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

XULIANG SHEN[1*], MEIXIANG ZHANG[2*], YIFAN SHEN[3*], WENZHI SHI[1], WEI LIU[1] and WU WEI[1]

[1]Department of Hematology, Heping Hospital of Changzhi Medical College, Changzhi, Shanxi 046000;
[2]Department of Hematology, Beijing Rehabilitation Hospital of Capital Medical University, Beijing 100144;
[3]Department of Hematology, Heji Hospital of Changzhi Medical College, Changzhi, Shanxi 046000, P.R. China

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, the rearrangement 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

Correspondence to: Dr Wu Wei, Department of Hematology, Heping Hospital of Changzhi Medical College, 110 Yan'an Road, Changzhi, Shanxi 046000, P.R. China
E-mail: wweicn@126.com

*Contributed equally

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-banding (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.

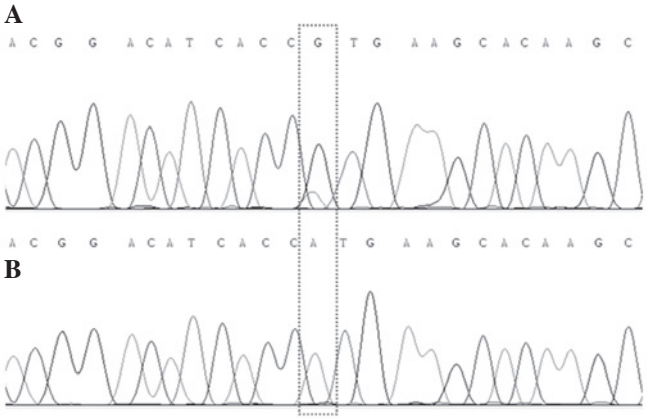

Figure 1. (A) *ABL* kinase region mutation, c.730 A>G (p.244V). (B) *ALB* kinase polymerase chain reaction analysis and sequencing found no mutations. *ABL*, Abelson oncogene.

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

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

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

BCR-ABL, breakpoint cluster region-Abelson oncogene.

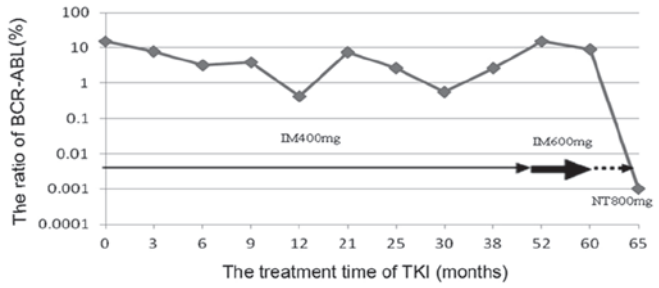

Figure 2. Correlation between the application of imatinib (IM), nilotinib (NT) and the *BCR-ABL/ABL* copy number ratio. *BCR*, breakpoint cluster region; *ABL*, Abelson oncogene; TKI, tyrosine kinase inhibitor.

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.

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).

References

1. Rowley JD. Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature 243: 290-293, 1973.
2. Jabbour E and Kantarjian H: Chronic myeloid leukemia: 2014 update on diagnosis, monitoring and management. Am J Hematol 89: 547-556, 2014.
3. Kantarjian H, Shah NP, Hochhaus A, *et al*: Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med 362: 2260-2270, 2010.
4. Saglio G, Kim DW, Issaragrisil S, *et al*; ENESTnd Investigators: Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. N Engl J Med 362: 2251-2259, 2010.
5. Druker BJ, Guilhot F, O'Brien SG, *et al*; IRIS Investigators: Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. N Engl J Med 355: 2408-2417, 2006.
6. Osborn M and Hughes T: Managing imatinib resistance in chronic myeloid leukaemia. Curr Opin Hematol 17: 97-103, 2010.
7. Jabbour E, Branford S, Saglio G, *et al*: Practical advice for determining the role of BCR-ABL mutations in guiding tyrosine kinase inhibitor therapy in patients with chronic myeloid leukemia. Cancer 117: 1800-1811, 2011.
8. Hochhaus A, La Rosée P, Müller MC, *et al*: Impact of BCR-ABL mutations on patients with chronic myeloid leukemia. Cell Cycle 10: 250-260, 2011.
9. Kim WS, Kim D, Kim DW, *et al*: Dynamic change of T315I BCR-ABL kinase domain mutation in Korean chronic myeloid leukaemia patients during treatment with Abl tyrosine kinase inhibitors. Hematol Oncol 28: 82-88, 2010.
10. Qin Y, Chen S, Jiang B, *et al*: Characteristics of BCR-ABL kinase domain point mutations in Chinese imatinib-resistant chronic myeloid leukemia patients. Ann Hematol 90: 47-52, 2011.
11. Ernst T, Erben P, Müller MC, *et al*: Dynamics of BCR-ABL mutated clones prior to hematologic or cytogenetic resistance to imatinib. Haematologica 93: 186-192, 2008.
12. Strhakova L, Bujalkova MG, Hojsikova I, *et al*: Use of direct sequencing for detection of mutations in the BCR-ABL kinase domain in Slovak patients with chronic myeloid leukemia. Neoplasma 58: 548-553, 2011.
13. Bagadi S, Saikia T, Pany A and Das B: Analysis of ABL kinase domain mutations conferring resistance to tyrosine kinase inhibitors in chronic myeloid leukemia cases from India. Clin Lab 57: 619-623, 2011.
14. Awidi A, Ababneh N, Magablah A, *et al*: ABL kinase domain mutations in patients with chronic myeloid leukemia in Jordan. Genet Test Mol Biomarkers 16: 1317-1321, 2012.
15. Huang Q, Du X, Zhang QX and Zhuo JC: Establishment of real-time fluorescent quantitative polymerase chain reaction for rapid detection of M244V mutation in kinase domain of BCR-ABL fusion gene. Zhongguo Shi Yan Xue Ye Xue Za Zhi 22: 1728-1734, 2014 (In Chinese).
16. Tanneeru K and Guruprasad L: Ponatinib is a pan-BCR-ABL kinase inhibitor: MD simulations and SIE study. PLoS One 8: e78556, 2013.
17. Preuner S, Mitterbauer G, Mannhalter C, *et al*: Quantitative monitoring of BCR/ABL1 mutants for surveillance of subclone-evolution, -expansion and -depletion in chronic myeloid leukaemia. Eur J Cancer 48: 233-236, 2012.
18. Florek I, Sacha T, Zawada M, *et al*: Implementation of direct sequencing as a method of ABL gene mutations analysis in patients with chronic myeloid leukemia treated with tyrosine kinase inhibitor. Przegl Lek 67: 1292-1297, 2010 (In Polish).
19. Soverini S, Colarossi S, Gnani A, *et al*; GIMEMA Working Party on Chronic Myeloid Leukemia: Contribution of ABL kinase domain mutations to imatinib resistance in different subsets of Philadelphia-positive patients: by the GIMEMA Working Party on Chronic Myeloid Leukemia. Clin Cancer Res 15: 7374-7379, 2006.
20. Anand M, Khorashad J, Marin D, Apperley JF, Goldman JM and Kaeda JS: Varying response to escalating the dose of imatinib in patients with CML who 'acquire' a BCR-ABL M244V mutant allele. Blood 108: 2881-2882, 2006.