Nilotinib rapidly reverses breakpoint cluster region-Abelson oncogene fusion gene and M244V mutations in a patient with chronic myelogenous leukemia: A case report

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Received June 11, 2014; Accepted February 5, 2015

DOI: 10.3892/etm.2015.2707

Abstract. Chronic myelogenous leukemia (CML) is a condition characterized by a balanced genetic translocation, t (9;22) (q34;q11.2), which leads to a fusion of the Abelson oncogene (ABL) from chromosome 9q34 with the breakpoint cluster region (BCR) gene on chromosome 22q11.2. This rearrangement is referred to as the Philadelphia chromosome. At a molecular level, this translocation results in the formation of the BCR-ABL fusion oncogene, which translates into a BCR-ABL oncoprotein. Imatinib, nilotinib and dasatinib are three tyrosine kinase inhibitors that have been approved by the US Food and Drug Administration for the treatment of patients diagnosed with CML in the chronic phase (CML-CP). The present study describes the case of a patient with imatinib-resistant CML who, following two months of treatment with nilotinib, no longer exhibited detectable BCR-ABL fusion genes or M244V mutations. This suggests that nilotinib may be effective for treating CML cases in which the BCR-ABL fusion protein has an M244V mutation.

Introduction

Chronic myelogenous leukemia (CML) is a cancer of the white blood cells characterized by a balanced genetic translocation, t (9;22) (q34;q11.2), which leads to a fusion of the Abelson oncogene (ABL) from chromosome 9q34 with the breakpoint cluster region (BCR) gene on chromosome 22q11.2. This chromosomal translocation is known as the Philadelphia chromosome. At a molecular level, this translocation results in the formation of the BCR-ABL fusion oncogene, which translates into a BCR-ABL oncoprotein (1).

The US Food and Drug Administration has approved three tyrosine kinase inhibitors (TKIs), imatinib, nilotinib and dasatinib, as first-line treatments for patients diagnosed with CML in the chronic phase (CML-CP) (2-5). Imatinib mesylate, otherwise known as Gleevec® (Novartis Pharmaceuticals Corp., East Hanover, NJ, USA), was the first of the TKIs to receive approval; however, 20-40% of patients receiving imatinib as a first-line therapy are likely to eventually require an alternative treatment, due to intolerance or resistance to imatinib (5). It is recommended that, upon failure of imatinib treatment, patients with CML should be assessed for BCR-ABL kinase domain mutations, as this can indicate which TKI should be selected for continued therapy. Dasatinib and nilotinib have been demonstrated to retain efficacy against several of the mutations known to confer resistance to imatinib (6). Notably, a number of distinct mutations leading to decreased sensitivity to dasatinib and nilotinib have been found in in vitro and in vivo studies (7,8). Dasatinib is favored when patients have Y253H, E255K/V or F359C/V mutations in BCR-ABL. By contrast, nilotinib is more effective when V299L or F317L mutations are present (2). Despite this evidence, it remains unclear how the M244V mutation to the BCR-ABL fusion protein should affect the choice of treatment. The present study describes the effect of nilotinib therapy in a patient with imatinib-resistant CML.

Case report

This study was conducted in accordance with the Declaration of Helsinki and with approval from the Ethics Committee of Changzhi Medical College (Changzhi, China). Written informed consent was obtained from the patient.

The patient was a 43-year-old female. Three months prior to diagnosis, the patient began sweating at night, but did not receive any special treatment. One month prior to diagnosis, the patient developed a rash on her face and neck, accompanied by itching. This was diagnosed as allergic dermatitis.

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Key words: chronic myeloid leukemia, M244V mutation, breakpoint cluster region-Abelson oncogene, tyrosine kinase inhibitors, nilotinib
by her doctors, and was treated with oral tablets, including cetirizine. Despite treatment, there was no notable relief of her symptoms. Further examination found that her white blood cell (WBC) count was significantly higher than normal. The patient came under the care of Heping Hospital of Changzhi Medical College as of August 22, 2008.

Physical examination of the patient revealed scattered red papules on her face and neck, superficial swelling of the lymph nodes and sternum tenderness. No yellowing of the skin or mucous membranes or formation of hemorrhagic spots were observed. The patient additionally underwent a cardiopulmonary examination, as well as an examination of the liver and spleen under the ribs and the abdomen. Laboratory studies revealed the patient had a WBC count of 112.7x10^9 cells/l, a red blood cell (RBC) count of 3.72x10^{12} cells/l, hemoglobin (Hb) levels of 116 g/l and a platelet (PLT) count of 445x10^9 cells/l. Peripheral blood smears consisted of granulocytes (1%), neutrophilic myelocytes (20%), neutrophilic metamyelocytes (17%), banded neutrophils (13%), neutral lobocytes (39%) and leukomonocytes (10%), with approximately normal mature erythrocyte levels and a normal distribution of PLTs. A significant reduction in neutrophil alkaline phosphatase was observed, as assessed by staining. Routine tests of the urine and of liver and renal function were normal. Furthermore, chest X-rays revealed no evident abnormalities, and the echocardiogram (ECG) was normal. Abdominal ultrasound indicated that there was a 0.9x0.9-cm swollen lymph node in the first hepatic portal. Additionally, the distance between the spleen and rib was 4.3 cm. Level I bone marrow hyperplasia was observed, with the cells of the immune system accounting for 87%. The following cells were present: Original granulocytes (5%), neutrophilic myelocytes (18%), neutrophilic metamyelocytes (31%), banded neutrophils (16%), neutral lobocytes (25%) and eosinophilic polymorphonuclear cells (2%). Erythroid proliferation was inhibited (3%), of which rubricytes composed 1% and metarubricytes composed 2% (granulocytes versus erythrocytes, 97:3). The entire staining smear contained 219 megakaryocytes, of which 210 had no PLTs and nine contained PLTs.

Chromosome karyotype was assessed by Giemsa-bandng (G-banding). G-banding analysis revealed two subsets of cells: Those with 46 total chromosomes (XX) containing a t(9;22) translocation or those with 46 total chromosomes (XX) with no translocation (2.9). Quantitative polymerase chain reaction (qPCR) analysis was used to assess the ratio of the copy numbers of BCR-ABL and ABL (BCR-ABL copy number/ABL copy number). The patient exhibited an initial ratio of 101,993/665,053 (15.3%).

During the CML-CP, the patient was prescribed hydroxyurea (1.0 g, three times per day) and allopurinol (0.1 g, three times per day) for one week. Beginning in September 2008, imatinib (0.4 g) was administered once daily. The response to the imatinib treatment was assessed via peripheral blood cell counts and classification of peripheral blood once a week until complete hematological remission (CRH) was achieved. Following CRH, these assays were performed once per month, and bone marrow cytogenetic analysis and/or fluorescence in situ hybridization (FISH) was performed once every 3-6 months, until complete cytogenetic remission (CCyR) was confirmed. To detect the BCR-ABL fusion gene, qPCR was performed once every three months until CRH was achieved. Following CRH, qPCR was performed once every 3-6 months. Biochemical tests, liver and kidney function and ECG were evaluated once a month.

Following three months of treatment with imatinib, the WBC count was 6.1x10^9 cells/l, RBC count was 3.8x10^{12} cells/l, Hb levels were 117 g/l and PLT count was 175x10^9 cells/l. The peripheral blood smear contained 2% banded neutrophils, 54% neutral lobocytes, 40% lymphocytes and 4% monocytes. Mature erythrocyte levels were approximately normal, and the distribution of PLTs was normal. The copy number ratio of BCR-ABL to ABL was 9,740/124,247 (7.8%).

After six months of treatment, the BCR-ABL/ABL copy number ratio was reduced to 2,383/73,403 (3.2%). Analysis of 300 interphase cells by FISH revealed that 70 visibly expressed BCR-ABL. The remaining 230 cells did not visibly contain the BCR-ABL fusion.

After nine months of imatinib treatment, G-banding analysis indicated that the karyotype of the cells was 46 chromosomes, XX. FISH analysis of 300 interphase cells revealed that eight contained the BCR-ABL fusion, while the remaining 292 did not contain the BCR-ABL fusion. The BCR-ABL/ABL copy number ratio was 3,355/88,250 (3.8%).

Following twelve months of imatinib treatment, the BCR-ABL/ABL copy number ratio was 414/98,693 (0.42%). After 52 months of imatinib treatment (0.6 g, once daily), the BCR-ABL/ABL copy number ratio was 1,002/6,557 (15.3%). At 60 months of treatment, the BCR-ABL/ABL copy number ratio was 7,103/77,370 (9.2%). PCR sequencing of the ABL kinase region of BCR-ABL revealed a mutation at nucleotide 730 (A to G), resulting in the point mutation M244V (Fig. 1A).

Sixty-two months after the diagnosis, the patient began receiving nilotinib (Tasigna®; Novartis Pharmaceuticals Corp.) at a dose of 0.4 g twice per day. Following two months of nilotinib therapy (64 months post-diagnosis), the BCR-ABL/ABL copy number ratio was 0/7,710 (0%). PCR sequencing detected no BCR-ABL or ABL kinase region mutations (Fig. 1B). The application of TKIs, such as imatinib and nilotinib, was
correlated with the BCR-ABL/ABL copy number ratio (Fig. 2). Over the course of the imatinib therapy, the patient experienced mild edema of the face, with no other obvious side effects. The patient suffered one urinary tract infection over the course of the disease, which was treated with antibiotics. Sixty-one months after the diagnosis of CML, the patient was diagnosed with type 2 diabetes and was prescribed insulin to regulate her blood sugar levels. Over the course of the nilotinib treatment, the patient also experienced mild edema of the face with headache and rash, which disappeared following symptomatic treatment.

Discussion

Resistance to TKIs in patients with CML is often the result of mutation to the tyrosine kinase domain of the BCR-ABL protein. To date, there have been 11 reports of the M244V mutation to the BCR-ABL fusion in the PubMed database (9-19).

There are only five references to imatinib resistance following BCR-ABL mutations in patients with CML (9-13). Among the 362 reported cases of resistance to imatinib in patients with CML, genetic mutations to the BCR-ABL fusion were observed in 192 cases (53%). Of these 192 cases, 26 (13.5%) had the M2344V mutation (Table I). Furthermore, three groups have shown that the first genetic mutation to BCR-ABL is M244V (10,11,13). Qin et al (10) found that genetic mutations to BCR-ABL occurred in 74 out of 127 cases (58%) of imatinib resistance in patients with CML. Of these, the M244V mutation occurred in 12 cases (16%). Additionally, one patient exhibited E355G and Y253H mutations (7). Ernst et al (11) analyzed 95 cases of imatinib resistance in patients with CML and identified 53 cases (56%) with BCR-ABL mutations, including six cases (11%) of M244V mutations. Finally, Bagadi et al (13) analyzed 24 cases of imatinib resistance in patients with CML and found 14 cases (58%) of BCR-ABL mutations, including four cases (29%) with M244V mutations. These data suggest that M244V may play an important role in imatinib resistance in patients with CML.

To analyze the response of patients with the M244V mutation to imatinib, Anand et al (20) reviewed the cases of six patients. Among these patients, increasing doses of imatinib (600-1,000 mg/day) resulted in three patients achieving CCyR. Of these, two patients were M244V-negative following treatment; however, ~1% of genetic transcripts contained the BCR-ABL fusion gene. Furthermore, imatinib was ineffective in the other three cases. This suggests that the M244V mutation gives rise to imatinib resistance. Kim et al (9) observed 55 cases of imatinib resistance in patients with CML, of which 32 cases (58%) had mutations to the BCR-ABL gene and three cases (9%) had the M244V mutation. The first patient had both the M244V and G250E mutations. These mutations disappeared following treatment with dasatinib for six months. The second case was a patient with the M244V mutation only. Treatment with nilotinib for nine months had no effect on the M244V mutation; however, it did give rise to a T315I mutation. The third case was a patient that had only the M244V mutation, which had not disappeared after nine months. No additional mutations were observed in this patient. Awidi et al (14) reported 185 cases of CML initially treated with imatinib, of which 21 cases had mutations to BCR-ABL. Of these 21 cases, two (10%) had the M244V mutation. In one case, the imatinib treatment was invalid. In the second case, in which both the M244V and G250E mutations were present, the M244V status was negative following treatment with nilotinib. The present study described the case of a patient with imatinib-resistant CML who, after two months of treatment with nilotinib, no longer had detectable BCR-ABL fusion genes or M244V mutations. This suggests that nilotinib may be effective for treating CML cases in which the BCR-ABL fusion has a M244V mutation; however, the mechanism underlying the action of nilotinib requires further study.

Table I. M244V BCR-ABL genetic mutations among patients with imatinib-resistant chronic myelogenous leukemia.

<table>
<thead>
<tr>
<th>First author (reference)</th>
<th>Cases, n</th>
<th>Cases with BCR-ABL genetic mutations, n/total n (%)</th>
<th>Cases with M244V mutations, n/total n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kim (9)</td>
<td>55</td>
<td>32/55 (58)</td>
<td>3/32 (9)</td>
</tr>
<tr>
<td>Qin (10)</td>
<td>127</td>
<td>74/127 (58)</td>
<td>12/74 (16)</td>
</tr>
<tr>
<td>Ernst (11)</td>
<td>95</td>
<td>53/95 (56)</td>
<td>6/53 (11)</td>
</tr>
<tr>
<td>Strhakova (12)</td>
<td>61</td>
<td>19/61 (31)</td>
<td>1/19 (5)</td>
</tr>
<tr>
<td>Bagadi (13)</td>
<td>24</td>
<td>14/24 (58)</td>
<td>4/14 (29)</td>
</tr>
<tr>
<td>Total</td>
<td>362</td>
<td>192/362 (53)</td>
<td>26/192 (14)</td>
</tr>
</tbody>
</table>

BCR-ABL, breakpoint cluster region-Abelson oncogene.

Figure 2. Correlation between the application of imatinib (IM), nilotinib (NT) and the BCR-ABL/ABL copy number ratio. BCR, breakpoint cluster region; ABL, Abelson oncogene; TKI, tyrosine kinase inhibitor.
Acknowledgements

The present study was supported by the Natural Science Foundation of Shanxi Province (grant no. 2013011056-3); the Science and Technology Development Project of colleges and universities of Shanxi Province (grant no. 20121013); and the Scientific Research Subject of the Health Department of Shanxi Province (grant no. 201202008).

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