Chronic Myeloid Leukemia

The role of the K247R substitution in the ABL tyrosine kinase domain in sensitivity to imatinib

Imatinib mesylate has become the gold standard front-line treatment of chronic myelogenous leukemia through its ability to inhibit ABL tyrosyne kinase. Resistance to this inhibition may occur. We investigated the role of the K247R polymorphism in persistent sensitivity.

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Because of its ability to inhibit ABL tyrosine kinase with minimal toxicity, imatinib mesylate became the gold standard for front-line therapy of chronic myelogenous leukemia (CML). Unfortunately, increasing numbers of patients become resistant and a mathematical model suggests that imatinib is an inhibitor of differentiated leukemic cells, but does not deplete the leukemic stem cell compartment.[1]

The major mechanism altering this inhibition is the emergence of ABL mutations which may be present at very low levels at diagnosis.[2] To date, more than 30 mutations have been identified and are responsible for various degrees of resistance and could (according to their location) be correlated to disease progression.[3] While our work was underway, Crossman *et al.*[4] reported the screen of CML patients and healthy donors, conducted in order to determine the allelic frequency of the K247R polymorphism previously described in a normal control.[5] They showed that three out of five CML patients with the K247R failed to achieve a major cytogenetic response to imatinib and suggested that the allele may result in reduced sensitivity. In this study, we investigated the status of the K247R ABL substitution identified in two CML patients.

These two patients were diagnosed with CML based on peripheral blood findings, karyotypic analysis (t(9;22)(q34;q11)) and on molecular analysis (BCR-ABL transcript) in 2000 and 1994 respectively. Patient #1, is a 54-year old male who was enrolled in the interferon + AraC arm, of the IRIS study,[6] in 2000. He achieved a complete hematologic remission at 3 months, and a complete cytogenetic response at 24 months. At the time of cytogenetic relapse (2 years later), and although he had never received imatinib, the *BCR-ABL* transcript sequences revealed the nucleotide exchange (A740G) leading to the K247R amino acid substitution. Despite that, imatinib was introduced in April 2005 and he achieved a complete hematologic response, a complete cytogenetic response and a good molecular response, quantified as previously described[7] (one BCR-ABL/ABL log reduction after 2 months of imatinib). Therefore, the K247R appears not to be a mutation leading to reduced sensitivity or resistance to imatinib. Patient #2 received imatinib for 82 months and had shown only a complete hematologic rsponse for 14 months, with no major cytogenetic response. Sequencing of the *BCR-ABL* transcripts revealed the K247R associated with a F317L mutation in the ABL protein. Retrospective investigation of the K247R during disease progression in these two patients showed that it accounted for 100% of the BCR-ABL transcripts at CML diagnosis in patient #1 and in all the further samples from both patients (no material was available from patient #2 at diagnosis). In order to show that the K247R was constitutional, we investigated its presence in a cell type other than leukemic cells. The substitution was found in 50% of the *ABL* gene from DNA extracted from the two patients' epithelial mouth cells (Figure 1). For patient #2, the F317L, accounting for 100% of the BCR-ABL in the leukemic clone, was not present in epithelial cells (*data not shown*), strongly suggesting that (i) the K247R is indeed a polymorphism and (ii) the imatinib resistance observed in patient #2 was probably subsequent to the additional F317L mutation, known to induce a strong resistance to imatinib.[8] To demonstrate that K247R was a polymorphism, 255 unrelated individuals including 133 with CML, 76 with acute myeloid leukemia and 46 healthy donors were screened for the polymorphism. We could not detect the K247R in any of the acute myeloid leukemia samples but one healthy donor was positive in a heterozygous state from DNA

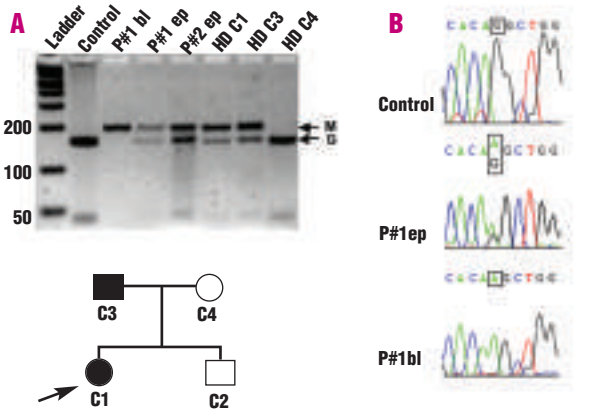

Figure 1. cDNA and DNA polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) analysis and primary sequence data for the Abl K247R polymorphism. (A) Upper: the K247R mutation was investigated by studying the loss of the Alu I restriction enzyme site (New England Biolabs, Beverly, MA, USA). Corresponding RFLP pattern on 4% ethidium-bromide–stained Nusieve 3:1 agarose gel after complete Alu I digestion. For CML patients, in order to amplify only the *BCR-ABL* fusion transcript (and not the normal *ABL* transcript), a first round of PCR was performed on cDNA using a forward specific primer for the BCR gene and a reverse primer, A9, in exon 9 of the *ABL* gene, as described previously.[9] A part of ABL exon 4 was amplified by PCR from one fiftieth of the first *BCR-ABL* PCR product for CML blood samples, from 25 ng DNA extracted from AML and healthy donor blood samples (High Pure PCR Template Preparation Kit, Roche Diagnosis, Mannheim, Germany) or directly from buccal epithelial cells from patients #1 and #2. The forward primer was 5'-GAGTTGGTTCATCATCATTC-3', and the reverse primer was 5'-GAGGTGTACGAGGGCGTGTGG-3'; the thermocycling conditions have been previously described.[9] RFLP patterns were obtained from patient #1, patient #2 and a healthy donor; bl: blood, ep: epithelial cell, M: mutated band, G: germinal band, HD C1: K247R positive HD. Bottom : A part of the pedigree of the HD C1 family carrier of the K247R (black arrow). C2: his brother, C4: his mother, C3: his father, a carrier of the K247R (in black). (B) The mutation was confirmed by direct sequencing. Upper reading frame: wild-type sequence. Middle reading frame: DNA from the epithelial cells of patient #1. Lower reading frame: mutated sequence from patient #1 *BCR-ABL* transcript at diagnosis. Black indicates guanine (G); blue, cytosine (C); red, thymydine (T); green, adenosine (A).

extracted from blood (Figure 1). Analysis of this subject's parents showed that the K247R had been inherited from his father (Figure 1). In view of these results, we can conclude that (i) the K247R is not a mutation leading to imatinib resistance and moreover, seems to have no impact on the sensitivity of the disease to imatinib (ii) the K247R is indeed a rare polymorphism, since it is also present in other cell types and in healthy donors, and it has a hereditary transmission. The incidence of its allele reaches nearly 0.8%, as Crossman *et al.*[4] described if all the K247R positive individuals are added. Furthermore, this substitution involves two basic aminoacids and modification of the protein, even if it is close to the P-loop, should not have any functional impact as was suggested by the good response to imatinib of patient #1. Each new ABL mutation found in imatinib-resistant patients should be carefully studied especially when the amino-acids involved are closely related. In conclusion, we strongly suggest that K247R is not a mutation requiring a modification of therapeutic strategy.

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