Malaysian J Pathol 2017; 39(2) : 107 – 113

ORIGINAL ARTICLE

Primary imatinib resistance in chronic myeloid leukemia patients in a developing country: *BCR-ABL* kinase domain mutations or *BCR-ABL* independent mechanisms?

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Abstract

Clinical resistance to imatinib (IM) in chronic myeloid leukemia (CML) carries adverse consequences. We investigated 22 CML patients who developed IM-resistance for *BCR-ABL* kinase domain (KD) mutations. The median follow-up for this study was 101.9 months (range: 22.2 to 176.5 months) and the estimated mean overall survival was 150.87 months (95% CI: 130.0 to 171.0). Five out of 22 patients tested positive for *BCR-ABL* KD mutations: 2 had T315I, 2 had E255K and 1 had V289F mutations. Of the remaining 17 patients who did not harbor *BCR-ABL* KD mutations, 11 patients received nilotinib while the rest continued on IM. All 17 achieved haematological remission but only 5 patients achieved complete cytogenetic remission, 4 of whom did so after switching to nilotinib. Our study shows that most of our IM-resistant patients do not test positive for *BCR-ABL* KD mutations by available testing methods and the role of second generation tyrosine kinase inhibitors remains undetermined. A critical analysis of the *BCR-ABL* KD mutations and the underlying mechanisms/ pathways of *BCR-ABL* independent IM-resistance along with potential treatments in the horizon will be discussed.

Keywords: imatinib, chronic myeloid leukemia, BCR-ABL mutations, nilotinib

INTRODUCTION

Chronic myeloid leukemia (CML) is caused by the chromosomal translocation t(9;22) which results in the Philadelphia (Ph) chromosome. This aberrant *BCR-ABL* fusion gene results in the translation of a constitutively active tyrosine kinase, *BCR-ABL* that plays a central role in the pathogenesis of this disease. Imatinib (IM) is a *BCR-ABL*-targeted agent that acts as a tyrosine kinase inhibitor (TKI), the use of which has significantly altered the treatment landscape and improved the prognosis of this disease.[1] Point mutations in the *BCR-ABL* kinase domain (KD) are known to be responsible for most cases of clinical resistance to IM. Different *BCR-ABL* KD mutations are known to confer variable degrees of resistance, but generally (exceptions include the T315I mutation) maintain non-overlapping sensitivity to second generation TKIs such as nilotinib and dasatinib,[2] of which only the former is available in Malaysia. Various international organizations have established guidelines for *BCR-ABL* KD mutation testing and utilization of second generation TKIs[3]. Although the amino acid substitutions that are responsible for *BCR-ABL* KD mutations and their *in-vitro* sensitivities to TKIs are increasingly known and documented, this database is far from complete. IM-resistant disease with no detectable *BCR-ABL* KD mutations are believed to involve *BCR-ABL* independent pathways such as drug influx-efflux, alternative signaling pathways, DNA hypermethylation, microRNA (miRNA) dysfunction and as such, may require a non-TKI

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centered treatment approach. In this study, we retrospectively analyzed the clinical behaviour of 22 IM-resistant CML patients with and without *BCR-ABL* KD mutations.

PATIENTS AND METHODS

Study design

Twenty-two CML patients from Universiti Kebangsaan Malaysia Medical Centre who demonstrated IM-resistance from January 2000 until February 2015 were investigated for *BCR-ABL* KD mutations. Case notes from all patients were critically analysed retrospectively. Baseline clinical characteristics (demographics, presence/absence of *BCR-ABL* KD mutation, disease phase at diagnosis and maximum IM dose tolerated) (Table 1), reported dose-limiting toxicities of IM, clinical characteristics/outcomes of patients with (Table 2) and without *BCR-ABL* KD mutations (Table 3) are summarized in Tables 1 to 3.

Diagnosis and treatment

The patients were diagnosed with CML based on bone marrow morphology and cytogenetics, i.e. detection of the Ph chromosome via karyotyping using chromosome banding analysis (CBA) and fluorescent *in-situ* hybridization (FISH) using dual probes for *BCR* and *ABL1* genes as described in published guidelinesl.[3,4] All patients were treated with hydroxyurea prior to receiving IM. Once warning/failure was documented, IM was dosed incrementally until maximum tolerated levels. Treatment was changed to nilotinib based on suboptimal/loss of responses despite increasing IM doses and accessibility to nilotinib.

Response to treatment

Following initiation of treatment, the patients were initially assessed for hematological response. Cytogenetic response was subsequently assessed with CBA of peripheral blood. If negative or unsuccessful, FISH will follow. Once complete cytogenetic response is achieved, patients will undergo surveillance with standardized reverse-transcriptase polymerase chain reaction (RT-PCR). Definitions of responses are as outlined in the National Comprehensive Cancer Network[4]:

Complete hematological response (CHR) - complete normalization of peripheral blood counts with leucocyte count < 10 x 10^9/L and platelet count < 450 x 10^9/L; no blasts or other immature cells in the blood; absence of signs and symptoms of disease and disappearance of palpable splenomegaly.

TABLE 1: Baseline characteristics of CML-resistant patients at diagnosis

	***BCR-ABL* KD mutation (n = 5)**	**No *BCR-ABL* KD mutation (n = 17)**
Median age, years	54 (39-69)	48 (28-75)
Sex		
Male	2	7
Female	3	10
Ethnic group		
Malay	4	10
Chinese	1	6
Indian	0	1
Disease phase at diagnosis		
Chronic phase	4	17
Accelerated phase	1	0
Maximum IM dose, mg		
300	0	1
400	0	1
500	0	0
600	1	6
800	4	9

TABLE 2: Clinical characteristics of 5 patients with *BCR-ABL* KD mutations and their best cytogenetic response

Patient	Age at diagnosis	Sex	Mutations	Time to CHR (months)	Best CyR	Follow-up duration (months)
1	39	M	T315I	3	Ph+ 66-95%	136
2	57	M	T315I	2	Ph+ 35-65%	73
3	54	F	E255K	1	Ph+ 0	105
4	69	F	E255K & CCA	5	Ph+ 1%	70 (deceased)
5	39	F	V289F	2	Ph+ 35-65%	109

CHR – complete hematological response
CyR – cytogenetic response
CCA – clonal cytogenetic abnormality

Complete cytogenetic response (CCyR) – no Ph-positive metaphases
Partial cytogenetic response (PCyR) – 1-35% of cells have Ph-positive metaphases
Major cytogenetic response (MCyR) – 0 to 35% of cells have Ph-positive metaphases
Minor cytogenetic response – >35% Ph-positive metaphases

Mutational analysis
The *BCR-ABL* KD mutational analysis was available from a research project undertaken by Universiti Sains Malaysia from June 2008 to September 2013 utilizing denaturing high performance liquid chromatography and direct DNA sequencing method.[5]

Statistical methods
Overall survival was defined as the time from diagnosis to time of analysis or death, whichever occurred first. Overall survival were estimated using the Kaplan-Meier method. All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 22.0.

RESULTS

Patients' characteristics are outlined in Table 1. IM was started at 400 mg daily and escalated as tolerated to a maximum dose of 800 mg daily.

TABLE 3: Clinical characteristics of 5 patients without *BCR-ABL* KD mutations who attained complete cytogenetic remission. Four patients (Patients 1 to 4) switched to nilotinib and 1 patient (Patient 5) remained on IM.

	Age at diagnosis	Sex	On nilotinib	Duration on IM prior to switching to nilotinib (months)	Time to CCyR from diagnosis (months)	Follow up duration (months)
1	31	F	Y	79.1	145.5	176
2	49	F	Y	61.5	72.6	105
3	44	M	Y	54.3	25.3	86
4	55	M	Y	26.4	21.2	40
5	28	M	N	-	29.7	119

IM – imatinib
CCyR – complete cytogenetic response

Most of our patients were able to tolerate dose increments. Common dose-limiting adverse events were thrombocytopenia (4 patients) and neutropenia (3 patients).

Three different mutations were identified in 5 patients, summarized in Table 2. Two of these patients had T315I mutation (Patients 1 and 2). Patient 1 presented in accelerated phase (AP) with high Sokal score. Although CHR was documented at 3.4 months, this patient only achieved Ph+ 66-95% as best cytogenetic response (CyR) documented at 29 months. A sibling-matched allogeneic stem cell transplant (ASCT) was performed at 109 months after initial diagnosis. The patient relapsed 20 months post ASCT despite given multiple doses of donor lymphocyte infusion and succumbed to complications of disease 136 months after diagnosis. Patient 2 presented in chronic phase (CP) with low Sokal score and achieved CHR at 2 months and a best CyR of Ph+ 35-65% at 15 months. This patient was subsequently enrolled in a clinical trial involving homoharringtonine and ponatinib at 20 months when the T315I mutation was detected, and was alive at time of analysis.

Two patients tested positive for E225K mutation (Patients 3 and 4). They both presented in CP with low Sokal scores and achieved CHR at 1 and 5 months respectively. Patient 3 was started on IM 5.7 months after diagnosis. Treatment failure was noted and the patient was switched to nilotinib 45 months after initial diagnosis when access to the drug was obtained. This enabled attainment of CCyR and negative RT-PCR 14 months after that but the patient demonstrated cytogenetic relapse 12 months later; at which point she opted for palliative support only. Patient 4 was initiated on IM 8.1 months after initial diagnosis and achieved a best cytogenetic response of Ph+ 1%. However, this was not sustained and cytogenetic relapse was noted 11 months later. The patient obtained access to nilotinib 15 months after cytogenetic relapse. However, no further improvements were evident and the patient later developed clonal cytogenetic abnormalities [deletion 7p, trisomy 19 and +der(22)t(9;22)] and succumbed to complications of CML.

The remaining patient (Patient 5) had V289F mutation and presented in CP with low Sokal score. The patient obtained CHR at 2 months and documented a best CyR of Ph+ 35-65%. She was offered nilotinib 89 months from initial diagnosis. Nevertheless, she also went on to demonstrate cytogenetic progression to Ph+ 66-95% at time of analysis.

Of the remaining 17 patients who did not harbour *BCR-ABL* KD mutations, 2 were deceased at the time of analysis; one from non-CML related condition. 11 patients switched to nilotinib while the rest continued on IM. All 17 attained CHR while on IM at a median of 3.2 months (range: 0.92 to 41.69). Only 5 patients achieved CCyR, as summarised in Table 3. Four out of the 5 patients switched to nilotinib while 1 patient remained on IM. Estimated mean OS for all IM-resistant patients was 150.87 months (range: 130.01 to 171.72) (Fig. 1).

DISCUSSION

In the era of IM for the treatment of CML, it is becoming increasingly clear that IM-resistance represents an emerging issue. It has been estimated that a third of all newly diagnosed CML patients will invariably fail to achieve optimal response with IM.[6,7] IM-resistance due to *BCR-ABL* KD point mutations was almost immediately identified in the trial phases of the drug[8] and numerous other additional *BCR-ABL* KD mutations leading to amino acid substitutions have since been described. *BCR-ABL* KD mutations continue to represent the main cause of IM-resistant disease.[2,9] However, their clinical significance in an individual patient may vary; some *BCR-ABL* KD mutations such as the T315I mutation leads to drug resistant-disease and poorer survival while some *BCR-ABL* KD mutations have not been shown to play any role in drug resistant-disease, depending on the type of amino acid substitution and the loci of the substitution along the *BCR-ABL* domain.[10,11] The quantitative nature of *BCR-ABL* KD mutations has enabled mutation-tailored therapy; as mutations that confer low-level resistance can be overcome by IM dose escalation while those that confer high-level resistance mandate switching to a second generation TKI. Jabbour *et al* investigated 169 patients with IM-resistant disease and calculated a mutation score based on *in vitro* inhibitory concentration for each TKI-mutation pair. The investigators reported that hematologic and cytogenetic responses correlated with mutation score; patients with intermediate scores had lower response rates, worse event-free and overall survival compared to those with lower scores. However these correlations with overall survival were not seen in advanced phases of the disease.[12]

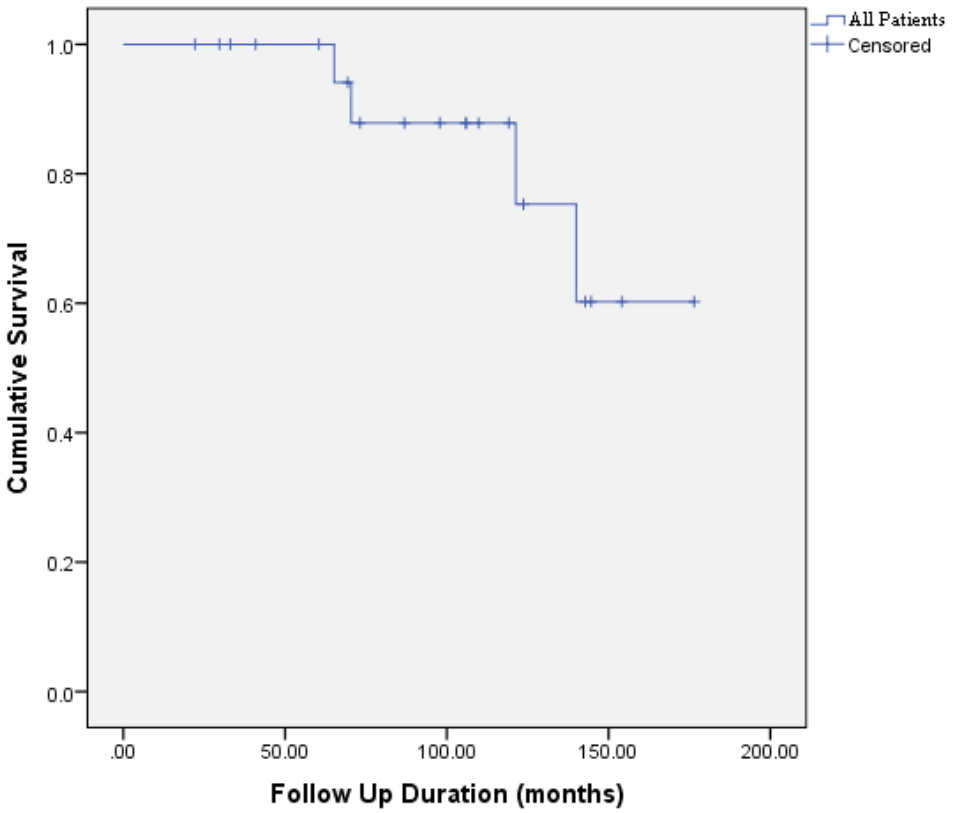

FIG.1: Overall survival of all IM-resistant CML patients

Our patient with V289F mutation only achieved minor cytogenetic response (Table 2) despite switching to nilotinib. More data regarding the relative strength of the V289F mutation and other rare *BCR-ABL* KD mutations such as TKI sensitivity can be useful in guiding second line therapy and until such information is available, patients with rare *BCR-ABL* KD mutations would probably benefit from switching early to a second generation TKI and close monitoring for response.

Both E255K and T315I are relatively common *BCR-ABL* KD mutations that have been shown to confer high-level resistance. Notably, both our patients with E255K developed resistant disease after achieving complete cytogenetic response. In an evaluation of 297 patients with IM-resistance, the E255K/V was one of the most common *BCR-ABL* KD mutations implicated with disease resistance, both in patients who developed primary and secondary resistance to IM; testifying to the high incidence and importance of this particular mutation.[13] It is known to have less favourable responses to Nilotinib,[14,15] as reflected by the clinical progression of our E255K-positive patients. This underscores the need to expand the TKI armamentarium in Malaysia. Early consideration for haematopoietic stem cell transplantation may be beneficial for these patients.

The T315I mutation has been estimated to occur in approximately 7% of IM-resistant patients[16] and may be more frequently detected in patients with advanced phases of the disease. The substitution of threonine with the more hydrophobic isoleucine disrupts a crucial hydrogen bond required for high-affinity inhibitor binding; thus rendering resistance against IM, Nilotinib and dasatinib.[17] Our experience confirms that patients with T315I mutations benefit most from early referral for HSCT or clinical trial enrollment. Intriguingly, Lange *et al* studied 36 patients with detectable T315I mutations and found that there was a correlation between *BCR-ABL* T315I levels and major molecular response at 12 months[18] suggesting a mutation-dose dependent effect even within a single *BCR-ABL* KD mutation. It has been speculated that prolonged duration of IM exposure could increase the rate of KD

mutation; probably due to sustained elimination of susceptible subclones and concomitant outgrowth of mutated subclones; although this would be difficult to demonstrate outside of a clinical trial context as current guidelines do not recommend routine testing for KD mutations at time of diagnosis.[19]

Our cohort showed that only a minority of our patients with IM-resistance tested positive for *BCR-ABL* KD mutations by available testing methods, indicating that other *BCR-ABL* independent factors may be contributing to IM-resistance; such as drug bioavailability,[20-24] alternative signaling pathways [25-29] and miRNA aberrations.[30-33] Perhaps more pertinently, clinical experience illustrates that patient adherence remains an important factor in promulgating the IM resistance phenotype. The concomitant administration of other medications/herbal supplements undoubtedly contributes to unpredictable drug-drug interactions that directly influence *in vivo* levels.

Our *BCR-ABL* KD mutation-negative cohort shows that the role of second generation TKIs on survival remains undefined. The 11 patients who switched to nilotinib did not seem to show better overall survival compared to the 6 patients who remained on IM. Of note, only 4 of 11 patients who switched to nilotinib achieved CCyR and interestingly, 1 of the 6 patients who remained on IM also achieved CCyR. Although much research with larger patient cohorts is still needed to further understand the biology of disease resistance, therapeutic approaches to overcome *BCR-ABL* independent resistance, such as combination therapies and development of novel TKIs that inhibit both *BCR-ABL* and Src kinases are already on the horizon.[34]

Our study has the following limitations: Firstly, it is a retrospective study that comprises a very small sample size. Therefore, no valid conclusions can be drawn with regards to the role of intervention on the results. Secondly, all the *BCR-ABL* KD mutation analyses were performed off-site, accounting for the long lag-time between testing and result availability. This resulted in a delay in switching patients to nilotinib, which is further worsened by the time needed to source for funding to facilitate the switch. As well, we did not incorporate molecular monitoring results using real-time quantitative reverse transcriptase polymerase chain reaction (RQ-PCR) using international scale; as it was not yet widely applied at our centre due to financial reasons. We acknowledge such information is crucial and the degree to which *BCR-ABL* transcripts are reduced by therapy have been proven to correlate with progression-free survival.[35,36] Surveillance by CBA of bone marrow and peripheral cells were often limited by lack of cells in metaphase. Nilotinib is now approved as front-line therapy for newly diagnosed patients in the US and in some countries in the European Union. This is not yet widely incorporated in our current practice, due to limited access to the drug. Longer follow-up is needed to study the impact of timely treatment with IM and various second generation TKIs in our patient cohort according to published guidelines, preferably in a clinical trial setting.

In conclusion, our experience underscores the laboratory and financial challenges in the management of IM-resistant CML in a developing country. Along with mutation analysis results, other factors such as patient characteristics, disease stage, adherence and co-morbidities should also influence the physician's decision in choosing the most appropriate TKI. Patients with IM-resistant disease, especially those without *BCR-ABL* KD mutations, remain a challenging clinical scenario and represent a critical unmet medical need.

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