Case Report

Leucine replaced by methionine at 273 position in chronic myeloid leukemia: Knowns and unknowns…

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ABSTRACT

Chronic myeloid leukemia is a clonal bone marrow stem cell disorder characterized by the presence of Philadelphia chromosome t(9;22)(q34;q11) leading to fusion oncogene BCR-ABL. Tyrosine kinase inhibitors (TKIs) act by competitively inhibiting BCR-ABL oncoprotein with significant response rates. However, up to 30% of patients fail to achieve complete cytogenetic remission on 1st line TKI imatinib, one of the reasons being mutations in BCR-ABL kinase domain leading to imatinib resistance. Over 80 such mutations have been documented in the literature; however, some of the rare mutations still remain to be studied for their impact in development of resistance and their responsiveness to currently available therapeutic options. Here, we report one such case of a rare mutation leucine replaced by methionine at 273 position and its clinical implications.

Key words: Chronic myeloid leukemia, imatinib resistance mutation analysis, leucine replaced by methionine at 273 position

INTRODUCTION

Chronic myeloid leukemia (CML) is a clonal hematopoietic stem cell disorder characterized by the presence of Philadelphia chromosome due to a reciprocal translocation between chromosome 9 and 22 leading to BCR-ABL fusion protein which has oncogenic property.

Over the last decade, there has been a significant revolution in therapy and outcomes of CML with advent of targeted therapy with tyrosine kinase inhibitors (TKIs) which act by competitive inhibition of BCR-ABL oncoprotein and inhibit BCR-ABL signaling. Among the TKIs, first generation TKI is imatinib, while second generation TKIs include nilotinib, dasatinib, and bosutinib; ponatinib being a third generation agent. They have effectively produced long-term remissions and disease control in CML.

However, around 30–90% of patients fail on imatinib depending on the phase of the disease due to imatinib resistance with one of the reasons being mutations in BCR-ABL kinase domain (KD) detected by imatinib resistance mutation analysis (IRMA).[1] With newer more sensitive diagnostic modalities, many new BCR-ABL KD mutations are detected and being studied in detail for their significance in clinical practice.[2] However, strong evidence is lacking for guiding therapeutic changes based on these novel mutations.

Here, we report one such rare mutation, leucine replaced by methionine at 273 position (L273M) found in our patient of CML-chronic phase (CP) and his clinical course with regards to the impact of this mutation for leading to imatinib resistance.

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CASE REPORT

A 20-year-old male had presented in 1993 with CML-CP. He was started on hydroxyurea; on which he attained complete hematological remission (CHR). He was later changed to interferon and ultimately to imatinib in 2002. He attained complete cytogenetic response at 12 months on imatinib; however, never achieved a major molecular response. In 2006, he lost cytogenetic response with a significant rise in BCR-ABL transcripts. IRMA was performed then, which revealed L273M mutation. His imatinib dose was escalated to 600 mg/day and later to 800 mg/day on which he maintained his CHR until April 2014; when he progressed to accelerated phase (CML-AP). He was changed over to nilotinib, but he developed persistent grade 4 thrombocytopenia. Bone marrow study done at this time revealed blast crisis (CML-BP), and he was started on dasatinib. IRMA analysis again revealed the same mutation. Allogeneic stem cell transplant was not attempted due to financial constraints. However, disease progressed rapidly over next 2 months, and ultimately, he expired in August 2014 [Table 1].

DISCUSSION

Imatinib resistance is emerging as an important problem in management of CML. Common causes of drug resistance are poor compliance, impaired absorption, activation of alternative signaling pathways, and mutation in BCR-ABL domain leading to imatinib resistance. Among them, imatinib-resistant mutations are a common and the best-characterized mechanisms of imatinib failure. Depending on the phase of CML, 30–90% of cases of CML who have failed on imatinib may show this mutation.[1] Current guidelines for management of CML thus recommend IRMA whenever there is a failure to achieve optimal response or progression of disease to change therapy accordingly.[3] There are studies suggestive of more frequent mutations seen for those with older age, with prior interferon therapy, patients presenting with CML-AP or CML-BP *de novo* and at the time of failure.[4]

More than 80 different amino acid substitutions are identified in literature associated with imatinib resistance.[5] With newer more sensitive techniques of DNA sequencing, more mutations are being identified in BCR-ABL KD. Apart from being predictive of response to TKIs, imatinib-resistant mutations also represent genetic instability and have shown prognostic significance. It has been observed that those patients with IRMA showing specific mutations have poorer outcomes than those without any detectable mutation.[6] Prognosis also depends on the site of mutation, for example, mutations in P-loop have poorer outcomes,[7] especially T315I mutation (also known as gatekeeper mutation) which has significant resistance to imatinib and all the three second-generation TKIs.

These mutations impair BCR-ABL oncoprotein inhibition by imatinib, which leads to varying degrees of resistance to imatinib. Second generation TKIs overcome most of the mutations, but they are not universally active. Thus, each mutation responds distinctly to the available TKIs. After studying inhibitory concentration of the drug at 50%, European leukemia net guidelines have proposed the sensitivity pattern of the mutations to the available TKIs.[5] One of the major limitation of this classification is that it has not addressed all the mutations [Figure 1]. Third generation TKI, ponatinib has shown efficacy against gatekeeper mutation T315I, and data are emerging for its efficacy and superiority in this setting over allogeneic stem cell transplant in CML-CP.[8]

L273M mutation found in our case is a very rare mutation. It suggests replacement of leucine with Methionine at

Table 1: Case capsule

Time line	Hematologic evaluation	Cytogenetic evaluation	Molecular - bcr-abl/ abl1 transcript	IRMA	Therapy started	Response to therapy
1993	CML-CP	t (9;22) (q43;q11)	ND	ND	Hydroxyurea	CHR at 6 months
1995	CHR	Ph 30%	ND	ND	Interferon-alpha	CHR maintained, stopped after 1 year due to logistic issues
1996	CHR	Ph 10%	ND	ND	Hydroxyurea	CHR maintained
2002	CHR	Ph 30%%	78%	ND	Imatinib 400mg OD	CCyR at 12 months, bcr-abl transcripts steadily decreased but No MMR
2006	CHR	Lost CCyR - PCyR (Ph 20%)	3% à 24% rise	L273M	Imatinib 600mg OD	Bcr-abl transcripts steadily decreased but No MMR
2008	CHR	PCyR (Ph 30%)	8% à 66% rise	L273M	Imatinib 800mg OD	Bcr-abl transcripts reached nadir of 1.7% but No MMR
April 2014	CML-AP (accelerated phase)	Ph 80%	81%	L273M	Nilotinib 400mg BD	Didn't tolerate due to frequent thrombocytopenia
July 2014	CML-BP (blast phase)	ND	ND	ND	Dasatinib 70mg BD	Disease progressed and patient Expired in Aug 2014

CHR: Complete hematological remission, CML-CP: Chronic myeloid leukemia-chronic phase

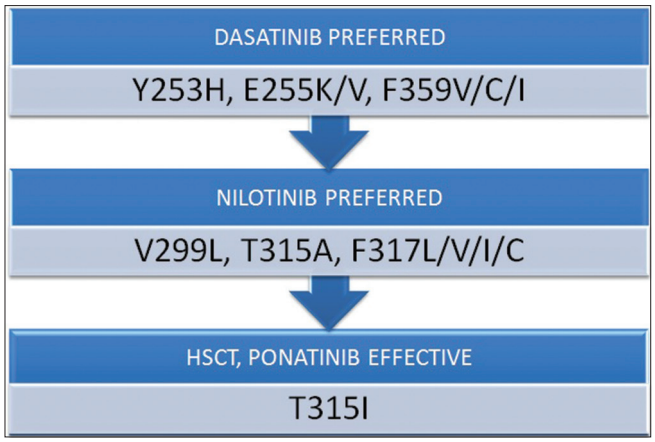

Figure 1: Tyrosine kinase inhibitor sensitivity according to mutational pattern

273rd position in BCR-ABL KD. This mutation lies in juxtaposition to P-loop. The mutation in not frequently reported with resistance to imatinib or nilotinib in a clinical setting. However, *in vitro* studies have suggested a possible partial resistance imparted by this mutation to imatinib. Our patient's clinical course suggests that this mutation imparts only partial resistance to imatinib considering prolonged maintenance of CHR for about 8 years after its detection.

Our case is an attempt to highlight three important facts about newer rare imatinib-resistant mutations. (1) With newer techniques of DNA sequencing such as mass spectroscopy and ultra-deep sequencing, many newer mutations are being identified; however, their clinical significance remains unknown, and more data regarding their utility in therapeutic decision making is required.[9,10] (2) For newer rare mutations, there is a need to differentiate them from single nucleotide polymorphisms (SNPs) which can be done by assessing normal ABL allele. This is important as SNPs do not implicate secondary imatinib resistance and do not need any change in therapy.[11] (3) Some of the newer mutations may still be partially or completely sensitive to imatinib, so there is a need for identifying biochemical and biological characterization of resistant phenotype before it can be ascribed as a mutant.[12]

Our patient survived with CML for 21 years and received multiple lines of therapies evolved in CML. He ultimately developed an uncommon mutation L273M for which no specific therapeutic guidelines are available. His clinical course suggests that L273M imparts only partial resistance to imatinib which could be overcome by higher doses of the drug. We want to emphasize the need of reporting such rare mutations and studying them in detail regarding their role in imatinib resistance and in formulating specific management guidelines for further therapy.

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Conflicts of interest
There are no conflicts of interest.

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