

CASE REPORT

H396P mutation in chronic myeloid leukaemia patient on nilotinib – A case report

Nor Syazana JAMALI¹, Raja Zahratul Azma RAJA SABUDIN^{1*}, Hafiza ALAUDDIN¹, Azlin ITHNIN¹, Nor Rafeah TUMIAN², Norunaluwar JALIL³, Rinie AWAI¹, Siti Hawa ABU AMIS³, Salwati SHUIB¹

¹Department of Pathology, ²Medicine, and ³Laboratory Diagnostic Services, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur.

Abstract

Introduction: The advent of *BCR-ABL1*-targeted therapy with the tyrosine kinase inhibitor (TKI), for example, imatinib and nilotinib, marked a turning point in the therapy of chronic myeloid leukaemia (CML). However, a substantial proportion of patients experience primary or secondary disease resistance to TKI. There are multifactorial causes contributing to the treatment failure of which *BCR-ABL1* kinase domain mutation being the most common. Here, we describe a case of a CML patient with H396P mutation following treatment with nilotinib.

Case: A 60-year-old woman presented with abdominal discomfort and hyperleukocytosis. She was diagnosed as CML in the chronic phase with positive *BCR-ABL1* transcripts. Due to the failure to obtain an optimal response with imatinib treatment, it was switched to nilotinib. She responded well to nilotinib initially and achieved complete haematological and cytogenetic responses, with undetectable *BCR-ABL1* transcripts. However, in 4 years she developed molecular relapse. Mutation analysis which was done 70 months after commencement of nilotinib showed the presence of *BCR-ABL1* kinase domain mutation with nucleotide substitution at position 1187 from Histidine(H) to Proline(P) (H396P). Currently, she is on nilotinib 400mg twice daily. Her latest molecular analysis showed the presence of residual *BCR-ABL1* transcripts at 0.22 %. **Discussion/conclusion:** This case illustrates the importance of *BCR-ABL1* mutation analysis in CML patients with persistent *BCR-ABL1* positivity in spite of treatment. Early detection and identification of the type of *BCR-ABL1* mutation are important to guide appropriate treatment options as different mutation will have different sensitivity to TKI.

Keywords: Chronic myeloid leukaemia, mutation analysis, H396P mutation, treatment failure, *BCR-ABL1*

INTRODUCTION

Chronic myeloid leukaemia (CML) is a clonal disorder of multipotent haematopoietic progenitor cells. It is characterised by a translocation between the long arm of chromosome 9 and 22 [t(9;22)(q34;q11)] that result in the production of the *BCR-ABL1* fusion protein with constitutive tyrosine kinase activity.¹ Inhibition of *BCR-ABL1* kinase activity by tyrosine kinase inhibitors (TKIs) will prevent disease progression in CML. Imatinib mesylate is the first TKI for the treatment of CML patients based on the landmark IRIS trial.² Despite the development of TKI therapy in the improvement of survival

in CML, treatment resistance and failure remain a significant challenge.³

Among various mechanisms of resistance identified, the dominant mechanism is point mutations in the kinase domain of *BCR-ABL1*. Until now, more than 90 different amino acid substitutions in the *ABL1* tyrosine kinase domain have been identified.⁴ They usually occur in four regions at the kinase domain region which includes ATP-binding P-loop, SH3 contact, SH2 contact and activation (A-) loop.⁵ Here, we described a case of a CML patient who developed an H396P mutation, which was detected by mutation analysis (gene sequencing) following treatment with imatinib and nilotinib.

*Address for correspondence: Professor Dr Raja ZahratulAzma Raja Sabudin, Department of Pathology, Faculty of Medicine, UKM Medical Centre, Jalan Yaacob Latif, 56000, Cheras, Kuala Lumpur, Malaysia. Tel No: +603-91459493. Fax No: +603-91456640. E-mail: zahratul@ppukm.ukm.edu.my

CASE HISTORY

We described a 60-year-old woman who was diagnosed with CML in a chronic phase 15 years ago. She initially presented with abdominal discomfort for a one-month duration associated with significant loss of appetite and weight. Physical examination revealed that she had mild pallor with hepatosplenomegaly. Full blood count result at presentation showed white blood cells (WBC) of $157.9 \times 10^9/L$, haemoglobin (Hb) of 11.2g/dL and platelet of $558 \times 10^9/L$ with an intermediate risk (Sokal score 0.9) disease. Peripheral blood film and bone marrow aspirate findings showed typical features of CML in the chronic phase. Neutrophils alkaline phosphatase (NAP) score was very low, 13/100 WBC (reference range 50-100/100 WBC). Reverse transcriptase-polymerase chain reaction (RT-PCR) detected e14a2 (b3a2) transcripts, thus confirmed the diagnosis of CML. Conventional cytogenetic analysis showed the presence of Philadelphia chromosomes (Ph) in only three metaphase spread. Fluorescence in situ hybridization (FISH) done on 166 nuclei and metaphase spread using Vysis LSI BCR/ABL ES Dual Colour Translocation Probe (Abbott Mol. Inc, Des Plaines, IL, USA). Fusion signals pattern were observed in 143 nuclei and metaphase spread, which were consistent with *BCR/ABL1* translocation (86%). She was initially started with hydroxyurea for 3 months before the commencement of imatinib at 400mg once daily. The imatinib dose was gradually increased to a maximum dose of 800 mg daily, as her complete haematological response (CHR) was not sustained and she never achieved complete cytogenetic response (CCyR) and *BCR-ABL1* transcripts were persistently present (Table 1).

Due to the failure to achieve both major molecular response and complete cytogenetic remission, the patient was switched to nilotinib 200mg twice a day after being treated with imatinib for about 54 months. Mutation analysis was not performed due to financial constraint. She responded well to nilotinib initially. She achieved CHR in a month and attained CCyR with undetectable *BCR-ABL1* transcripts three months later. However, she developed molecular relapse in 4 years after nilotinib treatment and it was later followed by loss of both CCyR and CHR. Nilotinib dosage was gradually increased to 600mg daily.

Mutation analysis was performed after 70 months on nilotinib by nested-PCR. The

RNAs was extracted using RiboPure™-Blood Kit (Ambion, Austin, TX). Then, complementary DNA (cDNA) was generated using the SuperScript III® RT cDNA synthesis kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions.

The patient's cDNA was amplified using semi-nested PCR. The sequence primers used in the PCR were as previously described (6). The sequence primers used for first step PCR were as follows; forward primer (5'-TGA CCA ACT CGT GTG TGA AAC TC-3') and reverse primer (5'-TCC ACT TCG TCT GAG ATA CTG GAT T-3'). The size of the final amplification of the first step PCR product was assessed by gel electrophoresis. Subsequent second steps using the semi-nested PCR technique were done using a forward primer sequence (5'-CGC AAC AAG CCC ACT GTC T-3'). The reverse primer sequence was the same as in the first step PCR. The PCR products were then re-assessed by gel electrophoresis.

The samples then were purified using a QIAquick PCR purification kit (Qiagen, Hilden, Germany) and then sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). The final sequences were then analysed using BioEdit Sequence Alignment Editor Version 7.2.6. Amino acid substitutions had been determined and compared with Abl-kinase domain reference sequence [National Centre for Biotechnology Information (NCBI) GenBank accession number M14752.1]. For this patient, there was nucleotide substitution noted at position 1187 from Histidine (H) to Proline (P) (H396P). The tests were repeated using a similar method with a new sample, which was taken 12 months from the previous sample, and the latest analysis confirmed the initial result. Currently, she is on nilotinib 400mg twice a day for 32 months. She had achieved CHR and CCyR but not major molecular response. Her latest *BCR-ABL1* quantitative test showed a transcripts level of 0.22% International Scale (IS) (Figure 1).

DISCUSSION

Balanced translocation between the long arm of chromosome 9 and 22 will produced a *BCR-ABL1* transcripts protein. This fusion protein is an active tyrosine kinase that gives continuous proliferative signals that cause the clinical manifestations of CML. The development of TKI enable it to selectively inhibit the tyrosine

Table 1. Haematological parameter, cytogenetic and molecular pattern and treatment medication

| Date | WBC (x10 ⁹ /L) | Hb (g/ dL) | Platelet (x10 ⁹ /L) | Basophil (%) | FISH % | <i>BCR-ABL1</i> transcripts* (IS)* | Medication |
|--------------------|------------------------------|---------------|-----------------------------------|-----------------|--------|--|---|
| Jan 2006 | 165.1 | 11.2 | 558 | 0.4 | 86 | Detected | T. Hydroxyurea 500mg BD |
| April 2006 | 16.6 | 12.1 | 691 | 1.2 | - | - | T. Imatinib 200mg per day |
| June 2006 | 4.9 | 11.3 | 369 | 1.2 | 53 | Detected | T. Imatinib gradually increased to 800mg per day |
| November 2007 | 6.0 | 11.3 | 263 | 0.2 | 2.5 | Detected (5.5%) | |
| November 2008 | 8.4 | 12.4 | 359 | 0.4 | 40 | Detected (35.47%) | |
| September 2009 | 8.3 | 12.5 | 456 | 7.9 | 47 | Detected | |
| October 2010 | 6.3 | 11.9 | 389 | 2.4 | 46.5 | Detected | T. Nilotinib 200mg then gradually to 400mg per day |
| April 2011 | 7.4 | 11.0 | 265 | 0.6 | 0 | Not detected | T Nilotinib 400mg per day |
| November 2013 | 7.1 | 11.3 | 235 | 0.0 | 0 | Not detected | |
| November 2014 | 8.3 | 11.5 | 245 | 0.9 | - | Detected | |
| March, 2015 | 8.8 | 12.0 | 288 | 1.0 | 0 | Detected | T. Nilotinib 600mg per day |
| August, 2016 | 12.4 | 12.0 | 520 | 5.5 | 41 | Detected | T. Nilotinib 800mg per day |
| September, 2017 | 10 | 12.1 | 340 | 0.9 | - | Detected (0.34%) | |
| February 2018 | 9.1 | 12.2 | 362 | 0.9 | 0 | - | |
| Mei 2018 | 8.8 | 12.8 | 321 | 1.3 | - | Detected (0.22%) | |

BCR-ABL1* transcript by RT-PCR (qualitative assay)BCR-ABL1* real-time PCR (quantitative assay)

kinase protein by binding to ATP-binding site of ABL-kinase domain plus. However, their potential to cure CML are facing challenges with the development of resistance.

There are various contributing factors that lead to resistance to imatinib or other tyrosine kinase inhibitors. It includes patients' compliance with treatment, bioavailability, pharmacodynamics, genetic changes, *BCR-ABL1* kinase domain mutations, or a combination any of these factors.⁷ It has been reported that, more than 95% of CML patients treated with imatinib who achieved complete cytogenetic respond had residual

BCR-ABL1-positive cells.⁸ The scenario was also being observed in this case, where *BCR-ABL1* transcript protein was still detected by RT-PCR even after she achieved CCyR.

BCR-ABL1 kinase domain mutations were observed in 63% of Korean and 58% of Chinese CML patients with Imatinib resistance.^{9,10} Based on the local study, the frequency of *BCR-ABL1* kinase domain mutations had been reported to occur in 22.4% of CML patients who were resistant to imatinib.¹¹ Mutation detection was higher in those patients who had the disease and had longer treatment. Patient treated with

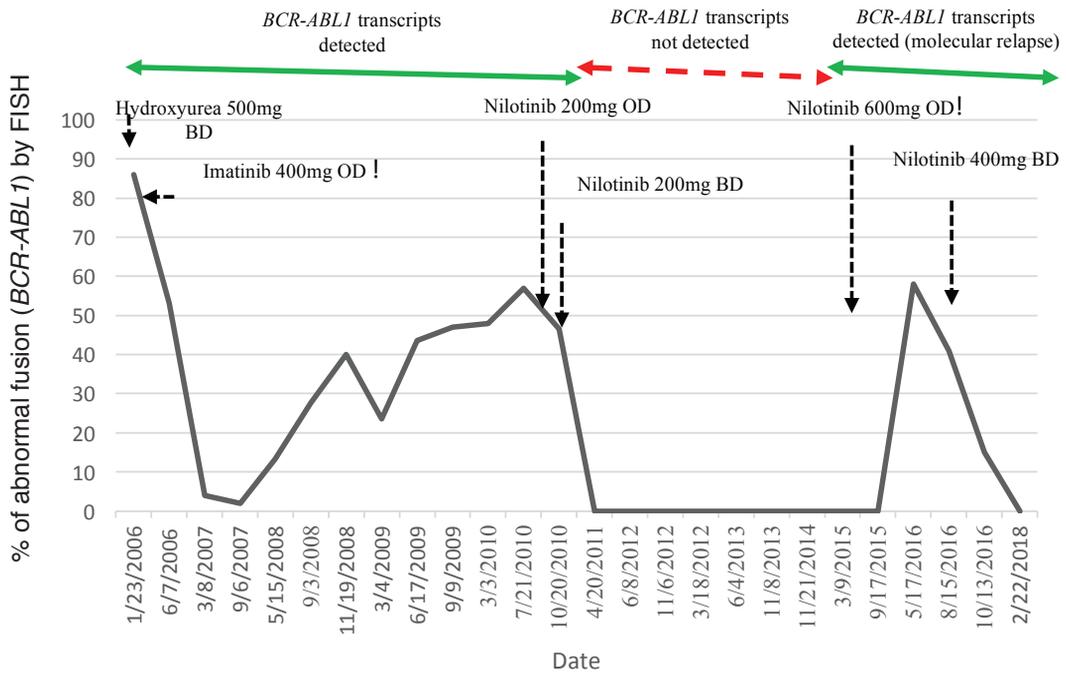


FIG. 1: Abnormal fusion signals of *BCR-ABL1* by FISH and detection of *BCR-ABL1* transcripts by qualitative PCR results with brief treatment summary of the patient.

imatinib therapy within 4 years duration, had less *BCR-ABL1* kinase domain mutation (9% of 100 patients), compared to patient who were on imatinib therapy for more than 4 years which account for 41% of 44 patients.¹²

Sanger based direct-sequencing is a reference method that is currently recommended for *BCR-ABL1* kinase domain mutation analysis.⁴ For this case, we used gene sequencing method (as previously described) which has 10-20% sensitivity detection rate of *BCR-ABL1* kinase domain mutations.⁶ Various other methods were available for mutations detection for example denatured high-performance liquid chromatography (d-HPLC), subcloning and sequencing, pyrosequencing and allele-specific oligonucleotide (ASO-PCR) which have

sensitivity of 0.1-10%, 9%, 5% and 0.01% respectively.¹³ Although Sanger based direct-sequencing is currently the recommended method for mutation detection, due to its low sensitivity, this method may not be able to detect or identify possibility of additional mutations that might be concomitantly present in this patient that contribute to the poor response to nilotinib.

According to the literature, most common mutations identified were M244V, G250E, Y253F/H, E255K/V, T315I, F317L, M351T, E355G, F359V and H396R/P.⁴ Four common mutations identified in our region are T315I (13.2%), G250E (11.4%), E255K (8.8%) and Y253H (7.9%).¹⁴ A local study reported that the most common mutations identified using d-HPLC and direct gene sequencing were T315I,

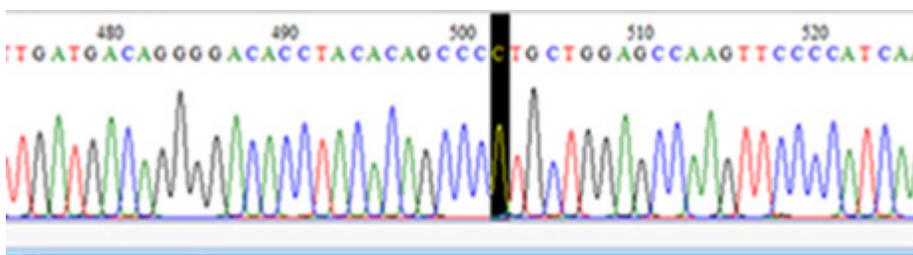


FIG. 2: Chromatogram of DNA sequencing. Noted nucleotide substitution from A>C at position 1187 from Histidine (H) to Proline (P) (H396P) by gene sequencing.

E255K, G250E, M351T and F359C.¹¹

Substitutions of nucleotide A>C at position 1187 lead to amino acid change from Histidine to Proline (H396P). This mutation located at A-loop region of kinase domain of *BCR-ABL1* gene. Imatinib does not interact directly in this region except for the anchor region. The anchor region is located at the amino-terminal of histidine 396. However, A-loop needs to be in inactive (closed) conformation so that imatinib binding will occur. Mutation in this region will destabilised the kinase and preventing inactive (closed) confirmation, thus inhibit imatinib binding.¹⁵ Fifty percent inhibitory concentration (IC_{50}) data have been published in most of the frequent mutations. Review of previous studies showed that wild type *BCR-ABL1* has IC_{50} ranging from 320-678nM with imatinib and maximum IC_{50} of 25nM. In comparison to that, H396P mutation has IC_{50} of 850-4300nM for imatinib with low IC_{50} with nilotinib and dasatinib (less than 43nM and less than 2.0nM, respectively).⁴ Our patient showed resistance to imatinib as the patient never achieved CCyR and major molecular response even with the highest dose imatinib. She achieved CHR, CCyR and MMR after she was given nilotinib at 200mg twice daily.

However, the patient developed molecular relapse and later loss her CCyR and CHR. This does not tally with above study in which H396P mutation is supposed to be sensitive to nilotinib. In vitro simulation for sequential TKI resistance in CML was studied and showed nilotinib as a second line treatment, often developed sequential resistance compare to dasatinib as second line especially at suboptimal drug concentration.¹⁶ This mechanism might contribute to the molecular relapse and loss of both CCyR and CHR since she was on nilotonib 200mg twice daily. This dose was lower as compared to established guideline.¹⁷

There are multiple factors that might contribute to the treatment failure other than presence of mutation, for example suboptimal dose due to non-compliance or intermittent interruption of the medication. However, our patient claimed to be fully compliant on treatment with no documented significant side effects of nilotinib, for example peripheral cytopenia and drug intolerance, that might contribute to the suboptimal dose.

CONCLUSIONS

Resistance to any drugs in general and any TKIs specifically were multifactorial. Mutation analysis should be done in CML patient who has suboptimal response and loss of cytogenetic or molecular response because present of *BCR-ABL1* kinase domain mutation is known to cause drug resistance. There are various methods of mutation analysis; but direct gene sequencing is the recommended method. However, due to its low sensitivity, it may miss the presence of mutation or additional mutation that contributed to the TKI resistance. Although H396P mutation was previously described to be sensitive to nilotinib, this case highlighted that it is not always true. Suboptimal treatment dosage due to various reasons may also contribute to the treatment failure.

Acknowledgement: This study was funded by UniversitiKebangsaan Malaysia-Research Grant (FF-2017-102) with ethic committee approval.

Authors' contribution: All authors contributed to the final preparation of manuscript. NSJ collected the case report history and initial drafting the manuscript. NJ, RA and SHAA involved in *BCR-ABL1* molecular and mutation analysis. RZARS and NRT contributed in final drafting of the manuscript. HA, AI and SS contributed in critical review of the manuscript. Finally, all authors have read and approved the manuscript.

Conflict of interest: The authors declare no conflict of interest to report.

REFERENCES

1. Sherbenou DW, Druker BJ. Applying the discovery of the Philadelphia chromosome. *J Clin Invest.* 2007;117(8):2067-74.
2. O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, *et al.* Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med.* 2003;348(11):994-1004.
3. Shah NP. Loss of response to imatinib: Mechanisms and management. *Hematology Am Soc Hematol Educ Program.* 2005:183-7.
4. Soverini S, Hochhaus A, Nicolini FE, *et al.* *BCR-ABL* kinase domain mutation analysis in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors: Recommendations from an expert panel on behalf of European LeukemiaNet. *Blood.* 2011; 118(5):1208-15.
5. Jones D, Kamel-Reid S, Bahler D, *et al.* Laboratory practice guidelines for detecting and reporting *BCR-ABL* drug resistance mutations in chronic

- myelogenous leukemia and acute lymphoblastic leukemia: A report of the Association for Molecular Pathology. *J Mol Diagn.* 2009;11(1):4-11.
6. Branford S, Hughes T. Detection of BCR-ABL mutations and resistance to imatinib mesylate. *Methods Mol Med.* 2006;125:93-106.
 7. Quintás-Cardama A, Kantarjian HM, Cortes JE. Mechanisms of primary and secondary resistance to imatinib in chronic myeloid leukemia. *Cancer Control.* 2009; 16(2):122-31.
 8. Hughes TP, Kaeda J, Branford S, *et al.* International Randomised Study of Interferon versus STI571 (IRIS) Study Group. Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. *N Engl J Med.* 2003; 349(15):1423-32.
 9. Kim SH, Kim D, Kim DW, Goh HG, Jang SE, Lee J, Kim WS, Kweon IY, Park SH. Analysis of BcrAbl kinase domain mutations in Korean chronic myeloid leukaemia patients: poor clinical outcome of P-loop and T315I mutation is disease phase dependent. *Hematol Oncol.* 2009; 27(4):190-7.
 10. Qin Y, Chen S, Jiang B, Jiang Q, Jiang H, Li J, Li L, Lai Y, Liu Y, Huang X. Characteristics of BCR-ABL kinase domain point mutations in Chinese imatinib-resistant chronic myeloid leukemia patients. *Ann Hematol.* 2011; 90(1):47-52.
 11. Elias MH, Baba AA, Azlan H, Rosline H, Sim GA, Padmini M, Fadilah SA, Ankathil R. BCR-ABL kinase domain mutations, including 2 novel mutations in imatinib resistant Malaysian chronic myeloid leukemia patients. Frequency and clinical outcome. *Leuk Res.* 2014;38(4):454-9.
 12. Branford S, Rudzki Z, Walsh S, *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(1):276-283.
 13. Hughes T, Deininger M, Hochhaus A, *et al.* Monitoring CML patients responding to treatment with tyrosine kinase inhibitors: Review and recommendations for harmonizing current methodology for detecting BCR-ABL transcripts and kinase domain mutations and for expressing results. *Blood.* 2006;108(1):28-37.
 14. Kim H, Kim S, Kim HJ, *et al.* Comparison of frequency and sensitivity of BCR-ABL1 kinase domain mutations in Asian and white patients with imatinib-resistant chronic-phase Chronic Myeloid Leukemia. *Clin Lymphoma Myeloma Leuk.* 2018;18(10):e391-9.
 15. Schindler T, Bornmann W, Pellicena P, Miller WT, Clarkson B, Kuriyan J. Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. *Science.* 2000; 289(5486):1938-42.
 16. Bauer RC, Sanger J, Peschel C, Duyster J, von Bubnoff N. Sequential inhibitor therapy in CML: In vitro simulation elucidates the pattern of resistance mutations after second- and third-line treatment. *Clin Cancer Res.* 2013;19(11):2962-72.
 17. Baccarani M, Deininger MW, Rosti G, *et al.* European LeukemiaNet recommendations for the management of chronic myeloid leukemia. *Blood.* 2013;122(6):872-84.