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Short communication

Dasatinib treatment can overcome imatinib and nilotinib resistance in CML patient carrying F359I mutation of BCR-ABL oncogene

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Abstract. Point mutations of bcr-abl tyrosine kinase are the most frequent causes of imatinib resistance in chronic myeloid leukaemia (CML) patients. In most CML cases with BCR-ABL mutations leading to imatinib resistance the second generation of tyrosine kinase inhibitors (TKI- e.g. nilotinib or dasatinib) may be effective. Here, we report a case of a CML patient who during imatinib treatment did not obtain clinical and cytogenetic response within 12 months of therapy. The sequencing of BCR-ABL kinase domains was performed and revealed the presence of a F359I point mutation (TTC-to-ATC nucleotide change leading to Phe-to-Ile amino acid substitution). After 1 month of nilotinib therapy a rapid progression of clinical symptoms was observed. In the presence of the F359I point mutation only dasatinib treatment overcame imatinib and nilotinib resistance.

Keywords: BCR-ABL oncogene, chronic myeloid leukemia, direct sequencing, F359I point mutation, kinase inhibitors.

We report the case of a 57-year-old patient with CML treated with tyrosine kinase inhibitors (TKI) due to the failure of interferon-alpha therapy**.** Imatinib at 400mg daily was initiated in May 2004 and complete haematological response (CHR) was achieved within 3 months. In October 2005 the patient lost haematological response and an accelerated phase of CML was recognized. The cytogenetic analysis of bone marrow cells revealed 45% Ph-positive metaphases, while BCR-ABL/ABL^{IS} ratio was 100%. The dose of imatinib was increased to 600mg orally daily. CHR was accompanied with a minor cytogenetic response. Unfortunately, in March 2007 a relapse of the disease was recorded (WBC 22.0 × 10^9 L^{-1} (myeloblasts 0%), haemoglobin 5.15mmol L^{-1}, PLT 262.0 10^9 L^{-1}, splenomegaly 3 cm below the costal margin). A repeated cytogenetic analysis of bone marrow revealed the presence of 100% Ph-positive metaphases. Also the real-time quantitative polymerase chain reaction (RQ-PCR) showed the presence of the BCR-ABL transcript and BCR-ABL/ABL^{IS} ratio was 83%. Direct sequencing of BCR-ABL kinase domains, including the nucleotide binding loop (P-loop), the catalytic domain and the activation loop, displayed a F359I point mutation - TTC-to-ATC nucleotide change leading to Phe-to-Ile amino acid substitution (Figure 1). The decision to administer second generation thyrosine kinase inhibitors was made. After 1 month of nilotinib therapy a rapid progression of clinical symptoms was observed with leukocyte count over 100 G L^{-1}. Due to the treatment failure repeated analyses for the BCR-ABL mutations were done. Direct sequencing confirmed the presence of F359I only. Therefore, the decision on dasatinib administration was undertaken. Dasatinib therapy (at the dose of 140mg d^{-1}) was initiated in August 2007. The patient achieved a CHR after 3 months of treatment. Moreover, the gradual reduction in the number of BCR-ABL transcript copies was observed.

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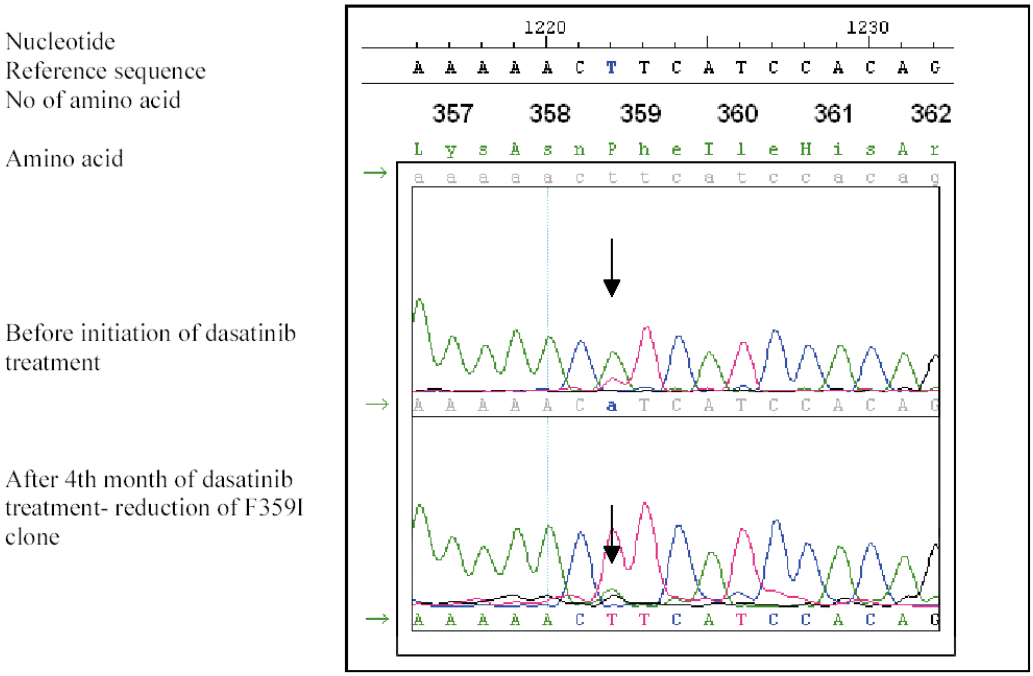

Figure 1. Patient E.K. Direct sequencing of BCR-ABL oncogene (nt 748-1140): TTC-to-ATC nucleotide change (nt1222) causes Phe-to-Ile amino acid substitution (F359I). Arrow indicated nucleotide substitution in F359I mutant clone (in green)

There is very limited information on the clinical outcome of CML patients carrying the F359I point mutation of the BCR-ABL oncogene. F359I is localized in the catalytic center of kinase and is associated with complete imatinib resistance (Talpaz et al. 2007). The F359 residue neighbors the activation loop and is critical for maintaining the particular inactive imatinib-bound conformation. F359 is also involved in van der Waals contacts with both imatinib and the activation loop in the imatinib-bound form (Tokarski et al. 2006). Both imatinib and nilotinib bind to inactive conformation of the Abl tyrosine kinase, with the P-loop folding over the ATP-binding site, and the activation-loop blocking the substrate binding site, to disrupt the ATP-phosphate-binding site and inhibit the catalytic activity of the enzyme (Weisberg et al. 2006).

In available publications other point mutations in the F359 position of the BCR-ABL oncogene were described. According to the literature data F359C and F359V are more frequent and F359D and F359A occur rarely. All these mutations caused imatinib resistance in CML patients treated with standard doses of drugs. In some cases imatinib dose escalation to 800mg daily may overcome resistance and improve treatment outcome (achieving complete hematologic response or major cytogenetic response) (Kantarijan et al. 2004). The data concerning the second TKI treatment results of patients carrying F359 mutations are limited. However, greater efficacy of alternative bcr-abl kinase inhibitors in CML patients with F359C and F359V mutations was confirmed (Guilhot et al. 2007; Kantarjian et al. 2007; Ray et al. 2007; Talpaz et al. 2007).

According to laboratory data mutations in the F359V/C/I position were moderately sensitive to nilotinib (IC^{50} <=200 nM, IC_{90} <=485 nM) (Jakubowska et al. 2006). Moreover, in vitro analysis showed that treatment with nilotinib can induce the F359I point mutation, which can be suppressed at clinically achievable concentrations of nilotinib (von Bubnoff et al. 2006). There are only limited data concerning in vivo efficacy of nilotinib in CML patients carrying the F359I mutation of the BCR-ABL oncogene (Ray et al. 2007). In the presented case the F359I mutation was associated with complete imatinib and nilotinib resistance. Therefore, our data confirm observations of those who reported resistance of the F359I mutant to imatinib and nilotinib (O'Hare et al. 2007).

Many more data confirm the efficiency of dasatinib (Sprycel, BMS-354825) in patients with the F359I point mutation (O'Hare et al. 2007). Dasatinib does not reside near the F359 residue, explaining its efficacy against BCR-ABL with mutations at this side. Alfonso Quintás-Cardama et al. (Quintás-Cardama et al. 2007) reported the

successful outcome of a patient carrying double mutations G250E+F359I, who was treated with dasatinib after a sequential failure of both imatinib and nilotinib. The presence of two mutations was identified prior to dasatinib therapy initiation. During therapy CHR was achieved. Re-sequencing of the BCR-ABL transcript showed the disappearance of F359I with G250E persistence, which may confirm the sensitivity of the F359I mutant to the drug. In the presented case, a reduction of the BCR-ABL F359I positive clone was noted after 3 months of the treatment, together with the confirmation of CHR. This was visible in the sequencing study of the BCR-ABL transcript (Figure 1).

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