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ORIGINAL ARTICLE

Detection of *BCR-ABL* T315i Mutation in Imatinib Resistant Chronic Myeloid Leukemia Patients

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ABSTRAK

Pesakit myeloid leukemia kronik (CML) bermutasi BCR-ABL T315I sering menunjukkan kelangsungan hidup keseluruhan yang lebih pendek berbanding mereka yang tidak mempunyai mutasi tersebut. Tujuan kajian ini adalah untuk mengenalpasti prevalens mutasi T315I dikalangan pesakit CML yang menunjukkan rintangan terhadap imatinib mesylate (IM) dan membandingkan fasa penyakit serta kelangsungan hidup antara pesakit CML bermutasi T315I dan yang tidak. Enam puluh pesakit CML yang telah dirawat dengan IM untuk sekurang-kurangnya 18 bulan dan tindak-balas rawatan mereka direkodkan. Analisis mutasi T315I telah dilakukan menggunakan allele khusus oligonucleotide reverse transcriptase polymerase chain reaction (RT-PCR) diikuti oleh teknik direct sequencing. 42 (70%) orang pesakit didapati mempunyai rintangan terhadap IM dan daripada para pesakit tersebut, lima (12%) telah dikesan mempunyai mutasi T315I. Keseluruhan kelangsungan hidup untuk pesakit CML yang menunjukkan rintangan terhadap IM dengan mutasi T315I adalah 96 bulan (95% CI:54-138) berbanding mereka yang tidak mempunyai mutasi iaitu 84 bulan (95% CI:48-120) walaupun nilai ini tidak signifikan secara statistik [nilai p=0.43]). Kajian ini menunjukkan kadar kekerapan mutasi T315I (12%) di kalangan pesakit kohort kami adalah lebih tinggi. Median kelangsungan hidup keseluruhan bagi mereka yang mempunyai mutasi T315I adalah lebih panjang berbanding daripada mereka yang tidak mempunyai mutasi. Kajian lebih lanjut melibatkan bilangan pesakit yang lebih ramai adalah diperlukan untuk mengesahkan pemerhatian tersebut.

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Kata kunci: BCR ABL, chronic myeloid leukemia, mutasi T315I

ABSTRACT

Chronic myeloid leukemia (CML) patients who have *BCR-ABL* T315I mutation, usually present in the advance phase of the disease with overall survival (OS) shorter than those without the mutation. This study aimed to determine the prevalence of T315I mutation amongst imatinib mesylate (IM) resistant CML patients and to compare the OS between T315I-mutated and non-T315I-mutated patients. Sixty consecutive CML patients who were treated with IM for at least 18 months and their treatment responses, were recorded. The mutation analysis was done using allele-specific oligonucleotide reverse transcriptase-polymerase chain reaction (RT-PCR) assay followed by direct sequencing technique. Forty-two patients (70%) were found to have IM-resistance. Five out of 42 patients had detectable T315I mutation. Median OS of IM-resistant T315I-mutated patients was 96 months (95% CI:54-138) compared to 84 months (95% CI:48-120) in non T315I-mutated patients, although this was found to be statistically insignificant (p = 0.43). The present study showed a higher prevalence of T315I mutation as compared to a few local studies. Median OS of T315I-mutated patients were observed to be longer than non-T315-mutated patients. Further studies encompassing larger cohort of patients are required to confirm this finding.

Keywords: BCR ABL, chronic myeloid leukemia, T315I mutation

INTRODUCTION

Despite the good response of chronic myeloid leukemia (CML) patients to tyrosine kinase inhibitors (TKI), studies have shown that they do eventually develop resistance towards the treatment (Vaidya et al. 2011). Resistance to TKIs were reported in some CML patients in chronic phase (CP) and in most patients in more advanced phases of the disease (accelerated and blast phase) (Soverini et al. 2007).

Detection of several different point mutations in the amino acids of the *BCR-ABL* tyrosine kinase ATP binding site or other regions of the tyrosine kinase domain is the most dominant mechanism of resistance (Valent et al. 2008). These *BCR-ABL* mutations were reported to occur in approximately 50% of patients who developed resistance to imatinib mesylate (IM) (Jabbour et al. 2006). These mutations inhibit IM or other TKI drugs from binding to the ATP binding site of the tyrosine kinase domain, thus inhibiting the tyrosine kinase activity (Vaidya et al. 2011).

Among all the mutations in the *BCR-ABL* kinase domain, the greatest degree of resistance was associated with the T315I mutation and point

mutations in the P-loop domain which are G250E, Q252H, Y253F and E255K/V (Vaidya et al. 2011). T315I mutation was reported to occur in 7% of CML patients with IM resistance (Chahardouli et al. 2013). It is also called the mutation of the gatekeeper position as it prevents binding of TKIs. It represents a substitution of the amino acid isoleucine for threonine in the *BCR-ABL* kinase domain. Threonine 315 forms a crucial hydrogen bond with IM and the absence of an oxygen atom in the substituted isoleucine prevented bond formation. Additionally, the bulkier isoleucine was predicted to induce a steric clash with IM, which led to the designation of the 315 residues (Gorre et al. 2001). T315I mutation was found to affect a common *ABL* kinase contact residue and confers complete resistance to all known ATP-competitive *BCR-ABL* inhibitors (Chahardouli et al. 2013).

Accordingly, T315I mutation is more commonly detected in patients with progression of the disease phase /and is associated with severe IM resistance, disease progression and poor clinical outcome. This mutation was also shown to have poor overall survival (OS) and progression free survival (Kim et al. 2009).

The aim of the present study was to determine the presence of T315I mutations in CML patients with IM-resistance using allele-specific oligonucleotide-reverse transcriptase polymerase chain reaction (ASO-RT-PCR) method which was subsequently confirmed by direct sequencing method. We investigated the difference between the demographic data of CML patients with and without T315I mutation and correlated the mutation status with OS.

MATERIALS AND METHODS

STUDY DESIGN

In this comparative cross-sectional study, 60 consecutive patients who were diagnosed from January 2002 until May 2014 were recruited from Universiti Kebangsaan Malaysia Medical Centre (UKMMC). The patients were in different (chronic, accelerated and blast) phases of the disease and were being treated with IM with doses between 200-800 mg/day for at least 18 months.

The basic demographic, disease characteristics and treatment details were retrieved from patients' medical records. For each patient, diagnosis was confirmed by hematological, cytogenetics and molecular analysis of the bone marrow. Hematological, cytogenetic and molecular responses were evaluated every 3 months to determine the response to IM treatment using peripheral blood.

Patients who developed primary and secondary resistance according to the hematologic, cytogenetic and molecular response criteria in the 2013 European LeukemiaNet (ELN) guidelines were identified (Baccarani et al. 2013).

T315I MUTATION ANALYSIS

RNA was extracted from samples obtained from peripheral blood or bone

marrow which contained leukocytes using RiboPure™-Blood Kit (Ambion USA). From the RNA, complementary DNA (cDNA) was generated using SuperScript II cDNA synthesis kit (Invitrogen, USA) according to manufacturer's instructions.

T315I mutation was detected using allele specific-polymerase chain reaction (AS-PCR) method. Three sets of primers which consist of mutant primers, forward (5′-gcc ccc gtt cta tat cat aat-3′) and reverse (5′-gga tga agt ttt tct tct cca g-3′), wild type primers with forward (5′-tgg ttc atc atc att caa cgg tgg-3′) reverse (5′-gtt ccc gta ggt cat gaa ctc ag- 3′) as well as internal control primers with forward (5′-gtg ggg cgc ccc agg cac ca-3′) and reverse (5′-gtc ctt aat gtc acg cac gat ttc-3′) were used. All of these sequences were adapted from the previously published studies (Roche et al. 2002; Auewarakul et al. 2006).

The reaction took place in a thermal cycler (ABI 9700, Applied Biosystems Geneamp, USA) in a reaction mixture containing 1.0 µL of cDNA, 0.625 unit of Taq DNA polymerase (Invitrogen, USA), 14 pmol of mutant primers, 6 pmol of wild type primers, 1 pmol of internal control primers, 2.5 mmol/L $MgCl_2$, 0.2 mmol/L of Deoxynucleotide Triphosphate (dNTP), 3% Dimethyl sulfoxide (DMSO) and 0.625 unit of Taq DNA polymerase (Invitrogen, USA).

The thermal profile used for amplification were as follows: 95˚C of initial denaturation for 5 minutes (min), followed by 35 cycles of denaturation for 45 seconds at 94˚C, primer annealing at 57˚C for 30 seconds, extension for 1 minutes at 72˚C, and final extension for 5 minutes at 72˚C. These sets of PCR primers generate three PCR products of mutant T315I, wild type T315I, and internal control band at 158, 374 and 540 basepairs (bp), respectively. The products were visualized under ultraviolet light by electrophoresis on a 1.5% agarose gel stained using Olerup SSP® Gel Red stains. Ten RNA samples from non-leukemic patients were used as a normal control and samples with only added distilled water without cDNA, was used as a negative control.

DNA SEQUENCING METHOD

Bidirectional sequencing method was used to detect T315I mutation for ASO-RT-PCR-positive patients (Branford et al. 2002). Samples of RT-PCR-negative patients were also sequenced for comparison. The samples were purified using PCR purification kit (QIAquick, Qiagen) and then sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Finally, the sequences were compared with the wild-type *ABL* sequence (GenBank accession number NM 005157.5) and analyzed using Finch TV analyzer.

STATISTICAL ANALYSIS

Statistical analysis was conducted using Mann-Whitney U and Kruskal-Wallis tests to assess the differences of measurable variation among the patients. Kaplan-Meier survival curves were plotted for analysis between presence of mutation and the time from

diagnosis to last follow-up or death and the differences were estimated using the log rank test. The p value was two-sided and the level of significance was chosen at 0.05.

RESULTS

BASELINE CHARACTERISTICS OF THE CML PATIENTS

The basic demographic data (gender, race and age) of the 60 CML patients were shown in Table 1. The median age of the patients was 45 years (range: 29.5-61 years) and the median time from diagnosis to last follow-up/death was 72 months (range: 18-204 months). Out of the 60 patients, 42 (70%) were resistant to IM in which primary and secondary resistant patients were 29 and 13, respectively (Table 1).

TREATMENT RESPONSE IN IM-RESISTANT PATIENTS

Thirty-two (76.2%) patients were able to achieve complete haematological remission (CHR) after 18 months of

Table 1: Baseline characteristic of all CML patients and response to IM treatment (CP=Chronic phase; AP=accelerated phase; BC=blast crisis, CHR=complete hematological remission; CyR=cytogenetic remission)

Total number of CML patients	60
Gender:	
Male	38 (63.3%)
Female	22 (36.7%)
Race:	
Malay	38 (63.3%)
Chinese	15 (25%)
Indian	4 (6.7%)
Others	3 (5.0%)
Median age at diagnosis, years	45 (29.5-61)
Disease phase:	
CP	56 (93.3%)
AP	4 (6.7%)
BP	0
Median time from diagnosis to last follow-up/death, months	72 (18-204)
Response to IM treatment:	
Not IM-resistant	18 (30%)
IM-resistant	42 (70%)
-Primary resistance:	
No CHR	4 (6.7%)
No CyR	25 (41.7%)
-Secondary resistance:	
Loss of CHR	2 (3.33%)
Loss of CyR	11 (18.3%)

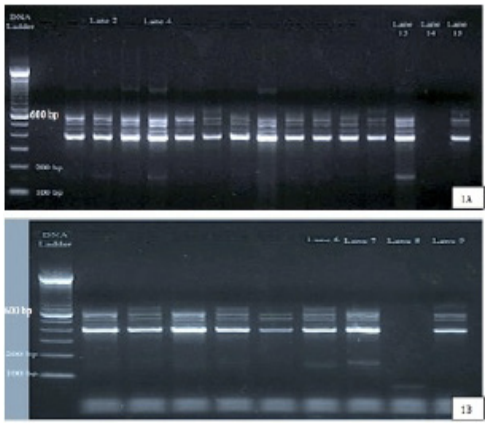

Figure 1A and 1B: Gel electrophoresis results of patients with T315I mutation. In both figures, lane 2,4, 13, 6 and 7 represent the patients who have the T315I mutation which show presence of three disinct bands including mutant T315I band (indicate T315I mutation), wild type T315I band and internal control band at 158, 374 and 540 base pairs respecticely. The rest of the unlabeled lanes represent the patients who do not have the mutation and shows presence of wild type T315I band and internal control band at 372 and 540 base pairs respectively. Lane 14 and lane 8 represent the non-template control or negative control while lane 15 and lane 9 represent normal control

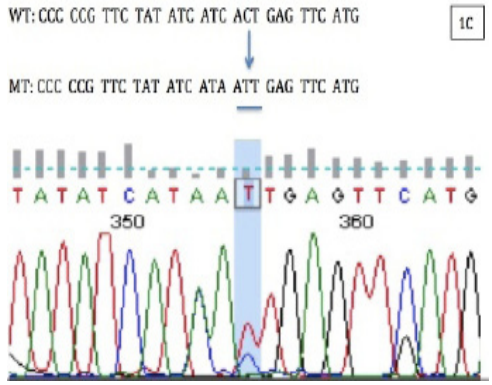

Figure 1C: DNA sequencing analysis showing the changes made by C1308T whre an 'ACT' coding for Threonine mutate to an 'ATT' coding for Isoleucine (T315I) in exon 7. WT (Wild type) represent the nucleotide sequence of a normal patient while MT (mutant type) represents the nucleotide sequence of the patient who is positive for T315I mutation

treatment with IM. Out of the 32 patients, 4 (9.5%) achieved complete cytogenetic remission (CCyR) while 16 (38.1%) had incomplete cytogenetic response at the time of analysis (Table 2). The demographic data of IM-resistant patients are shown in Table 3.

T315I MUTATION ANALYSIS AND DIRECT SEQUENCING

Qualitative results of T315I mutation analysis using gel electrophoresis could be seen in Figures 1A and 1B. In both figures, lane 2, 4, 13, 6 and 7 represent the patients who had the T315I mutation which showed the presence of three distinct bands including mutant T315I band (indicate T315I mutation), wild type T315I band and internal control band at 158, 374 and 540 base pairs, respectively. The rest of the unlabelled lanes represent the patients who did not have the mutation and showed the presence of wild type T315I band and internal control band at 374 and 540 base pairs, respectively. Lane 14 and lane 8 represented the non-template control or negative control while lane 15 and lane 9 represented normal control.

This mutation was later confirmed by direct sequencing analysis showing the changes made by C1308T where an 'ACT' coding for Threonine mutate to an 'ATT' coding for Isoleucine (T315I) in exon 7 (Figure 1C).

SUMMARY OF IM-RESISTANT CML PATIENTS WITH T315I MUTATION

Table 2: Response to TKI treatment in 42 IM-resistant CML patients after 18 months follow-up period (CHR=complete hematological response; CyR=cytogenetic response; CCyR=complete cytogenetic response).

Total number of IM-resistant patients	42
Response to IM treatment after 18 months	
- Haematological response:	
CHR	32 (76.2%)
No CHR	10 (23.8%)
- Cytogenetic response:	
CCyR	4 (9.5%)
Incomplete CyR	16 (38.1)
(No Cyr, Minimal CyR, Minor CyR, Partial CyR)	
Unknown	12 (28.6)

Table 3: Demographic data and disease phase in 42 IM-resistant CML patients and the prevalence of T315I mutation in these patients(AP=accelerated phase; BC=blast crisis; CP=chronic phase).

		Total no.(%)	T315I mutation	No T315I mutation	p value
No. of patients with IM resistance		n=42	n=5	n=37	
Age at Dx	Median (Range)	44(23-56.75)	45 (41-60)	40 (23-57)	0.36
Time from diagnosis to last follow-up/death (months)	Median (Range)	84 (48-123)	96 (54-138)	84 (48-120)	0.63
Race	Malay	27 (64.3)	3 (60.0)	24 (64.9)	0.71
	Chinese	11 (26.2)	2 (40.0)	9 (24.3)	
	Indian	3 (7.1)	0	3 (8.1)	
	Others	1 (2.4)	0	1 (2.7)	
Gender	Male	25 (59.5)	3 (60)	22 (59.5)	1.00
	Female	17 (40.5)	2 (40)	15 (40.5)	
Disease phase during analysis	AP	5 (11.9)	1 (20.0)	4 (10.8)	0.46
	BC	3 (7.1)	1 (20.0)	2 (5.4)	
	CP	34 (81.0)	3 (60.0)	31 (83.8)	
Range of dose for IM given per day		400-800mg	200-800mg		

Table 4 summarized the clinical data of patients with T315I mutation. Two of them were in accelerated phase (n=1) and blast crisis (n=1), respectively. Three achieved CHR but all never achieved CCyR. The two patients who were in advanced phase of the disease died from progression to blast crisis (n=1) and sepsis (n=1).

Patient 1 was a 39-year-old man who was diagnosed with CML in accelerated phase with 17% blasts detected in the bone marrow. His initial *BCR-ABL* copy numbers were 130.64%. He was started on hydroxyurea (HU) for 4 years before

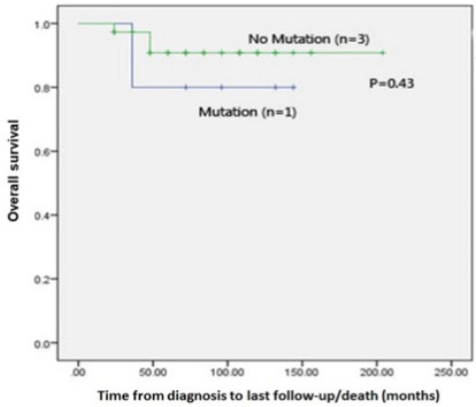

Figure 2: This figure illustrates the overall survival of 42 IM resistance CML patients according to T315I mutation. The median OS among IM-resistant CML patients who have T315I mutation was 96 months with 1 patient died (n=1) while those without the mutation was 84 months with 3 patients (n=3) died along the course of the treatment. Majority of the patients with T315I mutation had changed to nilotinib after at least 2 years of IM therapy as they never reached CCyR

the availability of IM therapy. He failed to achieve hematological and cytogenetic response after 12 months of initiation of IM and unfortunately developed double Ph chromosome on subsequent follow-up. The IM therapy was stopped after 3 years due to intolerance as he complained of severe headache. Eventually, he underwent an allogeneic stem cell transplant but the disease relapsed 2 months, post-transplant. Despite donor lymphocytes infusion and subsequent intravenous Cytarabine for cytoreduction, his disease progressed and he succumbed to the disease 2 years later.

Patient 2 was a 45-year-old man who was diagnosed with CML in chronic phase. He was on IM 400-800 mg daily for 4 years. As he never reached CCyR, the treatment was subsequently changed to nilotinib 200 mg daily which she failed to respond to.

Patient 3 was a 43-year-old lady who was diagnosed with CML in chronic phase. She was treated with IM 400 mg daily which was switched to nilotinib 400 mg daily 2 years later when she remained in minor cytogenetic response. She also had treatment failure with nilotinib.

Patient 4 was a 64-year-old man who was diagnosed with CML in chronic phase. He was given 800mg IM per day for more than 2 years. However, he only remained in minimal CyR. As he never reached CCyR, the treatment was subsequently changed to nilotinib but unfortunately, he also

Table 4: Summary of patients with T315I mutation (CP=chronic phase; AP=accelerated phase; BC=blast crisis; HR=hematological response; CyR=cytogenetic response; CCyR=complete cytogenetic response).

Patients	Sex	Race	Age at detection of T315I mutation	Disease phase during analysis	Response to imatinib at the time of analysis	Time from diagnosis to last follow-up/death (months)
Patient 1	Male	Malay	49	AP	No HR, No CyR	144
Patient 2	Male	Malay	55	CP	CHR, Minor CyR	132
Patient 3	Female	Chinese	51	CP	CHR, Minor CyR	72
Patient 4	Male	Malay	68	CP	CHR, Minor CyR	96
Patient 5	Female	Chinese	58	BC	No HR, No CyR	36

showed treatment failure.

Patient 5 was a 56-year-old lady who was diagnosed with CML in chronic phase. She was given hydroxyurea for 11 months before starting IM therapy 400 mg daily. She only achieved minimal cytogenetic response after 1 year of treatment and transformed into acute myeloid leukemia with double Ph chromosome after 2 years of treatment. She eventually died due to blast crisis.

OVERALL SURVIVAL DATA ANALYSIS

The overall survival of 42 CML patients with IM resistant was analyzed based on the absence or presence of T315I mutation (Figure 2). The median OS among IM-resistant CML patients who had T315I mutation was 96 months (CI:41.25-150.75) while those without T315I mutation was 84 months (CI:72.37-102.77). Even though the median OS among CML patients with T315I mutation were longer, the results was statistically insignificant (p =0.43) as these patients may have other types of *BCR-ABL* mutation that was not tested in this study (Figure 2).

DISCUSSION

The aimed of the study was to analyze IM treatment response of CML patients, examine the demographic data of IM-resistant patients, determine the prevalence of T315I mutation in these patients and compare their overall survival with those without T315I mutation. Resistance towards IM therapy in CML patients has been associated with *BCR-ABL* kinase domain mutations (Jabbour et al. 2006). Among all the mutations in the *BCR-ABL* kinase domain, T315I mutation is the most frequently identified. The mechanism of the mutation in T315I is point mutation at amino acid 315 in which the 'ACT' codon that codes for threonine is switched to the 'ATT' codon which codes for isoleucine (Vaidya et al. 2011). This mutation inhibits IM from binding to the ATP binding site of the tyrosine kinase domain and thus inhibiting the tyrosine kinase activity.

At a median of 18 months follow-up, 32 out of 42 (76%) patients achieved CHR and only 4 (12.5%) out of these 32 patients achieved CCyR (Table 2). The study showed a lower number of patients responding to IM as compared to a study by Druker et al. (2006) on IM efficacy in 553 CML patients in which 98% of patients who received IM achieved CHR and 87% achieved CCyR (Druker et al. 2006). Poor CyR to IM was also noted at a median period of 72 months from diagnosis to last follow-up/death (Table 1): 25 (41.7%) developed primary IM-resistance and 11 (18.3%) developed secondary IM-resistance with loss of CyR and relapsed at the time of analysis (Table 1). According to the International Randomized Study of Interferon and STI571 (IRIS) in investigating the imatinib efficacy in 553 patients, 5% patients developed primary IM-resistance with failure to achieve a CHR after 18 months and the estimated rate of failure to achieve partial CyR was only 12% after 24 months of follow-up. The estimated

rate of relapse was 10% (O'Brien et al. 2003). Our study showed poorer CyR towards IM treatment which can be attributed to poor compliance to IM treatment.

Tables 3 summarized the characteristics of 42 IM-resistant CML patients: 25 males (59.5%) and 17 females (40.5%). Similar findings were observed in a study on 60 CML patients with IM-resistance in Iran (Chahardouli et al. 2013). However, Elias et al. reported that in 125 CML patients with IM-resistance in peninsular Malaysia, the male to female ratio was almost similar (Elias et al. 2014). The median age at diagnosis was 44 years and it was predominated by Malays followed by Chinese, Indians and other races which was 27 (64.3%), 11 (26.2%), 3 (7.1%) and 1 (2.4%), respectively. The racial distribution was comparable with the study done by Elias et al. in which the prevalence was dominated by Malays and Chinese population. For the disease phase, 34 (81%) were in chronic phase, followed by 5 (11.9%) and 3 (7.1%) patients in accelerated and blast crisis, respectively. This was comparable with the local study which showed that 75% of the 125 IM-resistant CML patients were in the chronic phase (Elias et al. 2014). Another study by Chahardouli et al. showed that 71% of the 60 IM-resistant CML patients were reported to be in late chronic phase of the disease (Chahardouli et al. 2013).

In comparing the prevalence of T315I mutation, a study done by Elias et al. covering the CML patients in peninsular Malaysia showed presence of 7.2% T315I mutation. This low level was supported by a recent study by Mat Yusoff et al. on 285 IM resistant CML patients involving peninsular and east of Malaysia which demonstrate presence of 5.3% mutations. Our study reported to have 12% of patients with the similar mutation. However, a study done on 137 Korean CML patients with IM-resistance showed that most of them were in advanced phase of the disease: 83 (75%) patients versus only 28 (25%) in chronic phase of the disease (Kim et al. 2009). The prevalence of T315I mutation among their patients is 23% which is higher compared to the present study and the study done by Elias et al. and Chahardouli et al. It is possible that the low frequency of T315I mutation identified in our study may be due to more CML patients who were in chronic phase of the disease at the time of analysis.

For clinical outcome of IM-resistance patients harboring the T315I mutation, previous studies showed that the prevalence of T315I mutation increased as the disease progressed into advanced phase (accelerated or blast crisis). In the study by Chahardouli et al., out of 4 patients with T315I mutation, only one of them was in late chronic phase of the disease and the patient subsequently progressed into the accelerated phase 4 months later with increasing number of *BCR-ABL* copies. All of these 4 patients had lost all responses (HR and CyR) at the time of mutation analysis (Chahardouli et al. 2013). Apart from that, another study by Kim et al. showed that none of their chronic phase CML patients harboured T315I mutation while 8 and 11 of them were in accelerated

and blast crisis respectively (Kim et al. 2009). In the present study, out of 5 patients who had T315I mutation, only 2 patients were in accelerated phase/blast crisis at the time of analysis while the remaining 3 patients were in chronic phase of the disease. The lower number of patients harbouring T315I mutation in accelerated phase/ blast crisis might be due to the low sample size involved in this study.

In our study, the median OS of patients with T315I mutation was slightly longer than those without the mutation (Figure 2). There may be other factors that may contribute to this finding. For example, those who do not have T315I mutation might harbor other *BCR-ABL* mutation, such as E255K/V and Y253F/H (Kim et al. 2009), which were not tested in this study that can affect the OS. IM-resistance can also occur in the absence of T315I mutation or any other kinase domain mutation although acquisition of mutation in the kinase domain is the most frequently identified mechanism of resistance (Vaidya et al. 2011). Another mechanism is presence of immature leukemia cells (stem cells) that may exhibit *BCR-ABL* independent mechanism of resistance. Previous study showed that these CML stem cell populations expressed high level of *BCR-ABL* mRNA, protein and kinase activity as well as elevated expression of IL-3 and G-CSF which cause these stem cells to be insensitive to IM as well as other TKI treatment (Jiang et al. 2007). A number of cellular molecules that are involved in the regulation of drug uptake, drug metabolism or drug efflux may also influence the bio-availability of TKI treatment (Wang et al. 2007).

CONCLUSION

Our study showed a higher prevalence of T315I mutation compared to other local studies although there were no significant differences in the baseline characteristics and disease phase between T315I and non-T315I mutated CML patients. However, median OS of those with T315I mutation was slightly longer than those without the mutation. This could be due to limited number of patients involved in this analysis. Further studies involving a larger number of patients are needed to confirm this. It would be useful to investigate other mutations in the *BCR-ABL* kinase domain in order to determine the cause of IM-resistance as presence of mutations in different regions of the kinase domain may lead to different levels of resistance.

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