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Imatinib resistance mutation analysis: Experience from a tertiary oncology center

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Original Article

Abstract

Purpose: BCR-ABL kinase domain (KD) mutations account for 50-90% of the imatinib resistance observed in patients of CML-chronic phase. In CML-CP patients receiving imatinib first-line, mutation analysis is recommended in case of failure or suboptimal response using European LeukemiaNet (ELN) criteria. The present study was carried out at a tertiary oncology centre in south India to assess which mutations accounted for resistance to imatinib among patients of chronic phase CML being treated with imatinib. **Methods:** This was a retrospective observational study. We analyzed patients who were tested for imatinib resistance mutation in view of suboptimal responses while on imatinib or imatinib failure. Direct sequencing of the BCR-ABL transcript by the Sanger method was used for IRMA testing. **Results:** Out of 120 tested for IRMA, 36 (30%) had detectable mutations. We observed a higher frequency of mutations at amino acids T315, F359 and M351T. **Conclusions:** Among the patients who were tested for imatinib resistance mutation in view of suboptimal responses while on imatinib or imatinib failure, 30% had IRMA +ve mutations. The high incidence of imatinib resistance in present study may be attributed to the fact that our patients were given higher dose of imatinib (600 mg), if they failed to achieve CCyR at 12 months or CHR at 3 months as they could not afford second generation TKIs.

Keywords: Imatinib; IRMA; Mutation

Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by the presence of the Philadelphia (Ph) chromosome resulting from the reciprocal translocation t (9; 22) (q34; q11).[1, 2] The molecular consequence of this translocation is the generation of the BCR-ABL fusion gene, which encodes a constitutively active protein tyrosine kinase. Typically, CML has 3 clinical phases: chronic stable phase, accelerated phase and blast crisis phase with 90% of patients being diagnosed in the chronic phase. The standard treatment of choice in chronic stable phase has been imatinib mesylate 400 mg OD. Imatinib mesylate is tyrosine kinase inhibitor that inhibits the abnormal bcr-abl tyrosine kinase created by the Philadelphia (Ph1) chromosome translocation abnormality. Imatinib inhibits proliferation and induces apoptosis in cells positive for *BCR/ABL*.[3-5]

With 5 years of follow-up, the overall survival of patients randomized to imatinib as initial therapy is 89% and with 8 years, it is 85%.[6, 7] Second generation tyrosine kinase inhibitors like Nilotinib and Dasatinib are also approved for first line treatment in chronic phase CML and have shown better cytogenetic and molecular responses and improvements in progression-free survival as compared to Imatinib.[8, 9] However considering their high cost, imatinib still remains the gold standard for frontline treatment of CML, especially in low income countries.

Although imatinib is a major breakthrough in treatment of chronic phase CML, every third or fourth patient has to come off this therapy due to resistance or intolerance. BCR-ABL kinase domain (KD) mutations account for 50-90% of the imatinib resistance observed in patients of CML-CP.[10] Till-date, more than 90 discrete resistance conferring point mutations at 57 residues in the ABL kinase have been documented.[11] The present study was carried out at a tertiary oncology centre in south India to assess which mutations accounted for resistance to imatinib among patients of chronic phase CML being treated with imatinib.

Methods and Materials

This was a retrospective observational study. We analyzed patients who were tested for imatinib resistance mutation in view of suboptimal responses while on imatinib or imatinib failure (loss of achieved milestones). Responses were being assessed using ELN 2009 guidelines for management of

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CML.[12] Complete haematologic response (CHR) was defined as white blood count < 10×10^9 /L; basophils <5%; no myelocytes, promyelocytes, myeloblasts in the differential; platelet count <450×10^9 /L and no palpable spleen. Major cytogenetic response (MCyR) was defined as 0% - 35% Ph+ metaphases in bone marrow aspirates. Complete CyR (CCyR) was defined as no Ph+ metaphases, partial CyR (PCyR) was defined as 1% to 35% Ph+ metaphases, minor CyR was defined 36% to 65% Ph+ metaphases, minimal CyR was taken with 66% to 95% Ph+ metaphases and presence of >95% Ph+ metaphases confirmed no CyR. Complete molecular response was considered with undetectable BCR-ABL messenger ribonucleic acid transcript by real time quantitative and/or nested polymerase chain reaction in two consecutive blood samples of adequate quality and major molecular response (MMolR) was defined as ratio of BCR-ABL to ABL ≤ 0.1% on the international scale. Response was considered as optimal in patients who achieved CHR and at least minor CyR at 3 months, PCyR at 6 months, CCyR at 12 months and MMolR at 18 months (**Table 1** shows response definitions). IRMA was done if patients failed to achieve CHR at 3 months or CcyR at 18 months or if they had loss of CHR/MMolR/ progression to accelerated phase at any time (**Table 2** shows patient criteria for IRMA).

TABLE 1: Response recommendations of European LeukemiaNet 2009.

Time	Optimal response	Suboptimal response	Failure
3 months	CHR and at least minor CyR (Ph+ ≤ 65%)	No cytogenetic response (Ph+ > 95%)	Less than CHR
6 months	PCyR(ph+ 1-35%)	Less than PCyR (Ph+ > 35%)	No cytogenetic response (Ph+ > 95%)
12 months	CCyR and MMolR	PCyR (Ph+ 1-35%)	Less than PCyR (Ph+ > 35%)
18 months	CCyR and MMolR	Less than MMolR	Less than CCyR
Any time	Stable CMolR, MMolR	Loss of MMolR	Loss of CHR, Loss of CCyR, Cytogenetic clonal evolution

Abbreviation: CHR = Complete hematologic response; CyR = Cytogenetic response; PCyR = Partial cytogenetic response; CCyR = Complete cytogenetic response; CMolR = Complete molecular response; MMolR = Major molecular response.

TABLE 2: Patient criteria for IRMA.

Indication for IRMA	Number of patients (n= 36)
Failure to achieve CHR at 3 months	4
Failure to achieve CCyR at 18 months	10
Loss of CHR	11
Loss of MMolR	5
Progression to accelerated phase/ blast crisis	6

Results

Between January 2007 and January 2013, a total of 120 patients were tested for IRMA. Direct sequencing of the BCR-ABL transcript by the Sanger method was used for IRMA testing.[13] Out of 120 patients, 84 patients did not have any mutations. Rest of the 36 patients various mutations rendering them resistant to imatinib (**Table 3**). Among these 36 patients, 11 patients had loss of CHR; 5 patients had loss of MMolR; 4 patients had failure to achieve CHR at 3 months; 10 patients had failure to achieve CCyR at 12 months and 6 patients had progression to accelerated phase/ blast crisis. We observed a higher frequency of mutations at amino acids T315, F359 and M351T. In contrast to GIMEMA study we had low frequency of M244V, Y253H and E255V.[14]

TABLE 3: Mutations detected by IRMA in present study.

Mutation	Number of patients
T315I	6
F359I	4
M351T	4
V253F	3
G250E	3
M244V	2
E275K	2
H395R	2
F359C	2
Y253H	1
F311L	1
F517L	1
Y253F	1
E255V	1
E355A	1
E355D	1
I418V	1

Discussion

The present study was done to assess which mutations accounted for resistance to imatinib among patients of chronic phase CML being treated with imatinib. Out of 120 patients tested for IRMA, nearly one-third (30%) had mutations detected by IRMA. Our study results are in parallel with other reported study in India in which 29 out of 90 (32.2%) patients had detectable KD mutations.[14] A considerably higher percentage of mutations (43%) was reported in the GIMEMA study.[15] The lower frequency of mutations in our study as compared to GIMEMA study may be attributed to the fact that while our study primarily focused on CP patients, the GIMEMA study had patients from all stages of CML. We observed a higher frequency of mutations at amino acids T315, F359 and M351T. In contrast to GIMEMMA study we had low frequency of M244V, Y253H and E255V.

Apart from imatinib, specific mutation types are also closely associated with resistance to 2nd generation TKIs and this information is useful in directing the choice of TKI after

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imatinib failure. (**Table 4**) Resistance to dasatinib often manifests as mutations at amino acids 299 (V299L), 315 (T315I) and 317 (F317 L/I) and resistance to nilotinib preferentially results from mutations such as G250E, Y253H, E255K, T315I, or F311I.[16]

TABLE 4: Selection of therapy following particular mutation.

Type of mutation	Appropriate therapeutic option
T315I	Stem cell transplantation/experimental drug
V299L,T315A, F317L/V/I/C	Nilotinib
Y253H, E255K/V, F359V/C/I	Dasatinib
Any other mutation	High dose imatinib/nilotinib/dasatinib

Conclusion

Among the patients who were tested for imatinib resistance mutation in view of suboptimal responses while on imatinib or imatinib failure, 30% had IRMA +ve mutations. The high incidence of imatinib resistance in present study may be attributed to the fact that our patients were given higher dose of imatinib (600mg), if they failed to achieve CCyR at 12 months or CHR at 3 months as they could not afford second generation TKIs.

Conflict of interest

The authors declare that they have no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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