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Original article

Molecular response to imatinib mesylate of Brazilian patients with chronic myeloid leukemia

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ABSTRACT

Background: Imatinib mesylate has revolutionized the treatment of chronic myeloid leukemia leading to significant reductions of *BCR-ABL1* transcript levels in peripheral blood.
Objective: To evaluate the response to imatinib mesylate treatment (400 mg/day) in Brazilian patients in the chronic phase of chronic myeloid leukemia monitored by quantitative real time polymerase chain reaction.
Methods: Between October 2002 and October 2010, 3169 peripheral blood samples were collected from 1403 patients from 3 to 5 months, 6 to 11 months, 12 to 17 months, 18 to 23 months and ≥24 months after beginning imatinib treatment. Eighty-two patients had samples available and analyzed for all time intervals. *BCR-ABL1* quantification was performed by quantitative real time polymerase chain reaction using the *ABL1* gene as the control. Results of the *BCR-ABL1* ratio as a percentage were reported by the international scale (IS) using the laboratory conversion factor (0.51).
Results: In the first interval, 80.8% of patients achieved the optimal response (*BCR-ABL1*^{IS} ≤ 10%). In the second period, 69.1% achieved optimal response (*BCR-ABL1*^{IS} ≤ 1%) and, between 12 and 17 months, 47.3% achieved major molecular response (*BCR-ABL1*^{IS} ≤ 0.1%).
Conclusions: The results of this retrospective study show that the response to imatinib treatment (400 mg/day) of Brazilian patients in the chronic phase of chronic myeloid leukemia is within the expected profile when compared to patients reported in international prospective randomized studies.

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Introduction

The use of imatinib mesylate (IM), a first generation tyrosine kinase inhibitor, has revolutionized the treatment of chronic myeloid leukemia (CML) leading to a significant reduction of the *breakpoint cluster region-Abelson murine leukemia 1* (*BCR-ABL1*) transcript levels in peripheral blood.[1,2] The International randomized study of interferon vs. STI571 (IRIS) study showed that IM is able to reduce the amount of leukemic cells quickly and in large proportions in most patients with this disease.[1–6] The intensity and speed of response to treatment are prognostic and treatment planning parameters. The hematological, cytogenetic and molecular responses, the latter based on the number of *BCR-ABL1* transcripts in peripheral blood, are used to quantify the level of reduction of leukemic cells during therapy.[5,6] By analyzing the IRIS study data, the European Leukemia Network (ELN) established response goals to be achieved in different intervals of drug exposure, particularly in the chronic phase of CML.[5] Achieving major molecular response (MMR), defined as *BCR-ABL1* transcripts $\leq$0.1%, is the goal of treatment with IM due to the association between this level of response and the higher likelihood of disease-free progression.[7,8]

The lack of data on the molecular response to treatment with IM in Brazilian patients and the availability of a large number of samples tested by quantitative real time polymerase chain reaction (Q-PCR) in the Immunogenetics Laboratory of Hospital de Clinicas, Universidade Federal do Paraná (HC-UFPR) motivated us to evaluate the results in this population to indirectly demonstrate the response of Brazilian patients to this drug in different time intervals after the beginning of therapy.

Methods

The Immunogenetics Laboratory at HC-UFPR received samples from 26 Brazilian centers (23 public institutions) from October 2002 to October 2010. A total of 3169 samples were collected from 1403 patients from 3 to 5 months, 6 to 11 months, 12 to 17 months, 18 to 23 months and $\geq$24 months after the initiation of IM treatment. These samples were selected because they met the inclusion criteria for this study: Brazilian patients aged $\geq$18 years in the chronic phase of CML under treatment using IM (400 mg/day) and being monitored by Q-PCR. Among the 1403 patients, only 82 had samples available for all time intervals. This study was approved by the Ethics Committee of HC-UFPR.

RNA stabilization and extraction

Sixteen to 20 mL of peripheral blood collected in ethylenediaminetetraacetic acid (EDTA) was treated with red blood cell lysis buffer (0.144 M of NH_4Cl and 0.01 M of NH_4HCO_3) within 24 h of collection.[9] For samples processed until September 2006, RNA from 1×10^7 leukocytes was stabilized using GTC solution (4M guanidine thiocyanate, 5 mM EDTA, 0.5% n-laurilsarcosil, 25 mM sodium citrate, pH 7.0) with 7.1% β-mercaptoethanol and extracted with RNeasy Mini™ Kits (Qiagen, UK) according to the manufacturer's instructions.[10,11]

After September 2006, RNA from 1×10^7 leukocytes was stabilized using Trizol® (Invitrogen, USA) and isolated with isopropanol, chloroform and ethanol according to the manufacturer's instructions. Complementary DNA was synthesized from total RNA using the enzyme, Moloney murine leukemia virus (M-MLV) reverse transcriptase (Invitrogen, USA) and randomized hexamer as previously described.[11]

Identification of the type of BCR-ABL1 transcript

The type of *BCR-ABL1* transcript was identified by multiplex polymerase chain reaction (PCR) as optimized by Cross et al.,[10] except the ice bath was replaced for Platinum *Taq DNA polymerase* (Invitrogen, USA). The samples with negative results by multiplex PCR were amplified by nested PCR as previously described.[11]

Quantification of BCR-ABL1 transcripts

The transcripts of all samples were quantified in duplicate by Q-PCR (ABIPRISM 7500, Life Technologies, USA) using the hydrolysis TaqMan™ probe system.[12] The copy numbers of *BCR-ABL1* and the control gene *ABL1* were calculated by comparing the results with a standard curve based on serial dilutions of the linearized plasmid with the *BCR-ABL1* insert (pNC210/G) engineered by Cross et al.[13]

Results are reported as a ratio (%) of the *BCR-ABL1* to *ABL1* copy numbers (BCR-ABL1/ABL1 $\times$ 100). The ratio was multiplied by the conversion factor (CF) to report values in the international scale (IS). The conversion factor of the Immunogenetics Laboratory at HC-UFPR is 0.51, which was determined by comparing the results of *BCR-ABL1* transcript quantification in 30 samples analyzed in both the HC-UFPR laboratory and the reference laboratory at the Institute of Medical and Veterinary Science, Adelaide, Australia. The CF value was confirmed using a second set of 30 samples from the same reference laboratory. Samples were considered acceptable for analysis when the number of *ABL1* copies was $\geq$10.000. Nested PCR was performed on all samples that had no transcripts detected by Q-PCR in order to confirm the results.

Statistical analysis

Descriptive statistical analysis was performed for the general parameters of the sample: number of samples per patient, type of transcript, age at diagnosis and gender.

Results

Patients' demographics (n = 1403) are detailed in Table 1. The age at diagnosis, gender, stage of disease, treatment and IM dose were obtained from the request form sent with the samples to the Immunogenetics Laboratory at HC-UFPR.

The number of samples per patient ranged from 1 to 5 (median = 2). Samples were grouped into five time intervals close to those set by the ELN to assess response to IM treatment (Table 2); the responses of patients in each interval are shown in Table 3.

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