Original Article

H396R, F359V and E255K mutations of the Abl kinase domain in imatinib-resistant Nigerian patients with chronic myeloid leukemia

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ABSTRACT

AIM We have observed therapy failure in some Nigerian patients with chronic myeloid leukemia (CML). We therefore set out to determine the causes of imatinib resistance in these patients.

METHODS From August 2003 to July 2010, we registered and commenced 266 consenting CML patients on imatinib (IM) under the Glivec International Patient Assistance Program (GIPAP), sponsored by Novartis Pharma. We investigated the occurrence of Abl kinase domain mutations (KDM) among a cohort of these patients, with features of imatinib resistance or intolerance. Peripheral blood samples were collected from 14 patients, after informed consent was obtained for Bcr-Abl quantitative PCR assessment and Abl KDM screening.

RESULTS The chimeric Bcr-Abl gene was detectable in all patients, (range of 0.18 – 141.9%), when compared with the Abl gene. Sequencing analysis was done in 12 out of the 14 patients and Abl KDM were identified in three of the 12 patients (one quarter). One mutant (H396R) is known to retain intermediate sensitivity to imatinib, while the other two (E255K and F359V) are insensitive; all are sensitive to nilotinib. Four of these patients have since been commenced on nilotinib and are responding well to therapy, while three have died of disease progression.

CONCLUSION An increasing number of Nigerian CML patients on imatinib are developing resistance or intolerance to the drug, and some are due to kinase domain mutations, while others may have other yet unexplained reasons for suboptimal response. Continuous monitoring is mandatory in the care of CML patients, to aid early detection of suboptimal therapy outcomes, necessitating further molecular studies. Additionally, more work needs to be done to fully understand the mechanisms for these therapy failures in Nigerian CML patients.

Keywords: Leukemia, Myelogenous, Chronic, BCR-ABL Positive ; imatinib; nilotinib; Polymerase Chain Reaction; mutation; Drug Resistance.

INTRODUCTION

Chronic myeloid leukaemia was the first human malignancy to be linked to a specific acquired genetic abnormality, the Philadelphia chromosome which is formed as a reciprocal translocation between the long arms of chromosomes 9 and 22.[1] This translocation produces the Bcr-Abl fusion gene on chromosome 22 that encodes an Abl tyrosine kinase with increased activity. The discovery of imatinib, the first molecularly-targeted drug directed at this aberrant tyrosine kinase, has revolutionized the management of CML.[2] The clinical and cytogenetic response to imatinib was so remarkable that it soon became the first-line therapy for all stages of CML, effectively relegating allogeneic stem cell transplantation to a second-line option.[3-5]

While most patients achieved significant hematologic and cytogenetic improvement with imatinib, resistant CML clones soon emerged,[6] leading to the development of other tyrosine kinase inhibitors (TKIs).[7] Also, some patients experience disease progression during imatinib therapy.[8] The most common cause of imatinib resistance is the development of point mutations in the Abl kinase imatinib-binding domain.[9] Imatinib (Glivec, Novartis) became available in Nigeria in 2003 under the patient assistance programme of Novartis, and the local patient outcomes have since improved tremendously.[10] However, we have also been recording cases of imatinib resistance. We therefore examined our imatinib-resistant patients for the presence of kinase domain mutations.

PATIENTS AND METHODS

From August 2003 to July 2010, 266 consenting CML patients have been registered with the Glivec International Patient Assistance Program (GIPAP), and commenced on Imatinib (IM). We investigated the occurrence of Abl kinase domain mutations (KDM) among a cohort of these patients, with features of imatinib resistance or intolerance. Peripheral blood samples were collected from 14 patients, after informed consent was obtained for Bcr-Abl quantitative PCR (qPCR) assessment as well as Abl KDM screening which was done in 12 of these 14 patients.

This prospective study included all confirmed cases of Philadelphia chromosome-positive (Ph+) and/or Bcr-Abl-positive CML patients in all disease phases managed in our centre over a seven-year period. At every clinic visit, patients are examined to identify those with features suggestive of suboptimal response to IM, and thus candidates for possible resistance. Local institutional approval was obtained for this study. Written informed consent was obtained from all patients according to the Helsinki declaration.

Total RNA was extracted from mononuclear cells from the peripheral blood samples, and subjected to reverse-transcription and quantitative PCR. The resulting amplicon was sequenced and the Abl kinase domain examined for mutations. All qPCR and KDM screening were carried out at the Hammersmith Hospital, London.

RESULTS

Fourteen imatinib-resistant or intolerant patients were screened (male/female ratio was 6/8), mean age was 38.1 ± 22.9. The chimeric Bcr-Abl gene was detectable in all patients, (range of 0.18 – 141.9%) when compared with the Abl gene. Of the 12 patients in whom KDM screening was done, three (one quarter) had mutations. Two mutations (H396R and F359V) are known to retain intermediate sensitivity to imatinib, while the third (E255K) is insensitive; though all are sensitive to nilotinib.[11]

All 14 patients were recruited to the follow-up program of compassionate use of nilotinib (Novartis), and four of them have since been commenced on nilotinib. Two of the four commenced have been on nilotinib for >16 months and are in major molecular remission. Four of the 14 patients have since died, from disease progression.

There was an average of 313 ± 984 days from diagnosis to commencement of imatinib in all 14 patients, though this was heavily skewed by five patients with a range of 375 – 1597 days, while the remaining ranged from 0 – 71 days. They had been on imatinib for an average of 43.5 ± 46.2

months before showing features of therapy failure, necessitating molecular testing (Table 1).

Of the five patients starting imatinib more than one year after diagnosis, three of them have died, representing three fifths of all deaths from this cohort. Using the Kaplan-Meier survival model, commencement of imatinib more than one year after diagnosis ($p = 0.028$) and advanced Hasford risk group (i.e. intermediate and high grade disease) at diagnosis ($p = 0.027$) were found to be statistically significant in predicting for death events. The presence of Abl kinase domain mutations and Sokal risk groups were statistically insignificant in predicting death events in this study ($p > 0.05$).

DISCUSSION

Imatinib has been shown to act by binding to the ATP-binding site of the Abl catalytic domain, resulting in the inhibition of phosphorylation of Bcr-Abl, thereby preventing its kinase activity and making the cell susceptible to apoptosis. Imatinib resistance is due to the interactions between several factors, some Abl-dependent and others Abl-independent: treatment compliance, bioavailability, pharmacodynamics, genetic changes and Bcr-Abl kinase domain mutations.[11] Plasma levels of imatinib are reduced in individuals with increased activity of cytochrome p450 isoenzymes p3A4 and p3A5 or use of enzyme inducing drugs leading to reduced efficacy of imatinib.[12,13] Resistance to imatinib is sometimes primary i.e. no response at the beginning of therapy, or secondary i.e. loss of initial response. There are also variations in the manifestation of resistance: some patients present with hematologic - lack or loss of complete haematological remission (CHR), cytogenetic - persistence or recurrence of Philadelphia chromosome, or molecular - persistence or recurrence of Bcr-Abl transcripts on qPCR.

Unfortunately, in the clinical setting and for individual patients, only the assessment of changes in the Abl kinase domain is feasible. In this study, we have found that only about 25% of our IM-resistant or -intolerant patients have a resistance-conferring mutation. The values reported by different workers have unfortunately varied widely from 20% to 90%, due largely to differences in the stringency criteria for detection. In fact, it has been claimed that the ability to detect KDMs in these patients is highly dependent on patient selection and the assay methods, and particularly on the sensitivity of the method used. It has been estimated that 20-30% of CML patients will eventually develop resistance to

Table 1. Detailed summary of Abl-Bcr kinase domain mutations in all patient

SNo.	Age Sex	Time from diagnosis to starting imatinib (d)	Duration of imatinib treatment at time of screening (mo)	Overall survival on imatinib (mo)	Bcr-Abl kinase domain mutation	Abl-bcr/abl ratio	Clinical State
1	31 F	62	20	35	None	23.069	Alive on Nilotinib
2	37 F	1227	45	48	H396R	108.132	Dead
3	43 M	50	48	59	E255K	66.725	Alive on Nilotinib
4	26 F	392	32	39	None	141.895	Dead
5	20 F	70	55	60	Not done	0.189	Alive
6	17 F	375	44	47	None	120.470	Dead
7	45 M	71	19	23	None	4.584	Alive
8	37 F	13	28	32	None	0.715	Alive
9	42 F	16	93	95	None	57.736	Dead
10	50 M	0	84	84	Not done	53.000	Alive on Nilotinib
11	45 F	1597	52	63	F359V	21.885	Alive
12	59 M	390	16	22	None	0.368	Alive
13	41 M	57	26	32	None	14.294	Alive on Nilotinib
14	41 M	63	46	47	None	81.028	Alive

imatinib,[11] and up to 90% of these are probably due to kinase domain mutations.[14]

Some studies on the CML stem cell have shown them to be insensitive to Imatinib, and they thus persist in a quiescent stage [15] despite adequate clinical response, hence they can proliferate when the drug is discontinued and this can be a source of resistance.

Additionally, some novel mutations have been observed in some CML patients, which are associated with rapid proliferation of cells, blastic transformation and resistance to imatinib. Some patients have even been demonstrated by Gorre et al to have increased expression of the Bcr-Abl gene; the importance and clinical effects of which were explored in their report.[16] It was shown that the H396R (detected in patient 2) impairs conformational change of the activation loop, while the E255K (detected in patient 3) impairs conformational change of the P loop. The F359V (detected in patient 11) inhibits imatinib binding leading to resistance. Fortunately, all three mutations are known to retain sensitivity to Nilotinib and interestingly responded initially to escalated doses of Imatinib. Two of the patients died while awaiting drug supplies, while the third is doing well on nilotinib. Only the T315I mutation is known to confer resistance to imatinib, dasatinib, nilotinib and bosutinib.[17]

Our study also shows that there is a much higher risk of death in patients who were diagnosed more than one year before they commenced imatinib and patients with advanced disease using the Hasford scoring system. However, these results are to be interpreted with caution since our numbers are rather small, and hence with limited statistical power. In any case, it is prudent to commence patients on targeted therapy as soon as a diagnosis is made; and to mandatorily monitor for early detection of resistance i.e. complete blood counts every 4-6 weeks and bone marrow cytogenetic analysis every 6 months.[8] It is equally vital to properly counsel patients to ensure compliance and to withdraw imatinib in patients who for any reason are intolerant.

Whenever imatinib failure or resistance is suspected, quantitative molecular assessment of the Bcr-Abl transcript and Abl kinase domain mutations should be investigated.[18] Plans for second-generation TKI should be commenced at the same time as these tests are done. These agents have been proven to be effective in imatinib resistance.[19,20] Considering the favourable responses reported and also noted in this study, it is advisable to escalate imatinib doses to 600 mg/d and 800 mg/d, while other TKIs are being expected. This is particularly so, since most cases are negative for KDMs. The combination of imatinib with other drugs like hydroxyurea and cytarabine is also a good strategy.

Patients in blastic crises are to be treated with acute leukaemia induction regimen, in combination with imatinib. Allogeneic stem cell transplantation may be considered if T315I mutation is discovered, as this mutation does not respond to any of the TKIs. Supportive therapy should be given as necessary in form of blood products support, antibiotics and growth factors.

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FOOTNOTES

Conflicts of interest: The authors declare no competing conflicts of interest.

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