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).

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.

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%.

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. 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. Till-date, more than 90 discrete resistance conferring point mutations at 57 residues in the ABL kinase have been documented. 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...
CML. 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. 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.

**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                 |
| E278K    | 2                 |
| H395R    | 2                 |
| F359C    | 2                 |
| Y253H    | 1                 |
| F311L    | 1                 |
| F517L    | 1                 |
| Y253F    | 1                 |
| E255V    | 1                 |
| E355A    | 1                 |
| E355D    | 1                 |
| I1418V   | 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. A considerably higher percentage of mutations (43%) was reported in the GIMEMA study. 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 GIMEMA 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
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

| Type of mutation | Appropriate therapeutic option |
|------------------|--------------------------------|
| T315I            | Stem cell transplantation/experimental drug |
| V299L,T315A      | Nilotinib |
| F317L/V/E/C      | Dasatinib |
| Y253H, E255K/V   | Dasatinib |
| F359V/C/I        | 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.

### References

1. Jabbour E, Kantarjian H. Introduction: chronic myelogenous leukemia (CML). Semin Hematol 2007; 44:S1-3.
2. Rowley JD. Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature 1973; 243:290-3.
3. Druker BJ, Sawyers CL, Kantarjian H, et al. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N Engl J Med 2001; 344:1038-42.
4. Kantarjian H, Sawyers C, Hochhaus A, et al. Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. N Engl J Med 2002; 346:645-52.
5. Goldman JM, Druker BJ. Chronic myeloid leukemia: current treatment options. Blood 2001; 98:2039-42.
6. Druker BJ, Guilhot F, O’Brien SG, et al. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. N Engl J Med 2006; 355:2408-17.
7. Deininger MW, O’Brien SG, Guilhot F, et al. International randomized study of interferon vs STI571 (IRIS) 8-Year follow up: sustained survival and low risk for progression or events in patients with newly diagnosed chronic myeloid leukemia in chronic phase (CML-CP) treated with imatinib. Blood 2009; 114:1126.
8. Kantarjian H, Shah NP, Hochhaus A, et al. Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med 2010; 362:2260-70.
9. Saglio G, Kim DW, Issaragrisil S, et al. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. N Engl J Med 2010; 362:2251-9.
10. Ernst T, Erben P, Müller MC, et al. Dynamics of BCR-ABL mutated clones prior to hematologic or cytogenetic resistance to imatinib. Haematologica 2008; 93:186-92.
11. Melo JV, Chuah C. Resistance to imatinib mesylate in chronic myeloid leukaemia. Cancer Lett 2007; 249:121-32.
12. Baccarani M, Cortes J, Pane F, et al. Chronic myeloid leukemia: an update of concepts and management recommendations of European LeukemiaNet. J Clin Oncol 2009; 27:6041-51.
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; 108:28-37.
14. Rajappa S, Mallavarapu KM, Gundeti S, et al. Kinase domain mutations and responses to dose escalation in chronic myeloid leukemia resistant to standard dose imatinib mesylate. Indian J Med Paediatr Oncol 2013; 34:221-3.
15. Soverini S, Colarossi S, Gnani A, et al. 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 Can Res 2006; 12:7374-9.
16. Cortes J, Jabbour E, Kantarjian H, et al. Dynamics of BCR-ABL kinase domain mutations in chronic myeloid leukemia after sequential treatment with multiple tyrosine kinase inhibitors. Blood 2007; 110:4005-11.