

Original article

Mutations in the breakpoint cluster region-Abelson murine leukemia 1 gene in Brazilian patients with chronic myeloid leukemia



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ABSTRACT

Introduction: Mutations in the *breakpoint cluster region-Abelson murine leukemia 1* gene are the leading cause of resistance to treatment with tyrosine kinase inhibitors in chronic myeloid leukemia patients. Mutations have been detected throughout the extension of the kinase domain of this gene and it is important to investigate their positions because there may be a difference in clinical relevance.

Objective: To evaluate mutations in the transcripts of the BCR-ABL1 gene in Brazilian patients with chronic myeloid leukemia under tyrosine kinase inhibitor treatment in the Hospital de Clínicas of the Universidade Federal do Paraná.

Methods: This retrospective observational cross-sectional study analyzed mutation data of BCR-ABL1 gene transcripts. Three hundred and thirty peripheral blood samples from 193 patients were evaluated with the search for mutations being achieved by Sanger sequencing.

Results: Sixteen mutation types were identified in 48/193 (24.87%) patients with T315I (20.83%) being the most common. Furthermore, four polymorphisms (T240T, K247R, E275E and Y275Y) were identified. The highest incidence of mutations (19/53: 35.85%) occurred in the P-loop of the tyrosine kinase domain, whereas no mutation was found in the A-loop. In 43/48 (89.58%) patients only one mutation was found and more than one mutation was found in 5/48 (10.42%). The simultaneous presence of two mutations (E189G/V299L and E255K/T315I) was observed in 2/5 patients while the different mutations were seen in sequential samples of the other three patients (Y253Y/T315I, T315I/E255K and E255K/T315I).

Conclusions: This molecular characterization contributed to the identification of the resistance profile to tyrosine kinase inhibitors in Brazilian patients, thus enabling the use of adequate therapeutic strategies in a timely manner.

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Introduction

Point mutations in the kinase domain of the p210^{bcr-abl1} protein, the main mechanism of secondary resistance to tyrosine kinase inhibitors (TKI), interfere in the binding of the drug to the target protein leading to the reactivation of kinase activity.^{1,2} This domain is divided in four regions: P-loop (amino acids 248–255), TKI binding site (amino acids 315–317), catalytic domain (amino acids 350–363) and A-loop (amino acids 381–402).³ Depending on the mutation position, different drug resistance levels are observed, requiring changes in the therapeutic strategy.⁴ Some studies have reported a high incidence of mutations in the P-loop.^{3,5–8}

Imatinib mesylate (IM) (Gleevec; STI571), the first tyrosine kinase inhibitor developed, revolutionized the treatment of chronic myeloid leukemia (CML). However, some patients do not respond to this therapy. Second-generation inhibitors, such as Nilotinib (Tasigna; AMN107) and Dasatinib (Sprycel; BMS-354825), can overcome most of the IM-resistant mutations.⁴ Among the more than 100 types of mutations in the tyrosine kinase domain, T315I is the only one that has total resistance to first- and second-generation inhibitors and is reported as the most common in several studies.^{3,8–13} A third-generation inhibitor, Ponatinib (AP24534), approved by the US Food and Drug Administration (FDA),¹⁴ shows significant activity in the presence of the T315I mutation.^{15,16} The therapeutic strategy for patients who do not have a satisfactory response to the available TKI is hematopoietic stem cell transplantation (HSCT).^{12,13,17,18}

Objective

This study aimed at investigating the location and frequency of point mutations in the transcripts of the *breakpoint cluster region-Abelson murine leukemia 1* (BCR-ABL1) gene in patients with CML under tyrosine kinase inhibitor treatment in the Hospital de Clínicas of the Universidade Federal do Paraná (HC-UFPR).

Methods

This study investigated mutations in the tyrosine kinase domain of the BCR-ABL1 gene in 330 samples from 193 Brazilian patients with CML who underwent TKI therapy. One hundred and nineteen patients (61.7%) were males and 74 (38.3%) were females. Patients were attended in the HC-UFPR between August 2005 and April 2017 with the analyses of BCR-ABL1 mutations being performed in the Molecular Biology for Onco-Hematology Diseases/Immunogenetics Laboratory of the same institution. Brazilian patients with CML included in the study were over 18 years of age, undergoing TKI treatment and met the European LeukemiaNet (ELN) criteria for mutation search.¹⁹ This study was carried out by a retrospective cross-sectional analysis after being approved by the Research Ethics Committee of the HC-UFPR.

Peripheral blood collected in ethylenediaminetetraacetic acid (EDTA) was treated with red blood lysis buffer followed by stabilization and extraction of RNA according to Brand-

ford et al.²⁰ Complementary DNA was synthesized from total RNA by reverse transcription as described by Cross et al.²¹ The identification of BCR-ABL1 mutations was achieved by Sanger sequencing using a forward oligonucleotide on exon b2 of the BCR gene and a reverse oligonucleotide on exon 10 of the ABL1 gene according to Brandford and Hughes.²² Polymerase chain reaction (PCR) was carried out in an ABI PRISM 9700 thermocycler (Applied Biosystems/Thermo Fisher, USA) using the Expand Long Template PCR system (Roche Diagnostics, Germany) under the following conditions: 94 °C for 2 min; 10 cycles at 94 °C for 10 s, 60 °C for 30 s and 68 °C for 2 min; 20 cycles at 94 °C for 10 s, 60 °C for 30 s and 68 °C (the first cycle for 2 min with each subsequent cycle increasing by 20 s) and one final cycle at 68 °C for 7 min. PCR products were evaluated by electrophoresis on 2% agarose gel. Semi-nested PCR reactions, under the same conditions, were performed for samples that did not show amplification.

PCR products were purified with Exosap-It (USB Affymetrix Inc., USA) according to the manufacturer's recommendations. Direct forward and reverse sequencing reactions using Big Dye 3.1 Terminator Chemistry (Applied Biosystems/Thermo Fisher, USA) were performed under the following conditions: 96 °C for 10 s, and 25 cycles at 50 °C for 5 s and at 4 °C for 10 min in an ABI PRISM 9700 thermal cycler. The products of the sequencing reactions were separated by capillary electrophoresis in an ABI 3130 Genetic Analyzer (Applied Biosystems/Thermo Fisher, USA) with the results being analyzed using the Mutation Surveyor software (SoftGenetics, USA). The sample nucleotide sequences were compared to the GenBank accession number X16416.

Results

Mutations were identified in 66/330 (20%) samples from 48/193 (24.87%) patients, with 53 mutations of 16 different types being observed in this group (Fig. 1). Of these, only one mutation was found in 43/48 (89.58%) patients and two mutations were observed in five (10.42%). The different types of mutations and their respective frequencies are shown in Fig. 2. The region of the kinase domain of the p210^{bcr-abl1} protein with the highest incidence of mutations was the P-loop (19/53; 35.85%), followed by the TKI binding site (11/53; 20.75%), the catalytic domain (10/53; 18.87%) and other areas of this domain (12/53; 22.64%). No mutation was observed in the A-loop, and the E189G mutation, located upstream of the kinase domain, was seen only once (1.89%).

No mutation was detected in 80% (264/330) of the samples. Among these negative samples, four polymorphisms were identified in 8/264 samples (3.03%) that came from 5/145 patients: T240T (0.69%), K257R (1.38%), E275E (0.69%) and Y253Y (0.69%). The T240T is located upstream of the kinase domain of the p210^{bcr-abl1} protein, and the other three are located within this domain (Fig. 1).

Discussion

This retrospective study revealed the profile of mutations in the transcripts of the BCR-ABL1 gene in Brazilian patients with CML, who were being treated with TKIs in the HC-UFPR.

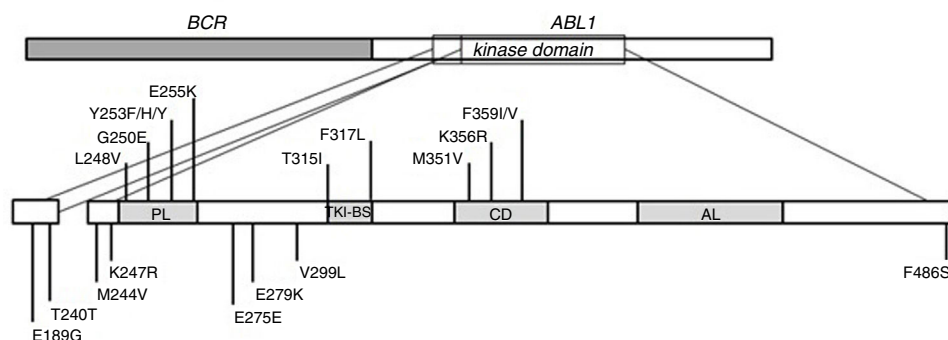


Fig. 1 – Identified mutations and polymorphisms according to their location in the tyrosine kinase domain and adjacent regions.

PL: P-loop; TKI-BS: tyrosine kinase inhibitor binding site; CD: catalytic domain; AL: A-loop.

Adapted from Srivastava and Dutt.²³

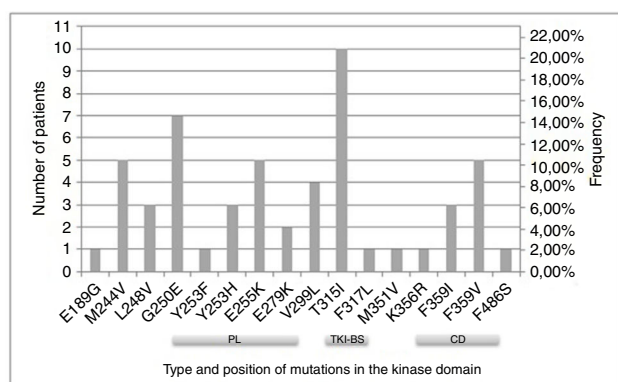


Fig. 2 – Frequency and location of identified mutations.

PL: P-loop; TKI-BS: tyrosine kinase inhibitor binding site; CD: catalytic domain.

Direct DNA sequencing was used to identify these point mutations with the primer oligonucleotides allowing an analysis of the entire extent of the kinase domain, as well as adjacent upstream and downstream regions. The design of the primers allowed the detection of the E189G mutation and the T240T polymorphism, both located outside the kinase domain.

This investigation of the genetic variability of the tyrosine kinase domain and adjacent regions revealed 16 types of mutations and four polymorphisms (T240T, K247R, E275E and Y275Y). The K247R was the most common polymorphism (1.38%) in this study as well as in the study of Crossman et al.²⁴ where 3.18% of the individuals were affected. On the other hand, the K247R was not detected in the study of Hochhaus et al.²⁵ who reported the E499E as being the most common polymorphism (6.3%).

The most frequently found mutation was T315I (10/48; 20.83% patients); this finding is in agreement with published Latin American data³ as well as other studies that evaluated patients from different geographic locations.^{8–13} However, some studies did not find that this mutation was the most common^{5,7} and one did not find this mutation at all.⁶ The

reason for this discrepancy among the results is still unclear, but Jabbour et al.⁷ argued that the increase in the frequency of T315I may have occurred due to the use of TKIs to which other mutations are sensitive or to the inclusion of patients at different stages of the disease.⁷

The T315I mutation is of great clinical relevance because it confers full resistance to first and second generation inhibitors with this effect being associated with poor prognosis.¹² Resistance to TKIs occurs due to the substitution of the amino acid threonine with isoleucine at position 315 of the p210^{bcr-abl1} protein. This mutation eliminates the critical interaction site with the target drug, as the presence of isoleucine in this position makes the formation of hydrogen bonds with TKIs impossible due to steric interference.²⁶

Inside the p210^{bcr-abl1} region, the P-loop was the one with the highest incidence of mutations (19/53: 35.85%) corroborating the findings of Branford et al.,⁵ Soverini et al.,⁶ Jabbour et al.,⁷ Nicolini et al.⁸ and Pagnano et al.³ The frequency of mutations in other regions, such as the inhibitor binding site (20.75%), catalytic domain (18.87%) and other areas of the kinase domain (24.53%) are in agreement with Nicolini et al.⁸ and Pagnano et al.³ except in the A loop, where these authors found 4% and 5.2% of mutations, respectively, while no mutations were detected in the present study. This contrast may reflect differences in the sample composition or the sample size.

More than one mutation type was identified in five (10.42%) patients. The simultaneous presence of two mutations (E189G/V299L and E255K/T315I) was observed in two out of five patients (4.17%); this finding may be explained by clonal evolution of the disease. It was not possible to differentiate between compound and polyclonal mutations since no cloning techniques were employed. Khorashad et al.²⁷ define compound mutations as two or more mutations occurring in the same sample and in the same BCR-ABL1 molecule while polyclonal are two or more mutations in the same sample but in distinct BCR-ABL1 molecules. According to the literature, the incidence of compound mutations occurs in more than 70% of the cases.²⁷ Information on the sensitivity/resistance of most mutants already identified is widespread. However,

in the presence of simultaneous mutations this rule does not seem to apply because it dramatically affects the sensitivity to TKIs and the catalytic capacity of the tyrosine kinase.²⁸ The other three (6.12%) patients showed different mutations in their sequential samples (Y253Y/T315I, T315I/E255K and E255K/T315I), but T315I was always present as one of them.

Conclusion

This study demonstrated that the highest incidence of mutations occurs in the P-loop of the p210^{bcr/abl1} protein. Also it shows that T315I, associated with the absence of response to first and second generation TKIs, was the most common mutation found, which is in agreement with most of the other populations reported. The detection of mutations that confer resistance to TKI is crucial for the definition of new therapeutic strategies in order to optimize treatment response.

Conflicts of interest

The authors declare no conflicts of interest.

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