Original Articles

C1236T polymorphism in *MDR1* gene correlates with therapeutic response to imatinib mesylate in Indian patients with chronic myeloid leukaemia

SUNITA CHHIKARA, SUDHA SAZAWAL, PRAVAS MISHRA, REKHA CHAUBEY, MANORANJAN MAHAPATRA, RENU SAXENA

ABSTRACT

Patients with chronic myeloid leukaemia show an excellent response to treatment with imatinib. However, in some patients, the disease is resistant to imatinib. This resistance may be related to the presence of genetic variations on the drug's pharmacokinetics and metabolism. We therefore studied three polymorphisms (C1236T, G2677T and C3435T) in the human multidrug-resistance gene (*MDR1*) in 86 patients with chronic myeloid leukaemia treated with imatinib. Imatinib resistance was more frequent in patients with TT genotype at locus 1236 than in those with CT/CC genotypes (p = 0.003). For the other two loci (G2677T and C3435T), resistance was seen to be higher for TT genotype when compared to GG/GT and CT/CC but it was not statistically significant (p = 0.13 and p = 0.099). In conclusion, determination of C1236T MDR1 genotype may help to predict response to imatinib therapy in patients with chronic myeloid leukaemia.

Natl Med J India 2015;28:272–5

INTRODUCTION

Imatinib (Gleevec or Glivec, Novartis, Basel, Switzerland), a competitive inhibitor of Bcr–Abl tyrosine kinase, is the current standard of care for patients with chronic myeloid leukaemia (CML).[1] The rearrangement of *Bcr–Abl* is associated with Philadelphia chromosome, which is formed by the reciprocal translocation of genetic material between chromosome 9 and chromosome 22, and contains a fusion gene called *Bcr–Abl*. The best method to evaluate the response to imatinib is the occurrence of major molecular response (MMR), defined as a 3.0 $\log_{10}$ reduction in the concentration of *Bcr–Abl* genetic rearrangement in blood, after 18 months of treatment.[2]

Though a majority of patients with CML respond well to imatinib, a subset of those in the advanced phase of the disease do not respond. Further, some patients who initially respond to this drug later develop resistance (secondary resistance). The proposed molecular mechanisms of secondary resistance include: amplification and over-expression of the *Bcr–Abl* gene, point mutations in the ATP-binding site of Bcr-Abl fusion protein with kinase reactivation, or over-expression of the multidrug resistance 1 (*MDR1*) gene.[3,4] The structure of Bcr-Abl contains two flexible loops, the ATP-binding P-loop and the activation loop. Mutations in these loops destabilize the arrangement of loops such that the kinase domain cannot assume the inactive conformation required for imatinib binding. The *MDR1* gene (also known as ABCB1), located on chromosome 7 (7q21.1) and consisting of 28 exons, codes for P-glycoprotein 1 (P-gp), a 170-kilodalton ATP-binding cassette transmembrane transporter.[5,6] Cells from human tumours that have developed multidrug resistance following cancer chemotherapy often show over-expression of P-gp, which in turn alters the pharmacokinetics of drugs that are P-gp substrates.[7]

Imatinib is a substrate of P-gp-mediated efflux. Thus, upregulation of *MDR1* results in increased clearance from the cells and reduced intracellular levels of imatinib, and hence imatinib resistance.[8] The *MDR1* gene is polymorphic, with more than 1279 known single nucleotide polymorphisms (SNPs), which may affect the expression and function of this protein.[9] This could explain, at least in part, the inter-individual variability in responses to this drug.[10,11] Three SNPs, namely C1236T, G2677T/A and C3435T, are the most prevalent variants in the coding region of *MDR1*.[12] Identifying influential SNPs in this gene may allow prediction of disposition of imatinib in individual patients and hence of optimal response to this drug in patients with CML.[8,13] We therefore examined the association of these SNPs with response to imatinib therapy in Indian patients with CML.

METHODS

In 2012, we prospectively included 100 patients who attended the outpatient clinic of the All India Institute of Medical Sciences, New Delhi with the diagnosis of CML. Patients with incomplete information (n=3), incomplete follow-up (n=5), or those who were already resistant to imatinib and now on follow-up (secondary resistance, n=2) were excluded from the study. Patients who did not achieve MMR were tested for mutations which were also responsible for imatinib resistance in CML patients, and the patients positive for kinase domain mutations were also excluded

All India Institute of Medical Sciences, New Delhi 110029, India
SUNITA CHHIKARA, SUDHA SAZAWAL, PRAVAS MISHRA, REKHA CHAUBEY, MANORANJAN MAHAPATRA, RENU SAXENA Department of Haematology

Correspondence to Renu Saxena; *renusaxena@outlook.com*

(*n*=4). The remaining 86 patients were included in this study to assign the role of *MDR1* polymorphisms in response prediction to imatinib therapy in patients with CML.

The diagnosis of CML was based on physical examination, blood counts, peripheral blood smear, bone marrow biopsy and presence of *Bcr–Abl* fusion gene by reverse transcription polymerase chain reaction (RT-PCR). The typical clinical course is triphasic: chronic phase (CP), accelerated phase (AP) and blastic phase (BP) or blast crisis (BC). AP is defined by 15%–29% blasts in the blood or bone marrow, >20% basophils in the blood, thrombocytosis, thrombocytopenia unrelated to therapy or clonal chromosome abnormalities in the Ph+ clone (CCA/Ph+). The BP/BC of the disease is characterized by ≥30% blasts in blood or bone marrow or extramedullary blastic infiltration.

All these patients received 400 mg daily and those with advanced disease received 600–800 mg daily of imatinib provided free by the Max Foundation as part of the Gleevec International Patient Assistance Program. Under this programme, patients initially received the drug for 4 months, after which they could obtain a refill on returning empty wrappers. The rest of the patients were also followed strictly at 3–4 months intervals. The patients were explained the need for compliance with the treatment regimen; no other measures were available for ensuring compliance. The study was approved by the Institutional Ethics Committee, and all patients provided a written informed consent.

Response assessment

The patients were monitored on an outpatient basis for clinical findings and blood counts (haematological response, HR) and quantitative assessment of *Bcr–Abl* transcripts (molecular response, MR).[14] In brief, patients underwent a physical examination every fortnight, and blood counts, initially every week for the first 4 weeks, then every 2 weeks in the second month, and thereafter every month. Real-time PCR was done every 3 months to assess the MR. Patients who showed no change in *Bcr–Abl* copies or higher *Bcr–Abl* copies after diagnosis were considered to have primary molecular resistance.

Complete haematological response (CHR) was defined as total leukocyte count 4–11×10^9/L, platelet count 150–450×10^9/L, normalization of blood cell differential count with no immature forms (myelocytes, metamyelocytes, promyelocytes and blasts), disappearance of all clinical signs and symptoms (including splenomegaly) and no evidence of extramedullary disease. MMR was defined as ratio of *Bcr–Abl* to *Abl* (or other housekeeping genes) ≤0.1% of the pretreatment baseline (or >3-log reduction from the level measured at the start of treatment) on the international scale. The international scale was specifically designed so that, by definition, 100% is the median pretreatment baseline level of *Bcr–Abl* RNA in early CP-CML, and a 1000-fold (3-log) reduction from baseline is defined as 0.1% (MMR).[15] Imatinib resistance was defined as failure to attain either CHR at 3 months or MMR at 18 months.

The responses to tyrosine kinase inhibitors in CML can be assessed either with molecular tests alone or with cytogenetic tests alone, depending on the local laboratory facilities. It is accepted that molecular responses have prognostic significance.[16]

Real-time PCR for BCR–ABL

To assess MRs, total RNA was extracted from the peripheral or bone marrow blood cells. *Bcr–Abl* and internal control transcript levels were quantified using real-time PCR Taqman assay.[17]

Bcr–Abl point mutation detection

During follow-up, samples from patients who did not achieve MMR were sequenced for mutation. Regions of interest were amplified in a 30 μL reaction mixture using 100 ng of cDNA.[18] Cycling conditions used were: 94 °C for 5 minutes, followed by 30 cycles of denaturation at 94 °C for 25 seconds, annealing at the indicated temperature (65 °C) for 25 seconds, extension at 72 °C for 30 seconds, and a final extension at 72 °C for 5 minutes.

Genotyping of MDR1 polymorphisms

From venous blood, DNA was extracted using the standard phenol/chloroform method. Genotype of each *MDR1* locus was determined using PCR–restriction fragment length polymorphism (RFLP). In brief, specific DNA fragments were amplified using a reaction mix with a PCR buffer (Roche, Mannheim, Germany) with 5 pmol of respective forward and reverse primers (Table I; TIB Molbiol, Berlin, Germany), 0.2 mmol/L of deoxyribonucleoside triphosphates (Roche), 2 mmol/L of magnesium chloride, and 0.5 units of AmpliTaq (Perkin-Elmer, Weiterstadt, Germany) in a total volume of 25 μL. PCR amplification consisted of an initial denaturation at 94 °C for 2 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 60 °C for 30 seconds and extension at 72 °C for 30 seconds each, and then a terminal elongation at 72 °C for 7 minutes. The DNA fragments were then digested with respective restriction enzymes separated using a 3.5% agarose gel (low temperature melting agarose-standard agarose ratio, 3:1) electrophoresis, and visualized after ethidium bromide staining.

Statistical analysis

Inter-group differences in allele and genotype frequencies in patients with and without imatinib resistance were compared using the chi-square test. All tests were two-sided, and differences were considered significant when the p value was <0.05. All analyses were done using STATA 11.

RESULTS

Our study included 86 Ph+ CML patients (median age 32 years; 52 men, CP 67, AP or BC 19; median follow-up 38 months).

At the *MDR1* 1236 locus, the CC, CT and TT genotypes were found in 27 (31.4%), 38 (44.2%) and 21 (24.4%) patients, respectively. GG, GT and TT genotypes at the *MDR1* 2677 locus

TABLE I. Primer sequences used for amplification of polymerase chain reaction (PCR) fragments, which contained distinct *MDR1* polymorphisms, restriction endonucleases, and restriction fragment length polymorphisms (RFLP) fragment sizes resolved in dependence of the wild type or variant allele

Locus	Primer sequences	Restriction enzyme	Product lengths observed after restriction digestions
C1236T	MDR-15 5'-TAT CCT GTG TCT GTG AAT TGC C MDR-16 5'-CCT GAC TCA CCA CAC CAA TG	*Hae*III	T: 269, 62, 35 C: 269, 97
G2677T	MDR-9 5'-TGC AGG CTA TAGGTT CCA GG MDR-10a 5'-TTT AGT, TTG ACT CAC CTT CCC G	*Ban*I	T: 198, 26 C: 224
C3435T	MDR-11 5'-TGT TTT CAG CTG CTT GAT GG MDR-12 5'-AAG GCA TGT ATG TTG GCC TC	*Sau*3AI	T: 158, 39 C: 197

TABLE II. Characteristics of patients according to *MDR1* polymorphisms

Locus	Genotype	Imatinib responders (*n*=30)	Imatinib non-responders (*n*=56)	p value
C1236T	CC	6 (20)	21 (37.5)	0.010
	CT	11 (36.6)	27 (48.2)	
	TT	13 (43.3)	8 (14.2)	
G2677T	GG	10 (33.3)	19 (33.9)	0.275
	GT	11 (36.6)	28 (50)	
	TT	9 (30)	9 (16)	
C3435T	CC	7 (23.3)	22 (39.2)	0.155
	CT	15 (50)	27 (48.2)	
	TT	8 (26.6)	7 (12.5)	

Values in parentheses are percentages

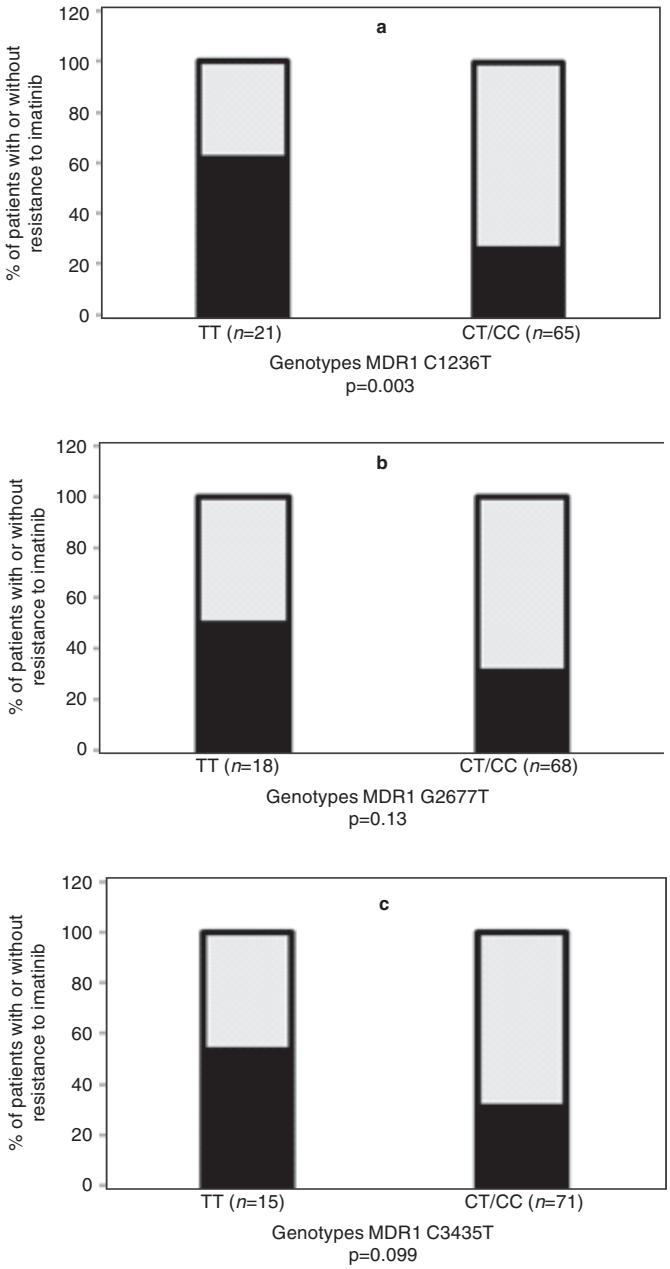

FIG 1. Genotypes of MDR1 (black indicates resistance and white indicates response to imatinib)

were identified in 29 (33.7%), 39 (45.3%) and 18 (20.9%) patients, respectively. At the *MDR1* 3435 locus, CC, CT and TT genotypes were found in 29 (33.7%), 42 (48.8%) and 15 (17.4%) patients, respectively (Table II).

Of the 86 patients, 56 (65.1%) patients who achieved MMR were categorized as responders, while 30 (34.8%) who did not achieve MMR were non-responders. For the C1236T polymorphism, resistance incidence correlated with the number of T alleles at locus 1236. Resistance was higher for TT genotype as compared to CT and CC genotype (p=0.003, Fig. 1a). However, for the *MDR1* C3435T polymorphism, resistance was seen to be higher for TT genotype when compared to CT and CC, but it was not statistically significant (p=0.099, Fig. 1c). Similarly, the treatment outcome also did not differ significantly when TT genotype was compared with GT and GG genotype for G2677T polymorphism (p=0.13, Fig. 1b).

The T allele was more common in patients who were non-responders than responders (C1236T, p=0.004; G2677T, p=0.36; and C3435T, p=0.06). The data for each of the three loci were in Hardy–Weinberg equilibrium (Table III).

DISCUSSION

The introduction of imatinib has markedly improved outcomes in patients with CML. However, patients vary in their response to imatinib, with some showing resistance to its beneficial effect. The molecular basis for this inter-individual variation is unclear. Genomic polymorphisms can influence an individual's response to a drug through an effect on the latter's pharmacokinetics or by altering the cellular response.[19] The *MDR1* polymorphisms might alter P-gp expression and activity towards specific anticancer agents, thereby influencing their therapeutic efficacy; the functional consequences of the changes at positions 2677 and 3435 are still controversial. P-gp is encoded by the *MDR1* gene, which is highly polymorphic, and inter-ethnic differences in the frequencies of several SNPs have been reported. Two of the most common SNPs, C3435T and G2677T, have been found to differ significantly in

TABLE III. Allelic and genotype frequencies of *MDR1* gene polymorphisms in imatinib responders (*n*=30) and imatinib non-responders (*n*=56)

Locus/ poly-morphism	Comparison	Imatinib responders (%)	Imatinib non-responders (%)	p value
C1236T	CC *v.*	6 (20)	21 (38)	0.096
	CT/TT	24 (80)	35 (62)	
	TT *v.*	13 (43)	8 (14)	0.003
	CT/CC	17 (57)	48 (86)	
	C allele *v.*	23 (38)	69 (62)	0.004
	T allele	37 (62)	43 (38)	
G2677T	GG *v.*	10 (29)	19 (34)	0.956
	GT/TT	20 (71)	37 (66)	
	TT *v.*	9 (30)	9 (16)	0.130
	GT/GG	21 (70)	47 (84)	
	G allele *v.*	31 (51)	66 (58)	0.360
	T allele	29 (48)	46 (41)	
C3435T	CC *v.*	7 (23)	22 (39)	0.136
	CT/TT	23 (77)	34 (61)	
	TT *v.*	8 (27)	7 (13)	0.099
	CT/CC	22 (73)	49 (87)	
	C allele *v.*	29 (48)	71 (63)	0.056
	T allele	31 (51)	41 (36)	

Values in parentheses are percentages

different ethnic groups.[20] We analysed the C1236T, G2677T and C3435T polymorphisms in patients who had resistance or were non-resistant to imatinib. For C1236T polymorphism we observed a significant difference between resistant and non-resistant patients (p=0.003). According to our data, we may be able to predict the response to imatinib in patients with CML.

Dulucq *et al*. reported a better MMR for patients with TT.[13] Similarly Gurney *et al*. reported that ABCB1 SNPs are associated with imatinib clearance, but its mechanism is still uncertain. Three SNPs (C1236T, G2677T and C3435T) are in strong linkage disequilibrium and are associated with the efficacy of response of patients with CML treated with imatinib.[8] Another study conducted by Kim *et al*. on CML patients did not find an association with G2677T polymorphism and MMR.[21]

We observed a significantly higher resistance to imatinib in carriers of the 1236TT genotype (61.9%) when compared to non-carriers 1236CC/CT genotype (26%) (p=0.003). This suggests that increased risk of resistance of patients with imatinib treatment may be associated with the T allele, particularly if homozygous. Ni *et al*. have also reported a significant correlation between SNPs and imatinib efficacy. One of the haplotypes (2677A-1236C genotype) or 3435C homozygote was statistically linked with less resistance to imatinib.[22]

Carriers of the TT genotype are at higher risk of developing acute lymphoblastic leukaemia than other individuals, whereas the CC genotype carrier exhibits a different prognosis.[23] Another study on patients with acute myeloid leukaemia reported that TT genotype was associated significantly with shorter relapse time and survival rates compared to heterozygotes.[24]

Consistent with our results, Dulucq *et al*. reported that the presence of C allele at 1236 positions was associated with a significantly worse response in CML patients receiving imatinib. The C allele was found to enhance P-gp expression and to be associated with increased efflux of P-gp substrates. Since imatinib is a substrate for P-gp, the presence of C allele was associated with a poorer response to imatinib in patients with CML.[13]

In line with previous data, studies on *MDR1* polymorphisms have yielded contradictory results, possibly due to small sample sizes and the different ethnic groups.[20,25] Further prospective studies in larger groups are needed to better define the effect of *MDR1* polymorphism (C1236T) on response to imatinib treatment.

In conclusion, we have shown a significant association between C1236T polymorphism and the efficacy of imatinib. Hence, pretreatment genotyping for this polymorphism may help in planning therapy.

ACKNOWLEDGEMENT

Sunita Chhikara acknowledges the Indian Council of Medical Research, New Delhi for a research fellowship.

REFERENCES

1 Druker BJ, Guilhot F, O'Brien S. Five-year follow-up of imatinib therapy for newly diagnosed myeloid leukemia in chronic-phase shows sustained responses and high overall survival. *N Engl J Med* 2006;**355:**2408–17.

2 Hughes TP, Kaeda J, Branford S. International Randomised Study of Interferon versus STI571 (IRIS) Study Group: Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. *N Engl J Med* 2003;**349:**1423–32.

3 Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN. Clinical resistance to STI-571 cancer therapy caused by BCR–ABL gene mutation or amplification. *Science* 2001;**293:**876–80.

4 Mahon FX, Belloc F, Lagarde V, Chollet C, Moreau-Gaudry F, Reiffers J, *et al*. MDR1 gene overexpression confers resistance to imatinib mesylate in leukemia cell line models. *Blood* 2003;**101:**2368–73.

5 Chen CJ, Clark D, Ueda K, Pastan I, Gottesman MM, Roninson IB. Genomic organization of the human multidrug resistance (MDR1) gene and origin of P-glycoproteins. *J Biol Chem* 1990;**265:**506–14.

6 Ueda K, Cornwell MM, Gottesman MM, Pastan I, Roninson IB, Ling V. The MDR1 gene, responsible for multidrug resistance, codes for P glycoprotein. *Biochem Biophys Res Commun* 1986;**141:**956–62.

7 Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: Role of ATP-dependent transporters. *Nat Rev Cancer* 2002;**2:**48–58.

8 Gurney H, Wong M, Balleine RL, Rivory LP, McLachlan AJ, Hoskins JM. Imatinib deposition and ABCB1(MDR1, Pglycoprotein) genotype. *Clin Pharmacol Ther* 2007;**82:**33–40.

9 Fung KL, Gottesman MM. A synonymous polymorphism in a common MDR1 (ABCB1) haplotype shapes protein function. *Biochim Biophys Acta* 2009;**1794:** 860–71.

10 Kimchi-Sarfaty C, Oh JM, Kim IW, Sauna ZE, Calcagno AM, Ambudkar SV, *et al.* A 'silent' polymorphism in the MDR1 gene changes substrate specificity. *Science* 2007;**315:**525–8.

11 Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmoller J, Johne A: Functional polymorphisms of the human multidrug-resistance gene: Multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity *in vivo*. *Proc Natl Acad Sci USA* 2000;**97:**3473–8.

12 Hamidovic A, Hahn K, Kolesar J. Clinical significance of ABCB1 genotyping in oncology. *J Oncol Pharm Pract* 2010;**16:**39–44.

13 Dulucq S, Bouchet S, Turcq B. Multidrug resistance gene (MDR1) polymorphisms are associated with major molecular responses to standard-dose imatinib in chronic myeloid leukemia. *Blood* 2008;**112:**2024–7.

14 Baccarani M, Castagnetti F, Gugliotta G, Palandri F, Soverini S. European Leukemia Net. Response definitions and European Leukemianet Management recommendations. *Best Pract Res Clin Haematol* 2009;**22:**331–41.

15 Druker BJ, Lee SJ. Chronic leukemias. In: Devita VT, Hellman S, Rosenberg SA (eds.) *Cancer, principles and practice of oncology*.7th ed. Philadelphia: Lippincott; 2005: 2124.

16 Baccarani M, Deininger MW, Rosti G, Hochhaus A, Soverini S, Apperley JF, *et al.* European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. *Blood* 2013;**122:**872–84.

17 Gabert J, Beillard E, van der Velden VJH. Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia – A Europe Cancer Program. *Leukemia* 2003;**17:**2318–57.

18 Branford S, Rudzki Z, Walsh S. High frequency of point mutation clustered within adenosine triphosphate binding region of BCR/ABL in patients with chronic myeloid leukemia or Ph-positive acute lymphoblastic leukemia who develop imatinib (STI571) resistance. *Blood* 2002;**99:**3472–5.

19 Sauna ZE, Kimchi-Sarfaty C, Ambudkar SV. Silent polymorphisms speak: How they affect pharmacogenomics and the treatment of cancer. *Cancer Res* 2007;**67:** 9609–12.

20 Kimchi-Sarfaty C, Marple AH, Shinar S. Ethnicity-related polymorphisms and haplotypes in the human ABCB1 gene. *Pharmacogenomics* 2007;**8:**29–39.

21 Kim DH, Sriharsha L, XuW, Kamel-Reid S, Liu X, Siminovitch K. Clinical relevance of a pharmacogenetic approach using multiple candidate genes to predict response and resistance to imatinib therapy in chronic myeloid leukemia. *Clin Cancer Res* 2009;**15:**4750–8.

22 Ni LN, Li JY, Miao K. Multidrug resistance gene (MDR1) polymorphisms correlate withimatinib response in chronic myeloid leukemia. *Med Oncol* 2010;**28:**265–9.

23 Pawlik A, Baskiewicz-Masiuk M, Machalinski B. Involvement of C3435T and G2677T multidrug resistance gene polymorphisms in release of cytokines from peripheral blood mononuclear cells treated with methotrexate and dexamethasone. *Eur J Pharmacol* 2005;**528:**27–36.

24 Van Den MM, Heuvel-Eibrink EA, Weimer MJ, De Boevere B, Van Der Holt PJ, Vossebeld R. MDR1 gene related clonal selection and P-glycoprotein function and expression in relapsed or refractory acute myeloid leukemia. *Blood* 2001;**97:** 3605–11.

25 Tang K, Ngoi SM, Gwee PC, Chua JM, Lee EJ, Chong SS. Distinct haplotype profiles and strong linkage disequilibrium at the MDR1 multidrug transporter gene locus in three ethnic Asian populations. *Pharmacogenetics* 2002;**12:**437–50.