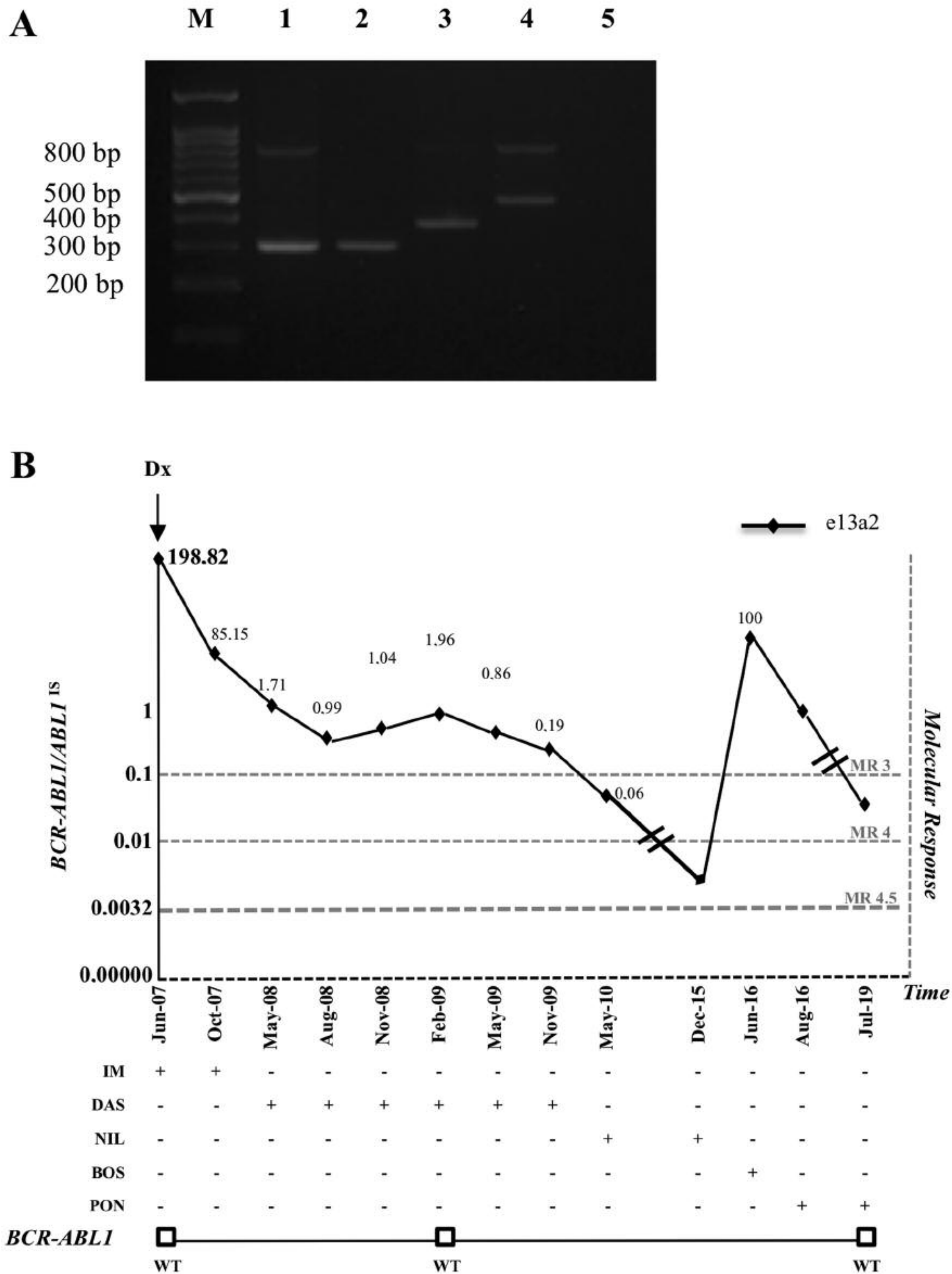

Figure 1. *Clinical evolution of the patient. A. Multiplex RT-PCR analysis of different BCR-ABL1 fusion transcripts. Lane M=Molecular size marker (100-bp ladder); lane 1=e13a2 (310 bp) from the patient; lane 2=e13a2 (310 bp) positive control; lane 3=e14a2 (385 bp) positive control; lane 4=e1a2 (481 bp) positive control; lane 5=negative control. B. Molecular response to different TKIs. Monitoring of the patient's disease evolution indicating variations in the e13a2 transcripts (top panel), drug treatments (middle panel) or BCR-ABL1 mutant clones (bottom panel). Dotted lines represent achievement of a major (MR3) or a deep molecular response (MR4; MR4.5). A white square indicates wild-type BCR-ABL1. IM: Imatinib; DAS: dasatinib; NIL: nilotinib; BOS: bosutinib; PON: ponatinib.*

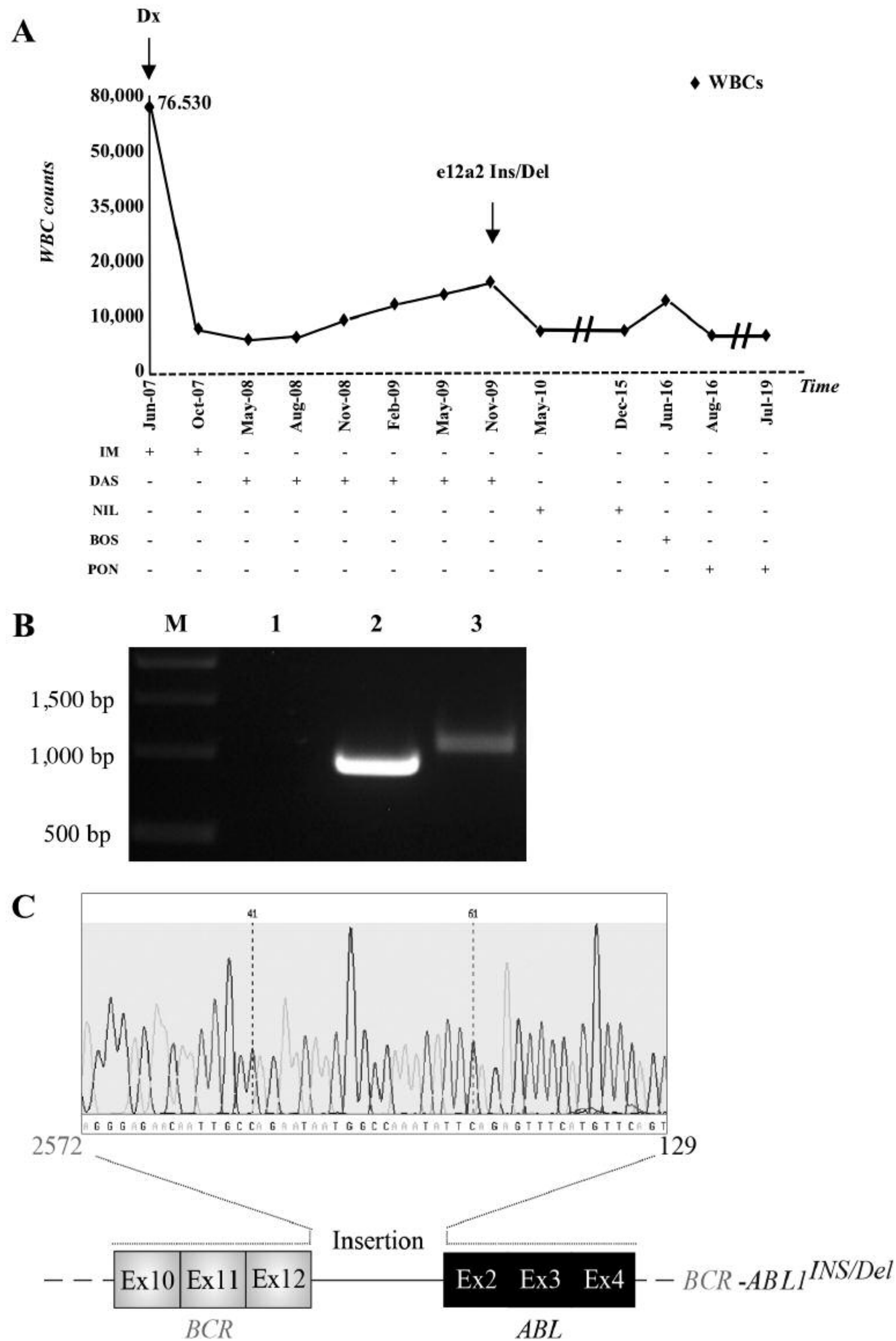

Figure 2. *Identification of the e12a2 Ins/Del fusion transcript. A. The graph depicts white blood cell (WBC) counts from the time of diagnosis (June 2007) to the last follow-up (July 2019) and indicates the time point of the e12a2 Ins/Del breakpoint detection. B. RT-PCR for the e12a2 Ins/Del rearrangement performed at the time point indicated by an arrow. Molecular size marker (100-bp ladder); lane 1=negative control; lane 2=e12a2 Ins/Del (850 pb) from the patient; lane 3=e13a2 (1079 pb) positive control. RT-PCR was employed using previously reported forward (BCR-10: 5'-TATGACTGCAAATGGTACATTCC-3') and reverse (ABL1-4: 5'-TCGTAGTTGGGGGACACACC-3') primers. C. Schematic representation of the e12a2 ins/Del BCR-ABL1 fusion transcript and a representative pherogram obtained after Sanger sequencing showing the BCRe12 and ABL1a2 insertion/delection junction. The numbers 2572 and 129 indicate the nucleotide position in BCR and ABL1 genes, respectively.*

had to suspend NIL because of a serious cardiovascular event (myocardial infarction) and - after three months - started bosutinib (BOS) 400 mg/day. Unfortunately, after three months he failed to attain any meaningful clinical benefit from this TKI [20/20 Ph-positive metaphases; *BCR-ABL1/ABL1*^{IS} (e13a2) 100%; WBC=6,123; Figures 1B and 2A] and therefore began the third-generation inhibitor PON at 45 mg/day. After 1 month, he achieved a PCyR and an MR^2 and, considering his cardiovascular comorbidities, we decreased PON to 15 mg/day. With this dose he achieved a CCyR and an MR^3 that are still ongoing, with failure to detect the e12a2 Ins/Del *BCR-ABL1* transcript.

Discussion and Conclusion

Most CML patients display the common e13a2 and/or e14a2 *BCR-ABL1* fusions. However, about 5% of these patients are diagnosed with an atypical *BCR-ABL1* transcript that involves alternative exons, insertions or breakpoints such as e19a2, e8a2, e13a3, e14a3, e1a3 and e6a2 (11-14). The breakpoint in these transcripts usually occurs in *ABL1* exon 2, but occasionally arises in *ABL1* exon 3. Moreover, different investigators have previously described the coexistence of two or more *BCR-ABL1* mRNAs in the same patient (20, 21), probably due to alternative splicing or phenotypic variations.

In the current report, we describe the case of a male patient diagnosed with CML where we identified an e12a2 Ins/Del, secondary to the presence of the common e13a2 *BCR-ABL1* transcript. However, we did not formally establish if the two different *BCR-ABL1* fusions were expressed by the same clone (*via* alternative splicing mechanisms) or represented two distinct cellular types each characterized by a specific rearrangement. It remains to be seen if co-expression of multiple *BCR-ABL1* transcripts in the same or in different leukemic clones modifies the prognosis of CML patients. For example, Chiarella and colleagues reported splicing events inducing deletions or insertions of nucleotides, as well as the activation of cryptic splicing sites leading to modifications in the translated protein (31).

The use of conventional multiplex RT-PCR may fail to detect uncommon *BCR-ABL1* rearrangements due to generation of atypical PCR products, which are often interpreted as non-specific. Therefore, in this study, we employed primers recognizing more distant exons from the common *BCR-ABL1* breakpoint region allowing the identification of the atypical e12a2 Ins/Del *BCR-ABL1* fusion and the molecular monitoring of the patient exhibiting this atypical transcript.

No clear evidence exists concerning the clinical and prognostic implications of infrequent *BCR-ABL1* rearrangements in Ph-positive leukemias. Moreover, the outcome of TKI therapy in patients with uncommon *BCR-ABL1* transcripts has not yet been defined. As previous reports suggested excellent efficacy of NIL in patients harboring atypical *BCR-ABL1* isoforms (30, 32), we wanted to employ this compound for the patient described in this manuscript. Indeed, we observed a rapid decline in the overall number of leukemic cells and Ph-positive metaphases, and the patient achieved a CHR, CCyR and MR^3 within 6 months of treatment.

According to the European Leukemia Net recommendations, 23% of CML patients discontinue 2G TKIs due to serious adverse events (33). In our case, NIL therapy was effective but the patient had to suspend the drug after developing a myocardial infarction. The shift to BOS was ineffective and, although the 3G TKI PON may induce cardiovascular events, the patient had no choice but to commence this drug that was promptly lowered to 15 mg/daily once he had shown initial signs of response. Currently, he continues PON with excellent clinical and molecular benefit.

In summary, the molecular characterization of atypical *BCR-ABL1* rearrangements is of pivotal importance to allow the correct diagnosis, the appropriate treatment and the timely monitoring of all CML patients. Hence, in case of a negative conventional multiplex RT-PCR, a reliable cytogenetic analysis is critical to identify the Ph chromosome and subsequently employ different primers to identify CML patients exhibiting rare transcripts.

We conclude that, in our experience, treatment with NIL and with low-dose PON may represent a highly effective therapy for CML patients with uncommon *BCR-ABL1* rearrangements like the e12a2 Ins/Del.

Therapeutic Implications

The reported patient received imatinib as first-line treatment of his chronic phase CML as was suggested in the 2006 ELN recommendations. However, he had to discontinue the drug due to his poor molecular response and the development of cutaneous toxicity. If he had been diagnosed in 2019, based on his intermediate ELTS score, his relatively young age and his lack of significant comorbidities, he would have probably been considered for a second-generation TKI in first line. When indeed he received such compounds in second line (dasatinib, followed by nilotinib and then by bosutinib) he experienced mixed results with TKIs targeting both ABL1 and SRC kinases (DAS and BOS) eliciting poor responses and an ABL-selective inhibitor (NIL) attaining a significant clinical benefit with a >6 year drug response. Unfortunately, the patient eventually developed a serious cardiovascular adverse event and was therefore switched to a full dose of ponatinib that promptly re-established disease control. Hence, after 1 month, PON was reduced to the safer 15 mg/daily dose that he continues to this day with excellent cytogenetic and molecular control of both his *BCR-ABL1* isoforms.

Informed Consent

Informed consent was received from the patient for the publication of the report. The patient gave his written consent to participate in the study, as specified in the Declaration of Helsinki.

Conflicts of Interest

The Authors declare that they have no competing interests regarding this study.

Authors' Contributions

SS (Stefania Stella), MM and ET designed and performed the experiments; SS (Stefania Stella), MM, ET, SRV, AP, MSP, SDG and CR analyzed and interpreted the data; SS (Stefania Stella) wrote the paper; VA, FDR, SS (Sergio Siragusa) and LM made a critical revision of paper; LM conceived the original idea and supervised the project.

References

1 Fujimaki K, Hattori Y and Nakajima H: 10-year complete remission in a philadelphia chromosome-positive acute lymphoblastic leukemia patient using imatinib without high-intensity chemotherapy or allogeneic stem cell transplantation. Int J Hematol *107(6)*: 709-711, 2018. PMID: 29188580. DOI: 10.1007/s12185-017-2382-2

2 Apperley JF: Chronic myeloid leukaemia. Lancet *385(9976)*: 1447-1459, 2015. PMID: 2548026. DOI: 10.1016/S0140-6736(13)62120-0

3 Massimino M, Consoli ML, Mesuraca M, Stagno F, Tirro E, Stella S, Pennisi MS, Romano C, Buffa P, Bond HM, Morrone G, Sciacca L, Di Raimondo F, Manzella L and Vigneri P: Irf5 is a target of bcr-abl kinase activity and reduces cml cell proliferation. Carcinogenesis *35(5)*: 1132-1143, 2014. PMID: 24445143. DOI: 10.1093/carcin/bgu013

4 Radujkovic A, Topaly J, Fruehauf S and Zeller WJ: Combination treatment of imatinib-sensitive and -resistant bcr-abl-positive cml cells with imatinib and farnesyltransferase inhibitors. Anticancer Res *26(3A)*: 2169-2177, 2006. PMID: 16827161.

5 Stagno F, Vigneri P, Del Fabro V, Stella S, Restuccia N, Giallongo C, Massimino M, Berretta S, Pennisi MS, Tibullo D, Tirro E, Buscarino C, Messina A and Di Raimondo F: Concomitant and feasible treatment with dasatinib and the anti-egfr antibody cetuximab plus radiotherapy in a cml patient with multiple squamous neoplasias. Acta Oncol *49(1)*: 109-110, 2010. PMID: 19842797. DOI: 10.3109/02841860903302913

6 Quintas-Cardama A and Cortes J: Molecular biology of bcr-abl1-positive chronic myeloid leukemia. Blood *113(8)*: 1619-1630, 2009. PMID: 18827185. DOI: 10.1182/blood-2008-03-144790

7 Preyer M, Vigneri P and Wang JY: Interplay between kinase domain autophosphorylation and f-actin binding domain in regulating imatinib sensitivity and nuclear import of bcr-abl. PLoS One *6(2)*: e17020, 2011. PMID: 21347248. DOI: 10.1371/journal.pone.0017020

8 Giallongo C, Parrinello N, Tibullo D, La Cava P, Romano A, Chiarenza A, Barbagallo I, Palumbo GA, Stagno F, Vigneri P and Di Raimondo F: Myeloid derived suppressor cells (mdscs) are increased and exert immunosuppressive activity together with polymorphonuclear leukocytes (pmns) in chronic myeloid leukemia patients. PLoS One *9(7)*: e101848, 2014. PMID: 25014230. DOI: 10.1371/journal.pone.0101848

9 Stella S, Tirro E, Conte E, Stagno F, Di Raimondo F, Manzella L and Vigneri P: Suppression of survivin induced by a bcr-abl/jak2/stat3 pathway sensitizes imatinib-resistant cml cells to different cytotoxic drugs. Mol Cancer Ther *12(6)*: 1085-1098, 2013. PMID: 23536723. DOI: 10.1158/1535-7163.MCT-12-0550

10 Manzella L, Tirro E, Pennisi MS, Massimino M, Stella S, Romano C, Vitale SR and Vigneri P: Roles of interferon regulatory factors in chronic myeloid leukemia. Curr Cancer Drug Targets *16(7)*: 594-605, 2016. PMID: 26728039.

11 Jinawath N, Norris-Kirby A, Smith BD, Gocke CD, Batista DA, Griffin CA and Murphy KM: A rare e14a3 (b3a3) bcr-abl fusion transcript in chronic myeloid leukemia: Diagnostic challenges in clinical laboratory practice. J Mol Diagn *11(4)*: 359-363, 2009. PMID: 19497989. DOI: 10.2353/jmoldx.2009.090008

12 Massimino M, Stella S, Tirro E, Consoli ML, Pennisi MS, Puma A, Vitale SR, Romano C, Zammit V, Stagno F, Di Raimondo F and Manzella L: Efficacy of dasatinib in a very elderly cml patient expressing a rare e13a3 bcr-abl1 fusion transcript: A case report. Anticancer Res *39(7)*: 3949-3954, 2019. PMI: 31262926. DOI: 10.21873/anticanres.13548

13 Tong YQ, Zhao ZJ, Liu B, Bao AY, Zheng HY, Gu J, Xia Y, McGrath M, Dovat S, Song CH and Li Y: New rapid method to detect bcr-abl fusion genes with multiplex rt-qpcr in one-tube at a time. Leuk Res *69:* 47-53, 2018. PMID: 29655153. DOI: 10.1016/j.leukres.2018.04.001

14 Qin YZ, Jiang Q, Jiang H, Lai YY, Shi HX, Chen WM, Yu L and Huang XJ: Prevalence and outcomes of uncommon bcr-abl1 fusion transcripts in patients with chronic myeloid leukaemia: Data from a single centre. Br J Haematol *182(5)*: 693-700, 2018. PMID: 29974949. DOI: 10.1111/bjh.15453

15 Hochhaus A, Larson RA, Guilhot F, Radich JP, Branford S, Hughes TP, Baccarani M, Deininger MW, Cervantes F, Fujihara S, Ortmann CE, Menssen HD, Kantarjian H, O'Brien SG, Druker BJ and Investigators I: Long-term outcomes of imatinib treatment for chronic myeloid leukemia. N Engl J Med *376(10)*: 917-927, 2017. PMID: 28273028. DOI: 10.1056/NEJMoa1609324

16 Stella S, Zammit V, Vitale SR, Pennisi MS, Massimino M, Tirro E, Forte S, Spitaleri A, Antolino A, Siracusa S, Accurso V, Mannina D, Impera S, Musolino C, Russo S, Malato A, Mineo G, Musso M, Porretto F, Martino B, Di Raimondo F, Manzella L, Vigneri P and Stagno F: Clinical implications of discordant early molecular responses in cml patients treated with imatinib. Int J Mol Sci *20(9)*, 2019. PMID: 31064152. DOI: 10.3390/ijms20092226

17 Stagno F, Stella S, Spitaleri A, Pennisi MS, Di Raimondo F and Vigneri P: Imatinib mesylate in chronic myeloid leukemia: Frontline treatment and long-term outcomes. Expert Rev Anticancer Ther *16(3)*: 273-278, 2016. PMID: 26852913. DOI: 10.1586/14737140.2016.1151356

18 Rosti G, Castagnetti F, Gugliotta G and Baccarani M: Tyrosine kinase inhibitors in chronic myeloid leukaemia: Which, when, for whom? Nat Rev Clin Oncol *14(3)*: 141-154, 2017. PMID: 27752053. DOI: 10.1038/nrclinonc.2016.139

19 Massimino M, Stella S, Tirro E, Romano C, Pennisi MS, Puma A, Manzella L, Zanghi A, Stagno F, Di Raimondo F and Vigneri P: Non abl-directed inhibitors as alternative treatment strategies

for chronic myeloid leukemia. Mol Cancer *17(1)*: 56, 2018. PMID: 29455672. DOI: 10.1186/s12943-018-0805-1

20 Agirre X, Roman-Gomez J, Vazquez I, Jimenez-Velasco A, Larrayoz MJ, Lahortiga I, Andreu EJ, Marquez J, Beltran de Heredia JM, Odero MD, Prosper F and Calasanz MJ: Coexistence of different clonal populations harboring the b3a2 (p210) and e1a2 (p190) bcr-abl1 fusion transcripts in chronic myelogenous leukemia resistant to imatinib. Cancer Genet Cytogenet *160(1)*: 22-26, 2005. PMID: 15949566. DOI: 10.1016/j.cancergencyto.2004.11.010

21 Stella S, Massimino M, Tirro E, Vitale SR, Scalise L, Leotta S, Pennisi MS, Puma A, Romano C, Stagno F, Sapienza G, Milone G and Manzella L: B-all relapses after autologous stem cell transplantation associated with a shift from e1a2 to e14a2 bcr-abl transcripts: A case report. Anticancer Res *39(1)*: 431-435, 2019. PMID: 30591491. DOI: 10.21873/anticanres.13130

22 Stagno F, Vigneri P, Consoli ML, Cupri A, Stella S, Tambe L, Massimino M, Manzella L and Di Raimondo F: Hyperdiploidy associated with a high bcr-abl transcript level may identify patients at risk of progression in chronic myeloid leukemia. Acta Haematol *127(1)*: 7-9, 2012. PMID: 21986290. DOI: 10.1159/000330607

23 Tirrò E, Massimino M, Stella S, Zammit V, Consoli ML, Pennisi MS, Vitale SR, Romano C, Pirosa MC, Martino E, Di Gregorio S, Puma A, Di Raimondo F, Manzella L and Stagno F: Efficacy of nilotinib in a cml patient expressing the three-way complex variant translocation t(2;9;22). Anticancer Res *39(7)*: 3893-3899, 2019. PMID: 31262918. DOI: 10.21873/anticanres.13540

24 Vigneri P, Stagno F, Stella S, Cupri A, Forte S, Massimino M, Antolino A, Siragusa S, Mannina D, Impera SS, Musolino C, Malato A, Mineo G, Tomaselli C, Murgano P, Musso M, Morabito F, Molica S, Martino B, Manzella L, Muller MC, Hochhaus A and Di Raimondo F: High bcr-abl/gus(is) levels at diagnosis of chronic phase cml are associated with unfavorable responses to standard-dose imatinib. Clin Cancer Res *23(23)*: 7189-7198, 2017. PMID: 28928163. DOI: 10.1158/1078-0432.CCR-17-0962

25 Sokal JE, Cox EB, Baccarani M, Tura S, Gomez GA, Robertson JE, Tso CY, Braun TJ, Clarkson BD and Cervantes F: Prognostic discrimination in “good-risk” chronic granulocytic leukemia. Blood *63(4)*: 789-799, 1984. PMID: 6584184.

26 Hasford J, Pfirrmann M, Hehlmann R, Allan NC, Baccarani M, Kluin-Nelemans JC, Alimena G, Steegmann JL and Ansari H: A new prognostic score for survival of patients with chronic myeloid leukemia treated with interferon alfa. Writing committee for the collaborative cml prognostic factors project group. J Natl Cancer Inst *90(11)*: 850-858, 1998. PMID: 9625174. DOI: 10.1093/jnci/90.11.850

27 Pfirrmann M, Baccarani M, Saussele S, Guilhot J, Cervantes F, Ossenkoppele G, Hoffmann VS, Castagnetti F, Hasford J, Hehlmann R and Simonsson B: Prognosis of long-term survival considering disease-specific death in patients with chronic myeloid leukemia. Leukemia *30(1)*: 48-56, 2016. PMID: 26416462. DOI: 10.1038/leu.2015.261

28 Pirosa MC, Leotta S, Cupri A, Stella S, Martino EA, Scalise L, Sapienza G, Calafiore V, Mauro E, Spadaro A, Vigneri P, Di Raimondo F and Milone G: Long-term molecular remission achieved by antibody anti-cd22 and ponatinib in a patient affected by ph’+ acute lymphoblastic leukemia relapsed after second allogeneic hematopoietic stem cell transplantation: A case report. Chemotherapy *63(4)*: 220-224, 2018. PMID: 30372691. DOI: 10.1159/000492941

29 Stella S, Tirrò E, Massimino M, Vitale SR, Russo S, Pennisi MS, Puma A, Romano C, S DIG, Innao V, Stagno F, Di Raimondo F, Musolino C and Manzella L: Successful management of a pregnant patient with chronic myeloid leukemia receiving standard dose imatinib. In Vivo *33(5)*: 1593-1598, 2019. PMID: 31471409. DOI: 10.21873/invivo.11641

30 Massimino M, Stella S, Tirro E, Consoli ML, Pennisi MS, Puma A, Vitale SR, Romano C, Zammit V, Stagno F, Di Raimondo F and Manzella L: Rapid decline of philadelphia-positive metaphases after nilotinib treatment in a cml patient expressing a rare e14a3 bcr-abl1 fusion transcript: A case report. Oncol Lett *18(3)*: 2648-2653, 2019. PMID: 31404304. DOI: 10.3892/ol.2019.10558

31 Chiarella P, Summa V, De Santis S, Signori E, Picardi E, Pesole G, Saglio G and Fazio VM: Bcr/abl1 fusion transcripts generated from alternative splicing: Implications for future targeted therapies in ph+ leukaemias. Curr Mol Med *12(5)*: 547-565, 2012. PMID: 22300134. DOI: 10.2174/156652412800619996

32 Liu B, Zhang W and Ma H: Complete cytogenetic response to nilotinib in a chronic myeloid leukemia case with a rare e13a3(b2a3) bcr-abl fusion transcript: A case report. Mol Med Rep *13(3)*: 2635-2638, 2016. PMID: 26847385. DOI: 10.3892/mmr.2016.4826

33 Baccarani M, Deininger MW, Rosti G, Hochhaus A, Soverini S, Apperley JF, Cervantes F, Clark RE, Cortes JE, Guilhot F, Hjorth-Hansen H, Hughes TP, Kantarjian HM, Kim DW, Larson RA, Lipton JH, Mahon FX, Martinelli G, Mayer J, Muller MC, Niederwieser D, Pane F, Radich JP, Rousselot P, Saglio G, Saussele S, Schiffer C, Silver R, Simonsson B, Steegmann JL, Goldman JM and Hehlmann R: European leukemianet recommendations for the management of chronic myeloid leukemia: 2013. Blood *122(6)*: 872-884, 2013. PMID: 23803709. DOI: 10.1182/blood-2013-05-501569

Received November 6, 2019
Revised November 14, 2019
Accepted November 19, 2019