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ORIGINAL RESEARCH

# Impact of *CYP3A4*18* and *CYP3A5*3* Polymorphisms on Imatinib Mesylate Response Among Chronic Myeloid Leukemia Patients in Malaysia

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## ABSTRACT

***Introduction***: Imatinib mesylate (IM), a selective inhibitor of the BCR-ABL tyrosine kinase, is a well-established first-line treatment for chronic myeloid leukemia (CML). IM is metabolized mainly by cytochrome P450 (CYP) in the liver, specifically the CYP3A4 and CYP3A5 enzymes. Polymorphisms in these genes can alter the enzyme activity of IM and may affect its response. In this study, the impact of two single-nucleotide polymorphisms (SNPs), *CYP3A5*3* (6986A>G) and *CYP3A4*18* (878T>C), on IM treatment response in CML patients ($n = 270$; 139 IM resistant and 131 IM good responders) was investigated.

***Methods***: Genotyping of *CYP3A4*18* and *CYP3A5*3* was performed using the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) technique. The association between allelic variants and treatment response was assessed by means of odds ratio (OR) with 95% confidence intervals calculated by logistic regression.

***Results***: Our results indicated that CML patients carrying the heterozygous (AG) and homozygous variant (GG) genotype of *CYP3A5**3 were associated with a significantly lower risk of acquiring resistance with OR 0.171; 95% CI: 0.090–0.324, $p < 0.001$ and OR 0.257; 95% CI: 0.126–0.525, $p < 0.001$, respectively. Although CML patients carrying the heterozygous (TC) genotype of *CYP3A4*18* showed a lower risk of acquiring resistance toward IM (OR 0.648; 95% CI: 0.277–1.515), the association was not statistically significant ($p = 0.316$). No homozygous variant (CC) genotype of *CYP3A4*18* was detected among the CML patients.

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***Conclusion***: It is concluded that polymorphism of *CYP3A5*3* is associated with IM treatment response in Malaysian CML patients with carriers of *CYP3A5*1/*3* and *CYP3A5*3/*3* genotypes posing lower risk for development of resistance to IM.

**Keywords:** Chronic myeloid leukemia; *CYP3A4*18*; *CYP3A5*3*; Imatinib mesylate; Single-nucleotide polymorphisms

## INTRODUCTION

Currently, imatinib mesylate (IM) is the frontline therapy for newly diagnosed chronic myeloid leukemia (CML) patients worldwide. IM is a synthetic tyrosine kinase inhibitor specifically designed to inhibit the breakpoint cluster region (BCR)-Abelson (ABL) fusion protein resulting from Philadelphia chromosome translocation t(9; 22)(q34, q11) [1]. Although IM is the gold standard drug for CML treatment, development of resistance to IM is a major clinical problem. The development of resistance to IM is a multifactorial phenomenon in patients with CML. Resistance may be mediated by a range of different mechanisms in which pharmacokinetic variability due to genetic polymorphisms in IM transport and metabolizing genes may be potential factors. Previously, the contribution of genetic variations in transporter genes *ABCB1* and *ABCG2* in mediating resistance/good response to IM among Malaysian CML patients was reported by our group [2]. Drug-metabolizing enzymes (DME) are involved in deactivating xenobiotics as well as biotransformation of drugs, and polymorphisms in DME coding genes have been reported to alter the activity of the enzymes for some substrates [3]. Therefore, genetic variation in drug metabolism can lead to therapeutic failures, adverse drug effects or even fatal drug intoxications [4].

Xenobiotic metabolism is mainly conducted by three main cytochrome P450 (CYP) gene families: *CYP1*, *CYP2* and *CYP3*, with the most highly expressed subfamily being the *CYP3A*. Genetic variation in *CYP3A* activity may influence the rate of metabolism and elimination of *CYP3A* substrates including IM. The metabolism of IM is mainly mediated by *CYP3A4* and *CYP3A5* [5]. It is suggested that *CYP3A4* and *CYP3A5* are important genetic contributors to inter-individual differences in *CYP3A*-dependent drug metabolism. A polymorphism of *CYP3A4*18*, located at exon 10, had been reported as the common allelic variation of *CYP3A4*. This polymorphism involves nucleotide change from tyrosine (T) to cytosine (C) transition at position 878 and results in an amino acid change from leucine to proline at codon 293 (Leu293Pro) [6, 7]. *CYP3A4*18* had been reported to be associated with high turnover of testosterone and chlorpyrifos when compared to the wild type [8]. *CYP3A5*3* is another common allelic variation in *CYP3A5*. Polymorphism in *CYP3A5*3* plays a crucial role in the pharmacokinetics of these *CYP3A* substrates. It is reasonable to assume that polymorphisms in the *CYP3A5* gene could lead to interindividual variability, which has been observed in the pharmacokinetic changes seen with *CYP3A* substrates [9]. This study was designed to investigate the frequency of single-nucleotide polymorphisms (SNPs) *CYP3A5*3* (6986A>G) and *CYP3A4*18* (878T>C) in Malaysian CML patients undergoing IM therapy and to determine their impact on IM's treatment response in these patients. To our knowledge, this is the first study to report on the allelic and

genotypic frequencies as well as the association of *CYP3A4*18* and *CYP3A5*3* with IM response among CML patients in the Malaysian population.

## METHODS

### Subjects and DNA Extraction

The study was approved by the Research and Ethics Committee of Universiti Sains Malaysia and the Ministry of Health, Malaysia (ethics no. USMKK/PPP/JEPeM [244.3.(4)] and KKM/NHSEC/08/0804/P12-687), which complies with the Declaration of Helsinki of 1964, as revised in 2013. Informed consent was obtained from all patients for being included in the study. Subjects were recruited from various hospitals in Malaysia including Hospital Universiti Sains Malaysia (HUSM), Hospital Raja Perempuan Zainab II (HRPZ), Hospital Pulau Pinang, Hospital Raja Permaisuri Bainun, Universiti Kebangsaan Malaysia Medical Center (PPUKM), Sime Darby Medical Centre and Hospital Umum Sarawak (HUS). In this study, 270 CML patients (139 IM resistant and 131 IM good responders) were involved who were all *BCR-ABL* non-mutated. The patients selected were Philadelphia chromosome-positive CML patients in chronic, accelerated or blast phase, treated for at least 12 months, with IM (400 and 600 mg, respectively) on frontline treatment. The patients' characteristics are shown in Table 1.

Evaluation of imatinib response: By referring to European LeukemiaNet recommendations for the management of chronic myeloid leukemia 2013 [10], hematologic, cytogenetic and molecular criteria were accessed. Based on this, patients were grouped into IM good responders and IM-resistant CML patients. Hematologic response was considered as complete when the platelet count was $<450 \times 10^9$/l; white blood cell count $<10 \times 10^9$/L; differential without immature granulocytes and with <5% basophils and nonpalpable spleen. The cytogenetic response was defined as complete (0% $Ph^+$ metaphases), partial (1–35% $Ph^+$ metaphases), minor (36–65% $Ph^+$ metaphases), minimal (66–95% $Ph^+$ metaphases) and none (>95–100% $Ph^+$ metaphases) [11]. Molecular response was best assessed according to the International Scale (IS) as the ratio of *BCR-ABL1%* on a log scale, where 10%, 1%, 0.1%, 0.01%, 0.0032% and 0.001% correspond to a decrease of 1, 2, 3, 4, 4.5 and 5 logs, respectively, below the standard baseline that was used in the IRIS study. A *BCR-ABL1* expression of ≤0.1% corresponds to major molecular response (MMR) [10]. CML patients who achieved the above-mentioned response criteria were categorized as IM good responders and those who did not achieve the above response criteria within the specified time frame were categorized under the IM non-responders/resistant group.

Peripheral blood (3 ml) was collected after obtaining written informed consents from the subjects. Genomic DNA was extracted using a DNA extraction kit, QIAGEN QIAamp® DNA Blood Mini kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. Genotyping was conducted at Human Genome Centre, Universiti Sains Malaysia.

### Genotyping of CYP3A4*18 Polymorphisms

Genotyping of *CYP3A4*18* was performed by using the polymerase chain reaction restriction fragment length polymorphism (PCR–RFLP) technique. Amplification of *CYP3A4*18* was performed by using forward (CACATCAGAATGAAACCACC) and reverse

**Table 1** Patients' characteristics

| | IM resistant $n = 139$ (%) | IM good response $n = 131$ (%) | $p$ value |
|---|---|---|---|
| Gender | | | |
| Female | 78 (56.12) | 51 (38.93) | **0.005** |
| Male | 61 (43.88) | 80 (61.07) | |
| Age (years) | | | |
| <50 | 88 (63.31) | 96 (73.28) | 0.079 |
| ≥50 | 51 (36.69) | 35 (26.72) | |
| Mean (mean ± SD) | 43.04 ± 14.63 | 41.99 ± 13.33 | |
| CML stages in response to IM | | | |
| Chronic phase | 94 (67.63) | 126 (96.18) | **<0.001** |
| Accelerated phase | 31 (22.30) | 5 (3.82) | |
| Blast phase | 14 (10.07) | – | |

Bold values indicate statistical significance, which is $p < 0.05$
*CML* Chronic myeloid leukemia, *IM* imatinib mesylate

(AGAGCCTTCCTACATAGAGTCA) primers. PCR reactions were conducted in a 25-μl volume 1× PCR buffer, 2.0 μM magnesium chloride ($MgCl_2$), 0.5 μM dNTPs, 0.4 μM of each primer and 1.0 U AmpliTaq Gold Polymerase. Denaturation was at 95 °C for 2 min, followed by 35 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s and a final extension step at 72 °C for 5 min. The 450-bp PCR products were electrophoresed on a 2% agarose gel at 100 V for 30 min.

Following PCR amplification, 4 μl of 450-bp PCR products were digested with 1.0 unit of a restriction enzyme (*Msp1)* for 1 h at 37 °C. The digested PCR products were analyzed by electrophoresis on a 2% agarose gel at 90 V for 50 min. The homozygous wild-type allele (TT) was identified by the presence of an undigested band (450 bp), while the heterozygous allele (TC) was confirmed by the presence of three fragments at 450, 282 and 168 bp. The homozygous variant allele (CC) was identified by the presence of two fragments at 282 and 168 bp.

## Genotyping of CYP3A5*3 Polymorphisms

Genotyping of *CYP3A5*3* was performed by using a PCR–RFLP. The 293-bp DNA fragment that contains the *CYP3A5*3* allele was amplified with the primer pair 5′-GGTCCAAACAGG GAAGAAATA-3′ (forward) and 5′-CATGACTTA GTAGACAGATGAC-3′ (reverse). The PCR reactions were conducted in a 25-μl volume of 1× PCR buffer, 2.0 μM $MgCl_2$, 0.5 μM dNTPs, 0.4 μM of each primer and 1.0 U AmpliTaq Gold Polymerase with a denaturation step of 95 °C for 2 min, followed by 35 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s and a final extension step at 72 °C for 5 min. The 293-bp PCR products were run on a 2% agarose gel electrophoresis at 100 V for 30 min.

Following PCR amplification, 4 μl of PCR products was digested by restriction enzyme

*Ssp1* for 15 min at 37 °C. The digested PCR products were analyzed by electrophoresis on a 3% agarose gel. The homozygous wild-type allele (AA) was identified by the presence of 148, 125 and 20 bp, whereas the homozygous variant allele (GG) was confirmed by the presence of fragments of 168- and 125-bp size. The heterozygous variant allele (AG) was identified by the presence of 168-, 148-, 125- and 20-bp fragments.

### Direct Sequencing

Following genotyping, a few samples from each different genotype were randomly selected for sequencing to confirm the expected sequences of each genotype. The PCR product was purified by using a QIAquick PCR purification kit (QIAGEN) before sending it to First BASE Laboratories (Kuala Lumpur, Malaysia) for sequencing.

### Statistical Analysis

The frequencies of polymorphic genotypes among IM-resistant and good-response CML patients were compared by using the chi-square test ($\chi^2$). The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using a binary logistic regression to investigate the risk association of genotypes with IM response. All statistical tests were two sided, and statistical significance was determined as $p < 0.05$. SPSS v.20.0 (SPSS Inc., Chicago, IL, USA) was utilized. Pair-wise linkage disequilibrium (LD) indices ($r^2$) were determined using Haploview v.4.2 [12].

## RESULTS

Two hundred seventy (270) CML patients (139 IM resistant and 131 good responders) were successfully recruited. Table 1 shows the patients' characteristics. In the present study, the gender, age and CML stages did not show any influence on IM response (data not shown). In the PCR-RFLP analyses, the restriction enzymes were successfully cut at the correct regions for *CYP3A4*18* and *CYP3A5*3* (Fig. 1), which was confirmed by the sequencing result (Fig. 2). Our study observed the allelic frequency of *CYP3A4*18* and *CYP3A5*3* in Malaysian CML patients as 4.44% and 47.41%, respectively. Among the CML patients, the allelic frequency of *CYP3A4*18* was lower (3.60%) among the IM-resistant group compared to good responders (5.34%), although this difference was not

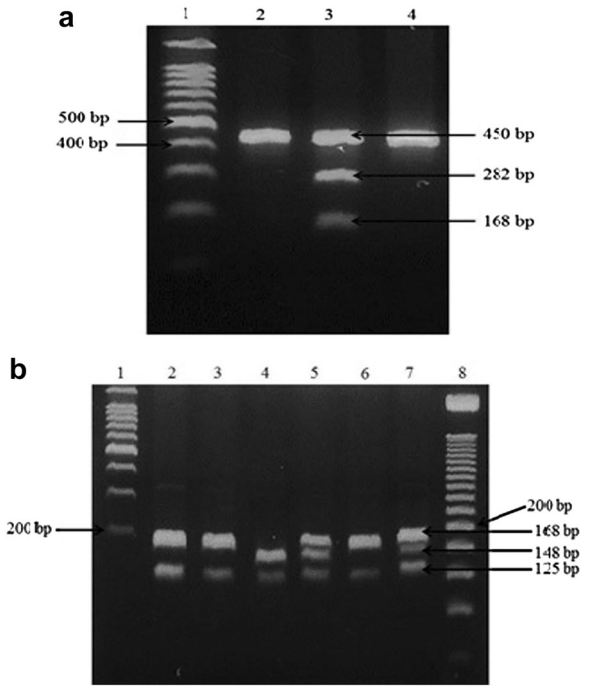


**Fig. 1** Gel electrophoresis after RFLP analysis for **a** *CYP3A4*18* (following digestion with *Msp1*) and **b** *CYP3A5*3* (following digestion with *Ssp1*). **a** *Lane 1* contained a 100-bp ladder. *Lanes 2* and *4* indicated a homozygous wild-type individual. *Lane 3* showed a heterozygous individual. **b** *Lane 1* contained a 100-bp ladder. *Lanes 2, 3* and *6* showed homozygous variants (GG). *Lane 4* indicated a homozygous wild type (AA). *Lanes 5* and *7* showed a heterozygous individual. *Lane 8* contained a 50-bp ladder

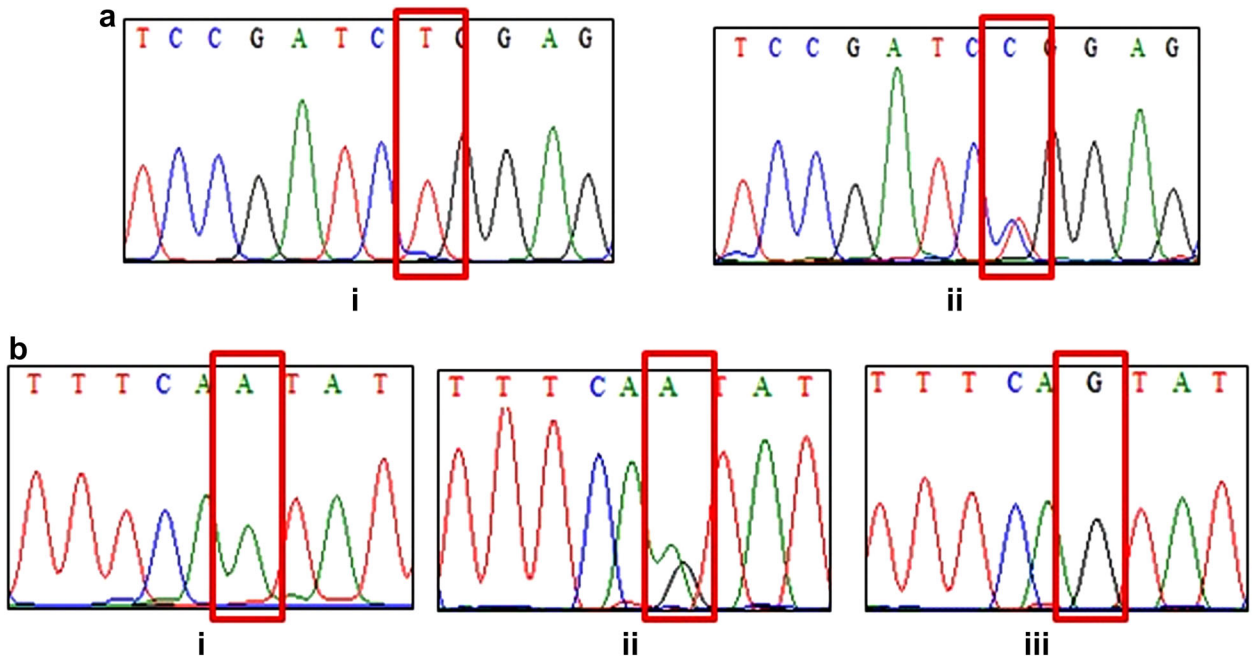


**Fig. 2** Direct DNA sequencing results showing part of the electropherogram for **a** *CYP3A4*18* and **b** *CYP3A5*3*. **a** *Top panel*: *i* a homozygous wild-type individual; *ii* a heterozygous individual. **b** *Bottom panel: i* homozygous wild-type, *ii* heterozygous and *iii* homozygous variants

significant. On the other hand, the allelic frequency of *CYP3A5*3* polymorphisms was significantly ($p < 0.001$) lower (38.85%) among the IM-resistant group compared to the good responders (56.49%).

The frequencies of the *CYP3A4*18* and *CYP3A5*3* genotypes in IM resistant and responders are shown in Table 2. For the *CYP3A4*18* polymorphism, only homozygous wild-type (TT) and heterozygous (TC) genotypes were observed among both IM-resistant and good-response CML patients. However, the frequency of *CYP3A4*18* homozygous wild type (TT) tended to be higher (92.81%) among the IM-resistant group although this difference was not statistically significant. No homozygous variant (CC) genotype was detected among both IM-resistant and good responders. As for the *CYP3A5*3* polymorphism, the genotypic frequencies of the homozygous wild type (AA) were significantly ($p < 0.001$) higher among the IM-resistant group (44.60% in IM resistant vs. 13.74% in IM good responders), but the frequency of heterozygous genotype (AG) was significantly ($p < 0.001$) higher in the IM good-responder group (59.54% in IM good responders vs. 33.09% in the IM-resistant group).

To establish the risk association of *CYP3A4*18* and *CYP3A5*3* polymorphisms with IM response, a binary logistic regression was performed (Table 3). For *CYP3A4*18*, there was no significant association between the polymorphism and the risk of acquiring resistance toward IM. However, for *CYP3A5*3* polymorphisms, both heterozygous (AG) and homozygous variants (GG) posed significantly ($p < 0.001$) lower risk for acquiring resistance toward IM. We determined that these two SNPs, rs28371759 (*CYP3A4*18*) and rs776746 (*CYP3A5*3*), are in low linkage disequilibrium ($r^2 = 0$) probably as a result of recombination occuring between the two markers (Fig. 3).

**Table 2** Genotype and allele frequencies of *CYP3A4*18* and *CYP3A5*3* polymorphisms in CML patients

| SNP | Rs number | Frequency (%) | | *p* value |
|---|---|---|---|---|
| | | IM resistance *n* = 139 (%) | IM good response *n* = 131 (%) | |
| *CYP3A4*18* | Rs28371759 | | | |
| (878T>C) | Genotype | | | |
| | Homozygous wild type (**1/*1*) | 129 (92.81) | 117 (89.13) | 0.313 |
| | Heterozygous (**1/*18*) | 10 (7.19) | 14 (10.69) | |
| | Homozygous variant (**18/*18*) | – | – | – |
| | Allele | | | |
| | **1* | 268 (96.40) | 248 (94.66) | 0.325 |
| | **18* | 10 (3.60) | 14 (5.34) | |
| *CYP3A5*3* | Rs776746 | | | |
| (6986A>G) | Genotype | | | |
| | Homozygous wild type (**1/*1*) | 62 (44.60) | 18 (13.74) | **<0.001** |
| | Heterozygous (**1/*3*) | 46 (33.10) | 78 (59.54) | **<0.001** |
| | Homozygous variant (**3/*3*) | 31 (22.30) | 35 (26.72) | 0.399 |
| | Allele | | | |
| | **1* | 170 (61.15) | 114 (43.51) | **<0.001** |
| | **3* | 108 (38.85) | 148 (56.49) | |

Bold values indicate statistical significance, which is $p < 0.05$

## DISCUSSION

To the best of our knowledge, this is the first study reporting the impact of polymorphism of *CYP3A4*18* and *CYP3A5*3* in IM treatment response in Malaysian CML patients. For *CYP3A4*18*, no homozygous variant *(*18/*18)* was detected among both IM resistant and good responders. This is similar to the findings among the Malaysian population reported by Ruzilawati et al. [13] who did not detect any homozygous variant, indicating that this genotype, which contributes to slower metabolism of most xenobiotics, is rather rare in the Asian population.

*CYP3A4*18* was detected at a frequency of 1.7% in a healthy Korean population [14], 2% in a healthy Chinese population [8], 1.3% in a healthy Japanese population [15] and 2.07% among Malaysian diabetics [13]. The allelic frequencies of *CYP3A4*18* among CML patients in Malaysia was almost double (4.44%) that reported among Malaysian diabetics indicating that the polymorphism may play a role in contributing to the disease. However, in another study among the Indian population to associate *CYP3A4*18* variant alleles with IM levels, although high inter-patient variability of IM levels were seen, no *CYP3A4*18* variants were detected to establish any significant correlation [16].

For *CYP3A5*, the mutant allele *CYP3A5*3* has been reported to contribute to the variable expression in the human liver. This mutation

**Table 3** Genotype frequencies and the risk association of *CYP3A4*18* and *CYP3A5*3* polymorphisms with IM response

| SNP | Rs number | Frequency (%) | | Risk association | | | *p* value |
|---|---|---|---|---|---|---|---|
| | | IM resistance $n = 139$ (%) | IM good response $n = 131$ (%) | OR (95% CI) | *p* value | Adjusted OR (95% CI)[a] | |
| *CYP3A4*18* | Rs28371759 | | | | | | |
| (878T>C) | Genotype | | | | | | |
| | Homozygous wild type (**1/*1*) | 129 (92.81) | 117 (89.13) | 1.000 | – | – | – |
| | Heterozygous (**1/*18*) | 10 (7.19) | 14 (10.69) | 0.648 (0.277–1.515) | 0.316 | – | – |
| | Homozygous variant (**18/*18*) | – | – | – | – | – | – |
| *CYP3A5*3* | Rs776746 | | | | | | |
| (6986A>G) | Genotype | | | | | | |
| | Homozygous wild type (**1/*1*) | 62 (44.60) | 18 (13.74) | 1.000 | – | 1.000 | – |
| | Heterozygous (**1/*3*) | 46 (33.10) | 78 (59.54) | 0.171 (0.090–0.324) | **<0.001** | 0.154 (0.076–0.312) | **<0.001** |
| | Homozygous variant (**3/*3*) | 31 (22.30) | 35 (26.72) | 0.257 (0.126–0.525) | **<0.001** | 0.203 (0.091–0.453) | **<0.001** |

Bold values indicate statistical significance, which is $p < 0.05$

[a] Adjusted for gender, age and CML stages in response to IM


**Fig. 3** LD blocks of rs28371759 (*CYP3A4*18*) and rs776746 (*CYP3A5*3*). The *plot* shows the $r^2$ value as pair-wise measure of LD

in intron 3, which creates a cryptic splice site, has been reported to cause a premature stop codon, thus resulting in the absence of the CYP3A5 protein [17]. In *CYP3A5*3,* a guanine (G) replaces an adenine (A) at position 6986. The *CYP3A5*1* allele appears to be the main allele associated with *CYP3A5* expression and activity. Individuals with at least one *CYP3A5*1* polymorphic allele tend to express higher amounts of *CYP3A5*. In the current study, CML patients who are carriers of the heterozygous *(*1/*3*) and homozygous variant *(*3/*3*) genotype of *CYP3A5*3* were associated with a significantly lower risk of acquiring resistance against IM, indicating that this variant allele has a protective effect against the development of IM resistance.

In the study conducted by Kim et al. [18] in a Canadian population, the *CYP3A5*1/*1* genotype had an adverse impact on achievement of a major cytogenetic response and complete cytogenetic response. Our findings, which indicate that CML patients who are carriers of *CYP3A5*1/*1* genotype tend to have a higher risk of developing resistance to IM, are in agreement with those of Kim et al. [18]. On the contrary, a study by Green et al. [19] on 14 Caucasian CML patients on IM therapy demonstrated that CML patients with high CYP3A activity tend to respond better to IM therapy than patients with low activity, indicating that the response may be variable from population to population based on the genetic background of the patients.

*CYP3A5*1/*1* genotype was reported to be associated with IM efficacy and **3/*3* genotype with inferior outcome among Egyptian CML patients [20] and also among Indian CML patients [21]. However, Takahashi et al. [22] on Japanese patients and Angelini et al. [23] on Caucasian patients reported no significant association of the *CYP3A5*3* polymorphism with IM response. Our findings showed that there is a significant association of *CYP3A5*3* polymorphism with IM response but with lower risk for development of resistance. Moreover, we also found that the *CYP3A5*1* genotype was higher in the IM-resistant group compared with the IM good-response group. Hence, it is reasonable to suggest that carriers of *CYP3A5*3* tend to have a protective effect against acquiring resistance toward IM therapy.

*CYP3A5* contributes substantially to the total metabolic clearance of many *CYP3A* substrates. Since individuals with at least one *CYP3A5*1* allele polymorphism express high amounts of *CYP3A5,* it is reasonable to predict that individuals with the highest clearance and lowest oral bioavailability of *CYP3A* substrates will be heterozygous or homozygous for *CYP3A5*1*. Hence, carriers of heterozygous and homozygous *CYP3A5*1* genotypes may be more likely to encounter a lack of efficacy from a standard dose of active parent drug. Likewise, the homozygous *CYP3A5*3* genotype can lead to a decrease in enzyme activity resulting in lowest clearance and high bioavailability of the

drug. Such CML patients are expected to have a better response to IM. As evidenced from our study, CML patients who are carriers of the *CYP3A5*1* genotype tend to acquire resistance toward IM treatment, and those with the *CYP3A5*3* genotype respond better to IM treatment.

The present study has some limitations in terms of sample size. Further studies are required to acquire a larger patient cohort for long-term IM response monitoring. It would also be worthwhile to estimate the intracellular and plasma levels of both IM and its active metabolites and to correlate their concentrations with the genotype pattern of *CYP3A4* and *CYP3A5*.

## CONCLUSION

Polymorphisms of *CYP3A4*18* are rather common among Malaysian CML patients, although they are not significantly associated with response to IM therapy. On the other hand, polymorphism of *CYP3A5*3* is associated with IM treatment response in Malaysian CML patients with individuals having the *CYP3A5*1/*3* and *CYP3A5*3/*3* genotypes posing lower risks of being resistant to IM treatment. Therefore, pretreatment genotyping of this SNP may be important in predicting IM response in CML patients.

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***Disclosures.*** Najlaa Maddin, Azlan Husin, Siew Hua Gan, Baba Abdul Aziz and Ravindran Ankathil declare no conflict of interest.

***Compliance with Ethics Guidelines.*** All procedures performed in the studies involving human participants were in accordance with ethical standards of the Research and Ethic Committee of Universiti Sains Malaysia and Ministry of Health, Malaysia, and with the Declaration of Helsinki of 1964, as revised in 2013. Informed consent was obtained from all patients for being included in the study.

***Data Availability.*** All data generated or analyzed during this study are included in this published article.



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