Sequential acquisition of tyrosine kinase domain mutations in a case of chronic myeloid leukemia: A dormant clone war against TKI

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Short Report

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Abstract

The tyrosine kinase inhibitor (TKI) therapy has a high response rate in chronic myeloid leukemia (CML). However majority of patients relapse due to high mutation susceptibility of tyrosine kinase domain (TKD) of *BCR/ABL* fusion gene. We report a case of CML which was diagnosed and monitored for 10 years as per the ELN guidelines. Mutational analysis using Sanger sequencing (SS) and Next generation sequencing (NGS) and In-silico study was performed. The present case describes the acquisition pattern of TKD mutation against the TKIs (Imatinib, Dasatinib and Nilotinib) at different time points. Interestingly, NGS identified a dormant mutant clone with p.G250E mutation in 2019 which was first detected by SS in 2011 along with one novel mutation p. Ala287Thr, which likely explain the dormant nature of these mutant clones. Bioinformatics analysis (Modelling and docking) of novel variant revealed that mutation detected through deep sequencing technique reducing the IM efficacy by increasing inhibition constant and suggests higher concentration of IM to overcome such mutations. The study highlights the importance of NGS in CML, as it can detects clinically relevant low level mutant clones in CML patients.

Introduction

The mutant clone and their allele burden are proved to be an important prognostic indicator in haematological disorder. However knowledge about the dormant mutant clone and its association with tyrosine kinase inhibitors (TKIs) resistance in chronic myeloid leukemia (CML) is limited and only few studies are reported in the literature [1, 2]. The low level dormant clone can only be detected by high sensitivity technique such as NGS [3, 4]. Recent studies have reported interesting data on CML and suggested that the high sensitivity techniques like NGS can help in detection of mutant clones which can be low in numbers but can undergo selective expansion if the TKI is not changed or an inappropriate TKI or TKI dose is chosen [1, 5, 6]. Here, we report a case of CML-CP who acquired mutations in sequential manner and progressed to accelerated phase and blast crisis due to presence of low level of mutant clone(s) (without additional chromosome abnormalities) during the TKI therapy.

Case Presentation

A female in late adolescent age referred to haematology clinic at KEM hospital, Mumbai, India with the complaints of easy fatigability and abdomen pain. On clinical examination, complete haemogram showed haemoglobin level of 12.3g/dl, WBC count of $314 \times 10^3/\mu\text{l}$ and platelets were in normal range. Ultrasonography showed mild splenomegaly. Complete haemogram suggestive of CML-chronic phase (CP) later it was confirmed by bone marrow analysis. Cytogenetic study using GTG-banding revealed 46,XX,t(9;22)(q34;q22.1) and fluorescence in-situ hybridisation (FISH) using dual colour dual fusion *BCR/ABL* probe (Vysis) showed double *BCR/ABL* fusion (3G3RDF) pattern in 91% of analysed cells. Further, molecular analysis using reverse transcription based pcr (RT-PCR) revealed presence of b3a2 transcript (p210). The Sokal score was calculated and the standard dose (400 mg) of Imatinib (Gleevec®) was started as a first line therapy [7]. The patient was monitored according to European leukemia net (ELN) guidelines [8]. She achieved complete haematological, cytogenetic and major

molecular response at 3, 12 and 18 months respectively. However the patient lost the major molecular response (MMR) at 36 months of IM treatment. Further the dose of IM was increased up to 600 mg and then 800 mg on subsequent follow-up, still she did not achieved MMR (Transcript level in international scale *BCR/ABL 1* IS% >0.1%) and considered as non- responder. The TKD mutation analysis was performed using SS and revealed presence of p.G250E mutation. As per the standard treatment guidelines the patient was shifted to Dasatinib (140mg/day) and the response was monitored according ELN guidelines. After 18 months, patient failed to achieve MMR and was detected with p.F359I (mosaic) mutation using SS. Further the patient was shifted to Nilotinib at standard doses and the response was monitored. However during the subsequent follow-up patient lost the response and progressed to CML-AP and mutational analysis using direct sequencing showed p.T315I mutation in mosaic condition. The patient was counselled for bone marrow transplantation; however patient was not given the consent for the transplantation. As p.T315I mutation is reported to be resistant for first and second generation TKI, the TKI therapy was stopped and Interferon alpha 100mg/week (with SOS Hydroxyurea 500mg) was prescribed [9]. But the patient's condition continued to be deteriorated and she progressed to CML-BC. Further the blood sample was collected and subjected to deep sequencing (NGS). Interestingly, we have identified the p-loop mutation p.G250E by NGS which was first detected by SS in 2011. In addition to p-loop mutation (p.G250E) we have identified one novel variation p.Ala287Thr in the tyrosine kinase domain. All the mutations detected in our case are presented in figure 1 according to their order of detection.

Further we have carried out bioinformatics analysis of these mutations (G250E and p.Ala287Thr) to identify the best TKI with better binding affinity after these mutations. The potential template of ABL1 protein was searched from BLAST-PDB (query coverage of 43% and an identity of 99.18%) and considering this as a template, the wild and the mutants models (mutant 1-Glu250, mutant 2-Tyr287and mutant 3 (combination of both mutant1 and 2) were generated [10]. The selected models were considered for docking studies [11–14]. The ABL1-wildtype when docked with Imatinib showed a binding energy of -7.24 kcal/mol with a least inhibitory constant. Although mutant 1, 2 and 3 showed better binding energy, still there was an upsurge in inhibitory constant value. With respect to Dasatinib, they bind with wild ABL1 with a binding affinity of -6.32 and an inhibitory constant value of 23.25 (nM). Most importantly, there were no hydrogen bond formation observed with mutant1, mutant2 and mutant3. Similar case was observed for drug Nilotinib with wild and the mutant ABL1 proteins (Table 1). Mostly Imatinib preferred to interact with charged and polar amino acids which were quite evident from the docking studies (Fig. 2A-D). The molecular modelling and docking studies revealed that mutation detected through deep sequencing technique reducing the IM efficacy by increasing inhibition constant and suggests higher concentration of IM to overcome such mutations. Further docking analysis of 2GTKI showed better binding affinity and reduce inhibition constant in mutant clone. However, due to lack of hydrogen bond formation the binding affinity has no value in these proteins.

Table 1

Wild and the mutant proteins with their binding energy, inhibitory constant values and number of hydrogen bonds.

Protein-drug complex	Binding energy (kcal/mol)	Inhibitory constant(nM)	Hydrogen bonds
ABL1wild-Imatinib	-7.24	4.93	3
ABL1mut1- Imatinib	-9.09	215.78	3
ABL1mut2- Imatinib	-8.99	259.02	1
ABL1mut3- Imatinib	-9.39	131.06	2
ABL1wild-Dasatinib	-6.32	23.25	1
ABL1mut1-Dasatinib	-5.72	64.58	No hydrogen bonds
ABL1mut2-Dasatinib	-7.63	2.57	No hydrogen bonds
ABL1mut3-Dasatinib	-6.58	15.12	No hydrogen bonds
ABL1wild-Nilotinib	-6.97	7.76	No hydrogen bonds
ABL1mut1-Nilotinib	-7.07	6.61	No hydrogen bonds
ABL1mut2-Nilotinib	-6.59	14.83	No hydrogen bonds
ABL1mut3-Nilotinib	-7.06	6.69	No hydrogen bonds

Material And Methods

RNA extraction and cDNA conversion

Total RNA was extracted from the peripheral blood samples and was reverse transcribed to cDNA using commercial available cDNA synthesis kit (Qiagen mini blood RNA extraction kit; Cat. No. 52304 and Verso cDNA synthesis kit; Cat. No. AB1453/A). The RNA extraction and cDNA preparation was performed according to the manufacturer's instructions.

PCR amplification of BCR/ABL gene, Direct (Sanger) and Next generation sequencing

Semi nested pcr was performed to amplify kinase domain of BCR/ABL fusion gene. Direct sequencing was performed on amplified product using Big Dye Terminator chemistry (Applied biosystem, USA) in ABI 3130 automated DNA analyser [9]. For Next generation sequencing sample was out sourced and it was performed by Medgenome India Pvt. Ltd.

Homology modelling

The potential template for human ABL1 protein sequence was identified using BLAST-PDB search [10]. Models were generated using commands like *align2d.py* and *model-single.py* wherein the former aligns the ABL1 protein sequence with the template structure and the latter helps in modelling the 3D structures

based on the query-template combo alignment. Of the ten generated models, the structure with least Discrete Optimized Protein Energy (DOPE) was selected and considered for energy minimization using GROMACS [11]. The ligand Imatinib was obtained from Protein Data Bank with a PDB id of 6HD4 and were separated from the protein using Swisspdbviewer[12,13]. Modelled structure was validated using ProSA-Web and Procheck-Ramachandran plot and later considered for docking studies [14]. Two mutations at position 250 and 287 (residues got renumbered after modelling) were introduced within the wild type protein to generate three mutants. After mutations, they were termed as *mut1 (Gly250Glu)*, *mut2 (Ala287Tyr)* and *mut3 (gly250glu and Ala287Tyr)*. All three mutants were energy minimized using GROMACS.

Protein-ligand Docking

Kollman charges were added to the modelled wild and mutant ABL1 protein and saved as *.pdbqt file* which were considered for docking with drugs like Imatinib, Dasatinib and Nilotinib. The prepared proteins and the ligand structures were considered for docking for which the grid box need to be generated. For the same, the active sites were much required which was obtained from the 3D structures available from Protein Data Bank. As per the PDB ids: 6HD4 and 6HD6 amino acids like Asp355, Glu260, Ile334, Thr289, Met 292, His335 (residues renumbered based on the modelled structures) interacts with imatinib drug molecule. Based on the drug binding site, the grid size was set to 82x 96x56 xyz points with grid spacing of 0.364 Å and grid center was designated at dimensions (x, y, and z): 5.367, 9.173 and 12.966.

Discussion

Early identification of mutation (s) helps in deciding effective treatment in CML. However detection of low level clone is challenging as it can be missed out by low sensitivity technique and expand in absence of appropriate TKI therapy in CML patients. If, any reason TKI treatment is discontinued or delayed, the low level mutant clones undergoes in selective expansion and contributes in disease progression [1]. We hypothesized that in our case the dormant clone (p.G250E) which was picked up with NGS, started expansion in selective manner and contributed in disease progression in the absence of TKI therapy. However the mechanism of specific mutant expansion and time for the disease progression is not clear. Recent study also showed importance of NGS in identifying dormant clone which supports our findings [4]. Many clinical trials based on the combinational therapy (TKI and non- TKI drug) are going on to overcome the disadvantages of TKI monotherapy [15–17]. Recent studies also highlighted the importance of NGS and combinational TKI therapy in such patients [6, 18]. Further our study highlighted that the disease progression in CML patients becomes faster after mutation but may show residual sensitivity to existing therapy due to presence of low level of mutant clones. This finding in agreement with an in vitro study conducted by Robert C. Bauer and his colleagues that shows sequential acquisition of *BCR/ABL* drug resistance mutations in CML might be underestimated and suggests close monitoring for the same [2].

Further molecular modelling and docking studies revealed that mutation detected through deep sequencing technique reducing the IM efficacy by increasing inhibition constant and suggests higher concentration of IM to overcome such mutations (Fig. 2A-D). The docking analysis of 2GTKI showed better binding affinity and reduce inhibition constant in mutant clone (Table 1). However, due to lack of hydrogen bond formation, the binding affinity has no value in these proteins. Hence based on the affordability of the patient, treatment with Bosutinib (2GTKI) or Ponatinib (3GTKI) may help in further management of such patients.

In conclusion the detection of clinically relevant low level mutant clones is challenging as it can expand in absence of TKI therapy in CML patients. Though the mechanism of specific mutant expansion and time is not clear. The NGS-in-CML is important to identify IM resistance dormant clone for proper management of the disease.

Declarations

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Author Contributions

S. Dhangar and J Ghatanatti carried out laboratory work. Clinical analysis was done by C. Shanmukhaiah. Bioinformatics analysis was carried out by Selvaa K. C. BR Vundinti designed the study and analysed the results. The manuscript was drafted by S. Dhangar, C. Shanmukhaiah and BR Vundinti.

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Availability of data and material (data transparency)

All the data related to this study are included in this manuscript.

Compliance with ethical standards

Conflict of interest: The authors have no conflicts of interests to declare.

Consent to participate: Consent was obtained from the patient.

Consent for publication: Consent was obtained from the patient.

Ethics approval: This case report was approved by the Institutional Ethics committee.

References

1. Soverini S, Bavaro L, De Benedittis C, Martelli M, Iurlo A, Orofino N et al (2020) Prospective assessment of NGS-detectable mutations in CML patients with nonoptimal response: the NEXT-in-CML study. *Blood, The Journal of the American Society of Hematology*. 20:534–5418
2. Bauer RC, Sanger J, Peschel C, Duyster J, von Bubnoff N (2013) Sequential inhibitor therapy in CML: in vitro simulation elucidates the pattern of resistance mutations after second-and third-line treatment. *Clinical cancer research*. Jun 1;19(11):2962-72
3. Hochhaus A, Baccarani M, Silver RT, Schiffer C, Apperley JF, Cervantes F et al (2020 Apr) European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. *Leukemia* 34(4):966–984
4. Rosso V, Petit J, Dragani M, Andreani G, Croce E, Iacono ML et al (2020 Feb) Clone wars: co-occurrence of IDH2 R140Q and R172K in myelodysplastic syndromes. *Ann Hematol* 4:1–3
5. Alikian M, Gale RP, Apperley JF, Foroni L (2017) Molecular techniques for the personalised management of patients with chronic myeloid leukaemia. *Biomolecular detection and quantification*. 1:4–20
6. Kizilers A, Cris E, Lea N, Passera R, Mian S, Anwar J et al (2019) Effect of low-level BCR-ABL1 kinase domain mutations identified by next-generation sequencing in patients with chronic myeloid leukaemia: a population-based study. *The Lancet Haematology*. May 1;6(5):e276-84
7. Hasford J, Pfirrmann M, Hehlmann R, Allan NC, Baccarani M, Kluin-Nelemans JC et al (1998) A new prognostic score for survival of patients with chronic myeloid leukemia treated with interferon alfa Writing Committee for the Collaborative CML Prognostic Factors Project Group. *JNCI: Journal of the National Cancer Institute*. Jun 3;90(11):850-9
8. Baccarani M, Cortes J, Pane F, Niederwieser D, Saglio G, Apperley J et al Chronic myeloid leukemia: an update of concepts and management recommendations of European LeukemiaNet. *Journal of clinical oncology*. 2009 Dec10;27(35):6041
9. Xu P, Guo D, Shao X, Peng M, Chen B (2017) Characteristics and mutation analysis of Ph-positive leukemia patients with T315I mutation receiving tyrosine kinase inhibitors. *OncoTargets and therapy* 10:4731
10. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ Basic local alignment search tool. *Journal of molecular biology*. 1990 Oct5;215(3):403–10
11. Van Der Spoel D, Lindahl E, Hess B, Groenhof G, Mark AE, Berendsen HJ (2005 Dec) GROMACS: fast, flexible, and free. *J Comput Chem* 26(16):1701–1718
12. Berman HM The protein data bank: a historical perspective. *Acta Crystallographica Section A: Foundations of Crystallography*. 2008 Jan 1;64(1):88-95
13. Guex N (1996) Swiss-PdbViewer: a fast and easy-to-use PDB viewer for Macintosh and PC. *Protein Data Bank Quarterly Newsletter* 77:7
14. Wiederstein M, Sippl MJ (2007) ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. *Nucleic acids research*. Jul 1;35(suppl_2):W407-10

15. Deenik W, Janssen JJ, van der Holt B, Verhoef GE, Smit WM, Kersten MJ et al Efficacy of escalated imatinib combined with cytarabine in newly diagnosed patients with chronic myeloid leukemia. haematologica. 2010 Jun 1;95(6):914-21
16. Westerweel PE, Te Boekhorst PA, Levin MD, Cornelissen JJ (2019) New approaches and treatment combinations for the management of chronic myeloid leukemia.Frontiers in oncology. ; 9
17. Preudhomme C, Guilhot J, Nicolini FE, Guerci-Bresler A, Rigal-Huguet F, Maloisel F et al Imatinib plus peginterferon alfa-2a in chronic myeloid leukemia. New England Journal of Medicine. 2010 Dec 23;363(26):2511-21
18. Soverini S, De Benedittis C, Machova Polakova K, Brouckova A, Horner D, Iacono M et al (2013 Aug) Unraveling the complexity of tyrosine kinase inhibitor–resistant populations by ultra-deep sequencing of the BCR-ABL kinase domain. Blood The Journal of the American Society of Hematology 29(9):1634–1648

Figures

Figure 1

Chromatogram showing acquired mutations in their order of appearance with respective TKI (Top to bottom) (left) and mutations detected by next generation sequencing (right).

Figure 2

Molecular modelling of Wild type and mutant protein and their docking with TKIs **a** Interaction of ABL1-wildtype with Imatinib **b** Interaction of ABL1-mutant1 with Imatinib **c** Interaction of ABL1-mutant 2 with Imatinib **d** Interaction of ABL1-mutant3 with Imatinib