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Positive response to imatinib mesylate therapy for childhood chronic myeloid leukemia

G.A.P. Oliveira^{1,2}, E.S. Costa³, M.S. Freitas², F.F. Dutra¹, S.F. Maia³, M.C. Guerra³, M.D. Taberner⁴, R. Borojevic¹, I.B. Otazu^{1,5} and J.L. Silva²

¹Hospital Universitário Clementino Fraga Filho and Departamento de Histologia e Embriologia, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brasil

²Programa de Biologia Estrutural, Instituto de Bioquímica Médica, Centro Nacional de Ressonância Magnética Nuclear Jiri Jonas, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brasil

³Instituto de Pediatria e Puericultura Martagão Gesteira/IPPMG and Departamento de Clínica Médica, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brasil

⁴IECSCYL, Unidad de Investigacion, Hospital Universitario de Salamanca, Salamanca, Spain

⁵Tawam Hospital, Al Ain, Abu Dhabi, United Arab Emirates (UAE)

Abstract

Chronic myeloid leukemia (CML) is rare in the pediatric population, accounting for 2-3% of childhood leukemia cases, with an annual incidence of one case per million children. The low toxicity profile of imatinib mesylate has led to its approval as a front-line therapy in children for whom interferon treatment has failed or who have relapsed after allogeneic transplantation. We describe the positive responses of 2 children (case 1 - from a 7-year-old male since May 2005; case 2 - from a 5-year-old female since June 2006) with Philadelphia-positive chromosome CML treated with imatinib (300 mg/day, orally) for up to 28 months, as evaluated by morphological, cytogenetic, and molecular approaches. Our patients are alive, are in the chronic phase, and are in continuous morphological complete remission.

Key words: Chronic myeloid leukemia; Imatinib mesylate; Minimal residual disease; Children

Introduction

Chronic myeloid leukemia (CML) is a rare childhood condition. The only known curative therapy for these children is allogeneic stem cell transplantation (SCT). When there are no matched donors for SCT, therapy with alpha-interferon (α -IFN) either alone or in combination with cytarabine results in a complete cytogenetic response (CCR) (1), with an outcome similar to that seen in adults (2). Recently, therapy with imatinib mesylate was found to be more effective in treating newly diagnosed adults in the chronic phase (CML-CP) than α -IFN, leading to complete hematological remission and CCR in almost all cases (3). However, detection of kinase domain mutations by direct sequencing techniques was almost always associated with imatinib resistance and a poor prognosis (4,5). To date, few studies have reported on the effects of imatinib in childhood Philadelphia-positive chromosome (Ph⁺) CML (6,7). This study reports on two cases of children with Ph⁺ BCR/ABL⁺ CML followed for up to 28 months after treatment with

imatinib. The patients showed favorable clinical and hematological responses, as demonstrated by the trends observed using interphase fluorescence *in situ* hybridization (iFISH), RT-PCR, and sequencing.

Patient presentation

All samples were obtained at the Pediatric Institute Martagão Gesteira/IPPMG from Federal University of Rio de Janeiro, Brazil. The persons responsible for the patients signed an informed consent to a research protocol that had been reviewed and approved by the Pediatric Institute Martagão Gesteira/IPPMG Ethical Committee (No. 44/07).

Case 1

In May 2005, a 7-year-old boy with splenomegaly was admitted to our hospital. His white blood count (WBC) was

Correspondence: J.L. Silva, Programa de Biologia Estrutural, Instituto de Bioquímica Médica, Centro Nacional de Ressonância Magnética Nuclear Jiri Jonas, UFRJ, 21941-590 Rio de Janeiro, RJ, Brasil. E-mail: jerson@bioqmed.ufrj.br

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$99.7 \times 10^9/L$ with granulocytosis and immature precursors. His hemoglobin level was 11.9 g/dL, and his platelet count was $350 \times 10^9/L$. Thorax radiography was normal. Abdominal ultrasonography showed splenomegaly (11.6 cm). Bone marrow evaluation revealed hypercellularity consistent with CML-CP. The karyotype was 46,XY,t(9;22)(q34;q11) in 20 metaphases. Conventional cytogenetics iFISH, which permits the distinction between Mbc and mbc gene rearrangements (8), revealed one clone (88.5%) with Mbc/abl gene rearrangements. The occurrence of Mbc (b3a2) instead of mbc rearrangements was confirmed by multiplex (9) and nested-PCR (Figure 1A). In addition, *bcr-abl/abl* ratios (10) showed high levels (up to a 100% increase) in the tumor (Figure 1B). Initially, hydroxyurea cyto-reduction (1 g/day) resulted in a decrease of WBC to $4.6 \times 10^9/L$. α -IFN treatment (5×10^6 U/day) was then started. High and persistent percentages of Mbc/abl gene rearrangements were detected by iFISH (up to 74%). During the 18th week of treatment, an abrupt increase of WBC to $247 \times 10^9/L$ occurred. With no matched donors for SCT, α -IFN was changed to imatinib, 300 mg/day, and the patient achieved normal levels of WBC without any adverse side effects (Figure 1C). Concomitantly, the response to imatinib was monitored with different cytomolecular assays (pre-imatinib and 1, 5, 8, 12, 16, 19, 22, 25, and 28 months after imatinib). A favorable decrease to <5% of cells with Mbc/abl gene rearrangement was observed by iFISH after 5 months of imatinib, coexisting with *bcr-abl/abl* levels below 10% and presenting b3a2 isoform (Figure 1A and B). Nested PCR still detected a residual *bcr-abl* transcript 28 months after the beginning of therapy. Nevertheless, iFISH no longer detected the Mbc/abl gene rearrangement after 19 months of therapy. Finally, direct sequencing of products was performed using 5 healthy individuals as controls (Figure 2). We did not detect any point substitutions or conflicting sites in any samples from this patient. Nested RT-PCR was first performed in the control samples, and negative *bcr-abl* findings were demonstrated. In fact, there was a clear positive concordance between a favorable clinical-hematological remission and an observed trend towards cytomolecular procedures.

Case 2

In June 2006, a 5-year-old girl was admitted with bone pain. WBC was $155 \times 10^9/L$ with granulocytosis and immature precursors. Hemoglobin was 10.8 g/dL, and platelet count was $559 \times 10^9/L$. Thorax radiography and abdominal ultrasonography were normal. Bone marrow evaluation revealed hypercellularity consistent with CML-CP. The karyotype showed a t(9;22)(q34;q11) translocation coinciding with 89% Mbc/abl hybridization by iFISH and b2a2 fusion transcripts by multiplex-PCR. Initially, hydroxyurea cyto-reduction (0.5 g/day) decreased the WBC count to $4.6 \times 10^9/L$. With no matched donors for SCT, the patient was treated with α -IFN, 5×10^6 U/day. On the 8th day of treat-

ment, the patient presented fever, monoarthritis, epistaxis, thrombocytopenia, and megakaryocytic hyperplasia in bone marrow. α -IFN therapy was changed to prednisone. The platelet count recovered, and the symptoms disappeared. Mbc/abl gene rearrangements decreased to 48% by iFISH, coexisting with a b2a2 transcript as determined by multiplex-PCR (Figure 1A). Imatinib was then initiated (300 mg/day), and hematological remission was maintained (Figure 1C). No side effects were observed, except for an evanescent cutaneous rash. Cytomolecular results were analyzed before and 6, 9, 12, 15, and 20 months after imatinib. Similarly to the first case, while residual b2a2 transcripts remained as determined by nested PCR, iFISH results were negative starting at 15 months after therapy. Other than this finding, we did not observe any substitution or conflicting sites related to resistance in two samples studied before imatinib and after 20 months therapy with the drug. In fact, the patient remains in clinical/hematological remission.

Discussion

While many adult cases of Ph+ CML have been successfully treated with imatinib (11), it remains unclear whether imatinib should be used as a front-line treatment for children with Ph+ CML. Nevertheless, a phase I study recently reported that imatinib was well tolerated by Ph+ children at doses ranging from 260 to 570 mg/m² (6). However, 60% of subjects in a phase II study with children in cryptic CP achieved CCR, while only 27% achieved molecular remission (7). Our current report shows that 2 children with Ph+ CML had a good response to imatinib. In both patients, an obvious association was found between a favorable clinical-hematological outcome and cytomolecular remission. While RT-PCR still detected residual BCR-ABL+ cells, they were not detected by iFISH 19 months (case 1) and 15 months (case 2) after the beginning of treatment. Patients with major clinical remission usually exhibit residual BCR-ABL by RT-PCR after imatinib treatment applied after the failure of α -IFN protocols and the presence of residual BCR-ABL has been associated with leukemic relapse (12). However, this was apparently not the case for the patients reported here.

In fact, the various methods and hematologic data described in this paper are well-defined tools for residual disease assessment (11). The simultaneous use of these assays is of great relevance for front-line monitoring of imatinib-based treatments (4,11,12). We have already analyzed drug resistance due to kinase mutations by sequencing and could not correlate the presence of imatinib resistance in either patient, unlike results reported in other studies (4). As expected, the positive drug response seen by iFISH evaluation was confirmed by the sequenced regions, in which the alignment of different donors and the patients did not show any point substitutions at least up to 28 months into imatinib treatment. This is good evidence that patients

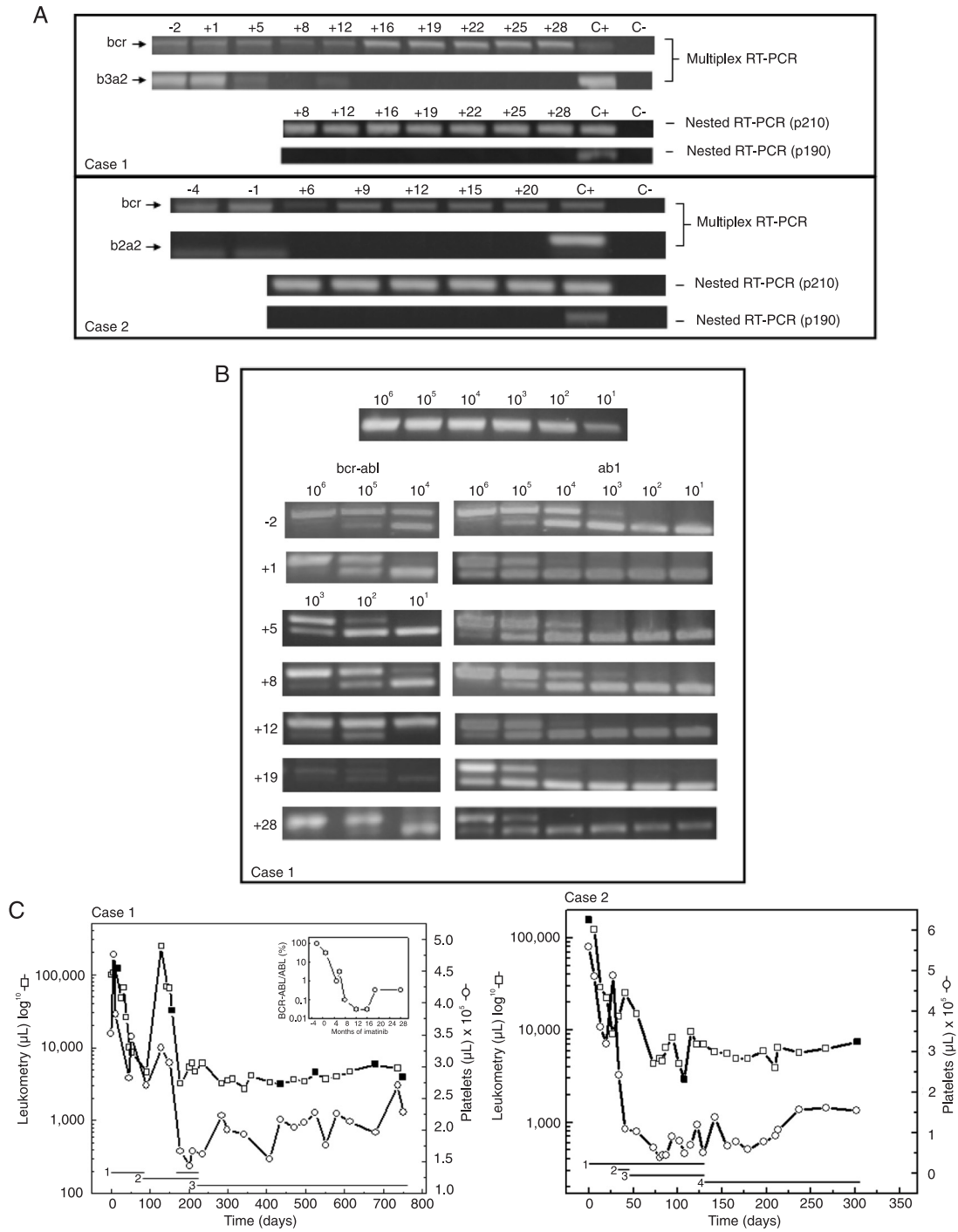


Figure 1. Analysis of the hematological responses to treatment in 2 children with chronic myeloid leukemia and molecular analysis. *A*, Multiplex and nested PCR for cases 1 and 2. Negative samples for multiplex were assessed for nested b3a2/b2a2 (p210) and e1a2 (p190). K562 cells were used as positive controls for multiplex and nested b3a2 and SUPB15 for nested e1a2. Samples were classified as months pre-imatinib (-) and post-imatinib (+) treatment. *B*, Competitive assays for *bcr-abl* transcripts in case 1; quality control for competitor template in the different dilutions used in the assays is shown on the top of Panel B. *C*, Results are shown from the beginning of treatment for cases 1 and 2. Squares and circles represent white blood cells and platelet counts, respectively. Filled squares indicate when bone marrow samples were also obtained. Numbered horizontal lines show the time of each treatment with hydroxyurea (1), α -IFN (2), and imatinib (3) for case 1 and hydroxyurea (1), α -IFN (2), prednisone (3), and imatinib (4) for case 2. The inset shows the percentage of *bcr-abl/abl* during imatinib treatment.

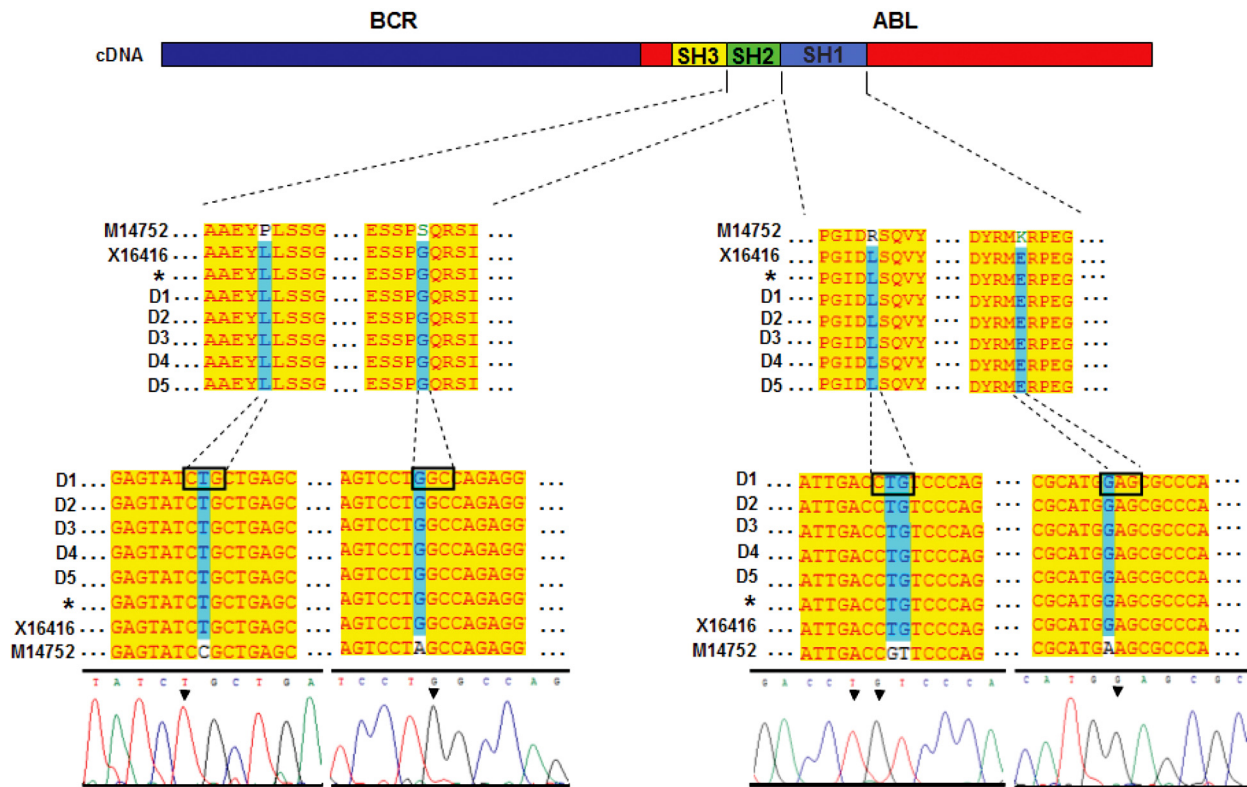


Figure 2. Sequence analysis of kinase regulatory domains (SH1 and SH2) at different times during treatment with imatinib did not detect substitutions before or after treatment. Our results were aligned with the *abl* sequences from Genbank (accession numbers X16416 and M14752). Samples from both cases are indicated with an asterisk and samples from different donors used as a positive control are indicated by D1-D5.

who achieved negative results by iFISH and sequencing after imatinib treatment are truly in continuous remission. We used two sequences of *c-abl* (GenBank accession No. M14752 and X16416) in our analysis. Curiously, sequence M14752 (13) showed a number of nucleotides that differed from other published sequences of *c-abl*, including X16416 and the sequence found in our patients and donors. These conflicting sites were also reported by others. Most interestingly, a point substitution (1375G>A) was seen in one of these discrepant nucleotides after imatinib treatment (5). One possible explanation for this is that the conflicting nucleotides in the M14752 sequence could have originated from mutated residual clones. This observation supports the theory of pre-existing mutated clones that expand due to drug selection (14). These clones likely appear in low frequency in patients as is now seen for 1375G>A (5,15). In our patients, these conflicting sites were not detected, nor were the substitutions related to imatinib resistance.

In summary, even though there is recent evidence (7) that children with Ph⁺-BCR-ABL+CML show positive responses to treatment with imatinib, the present study is the first to support this observation based on the use of

multiple cytomolecular methods. Interestingly, the cases reported here did not show mutations in the *abl* kinase domain of the *bcr-abl* allele that were previously found to be associated with a poor prognosis. Instead, CCR and major clinical remission were obtained, and these patients have remained in continuous remission for more than 28 (case 1) and 20 months (case 2).

Our intention was to show that sequence analysis and detection of residual tumor tissue by different cytomolecular assays, which include iFISH (8), multiplex and nested RT-PCR (9) and quantitative PCR (10), should be used to monitor the risk for relapse, and these results should be taken into account when considering alternative treatments.

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References

1. Dow LW, Raimondi SC, Culbert SJ, Ochs J, Kennedy W, Pinkel DP. Response to alpha-interferon in children with Philadelphia chromosome-positive chronic myelocytic leukemia. *Cancer* 1991; 68: 1678-1684.
2. Millot F, Brice P, Philippe N, Thyss A, Demeoq F, Wetterwald M, et al. Alpha-interferon in combination with cytarabine in children with Philadelphia chromosome-positive chronic myeloid leukemia. *J Pediatr Hematol Oncol* 2002; 24: 18-22.
3. Pulsipher MA. Treatment of CML in pediatric patients: should imatinib mesylate (STI-571, Gleevec) or allogeneic hematopoietic cell transplant be front-line therapy? *Pediatr Blood Cancer* 2004; 43: 523-533.
4. Lahaye T, Riehm B, Berger U, Paschka P, Müller MC, Kreil S, et al. Response and resistance in 300 patients with BCR-ABL-positive leukemias treated with imatinib in a single center: a 4.5-year follow-up. *Cancer* 2005; 103: 1659-1669.
5. Branford S, Rudzki Z, Walsh S, Parkinson I, Grigg A, Szer J, et al. Detection of BCR-ABL mutations in patients with CML treated with imatinib is virtually always accompanied by clinical resistance, and mutations in the ATP phosphate-binding loop (P-loop) are associated with a poor prognosis. *Blood* 2003; 102: 276-283.
6. Champagne MA, Capdeville R, Krailo M, Qu W, Peng B, Rosamilia M, et al. Imatinib mesylate (STI571) for treatment of children with Philadelphia chromosome-positive leukemia: results from a Children's Oncology Group phase 1 study. *Blood* 2004; 104: 2655-2660.
7. Millot F, Guilhot J, Nelken B, Leblanc T, De Bont ES, Békassy AN, et al. Imatinib mesylate is effective in children with chronic myelogenous leukemia in late chronic and advanced phase and in relapse after stem cell transplantation. *Leukemia* 2006; 20: 187-192.
8. Primo D, Tabernero MD, Rasillo A, Sayagués JM, Espinosa AB, Chillón MC, et al. Patterns of BCR/ABL gene rearrangements by interphase fluorescence *in situ* hybridization (FISH) in BCR/ABL+ leukemias: incidence and underlying genetic abnormalities. *Leukemia* 2003; 17: 1124-1129.
9. Otazú IB, Zalberg I, Tabak DG, Dobbin J, Seuáñez HN. Detection of BCR-ABL transcripts by multiplex and nested PCR in different haematological disorders. *Leuk Lymphoma* 2000; 37: 205-211.
10. Otazú IB, Tavares Rde C, Hassan R, Zalberg I, Tabak DG, Seuáñez HN. Estimations of BCR-ABL/ABL transcripts by quantitative PCR in chronic myeloid leukaemia after allogeneic bone marrow transplantation and donor lymphocyte infusion. *Leuk Res* 2002; 26: 129-141.
11. Hochhaus A, Druker B, Sawyers C, Guilhot F, Schiffer CA, Cortes J, et al. Favorable long-term follow-up results over 6 years for response, survival, and safety with imatinib mesylate therapy in chronic-phase chronic myeloid leukemia after failure of interferon-alpha treatment. *Blood* 2008; 111: 1039-1043.
12. Paschka P, Müller MC, Merx K, Kreil S, Schoch C, Lahaye T, et al. Molecular monitoring of response to imatinib (Gleevec) in CML patients pretreated with interferon alpha. Low levels of residual disease are associated with continuous remission. *Leukemia* 2003; 17: 1687-1694.
13. Shtivelman E, Lifshitz B, Gale RP, Roe BA, Canaani E. Alternative splicing of RNAs transcribed from the human abl gene and from the bcr-abl fused gene. *Cell* 1986; 47: 277-284.
14. Roche-Lestienne C, Soenen-Cornu V, Grardel-Duflos N, Lai JL, Philippe N, Facon T, et al. Several types of mutations of the Abl gene can be found in chronic myeloid leukemia patients resistant to STI571, and they can pre-exist to the onset of treatment. *Blood* 2002; 100: 1014-1018.
15. Khorashad JS, Anand M, Marin D, Saunders S, Al-Jabary T, Iqbal A, et al. The presence of a BCR-ABL mutant allele in CML does not always explain clinical resistance to imatinib. *Leukemia* 2006; 20: 658-663.