Case report

Successful treatment of a chronic-phase T-315I-mutated chronic myelogenous leukemia patient with a combination of imatinib and interferon-alfa

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Abstract

  BACKGROUND: The T315I BCR-ABL mutation in chronic myelogenous leukemia (CML) patients is responsible for up to 20% of all clinically observed resistance. This mutation confers resistance not only to imatinib, but also to second-generation BCR-ABL tyrosine kinases, such as nilotinib and dasatinib. A number of strategies have been implemented to overcome this resistance, but allogeneic stem cell transplantation remains the only established therapeutic option for a cure.

  CASE REPORT: A 61-year-old male was diagnosed with Philadelphia chromosome positive chronic phase CML in 2002. He was initially treated with imatinib and complete cytogenetic response (CCyR) was achieved 12 months later. However, after 18 months, a loss of CCyR was observed and a molecular study at 24 months revealed a T315I mutation of the BCR-ABL gene. At 30 months, imatinib/interferon-alfa (IFNα) -combination therapy was initiated in an effort to overcome the resistance. Thirty months later, he re-achieved CCyR, and the T315I BCR-ABL mutation disappeared at 51 months.

  Conclusion: To our knowledge, this is the first case report showing the effectiveness of imatinib/IFNα combination therapy for CML patients bearing the T315I BCR-ABL mutation.

1 Introduction

Chronic myelogenous leukemia (CML) is a clonal disease of the hematopoietic stem cell, which is characterized by an increased growth of predominantly myeloid cells in the bone marrow. The disease is associated with the Philadelphia chromosome, which arises by a reciprocal translocation between chromosomes 9 and 22 and harbors the BCR-ABL fusion oncogene [1]. Small molecules that specifically target the BCR-ABL gene product provide a successful treatment approach which can lead to a reduction in BCR-ABL transcripts below detectable levels. The drug imatinib, a rationally designed tyrosine kinase inhibitor (TKI), showed a superior response rate, improved progression-free survival, and overall survival, as compared with the previous standard therapy with IFNα [2-4].

Although high response rates are observed in patients who receive imatinib treatment, a small percentage of chronic phase (CP) CML patients are refractory to the therapy [2]. Patients develop imatinib resistance via multiple mechanisms, with some being BCR-ABL-dependent and others BCR-ABL-independent. To overcome the failure of imatinib, multiple strategies are under investigation. These strategies include a dose escalation of imatinib and switching to second-generation TKIs. Nilotinib and dasatinib are currently approved for the treatment of patients with CML who have developed resistance or intolerance to imatinib [5, 6].

The development of a T315I BCR-ABL mutation (threonine to isoleucine mutation at amino acid 315) is of particular concern as it confers resistance to all available TKIs [7-10]. The only established salvage option for patients harboring the T315I BCR-ABL mutation is allogeneic hematopoietic stem cell transplantation (allo-HSCT) [11-13]. However, allo-HSCT can be performed only in eligible patients [14]. For patients who could not receive allo-HSCT, new agents with activity against the T315I BCR-ABL mutation, such as danusertib and omacetaxin, have been developed [15, 16]. However, they are still in the clinical trial stage and it will take years before these agents can be put into use. Hence, patients harboring the T315I BCR-ABL mutation, who are not eligible for allo-HSCT, require treatment with combinations

of already approved drugs.

We report the successful treatment of a CML patient harboring the T315I BCR-ABL mutation with a combination of imatinib and IFNα.

2 Materials and Methods

2.1 total RNA extraction and cDNA synthesis

Total leukocytes in bone marrow and peripheral blood samples were isolated by centrifugation following red blood cell lysis and total RNA was extracted using TRIzol reagent (Invitrogen, CA, USA). cDNA was synthesized using oligo-dT primers and Super Script III Reverse Transcriptase (Invitrogen).

2.2 TaqMan quantitative reverse transcriptase-polymerase chain reaction

Quantitative reverse transcriptase-polymerase chain reaction (RQ-PCR) for BCR-ABL transcript levels were performed using the LightCycler (Roche Diagnostics, Mannheim, Germany) and LightCycler TaqMan Master (Roche Diagnostics). Primers and TaqMan probe sequences published in the EAC network protocol were used for RQ-PCR [17]. The amount of the fusion gene in the original sample was calculated by means of a standard curve (created with the BCR-ABL fusion gene or the ABL gene cloned in plasmids) and expressed as the BCR-ABL/ABL ratio.

2.3 Direct sequencing of ABL kinase domain

A nested PCR sequencing approach was used for direct sequencing of the ABL kinase domain, with a first-round amplification of the BCR-ABL transcript followed by two separate PCR reactions. For the nested PCR, the primers were used as described previously [18, 19]. To screen for mutations, the PCR products were sequenced in the both directions with the following: ABL-1F

(5'-ACAGGATCAACACTGCTTCTGA-3'), ABL-1R(5'-TGGCTGACGAGATCTGAGTG-3'), ABL-2F
(5'-ATGGCCACTCAGATCTCGTC-3'), and ABL-2R (5'-GATACTGGATTCCTGGAACA-3') using a
BigDye Terminator v3.1 Cycle Sequencing Kit and the ABI Prism 3100xl Genetic Analyzer (Applied
Biosystems, CA, USA).

2.4 Quantitative T315I BCR-ABL mutational analysis by pyrosequencing

  Quantitation of T315I BCR-ABL and un-mutated BCR-ABL transcript levels were performed using the
PyroMark ID Pyrosequencing system (QIAGEN). First-round PCR was carried out followed by
second-round PCR for T315I BCR-ABL mutation including one biotin-labeled primer. Primers and PCR
conditions were used as described previously [20]. The linearity of quantitative T315I BCR-ABL
mutation by pyrosequencing was confirmed by subjecting cDNA generated from graded mixes of Ba/F3
cell lines (RIKEN Cell Bank, Tsukuba, Japan) transfected with BCR-ABL cDNAs containing either the
un-mutated BCR-ABL sequence or the T315I BCR-ABL mutation.

3 Case report

  A 61-year-old male was referred to our hospital due to leukocytosis, thrombocytosis, and
hepatosplenomegaly (hypochondrial spleen size 8cm) in October 2002. Complete blood cell analysis
showed that the white blood cell count was 138,900/μl, with 36% neutrophils, 3% myeloblasts, 5%
promyelocytes, 5% myelocytes, 14% metamyelocytes, 6% lymphocytes, 5% monocytes, 5% basophils,
and 3% eosinophils; hemoglobin concentration was 11.2g/dl; and the platelet count was 122.1×10^4/μl.
Bone marrow analysis showed hypercellularity with significant myeloid hyperplasia with 3.0%
myeloblasts. Chromosomal analysis (G-banding) revealed that there were no additional chromosomal
abnormalities other than t(9;22)(q34;q11). No BCR-ABL kinase domain mutation was detected by direct

sequencing (Fig. 1a) and also by pyrosequencing. He was diagnosed with CP-CML. The Sokal score was 1.94, indicating high risk.

He was registered in the clinical trial (Japan Adult Leukemia Study Group, CML202 study) and imatinib was initiated with a dose of 400 mg/d in October 2002. A dose reduction (300mg/day) was necessary after 6 months due to muscle cramp, which was considered to be a side effect. Complete hematologic response (CHR) and complete cytogenetic response (CCyR) were achieved within 1 and 12 months of treatment, respectively. However, after 18 months of imatinib treatment, a loss of CCyR was observed and a direct sequencing study at 24 months revealed a T315I mutation of the BCR-ABL gene (Fig.1b). The earlier samples (at 18 months) were then analyzed retrospectively and the mutation was also identified. Even though pyrosequencing revealed that T315I transcripts increased over 2.5-fold during the 18 month to 24 month period (Fig. 1c), total BCR-ABL transcripts measured by a RQ-PCR remained unchanged: ratios of BCR-ABL to ABL were 10.1% at 18 months and 11.1% at 24 months, respectively. Because a loss of the major cytogenetic response occurred at 30 months, a combination therapy which consisted of imatinib and IFNα was initiated. IFNα was administrated at a dose of 6 million units/week. Thirty months after the initiation of the imatinib/IFNα combination therapy, he re-achieved CCyR. Forty-eight months after, the T315I BCR-ABL mutation remained detectable although CCyR was maintained. After 51 months, RQ-PCR revealed a reduction of BCR-ABL transcripts by 3 or more logs (i.e. major molecular response (MMR)), and the T315I BCR-ABL mutation was not detected by direct-sequencing and pyrosequencing (Fig. 1d). The MMR was still maintained at 75 months after the initiation of the imatinib/IFNα combination therapy without any signs of a recurrence of the T315I BCR-ABL mutation (Fig. 2). Although he experienced grade 2 anemia, grade 1 neutropenia, and thrombocytopenia according to the National Cancer Institute Common Terminology Criteria for Adverse Events version4.0, it was possible to continue the imatinib/IFNα combination therapy with no dose reduction.

4 Discussion

The current treatment algorithm for patients with CML suggests that if the patient develops a T315I BCR-ABL mutation, allo-HSCT or participation in clinical trials should be considered (new agents against the T315I BCR-ABL mutation [15, 16, 21-24] are still in trials). In our case, the imatinib/IFNα combination therapy used resulted in MMR, suggesting its effectiveness in patients harboring the T315I BCR-ABL mutation. De Lavallade et al have reported the clinical outcome for a CML patient who acquired the T315 BCR-ABL mutation while on imatinib, that was treated successfully with IFNα alone [25]. In their report, while the level of T315I BCR-ABL mutant transcripts decreased with the interferon therapy, the total amount of BCR-ABL transcripts was relatively stable, suggesting that the CML clone harboring an un-mutated BCR-ABL was expanding during that period. To prevent this phenomenon, we chose a combination therapy with imatinib and IFNα. This therapy theoretically seemed reasonable because it would inhibit both the T315I-mutated and the un-mutated BCR-ABL clone, and as shown in this report, it was quite successful. Determining whether or not the T315I BCR-ABL mutated clone is more susceptible to IFNα than an un-mutated clone would be of interest.

In conclusion, although our experience is limited to one patient, imatinib/IFNα combination therapy could be a viable treatment option for CP-CML patients with a T315I BCR-ABL mutation. Further studies are necessary to confirm the efficacy and applicability of imatinib/IFNα combination therapy.

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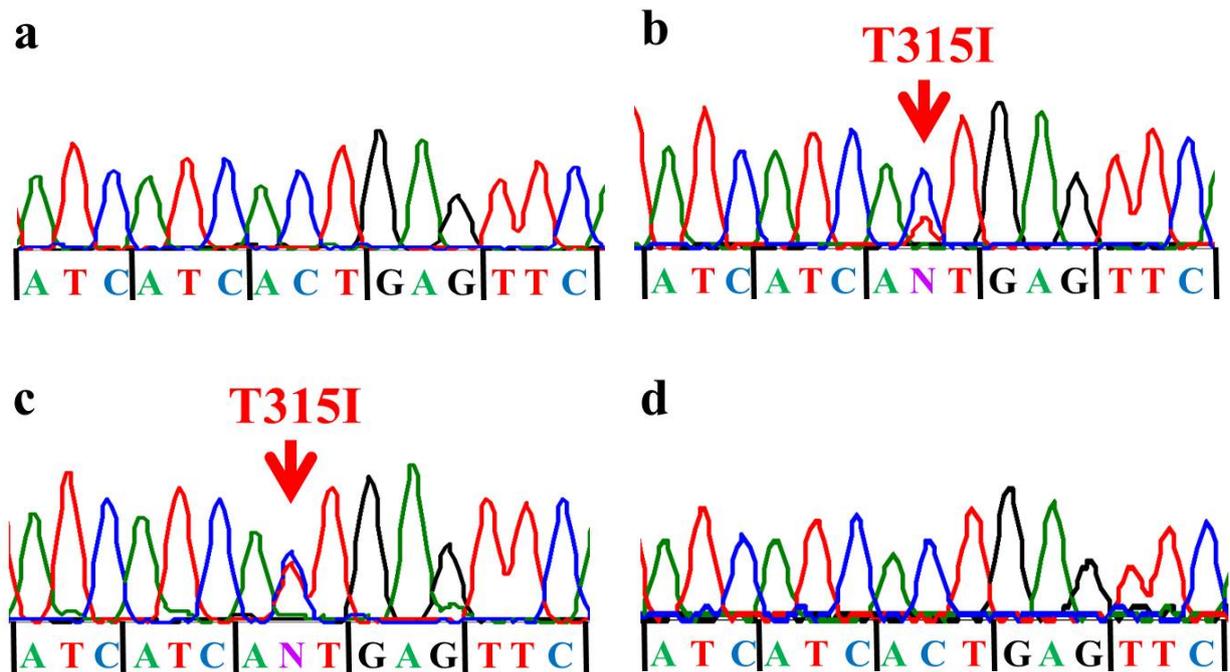
Fig. 1 T315I BCR-ABL mutation by direct sequencing.

a at diagnosis. **b** at 18 months after starting imatinib. **c** at 24 months after starting imatinib. **d** at 51 months after starting the combination therapy.
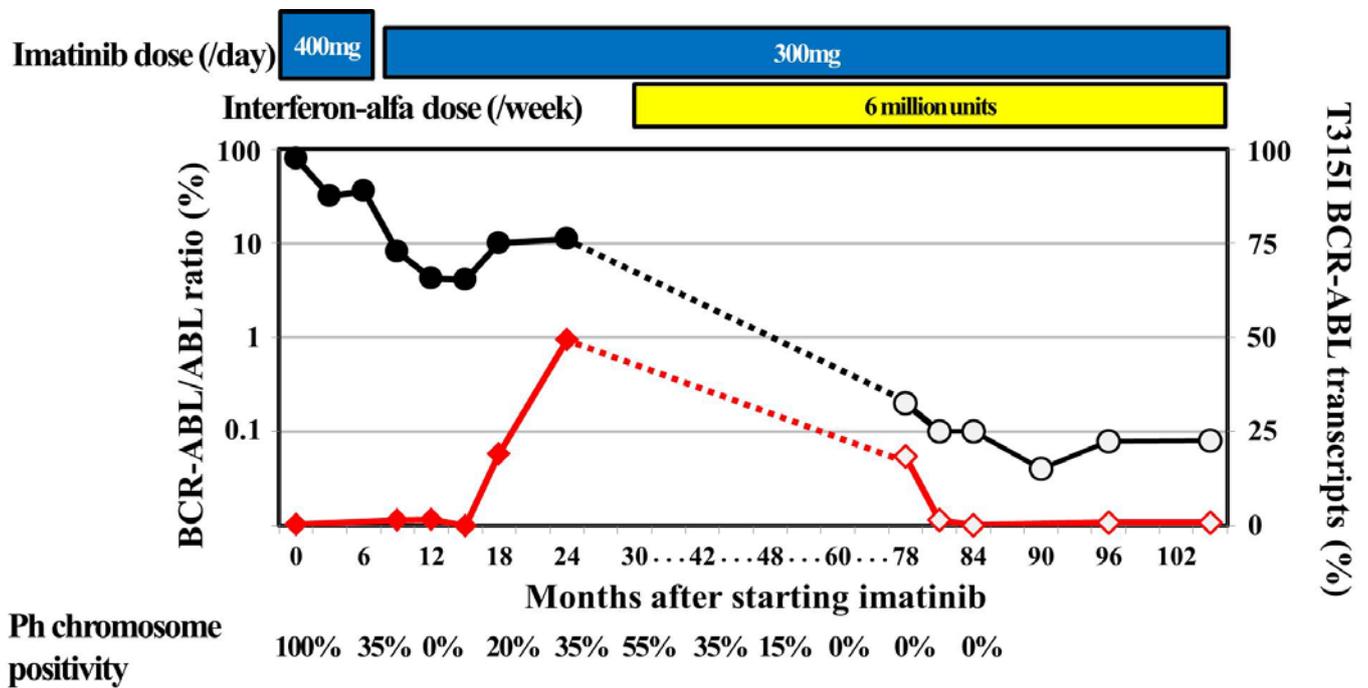
Fig.2 Clinical course of total and T315I BCR-ABL mutant transcript levels.

The figure shows total BCR-ABL transcript levels (solid line) measured by RQ-PCR and the relative size of T315I BCR-ABL mutant transcript levels (dotted line) by pyrosequencing. The filled-circle and -square represent that the samples are from bone marrow, and the open-circle and -square represent that the samples are from peripheral blood. Ph chromosome positivity (%) represents the ratio of Ph-positive cells in bone marrow cells determined by G-band chromosomal analysis.