Imatinib resistance and blast transformation of chronic myeloid leukemia associated with a novel tri-nucleotide insertion mutation of BCR-ABL kinase domain at position K294

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Dear Editor,

The introduction of tyrosine kinase inhibitors, in particular, imatinib mesylate (IM), has revolutionized the management of chronic myeloid leukemia (CML) and extended overall survival of patients who are treated early during the course of disease. The treatment is, however, occasionally complicated by the emergence of resistance that requires re-strategizing of treatment options. The most common mechanism of resistance is the acquisition of single nucleotide substitutions within the gene regions encoding for the kinase domain or regulatory regions of the abnormal BCR-ABL fusion protein [1, 2]. Activation of cryptic splice sites and frameshifts arising from insertion–deletion mutations of the gene has also been described [3–5]. Two cases of in-frame mutations arising from triplet nucleotide insertions within the ABL kinase domain have recently been reported [6, 7]. We now describe the third patient with IM-resistance transforming to lymphoid blast crisis of CML while harboring a tri-nucleotide insertion within the kinase domain.

The 18-year-old man presented in April 2008 with bleeding tendencies. His presenting white cell count was $319 \times 10^9/L$ with 3% peripheral blasts. Karyotyping confirmed the presence of $t(9;22)(q34;q11)$ consistent with diagnosis of CML, and the patient was promptly started on IM 400 mg daily. The patient, however, failed to achieve hematological remission despite drug compliance and was switched to nilotinib. No analysis for $BCR-ABL$ mutations was performed at that time as the patient was being treated in a peripheral center. He was subsequently referred to our center in July 2009 when his peripheral blood counts showed 53% blast and a bone marrow examination confirmed lymphoblastic transformation with CD45$^{dim}$, CD19$, CD10^+$, TdT$^+$, $c\mu^+$ blasts. Nilotinib was continued together with chemotherapy, but the patient failed to show an adequate response. Two further courses of chemotherapy were administered and allogeneic hematopoietic stem cell transplantation (HSCT) from his male sibling was performed in September 2010. Post-transplant recovery was uneventful and the patient is currently in complete cytogenetic remission with undetectable $BCR-ABL$ transcripts by quantitative RT-PCR.

Direct sequencing of the $BCR-ABL$ kinase domain prior to HSCT revealed the presence of a novel tri-nucleotide insertion after position K294 leading to the addition of histidine in between K294 and H295 with conservation of the open reading frame (Fig. 1). No other mutation within the kinase domain was identified. Sequencing of the normal $ABL$ kinase domain within somatic cells obtained from a buccal swab showed normal wild-type sequence confirming that the insertion was not a result of germline polymorphism.
The two previous reports of insertion mutations have also occurred adjacent to K294, the first involving a 12-nucleotide insertion between I293 and K294 [6], while the second was generated by two nucleotide mutations and an adjacent 6-nucleotide insertion resulting in a K294RGG [7]. K294 is positioned on the opposite face of the kinase domain to the IM binding site and makes SH3 contact by making a critical salt bridge to E98 of the SH3 domain [8]. SH3 negatively regulates the ABL kinase. Disruption of the SH3-kinase domain interaction in ABL by site-directed mutagenesis of K294 to glutamic acid leads to autophosphorylation and transforming activity [8]. Random mutagenesis analysis of K294R BCR-ABL expressing Ba/F3 cells, however, only shows weak resistance to IM [9]. All the three patients reported in the literature, including ours, have been resistant to IM and transformed to either biphenotypic or lymphoblastic leukemia. This suggests that amino acid substitutions at K294 are not adequately permissive for disruption of SH3 binding as compared to amino acid insertions which are likely to cause more severe destabilization of SH3 association and shift ABL towards the active kinase conformation that precludes IM drug binding. Both patients in the two previous reports were sensitive to dasatinib, while in our patient, nilotinib was ineffective, implying that only ABL inhibitors that are also capable of targeting the active conformational form, such as dasatinib, can effectively surmount the loss of inhibition resultant from destabilized SH3 interactions.

In summary, the three cases of in-frame insertion mutations at K294 described to date alludes to the importance of ABL kinase autoinhibition regulation by intramolecular interactions with SH3 domains. Destabilization of this autoinhibited state via disrupted SH3 contact may be an additional mechanism for IM resistance.

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References


Fig. 1 Upper frame shows nucleotide sequence trace of BCR-ABL kinase domain between K294 and P296 depicting the additional insertion of triplet nucleotides, coding for histidine at position 295. Amino acid residues are numbered according to the human c-Abl type la sequence. The lower panel shows the wild-type ABL sequence trace obtained from buccal smears of the same patient.
