Pharmacogenomics and Personalized Medicine

Dovepress

open access to scientific and medical research

Open Access Full Text Article

REVIEW

ABCB1 genetic variants in leukemias: current insights into treatment outcomes

This article was published in the following Dove Press journal:
Pharmacogenomics and Personalized Medicine
12 May 2017
Number of times this article has been viewed

Ravindran Ankathil

Human Genome Centre, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia

Abstract: Despite improvements in treatment of different types of leukemia, not all patients respond optimally for a particular treatment. Some treatments will work better for some, while being harmful or ineffective for others. This is due to genetic variation in the form of single-nucleotide polymorphisms (SNPs) that affect gene expression or function and cause inherited interindividual differences in the metabolism and disposition of drugs. Drug transporters are one of the determinants governing the pharmacokinetic profile of chemotherapeutic drugs. The *ABCB1* transporter gene transports a wide range of drugs, including drugs used in leukemia treatment. Polymorphisms in the *ABCB1* gene do affect intrinsic resistance and pharmacokinetics of several drugs used in leukemia treatment protocols and thereby affect the efficacy of treatment and event-free survival. This review focuses on the impact of three commonly occurring SNPs (1236C>T, 2677G>T/A, and 3435C>T) of *ABCB1* on treatment response of various types of leukemia. From the literature available, some of the genotypes and haplotypes of these SNPs have been found to be potential determinants of interindividual variability in drug disposition and pharmacologic response in different types of leukemia. However, due to inconsistencies in the results observed across the studies, additional studies, considering novel genomic methodologies, comprehensive definition of clinical phenotypes, adequate sample size, and uniformity in all the confounding factors, are warranted.

Keywords: leukemia, ABCB1 polymorphisms, chemotherapy response, survival

Introduction

Leukemia, a group of cancers that start in blood forming cells of the bone marrow, can affect anyone, including children. By considering whether leukemias are acute or chronic, and whether they are myeloid or lymphocytic in origin, they can be divided into four main types: acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL), and chronic lymphocytic leukemia (CLL). Acute leukemias have many subtypes that vary in the response to treatment. Treatment of leukemias depends on the type of leukemia, certain features of the leukemia cells, the extent of the disease, prior history of treatment, as well as the age and health of the patient.[1,2] The genetic profile or specific characteristics of the leukemia cells as determined in the laboratory are used to determine the type of treatment that may be most appropriate. Most leukemia patients are treated with chemotherapy, while some may also have radiation therapy, biological therapy, targeted therapy, and bone marrow transplantation.[1,2]

Correspondence: Ravindran Ankathil
Human Genome Center, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, Kubang Kerian, 16150, Kelantan, Malaysia
Email rankathil@hotmail.com

submit your manuscript | www.dovepress.com
Dovepress
http://dx.doi.org/10.2147/PGPM.S105208

Treatment challenges in leukemias

For most cases of AML, treatment is usually chemotherapy, which is divided into two phases – remission induction (often called induction therapy) and consolidation (postremission therapy). Patients with different subtypes of AML can have different outlooks and response to treatment. After diagnosis, AML patients are categorized into three outcome risk profiles (favorable prognosis, intermediate risk profile, and poor outcome risk profile) and treated accordingly. However, for some unknown reasons, some respond well and some do not. Accordingly, adult AML patients have a 25% 5-year overall survival (OS) rate, whereas children and teens younger than 15 years have 66% OS rate.[1] New discoveries are needed to increase the chances of survival for AML patients who do not respond well to treatment.

The understanding of aberrant tyrosine kinase activity as the molecular mechanism behind CML development led to the development of tyrosine kinase inhibitor (TKI) drugs such as imatinib mesylate (IM).[3,4] The molecularly targeted drug IM has become the gold standard drug for the first line treatment of CML. An event-free survival (EFS) of 86% and OS of 88% for CML patients on IM therapy was reported by a 6-year update of the IRIS study.[5] Furthermore, this resulted in overcoming the difficulties encountered with previous therapeutic approaches.[3,6] Despite these excellent results, approximately 30%–40% of CML patients on IM therapy develop resistance.[7] In a significant proportion of CML patients, achievement of prolonged response to IM is still a daunting problem due to development of resistance or suboptimal response to IM.

Treatments for ALL patients include chemotherapy, biological agents, and transplant. Most chemotherapy plans for ALL involve three steps which are induction, consolidation, and maintenance. Chemotherapy and corticosteroids are in the treatment protocols. Different types of ALL patients may be treated differently. Younger adults with ALL have better long-term survival rates than older adults with ALL. Nevertheless, survival rate for childhood ALL have risen dramatically during the last 10–15 years and more than eight out of ten children are now cured.[8] According to National Cancer Institute report, the 5-year survival rate for children and teens younger than 15 years is 92%, whereas the overall 5-year survival for ALL is 70%.[1]

CLL is treated by chemotherapy, radiation therapy, therapy using monoclonal antibodies, or bone marrow transplantation. Majority of patients follow an indolent clinical course with no or delayed treatment need and with a prolonged survival, while others experience aggressive disease requiring early treatment followed by frequent relapses. However, during the last few decades, several new chemotherapeutics drugs are being tried, and some of these are promising in targeting CLL. For CLL, an OS rate of 84% has been achieved with modern treatments.[1] Some of the available treatments can often induce disease remission, but they are not able to reach the cure, and hence CLL remains an incurable disease in virtually all cases. So, in CLL, further research on individual factors that can benefit treatment regimens need to be undertaken.

Pharmacogenomics of drug response

Earlier, most patients with a specific type of leukemia were given the same treatment. The realization that some treatments worked better for some patients than for others led to research into the genetic differences seen in patients and in tumors. The completion of the Human Project in 2003 had provided increasingly comprehensive information on the genetic variations among individuals that are responsible for this variation in treatment response. Genetic variations in the form of single-nucleotide polymorphisms that affect gene expression or function in both normal and cancer cells can cause inherited interindividual differences in the metabolism and disposition of medications. Single-nucleotide polymorphisms (SNPs) between individuals influence how effective and safe a drug is for a person.

Pharmacogenetics incorporates information on how inherited genetic variation in a gene can affect a patient's response to chemotherapeutic agents and aims to use this knowledge to tailor therapy for improved response and reduced toxicity. Meanwhile, pharmacogenomics is a broader strategy which elucidates the entirety of pharmacologically relevant genes, including the effects of genetic variation in single genes, the interaction among genes in biological and pharmacological pathways, the phenotype emerging from these variations, and the effect of the phenotype on drug response.[9] Studies of inherited variability in drug targets or target pathways, variation in genes encoding drug-metabolizing enzymes or drug transporters, and genetic polymorphisms in genes encoding proteins, all of which indirectly influence drug response, comprise the field of pharmacogenomics. It also includes how these genetic variations interact to produce inherited drug-response phenotypes.[10,11] Variations in drug metabolizing enzymes, drug transporters, and drug targets are the most practical aspects of pharmacogenomics. The approach of personalized medicine, which uses predictive and prognostic biomarkers to direct patient management, holds

great promise in improving the efficiency of treatment and outcomes of leukemia patients. In order to realize personalized medicine, there is the need to understand the molecular mechanisms underlying interindividual differences in drug response, including pharmacological effects and side effects.

Genetic variation and drug resistance

Development of resistance to chemotherapeutic drugs, which results in treatment failure, is a severe limitation of chemotherapy in leukemia patients. Factors that can influence plasma and intracellular levels of chemotherapeutic drugs may contribute to the development of resistance. Genetic polymorphisms in key genes encoding drug transporters and drug metabolizing and binding enzymes may influence the intracellular delivery and therefore the effectiveness and toxicity of chemotherapeutic drugs.[12,13] Genetic polymorphisms in the form of SNPs that affect gene expression or function in both normal and cancer cells can cause inherited interindividual differences in the metabolism and disposition of medications. Accordingly, genetic variations of candidate genes could affect expression of corresponding proteins and thus may bring about differences in response to chemotherapeutic drugs. Given that genetic differences between individuals or population can impact the efficacy of drugs, defining pharmacogenetic differences among patients is regarded as an important aspect which needs to be addressed in understanding the development of resistance to chemotherapeutic drugs in leukemia patients.

Accumulating evidence strongly suggests that drug transporters are one of the determinants governing the pharmacokinetic profile of chemotherapeutic drugs. Membrane transporters play an important role in acquired and de novo drug resistance. Based on the direction in which they transport, transporters are often classified as efflux, influx, or bidirectional. Drug resistance mechanism in anticancer therapy has been well established by drug transporter proteins. Expression of higher than normal levels of a transmembrane protein which serves as an energy dependent efflux pump causes a reduction in the amount of drug that accumulates within cancer cells and is considered a common mechanism of multidrug resistance in cancer cells.[14,15] Variation in drug transporters is one of the most clinically relevant pharmacogenomics aspect of some of the chemotherapeutic drugs for leukemias.

In this review, attention is focused on ABC transporters, and specifically on currently understood information on the impact of genetic variations in *ABCB1* gene on leukemia treatment that may suggest better strategies in future for the use of current therapeutic agents.

ABC superfamily

The ATP-binding cassette (ABC) transporter is a protein superfamily whose members are characterized by two highly conserved ATP binding cassettes. In the human genome, 48 different members, forming eight different subfamilies (A–G) have been identified, based on sequence similarities.[16] The ABC superfamily of proteins are involved in the transport of intrinsic and extrinsic molecules such as ions, sugars, glycans, phospholipids, amino acids, peptides, proteins, drugs, and toxins. Internalization of those substrates such as molecules and drugs occurs by active transport, which is dependent on the hydrolysis of ATP. All eukaryotic ABC proteins are efflux pumps.

ABCB1 protein

Within the ABC transporter superfamily, the subfamily B member 1 (ABCB1) appears to be most important in the human body, especially for the disposition of xenobiotics. ABCB1 transports a wide range of drugs and xenobiotics from the intra- to extracellular compartment at many biological interfaces such as the intestine, liver, blood–brain barrier, and kidney. As a transporter, ABCB1 has a broad affinity spectrum for different anticancer agents such as docetaxel, paclitaxel, irinotecan, vincristine, doxorubicin, vinblastine, mitoxantrone, teniposide, topotecan, etoposide, imatinib, sunitinib, etc.[17–19]

The *ABCB1* gene which codes the ABCB1 protein is located on chromosome 7q21.12. It spans 28 exons in a genomic region spanning 209.6 kb[20] and is one of the 49 putative members in the superfamily of human ABC transporters.[21] The messenger RNA (mRNA) is 4,872 bp in length, includes the 5' untranslated region (Ref seq accession NM_000 927.3), and can encode a protein of 1,280 amino acids in length, which is named the plasma membrane glycoprotein (P-glycoprotein [P-gp]) which is 170 kDa.[20] *ABCB1* was formerly termed multidrug resistance gene (*MDR1*) since P-gp was observed to be overexpressed in tumor cells and led to the commonly known phenomenon of multidrug resistance against certain antineoplastic agents.[21] Being involved in the extrusion of amphoteric compounds, it is also known as the Traffic ATPase.[22] Recently, the common ABC transporter nomenclature has been applied in naming the gene and protein of P-glycoprotein as ABCB1 (in italics and nonitalics, respectively).

One of the main functions of ABCB1 include first- pass elimination of orally administered drugs to limit their

bioavailability, by effluxing drugs from the lumen-facing epithelia of the small intestine and colon and from the bile-facing canaliculi of the liver. ABCB1 mediated drug disposition is influenced by modulation of *ABCB1* gene expression and/or ABCB1 activity by various mechanisms. Overexpression of these transporters on plasma membranes cause increased efflux and decreased intracellular accumulation of many anticancer drugs, leading to multidrug resistance.[23]

High levels of ABCB1 expression results in decreased intracellular concentration of drugs, and this will lead to development of cellular resistance to anticancer drugs. The expression level and functional integrity of ABCB1 may affect its pharmacogenetics and its interaction with drugs. Because of this, ABCB1 plays a significant role in drug efficacy and toxicity during treatment. Few studies have demonstrated that the level of ABCB1 activity determines the tissue distribution of drugs and affects the uptake from the gastrointestinal tract as well as elimination into urine or bile.[16] Many substrates of ABCB1 have been well documented to be potent ABCB1 inhibitors, including channel blockers, calmodulin antagonists, immunosuppressants, and protein kinase inhibitors.[24]

The discovery of ABCB1 provided a laboratory model that could explain the phenomenon of multidrug resistance. Cancer cells express ABCB1 proteins in different levels, thereby contributing to chemoresistance. For a better understanding of the significant variability in response to chemotherapeutics, polymorphisms in the ABC drug transporters have been extensively studied.

Genetic polymorphisms of *ABCB1*

Several publications have described polymorphisms of drug transporters as potential determinants of variability in drug disposition and efficacy. Polymorphisms in key drug transporter genes are known to influence intracellular drug delivery and, therefore, the effectiveness of drugs. With regard to *ABCB1* gene also, a number of SNPs have been identified that are likely to have an effect on P-gp expression levels and function. SNPs in *ABCB1* have been reported as modulators of ABCB1-mediated transport.

SNPs in *ABCB1* have the potential to alter *ABCB1* gene expression as well as P-gp function. Such SNPs are predicted to be associated with changes in both the pharmacokinetics and pharmacodynamics of several P-gp drug substrates, treatment response, as well as side effects.[25,26] According to NCBI SNP database, around 1,200 SNPs have been identified within the *ABCB1* gene, of which 66 SNPs have been identified in the coding sequence so far, while more than 20 are known to be silent.[27] There is considerable heterogeneity in the literature and across the populations regarding the frequencies and association of this transporter gene polymorphisms with drug resistance. Among the various population groups, three SNPs 1236C>T, 2677G>T/A, and 3435C>T of *ABCB1,* which are located in exons 12, 21, and 26, respectively, are the most widely investigated for their clinical implications.[28–30]

The SNPs C1236T, G2677T/A, and C3435T have been reported to be associated with altered mRNA expression levels, mRNA stability, and protein folding and influence drug pharmacokinetics.[31–33] However, another study found no association between these SNPs and *ABCB1* gene function.[34] Changes in P-gp expression and function would be expected to alter the absorption, plasma concentration, tissue distribution, and excretion of its drug substrates.

The silent 3435C>T (rs1045642) was the first polymorphism of *ABCB1* to be described and also modify P-gp expression.[35,36] This SNP modifies the gene expression of P-gp without altering the sequence of the protein (wobble mutation). However, it is probable that this SNP alters the mRNA stability,[37] as well as the folding of the protein, modifying its substrate specificity. The C3435T is the most widely investigated SNP of *ABCB1*. Differences in variant allelic frequency were observed among Caucasian, African, and Asian populations.[32]

The tri-allelic nonsynonymous SNP 2677G>T/A (rs2032582) changes the serine to either threonine or alanine. This SNP has been reported to be associated with drug response and various diseases and also affects posttranslational modifications.[38] Biochemical analysis has confirmed that the wild-type G allele of 2677 alters drug transport by affecting drug-induced ATPase activity.[39] This SNP has been detected in various ethnic groups, and the highest variant genotype frequency was reported among Japanese (34%) compared to South African population, which presented only 2%.[40,41]

ABCB1 1236T>C (rs1128503) encodes for the TM6 region, which is essential for substrate binding. This third most frequent SNP, 1236T>C, has also been reported to affect the expression and function of P-gp.[29] Highest frequency of 1236CC variant genotype was observed in German population, while lowest was among South Africans, where only 1% of variant CC genotype was detected.[41]

Genetic variants closely linked with other variants located on the same chromosome, known as haplotype, have also been documented to play an important role in drug response and disease susceptibility.[42] This nonrandom association of SNPs is called linkage disequilibrium (LD). Genetic

studies have identified a strong LD between SNPs in exons 12 (C1236T), 21 (G2677T), and 26 (C3435T).[43] Because of the significant LD, these SNPs are inherited to form two common haplotype patterns (T1236/T2677/T3435 or C1236/G2677/C3435).[29]

Frequencies of these SNPs and haplotypes have been reported to vary across races and populations.[32] Kroetz et al[44] reported 16 variants specific to African Americans, 8 to Caucasians, and 3 to Asian Americans. Despite the variant allele frequencies being higher in African Americans, the three SNPs were reported[37] to be twice as common in Caucasians as in African Americans. Therefore, *ABCB1* substrates may be transported differently depending on racial and genetic background of individuals.

Owing to the important role in the drug disposition process, focus is given on the role of these SNPs as potential determinants of interindividual variability in drug disposition and pharmacologic response in different types of leukemia.

ABCB1 variants and AML treatment

For all forms of AML in adult patients, a combination of cytarabine and various doses of different anthracyclines had been the mainstay of treatment in the last four decades. Addition of an occasional third agent to this combination chemotherapy regimen has been found to be effective for treatment of some AML patients. However, it is far from ideal. The traditional 'one size fits all' regimen is not appropriate for AML. With the current forms of treatment, nearly 35%–40% of patients younger than 60 years of age are likely to achieve long-term survival. However, wide variation in outcome among genetically distinguishable subsets of the disease has been encountered. Some subtypes show notoriously poor outcome. Likewise, the overall prognosis remains highly unsatisfactory for patients who are more than 60 years of age. Poor prognosis, development of drug resistance, and death within 2 years of remission are common presentations in most adult AML patients.[45] This warrants the need for urgent therapeutic improvements in AML.

For a better understanding of the significant variability in response to chemotherapeutics, polymorphisms in the ABC drug transporters have been extensively investigated in AML patients. P-gp expression and activity profile, which are influenced by *ABCB1* polymorphisms, importantly in exons 12 and 26, have been documented as factors that contribute to AML resistance to chemotherapy.[29,46] In AML patients, elevated P-gp expression and activity have been considered as adverse prognostic factors associated with refractory and relapsed disease.[47] Furthermore, dosage adjustment has been reported to be dependent on *ABCB1* polymorphisms, which in turn has been implicated to be related to P-gp status.[35,48]

In AML, complete remission rate and drug resistance are related to the function and expression of *ABCB1*.[49] The expression and functional drug efflux activity of *ABCB1* was reported to increase with patient age, from 17% in patients less than 35 years old to 39% in patients aged 50 years or older.[49] Seedhouse et al[50] studied the expression and genetic polymorphisms of *ABCB1* in 817 AML patients. These researchers observed that the 3435TT genotype (which results in unstable mRNA) of *ABCB1* had a significant effect on P-gp expression. But this was observed only in 40% of cases in which mRNA and protein were detectable. According to Seedhouse et al,[50] low white blood cell count, secondary AML, and poor-risk cytogenetics had a much higher impact on prognosis than genetic polymorphisms of *ABCB1* in AML blasts.

In addition, a strong link between *ABCB1* genetic variants and P-gp expression with poor survival in AML patients has been reported.[51,52] Scheiner et al[51] examined the relationship between *ABCB1* polymorphism (C1236T, C3435T) and P-gp expression activity in 109 Brazilian AML patients to understand the possible relationship between these factors and their clinical significance. They reported achievement of better 5-year OS and 5-year EFS rates in patients presenting with genetic variant CC in exon 12, followed by those presenting the variant CT in exon 26. According to Scheiner et al,[51] polymorphisms in the *ABCB1* gene and the levels of P-gp expression could be useful to identify prognosis in AML patients.

Green et al[52] and Falk et al[53] investigated the influence of SNPs 1199G>A, 1236C>T, 2677G>T/A, and 3435C>T in Swedish AML patients with de novo normal karyotype. Poorer OS was observed in patients with 1236 C/C or 2677 G/G genotypes than patients with other genotypes (p=0.03 and 0.02 respectively). Furthermore, leukemic cells from 1236 T/T and 2677 T/T patients demonstrated significantly higher susceptibility to mitoxantrone (p=0.02) and more susceptible to etoposide and daunorubicin (p=0.07–0.09) but not to cytarabine in in vitro studies.[52] In the subgroup analysis[53] based on FLT3 and NPM1 status of the patients, FLT3 wild-type 1236 C/C patients had significantly shorter OS compared to patients carrying variant allele (median OS 20 vs 49 months respectively, p=0.017). Also, those patients with FLT3 wild-type 2677 G/G genotype showed an inferior outcome, compared to patients carrying the variant allele (median OS 20 vs 35 months respectively, p=0.039). These researchers concluded that *ABCB1* 1236C>T and 2677G>T

might be used as prognostic markers to distinguish relatively high-risk patients in the intermediate-risk FLT3 wild-type group of AML patients as part of individualizing treatment strategies in future.

In 263 intermediate-risk Chinese AML patients treated with anthracycline and cytarabine, He et al[54] investigated the influence of polymorphisms G2677T/A, C1236T, and C3435T. This study reported that patients with TTT haplotype had a longer OS compared with those without TTT haplotype, and hence TTT haplotype was possibly related to the OS, EFS, and relapse in Chinese patients with AML. In a meta-analysis involving seven cohort studies with 1,241 AML patients undergoing standard chemotherapy (cytarabine plus anthracyclines), Megías-Vericat et al[55] reported significantly higher OS among carriers of the variant allele of 1236C>T, 2677G>T/A, and 3435C>T, with Caucasians showing consistent results in OS.

ABCB1 variants and ALL treatment

With the introduction of risk-directed therapy and current treatment protocols based on multiagent chemotherapy, the survival rates of ALL, especially of childhood ALL, has increased significantly. Nearly 85% of ALL patients achieve a long-term remission.[56] But still, nearly 15% of ALL children experience relapse due to substantial variation in treatment response.[57,58] There is still a group of patients for whom therapy fails, and some patients who experience severe toxicity.[59] Even though genomic alterations that are somatically acquired have long been recognized as hallmarks of ALL, inherited genetic variations (germ line) have also emerged as important determinants of interpatient variability in treatment response and toxicities of ALL patients. Increasing evidence that is emerging indicate that inherited genetic variations play significant roles in determining patient's risk of relapse. Treatment failures depend on inherited SNPs in genes affecting drug metabolism, transport, and binding site affinity.[60]

Treatment for childhood ALL involves complex combination of chemotherapy protocols, and hence individual SNPs might not have measurable effects on drug disposition and cure rates. Some adverse effects of ALL therapy have been linked to specific drugs. Candidate-gene and genome-wide approaches have identified inherited variants that may be associated with some of the risks of these drug-specific adverse effects in ALL.[61]

Earlier studies by Jamroziak et al[62] and Stanulla et al[63] reported better EFS and a lower rate of CNS relapse associated with the *ABCB1* T 3435 allele in childhood ALL. But Efferth et al[64] and Jamroziak et al[65] reported no association of this polymorphism with prognosis in adult ALL patients. Ceppi et al[66] observed that *ABCB1* 3435TT genotype had lower EFS in the discovery cohort in univariate and multivariate analysis. However, failure to subsequently replicate this finding in a validation patient set has raised arguments against the role of this polymorphism in modulation of ALL outcome. Erdélyi et al[67] examined the association of functional *ABCB* polymorphisms with acute side effects of chemotherapy in 138 Hungarian ALL children treated with the ALL-BFM-95 protocol. A higher proportion of patients who carried the *ABCB1* 3435TT genotype suffered excessive infectious complications than those harboring at least one C allele. These researchers concluded that *ABCB1* 3435T>C genotype was associated with the infectious complications of applied chemotherapy regimen.

However, single SNPs can have measurable effects if they either affect antileukemic agents such as 6-mercaptopurine or methotrexate (MTX) that are used extensively in the protocols,[68] or when the gene in question belongs to cytochrome P450 family,[69] or glutathione *S*-transferases,[70] and potentially the *ABCB1* gene.

Although MTX is not considered as a P-gp substrate, studies of patients on MTX monotherapy by Grabar et al[71] and Kato et al[72] showed that the silent *ABCB1* polymorphism 3435C>T might affect outcome and toxicity after MTX therapy. In a recent study on 522 Danish children with ALL, Gregers et al[73] explored the impact of *ABCB1* polymorphisms 1199G>A, 1236C>T, 2677G>T/A, and 3435C>T on the risks of relapse and toxicity. They reported that the genetic variants 1199G>A and 3435C>T were associated with outcome in childhood ALL. In the high-risk patients who were carriers of 1199 GA variant, an overall relapse rate of >29% and a relapse rate of >60% were observed. Gregers et al[73] concluded that 1199G>A might be a new possible predictive marker for outcome in childhood ALL and that patients with 1199G>A polymorphism should be observed more intensively. In pediatric ALL patients from China, Liu et al[74] studied the association of 12 SNPs in 4 candidate genes of the MTX/folate pathway with pharmacokinetics, toxicity, and outcome. They reported that long-term outcome was better in *ABCB1* rs1128503T and TC allele carriers than patients with C allele (92.7±1.6% vs 78.2±6.6%, p=0.020).

ABCB1 gene is involved in vincristine transport. A candidate gene study by Ceppi et al[66] demonstrated that variants in *ABCB1* were associated with vincristine neurotoxicity during ALL therapy. The two *ABCB1* variations (rs10264856 and rs4728709) were reported to be associated with increased risk of relapse in childhood ALL patients in a large genome-wide

association study.[75] Because these two SNPs are in LD, (but not in LD with SNPs at 3435, 2677, or 1236 positions), Ceppi et al[66] analyzed one of these two SNPs (rs4728709). Although no association with EFS or OS was found, they[66] observed protective effect of rs4728709 against lower grades of neurotoxicity. From these results, Ceppi et al.[66] concluded that rs4728709 of *ABCB1* (or other SNPs in LD) indeed has an impact on ALL treatment outcome, especially on vincristine-related neurotoxicity. Substitution (rs4728709) in the promoter of the *ABCB1* was reported to have a protective effect against lower-grade neurotoxicity, and C to A variation (rs3770102) located 17 nucleotides upstream from transcription start site had a protective effect against high-grade neurotoxicity.[66] In another study by Zqheib et al[76] on 127 Lebanese ALL patients, a statistically significant association was found among neutropenia (absolute neutrophil count <500) and variant allele carriers of *ABCB1* rs1045642 and *ABCB1* rs1128503. According to these authors, genotyping for *ABCB1* polymorphisms might be useful in identifying patients at risk of increased MTX toxicity, which warrants the need for dose optimization before treatment initiation. On the contrary, candidate gene studies by Kishi et al[77] and Guilhaumou et al[78] observed no association of *ABCB1* variants and vincristine neurotoxicity.

Hence, it is probable that polymorphisms in *ABCB1* gene may affect intrinsic blast resistance and pharmacokinetics of several drugs used in ALL protocols, thereby affecting the efficacy of treatment and EFS.

ABCB1 variants and CML treatment

Although targeted therapy with IM demonstrates high efficacy in most CML patients, nearly 35%–40% of CML patients on IM therapy develop resistance to IM. Resistance to IM could be due to a heterogeneous array of mechanisms involving BCR/ABL-dependent pathways and BCR/ABL-independent pathways.[78,79] BCR/ABL-dependent mechanism generally includes point mutations within the BCR/ABL kinase domain that interfere with IM binding and also overexpression or amplification of the BCR/ABL gene. Among BCR/ABL-independent mechanisms, a number of factors may influence the plasma and tissue levels of IM and, under certain circumstances, contribute to pharmacologic resistance. The efficacy and toxicity of IM seem to depend on both IM pharmacokinetics influenced by several enzymes and transporters, and IM pharmacodynamics influenced by mutational studies of the target. Recently, great attention has been focused on interpatient pharmacokinetic variability, which is due to patient's inherent genetic constitution, as a BCR/ABL-independent mechanism mediating resistance to IM.

Drug exposure below the target level could lead to IM levels that are insufficient to inhibit BCR/ABL and to achieve optimal response. Of the varied reasons, aberrant expression of drug transporters also accounts for IM resistance. Polymorphisms in *ABCB1* are likely to influence intracellular drug delivery, and therefore the effectiveness of IM which is a substrate of the P-gp-mediated efflux. Because of the same reason, *ABCB1* SNPs could affect IM's bioavailability and consequently the treatment outcome of IM therapy, which partially explains variable responses to IM.[80,81]

SNPs in *ABCB1* have been demonstrated to display high affinity for IM and confer IM resistance in vitro by extruding IM from hematopoietic cells.[82,83] *ABCB1* polymorphisms were hypothesized to be functional polymorphisms altering mRNA stability, modifying the P-gp expression and therefore reducing IM substrate specificity.

In Indian CML patients, Sailaja et al[84] reported a higher frequency of 3435TT genotype in minor/major cytogenetic response (CyR) group compared to non-CyR group, but the overrepresentation of 3435TT genotype was statistically not significant. Dulucq et al[85] reported that the distribution of 3435C>T genotypes was not significantly associated with MMR (p=0.20) in Caucasian CML patients. Angelini et al[86] also recapitulated that the 3435CC genotype was significantly associated with complete molecular response among Caucasian CML patients. In this sense, the T allele has lower *ABCB1* transcript levels compared to the C allele, and contributes to better IM response. On the contrary, a higher risk for IM resistance was reported for CML patients with homozygous T allele at 3435 locus.[87] Maffioli et al[88] also demonstrated inadequate response or failure to IM treatment associated with 3435TT genotype. Studies conducted on Malaysian CML patients had shown no relationship between 3435C>T polymorphism and response to a standard dose of IM.[89] In a recent study by Salimizand et al,[90] CML patients with C allele of *ABCB1* C3435T had poor cytogenetic response and TT3435 *ABCB1* diplotype was significantly associated with accelerated phase of CML. This study also indicated that CML patients with TT3435 *ABCB1* might be having weaker response to IM therapy.

It has been reported[90] that the *ABCB1* 2677 G>T/A single nucleotide substitution strongly affects the secondary structure of *ABCB1* mRNA. A decrease in P-gp expression could result in higher intracellular concentration of IM. Although Dulucq et al[91] observed a higher frequency of MMR in patients with non-G genotypes at position 2677, they could

not confirm these results in a larger patient cohort later,[85] The *ABCB1* 2677 variant was associated with MMR in Malaysian CML patients.[89] For ABCB1 2677 T/A polymorphism, a better complete cytogenetic response was observed for patients with variant TT/AT/AA genotypes compared to other genotype groups. Almost similar with the findings of Au et al,[89] a better CCyR rate was observed among patients with *ABCB1* 2677 GA/AT/AA genotype in the study by Ni et al.[87] This has been attributed to the fact that carriers of the 2677 variant genotype tend to have lower P-gp mRNA expression than those who had 2677 wild-type genotype[91] Galimberti et al[92] examined the role of *ABCB1* SNPs with IM resistance by conducting a study which comprised of 33 CML patients treated with IM. This study showed that CML patients who did not achieve at least major cytogenetic remission had higher levels of *ABCB1* expression.[92] Elghannam et al[93] investigated the association of G2677T SNP with IM response in Egyptian CML patients. Multivariate analysis showed GT genotype to be an independent risk factor for resistance, while TT genotype was found to be a protective factor against resistance to IM. So, G2677T polymorphism might be useful in response prediction to therapy with IM in CML patients.

In Malaysian CML patients on IM therapy,[89] resistance was significantly higher among patients homozygous for the *ABCB1* 1236CC genotype, compared to patients with good IM response. The result from the study by Au et al[89] is in accordance with the findings of Deenik et al,[94] in which patients with homozygous *ABCB1* 1236TT showed a higher probability of obtaining MMR.

In contrast, in the study by Ni et al[87] on the impact of these SNPs on IM response, the number of T alleles at loci 1236 and 3435 were found to correlate with resistance. Resistance was higher in those CML patients who were homozygous for the 1236T allele, compared to patients with CT/CC genotype groups. With regard to 2677T/A polymorphism, a better complete cytogenetic remission was observed for patients with genotypes AG/AT/AA compared to TT/GT/GG genotypes. In the case of C3435T polymorphism, patients with 3435TT/CT genotypes showed a higher resistance compared with patients with CC genotype. On the contrary, in the study by Maffioli et al,[88] the CC genotype of C3435T was associated with primary failure, whereas T allele of G2677T/A seemed to protect from priamry failure.

In a meta-analysis by Zu et al,[95] a significant association between C1236T polymorphism and increasing risk of IM resistance in Asian CML patients was observed. However, they noted no significant association for G2677T or C3435T polymorphism in Asian populations as well as Caucasian CML populations. Zheng et al[96] conducted a meta-analysis that combined data from 12 reports and included 1,826 patients. This meta-analysis showed that the 2677G allele or 3435T allele predicted a worse response to Imatinib in CML patients, whereas 1236CC genotype was associated with better response in CML patients from the Asian region. These reports suggest the usefulness of these three SNPs of ABCB1 as predictive markers for the therapeutic use of IM in CML patients.

ABCB1 haplotypes and IM resistance

At haplotypic level, 3435C>T is in strong LD with 1236C>T and 2677G>T/A, forming two major haplotypes of 1236C/2677G/3435C and 1236T/2677T/3435T with abundant frequencies. *ABCB1* 1236T/2677T/3435T haplotype was correlated to higher IM pharmacokinetics trough levels in CML patients.[91,97] In haplotype analysis of these three SNPs of *ABCB1* in Malaysian CML patients on IM therapy, Au et al[89] observed that the wild-type *ABCB1* haplotype 1236C/2677G/3435C was associated with IM resistance, which is in agreement with a report by Dulucq et al[91] in Caucasian population. On the other hand, Maffioli et al[88] found a correlation between 1236C/2677G/3435C haplotype and IM resistance. In yet another study,[98] none of the *ABCB1* haplotypes had any major influence on the efficacy of IM in K562 cells. An explanation to these contradictory results could be that these three ethnicity-related SNPs may have different distribution of genotype and haplotype frequencies when examined in different populations.

Ali and Elsalakawy[99] genotyped the three SNPs (C1236T, G2677T and C3435T) in 100 Egyptian CML patients undergoing IM therapy. They found that the optimal response rate did not differ significantly between C1236T, G2677T, or C3435T genotypes. However, optimal response was significantly different among patients with the CGC, TTT, TGC, CGT, TGT, CTC, CTT, and TTC haplotypes. The 1236T/2677G/3435T haplotype was significantly associated with lower probability of achieving optimal response. According to these authors, *ABCB1* SNPs haplotype analysis should also be taken into account in order to get clearer insights into who is likely to respond optimally to IM for identifying CML patients who may not respond optimally to standard dose IM therapy and potentially need an individualized therapeutic approach.

In another recent study, Eadie et al[100] investigated whether early increase in ABCB1 mRNA expression (fold change from diagnosis to day 22 of IM therapy) could predict patient

response. Patients exhibiting a high fold rise were significantly less likely to achieve early molecular response, and major molecular response, even when switched to nilotinib therapy. According to Eadie et al,[100] an increase in levels of *ABCB1* mRNA may serve as easily translatable early warning assay for loss of response/development of resistance to IM and could serve to identify poor responders who may benefit from the addition of ABCB1 inhibitor to their treatment regimen or from switching to alternate therapies. This study highlights the importance of drug efflux transporters and indicates that *ABCB1* mRNA levels may provide a valuable prognostic biomarker.

The most likely explanation for the association of *ABCB1* with IM resistance could be that *ABCB1* acted as the transporter for IM. Overexpression of P-gp at the cell surface reduces intracellular IM concentrations and leads to ineffective levels of the IM upon reaching its target.[101] Thus, increased *ABCB1* levels would lead to reduced IM intracellular levels, impaired BCR-ABL inhibition, and ultimately resistance to IM treatment.

There is paucity of information available regarding the impact of these SNPs on other tyrosine kinase inhibitor drugs used in CML treatment. Dessily et al[102] investigated the impact of expression of *ABCB1* 1236C>T, 2677G>T/A, and 3435C>T polymorphisms on the antiproliferative effects of imatinib, nilotinib, dasatinib, and ponatinib using K562 cell lines. They observed resistance of K 562 $_{C\text{-}G\text{-}C}$ to IM compared with K 562 $_{C\text{-}G\text{-}T,}$ K 562 $_{C\text{-}T\text{-}T}$ at clinically relevant concentrations. They demonstrated that the wild type protein (*ABCB1* $_{C\text{-}G\text{-}C}$) exported IM more efficiently and thus conferred higher resistance to IM compared to the variant protein (*ABCB1* $_{T\text{-}T\text{-}T}$). Consistently, in cells expressing the variant protein, IM intracellular concentrations were also significantly higher than in cells expressing wild-type protein. These results not only suggest that the variant haplotype decreases IM transport by *ABCB1* but also provide an explanation for previous studies that associated the wild-type haplotype (CGC) to IM resistance.[89,91,95,96] In contrast with IM, these polymorphisms did not affect intracellular concentrations of nilotinib and also demonstrated limited influence on the antiproliferative effects of nilotinib, dasatinib, and ponatinib. These results suggest that the *ABCB1* SNPs 1236C>T, 2677G>T/A, and 3435C>T significantly affect the antiproliferative activity and intracellular concentrations of IM, but not, or to a much lesser extent of nilotinib, dasatinib, and ponatinib.

In most of the studies, only the *ABCB1* 1236T>C and *ABCB1* 2677G>T/A genotypes and *ABCB1* 1236C/2677G/3435C haplotype were found to be significantly associated with IM response, whereas the other SNPs did not show any significant association. This could be attributed to several factors. ABC transporters are subjected to drug–drug interactions and to regulation by intracellular receptors, cytokines, and epigenetic factors. It has to be noted that overexpression of ABCB1 is partly mediated by nuclear receptors like the pregnane X receptor. Also, suppression of micro RNAs also have been shown to lead to an upregulation of *ABCB1*.

ABCB1 polymorphisms and CLL treatment

For CLL, there are many current first-line treatment options. The choice of treatment depends on the stage of the disease, the patient's symptoms, the age and overall health of the patient, and the benefits vs side effects of treatment. In a study on CLL patients, Jamroziak et al[103] found highest P-gp activity in the carriers of the 3435CC genotype followed by intermediate activity in 3435CT heterozygous subjects and the lowest activity in the carriers of 3435TT genotype. From the above findings, these authors concluded that genotype-related differences in P-gp activity in B-CLL tumor cells may have implications for response to chemotherapy with P-gp transported anticancer agents.

Dong et al[104] did not find any association of SNPs C1236T, G2677T/A, and C3435T of ABCB1 with clinical prognostic factors in Chinese CLL patients. Penna et al[105] evaluated whether the SNPs G2677T and C3435T provided any prognostic information on the clinical progression of B-CLL. The G2677T SNP was associated with the prognostic patients' characteristics and poor prognosis, whereas C3435T showed no association with CLL prognosis. According to Penna et al,[105] ABCB1 heterozygosis may lead to a different functional capacity of the encoded protein and to a different mRNA expression with respect to homozygous state. Moreover, mutant heterozygous G2677T genotype could be clustered nonrandomly and nonuniformly (LD) with other genes that are able to induce a worse prognosis. These findings support the importance of considering *ABCB1* polymorphisms as prognostic markers in patients with B-CLL in defining a more individualized prognosis and helping to identify patients who are at risk of rapid progression.

The conflicting findings reported in the literature could be attributed to several factors. Difference in demographic data from subjects selected for the various *ABCB1* SNPs, especially difference in genetic background of the study subjects across the populations worldwide, could be an important factor. Likewise, difference in study design and

sample size among the studies and different genotyping methods employed across studies also might be contributing. Moreover, strong LD between the SNPs and different unobserved causal SNPs in different study populations also may provide a plausible explanation for conflicting reports on association of the SNPs studied with IM response. According to Marchetti et al,[106] the methods used to measure P-gp expression in various studies, route of drug administration and extent of metabolism relative to P-gp-mediated transport, environmental factors including difference in dietary constituents among different populations that influence transporter function, involvement of other transporters, and associated genetic variability also contribute to the inconsistency in the reports worldwide. Noninclusion of haplotypes in several studies is another major factor of concern for contradictory reports. Therefore, more in-depth studies and increasing knowledge on function, regulation, and genetic variation of transporters are warranted, which can contribute to a better understanding on divergent results obtained.

Conclusion and future implications

There is accumulating evidence that treatment outcome in leukemias can be influenced by germ line polymorphisms that affect drug disposition and/or pharmacodynamics and that these effects may explain some of the variability in treatment outcome that cannot be explained by the genotype and phenotype variation in leukemic clone. Therefore, genetic variation in *ABCB1* is of tremendous clinical interest in the pharmacokinetics of commonly used antileukemic drugs and in multidrug resistance.

The World Health Organization (WHO) classification[107] has included an increasing amount of new clinically relevant genomic information for the implementation of precision medicine programs. Few polymorphisms and haplotypes of *ABCB1* have been associated with alterations in drug disposition and drug response, including adverse events with various ABCB1 substrates in different ethnic populations. These SNPs account for the interindividual differences in pharmacokinetics and clinical response of selected antileukemic drugs. But the data yielded are not in distinct and unconfined reproducible outcomes and are not yet conclusive enough to translate pharmacogenetic tests to clinical practice. In this context, *ABCB1* transporter polymorphisms are not yet suitable to be used as biomarkers to predict therapeutic response in leukemias and so have not been included in the WHO classification. New biomarkers and pharmacogenetic tests are emerging only, and based on these, novel treatment protocols that are personalized to the genotype needs to be designed.

For a complete understanding of the contribution of genetic variability in *ABCB1* and treatment response and toxicity in leukemias, additional studies involving larger sample sizes and stratification according to haplotype need to be carried out. Because of the known interpopulation differences in drug response, factors such as variability among ethnic groups, characterization of variability in haplotype structure, LD and recombination within and among ethnic populations, etc, should also be considered. It would also be ideal to carefully consider uniformity in demographic data of the subjects selected, sample size, environmental factors, and standardization of assays relating to *ABCB1* mRNA and protein detection and quantification. Hence, future research activities, considering novel genomic methodologies such as deep sequencing approaches (next-generation sequencing),[108] and a comprehensive definition of clinical phenotypes based on a representative and valid sample size calculation,[19,109] to elucidate the impact of rare *ABCB1* variants and their potential consequences for effect sizes are warranted. Individualized approaches based on pharmacogenomics profile of individual patients may offer more efficient and less toxic therapy to leukemias in the future and can lead to personalized approaches to diagnose and treat patients.

Disclosure

The author reports no conflicts of interest in this work.

References

1. National Cancer Institute. *Leukemia*. Bethesda, MD: National Cancer Institute; 2013. Available from: http://www.cancer.gov/cancertopics/types/leukemia.
2. National Comprehensive Cancer Network. *NCCN Clinical Practice Guidelines in Oncology: Chronic Myelogenous Leukemia Version 4*; 2013. Available from: http://www.nccn.org/professionals/physician_gls/pdf/cml.pdf.
3. Hamada A, Sahli Z, El Sabban M, Mouteirik M, Nasr R. Emerging therappeutic strategies for targeting chronic myeloid leukemia stel cell. *Stem Cell Int.* 2013;724360.
4. Baccarani M, Deininger MW, Rosti G, et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia. *Blood.* 2013;122(6):872–884.
5. Hochhaus A, O'brien S, Guilhot F, et al. Six-year follow-up of patients receiving imatinib for the first-line treatment of chronic myeloid leukemia. *Leukemia.* 2009;23(6):1054–1061.
6. Nasr R, Bazarbachi A. Chronic myeloid leukemia:" archetype" of the impact of targeted therapies. *Pathologie-biologie.* 2012;60(4):239–245.
7. Bixby D, Talpaz M. Mechanisms of resistance to tyrosine kinase inhibitors in chronic myeloid leukemia and recent therapeutic strategies to overcome resistance. *Hematology Am Soc Hematol.* 2009:461–476.
8. Pui CH, Cheng C, Leung W, et al. Extended follow-up of long-term survivors of childhood acute lymphoblastic leukemia. *N Engl J Med.* 2003;349(7):640–649.

9. Cheok MH, Lugthart S, Evans WE. Pharmacogenomics of acute leukemia. *Annu Rev Pharmacol Toxicol.* 2006;46:317–353.
10. Evans WE, McLeod HL. Pharmacogenomics – drug disposition, drug targets, and side effects. *N Engl J Med.* 2003;348(6):538–549.
11. Evans WE, Relling MV. Moving towards individualized medicine with pharmacogenomics. *Nature.* 2004;429(6990):464–468
12. Evans WE, Relling M V. Pharmacogenomics: translating functional genomics into rational therapeutics. *Science.* 1999;286(5439):487–491.
13. Relling MV, Dervieux T. Pharmacogenetics and cancer therapy. *Nat Rev Cancer.* 2001;1(2):99–108.
14. Borst P, Elferink RO. Mammalian ABC transporters in health and disease. *Annu Rev Biochem.* 2002;71(1):537–592.
15. Gottesman MM. Mechanisms of cancer drug resistance. *Annu Rev Med.* 2002;53(1):615–627.
16. Ambudkar SV, Kimchi-Sarfaty C, Sauna ZE, Gottesman MM. P-glycoprotein: from genomics to mechanism. *Oncogene.* 2003;22(47):7468–7485.
17. Clay AT, Sharom FJ. Multidrug resistance protein. In: You G, Morris ME, editors. *Drug Transporters: Molecular Characterization and Role in Drug Disposition.* 2nd ed. Hoboken, NJ: John Wiley & Sons; 2014:141–160.
18. Fromm MF. Importance of P-glycoprotein at blood–tissue barriers. *Trends Pharmacol Sci.* 2004;25(8):423–429.
19. Wolking S, Schaeffeler E, Lerche H, Schwab M, Nies AT. Impact of genetic polymorphisms of ABCB1 (MDR1, P-Glycoprotein) on drug disposition and potential clinical implications: update of the literature. *Clin Pharmacokinet.* 2015;54(7):709–735.
20. Bodor M, Kelley EJ, Ho RJ. Characterization of the humanMDR1 gene. *AAPS J.* 2005;7(1):E1–E5.
21. Sharom FJ. The P-glycoprotein multidrug transporter. *Essays Biochem.* 2011;50(1):161–178.
22. Efferth T. The human ATP-binding cassette transporter genes from the bench to the bedside. *Curr Mol Med.* 2001;1(1):45–65.
23. O'Connor R. The pharmacology of cancer resistance. *Anticancer Res.* 2007;27(3A):1267–1272.
24. Leveille-Webster CR, Arias IM. The biology of the P-glycoproteins. *J Membr Biol.* 1995;143(2):89–102.
25. Leschziner GD, Andrew T, Pirmohamed M, Johnson MR. ABCB1 genotype and PGP expression, function and therapeutic drug response: a critical review and recommendations for future research. *Pharmacogenomics J.* 2007;7(3):154–179.
26. Pauli-Magnus C, Kroetz DL. Functional implications of genetic polymorphisms in the multidrug resistance gene MDR1 (ABCB1). *Pharm Res.* 2004;21(6):904–913.
27. Wolf S, Bachtiar M, Wang J, Sim T, Chong S, Lee C. An update on ABCB1 pharmacogenetics: insights from a 3D model into the location and evolutionary conservation of residues corresponding to SNPs associated with drug pharmacokinetics. *Pharmacogenomics J.* 2011;11(5):315–325.
28. Ieiri I, Takane H, Otsubo K. The MDR1 (ABCB1) gene polymorphism and its clinical implications. *Clin Pharmacokinet.* 2004;43(9):553–576.
29. Marzolini C, Paus E, Buclin T, Kim RB. Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. *Clin Pharmacol Ther.* 2004;75(1):13–33.
30. Hodges LM, Markova SM, Chinn LW, et al. Very important pharmacogene summary: ABCB1 (MDR1, P-glycoprotein). *Pharmacogenet Genomics.* 2011;21(3):152.
31. Cascorbi I. Role of pharmacogenetics of ATP-binding cassette transporters in the pharmacokinetics of drugs. *Pharmacol Ther.* 2006;112(2):457–473.
32. Kimchi-Sarfaty C, Marple AH, Shinar S, et al. Ethnicity-related polymorphisms and haplotypes in the human ABCB1 gene. *Pharmacogenomics.* 2007;8(1):29–39.
33. Longo R, D'Andrea M, Sarmiento R, Gasparini G. Pharmacogenetics in breast cancer: focus on hormone therapy, taxanes, trastuzumab and bevacizumab. *Expert Opin Investig Drugs.* 2010;19(Suppl 1):S41–S50.
34. Sissung TM, Gardner ER, Piekarz RL, et al. Impact of ABCB1 allelic variants on QTc interval prolongation. *Clin Cancer Res.* 2011;17(4):937–946.
35. Hoffmeyer S, Burk O, Von Richter O, et al. 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.* 2000;97(7):3473–3478.
36. Hitzl M, Drescher S, van der Kuip H, et al. The C3435T mutation in the human MDR1 gene is associated with altered efflux of the P-glycoprotein substrate rhodamine 123 from CD56+ natural killer cells. *Pharmacogenetics.* 2001;11(4):293–298.
37. Wang D, Johnson AD, Papp AC, Kroetz DL, Sadee W. Multidrug resistance polypeptide 1 (MDR1, ABCB1) variant 3435C> T affects mRNA stability. *Pharmacogenetics.* 2005;15(10):693–704.
38. Ishikawa T, Hirano H, Onishi Y, Sakurai A, Tarui S. Functional evaluation of ABCB1 (P-glycoprotein) polymorphisms: high-speed screening and structure-activity relationship analyses. *Drug Metab Pharmacokinet.* 2004;19(1):1–14.
39. Sakurai A, Onishi Y, Hirano H, et al. Quantitative Structure – activity relationship analysis and molecular dynamics simulation to functionally validate nonsynonymous polymorphisms of human ABC transporter ABCB1 (P-Glycoprotein/MDR1). *Biochemistry.* 2007;46(26):7678–7693.
40. Komoto C, Nakamura T, Sakaeda T, et al. MDR1 haplotype frequencies in Japanese and Caucasian, and in Japanese patients with colorectal cancer and esophageal cancer. *Drug Metab Pharmacokinet.* 2006;21(2):126–132.
41. Dandara C, Lombard Z, Du Plooy I, McLellan T, Norris SA, Ramsay M. Genetic variants in CYP (-1A2, -2C9, -2C19, -3A4 and-3A5), VKORC1 and ABCB1 genes in a black South African population: a window into diversity. *Pharmacogenomics.* 2011;12(12):1663–1670.
42. Wu H, Kang H, Liu Y, et al. Roles of ABCB1 gene polymorphisms and haplotype in susceptibility to breast carcinoma risk and clinical outcomes. *J Cancer Res Clin Oncol.* 2012;138(9):1449–1462.
43. Kim RB, Leake BF, Choo EF, et al. Identification of functionally variant MDR1 alleles among European Americans and African Americans. *Clin Pharmacol Ther.* 2001;70(2):189–199.
44. Kroetz DL, Pauli-Magnus C, Hodges LM, et al. Sequence diversity and haplotype structure in the human ABCB1 (MDR1, multidrug resistance transporter) gene. *Pharmacogenetics.* 2003;13(8):481–494.
45. Ferrara F. Treatment of unfit patients with acute myeloid leukemia: a still open clinical challenge. *Clin Lymphoma Myeloma Leuk.* 2011;11(1):10–16.
46. Smith ML, Hills RK, Grimwade D. Independent prognostic variables in acute myeloid leukaemia. *Blood Rev.* 2011;25(1):39–51.
47. Wuchter C, Leonid K, Ruppert V, et al. Clinical significance of P-glycoprotein expression and function for response to induction chemotherapy, relapse rate and overall survival in acute leukemia. *Haematologica.* 2000;85(7):711–721.
48. Nakamura T, Sakaeda T, Horinouchi M, et al. Effect of the mutation (C3435T) at exon 26 of the MDR1 gene on expression level of MDR1 messenger ribonucleic acid in duodenal enterocytes of healthy Japanese subjects. *Clin Pharmacol Ther.* 2002;71(4):297–303.
49. Leith CP, Kopecky KJ, Chen IM, et al. Frequency and clinical significance of the expression of the multidrug resistance proteins MDR1/P-glycoprotein, MRP1, and LRP in acute myeloid leukemia. A Southwest Oncology Group Study. *Blood.* 1999;94(3):1086–1099.
50. Seedhouse CH, Grundy M, White P, et al. Sequential influences of leukemia-specific and genetic factors on p-glycoprotein expression in blasts from 817 patients entered into the National Cancer Research Network acute myeloid leukemia 14 and 15 trials. *Clin Cancer Res.* 2007;13(23):7059–7066.
51. Scheiner MA, da Cunha Vasconcelos F, da Matta RR, Dal Bello Figueira R Jr, Maia RC. ABCB1 genetic variation and P-glycoprotein expression/activity in a cohort of Brazilian acute myeloid leukemia patients. *J Cancer Res Clin Oncol.* 2012;138(6):959–969.

52. Green H, Falk IJ, Lotfi K, et al. Association of ABCB1 polymorphisms with survival and in vitro cytotoxicty in de novo acute myeloid leukemia with normal karyotype. *Pharmacogenomics J.* 2012;12(2):111–118.
53. Falk JI, Fyrberg A, Paul E, et al. Impact of ABCB1 single nucleotide polymorphisms 1236C> T and 2677G> T on overall survival in FLT3 wild-type de novo AML patients with normal karyotype. *Br J Haematol.* 2014;167(5):671–680.
54. He H, Yin J, Li X, et al. Association of ABCB1 polymorphisms with prognostic outcomes of anthracycline and cytarabine in Chinese patients with acute myeloid leukemia. *Eur J Clin Pharmacol.* 2015;71(3):293–302.
55. Megías-Vericat J, Rojas L, Herrero M, et al. Influence of ABCB1 polymorphisms upon the effectiveness of standard treatment for acute myeloid leukemia: a systematic review and meta-analysis of observational studies. *Pharmacogenomics J.* 2015;15(2):109–118.
56. Gatta G, Botta L, Rossi S, et al. Childhood cancer survival in Europe 1999–2007: results of EUROCARE-5—a population-based study. *Lancet Oncol.* 2014;15(1):35–47.
57. Pui CH, Evans WE. Treatment of acute lymphoblastic leukemia. *N Engl J Med.* 2006;354(2):166–178.
58. Dawidowska M, Kosmalska M, Sędek Ł, et al. Association of germline genetic variants in RFC, IL15 and VDR genes with minimal residual disease in pediatric B-cell precursor ALL. *Sci Rep.* 2016;6:29427.
59. López-López E, Gutiérrez-Camino Á, Piñán MÁ, et al. Pharmacogenetics of microRNAs and microRNAs biogenesis machinery in pediatric acute lymphoblastic leukemia. *PloS One.* 2014;9(3):e91261.
60. Davidsen ML, Dalhoff K, Schmiegelow K. Pharmacogenetics influence treatment efficacy in childhood acute lymphoblastic leukemia. *J Pediatric Hematol Oncol.* 2008;30(11):831–849.
61. Moriyama T, Relling MV, Yang JJ. Inherited genetic variation in childhood acute lymphoblastic leukemia. *Blood.* 2015;125(26):3988–3995.
62. Jamroziak K, Młynarski W, Balcerczak E, et al. Functional C3435T polymorphism of MDR1 gene: an impact on genetic susceptibility and clinical outcome of childhood acute lymphoblastic leukemia. *Eur J Haematol.* 2004;72(5):314–321.
63. Stanulla M, Schäffeler E, Arens S, et al. GSTP1 and MDR1 genotypes and central nervous system relapse in childhood acute lymphoblastic leukemia. *Int J Hematol.* 2005;81(1):39–44.
64. Efferth T, Sauerbrey A, Steinbach D, et al. Analysis of single nucleotide polymorphism C3435T of the multi-drug resistance gene MDR1 in acute lymphoblastic leukemia. *Int Journal Oncol.* 2003;23(2): 509–518.
65. Jamroziak K, Balcerczak E, Cebula B, et al. Multi-drug transporter MDR1 gene polymorphism and prognosis in adult acute lymphoblastic leukemia. *Pharmacol Rep.* 2005;57(6):882–888.
66. Ceppi F, Langlois-Pelletier C, Gagné V, et al. Polymorphisms of the vincristine pathway and response to treatment in children with childhood acute lymphoblastic leukemia. *Pharmacogenomics.* 2014;15(8): 1105–1116.
67. Erdélyi DJ, Kamory E, Zalka A, et al. The role of ABC-transporter gene polymorphisms in chemotherapy induced immunosuppression, retrospective study in childhood acute lymphoblastic lleukemia. *Cellular Immunol.* 2016;244:121–124.
68. Gregers J, Christensen IJ, Dalhoff K, et al. The association of reduced folate carrier 80G> A polymorphism to outcome in childhood acute lymphoblastic leukemia interacts with chromosome 21 copy number. *Blood.* 2010;115(23):4671–4677.
69. Borst L, Wallerek S, Dalhoff K, et al. The impact of CYP3A5* 3 on risk and prognosis in childhood acute lymphoblastic leukemia. *Eur J Haematol.* 2011;86(6):477–483.
70. Borst L, Buchard A, Rosthøj S, et al. Gene dose effects of GSTM1, GSTT1 and GSTP1 polymorphisms on outcome in childhood acute lymphoblastic leukemia. *J Pediatr Hematol Oncol.* 2012;34(1): 38–42.
71. Grabar PB, Rojko S, Logar D, Dolžan V. Genetic determinants of methotrexate treatment in rheumatoid arthritis patients: a study of polymorphisms in the adenosine pathway. *Ann Rheum Dis.* 2010; 69(5):931–932.
72. Kato T, Hamada A, Mori S, Saito H. Genetic polymorphisms in metabolic and cellular transport pathway of methotrexate impact clinical outcome of methotrexate monotherapy in Japanese patients with rheumatoid arthritis. *Drug Metab Pharmacokinet.* 2012;27(2):192–199.
73. Gregers J, Green H, Christensen IJ, et al. Polymorphisms in the ABCB1 gene and effect on outcome and toxicity in childhood acute lymphoblastic leukemia. *Pharmacogenomics J.* 2015;15(4):372–379.
74. Liu S, Gao C, Zhang R, et al. Germline genetic variations in methotrexate candidate genes are associated with pharmacokinetics and outcome in paediatric acute lymphoblastic leukemia in China. *Blood.* 2016;128:1595.
75. Yang JJ, Cheng C, Devidas M, et al. Genome-wide association study identifies germline polymorphisms associated with relapse of childhood acute lymphoblastic leukemia. *Blood.* 2012;120(20):4197–4204.
76. Zgheib NK, Akra-Ismail M, Aridi C, et al. Genetic polymorphisms in candidate genes predict increased toxicity with methotrexate therapy in Lebanese children with acute lymphoblastic leukemia. *Pharmacogenet Genomics.* 2014;24(8):387–396.
77. Kishi S, Cheng C, French D, et al. Ancestry and pharmacogenetics of antileukemic drug toxicity. *Blood.* 2007;109(10):4151–4157
78. Guilhaumou R, Solas C, Bourgarel-Rey V, et al. Impact of plasma and intracellular exposure and CYP3A4, CYP3A5, and ABCB1 genetic polymorphisms on vincristine-induced neurotoxicity. *Cancer Chemother Pharmacol.* 2011;68(6):1633–1638.
79. Jabbour E, Parikh SA, Kantarjian H, Cortes J. Chronic myeloid leukemia: mechanisms of resistance and treatment. *Hematol Oncol Clin North Am.* 2011;25(5):981–995.
80. Gardner ER, Burger H, Schaik RH, et al. Association of enzyme and transporter genotypes with the pharmacokinetics of imatinib. *Clin Pharmacol Ther.* 2006;80(2):192–201.
81. Gurney H, Wong M, Balleine R, et al. Imatinib disposition and ABCB1 (MDR1, P-Glycoprotein) genotype. *Clin Pharmacol Ther.* 2007;82(1):33–40.
82. Mahon FX, Belloc F, Lagarde V, et al. MDR1 gene overexpression confers resistance to imatinib mesylate in leukemia cell line models. *Blood.* 2003;101(6):2368–2373.
83. Burger H, van Tol H, Boersma AW, et al. Imatinib mesylate (STI571) is a substrate for the breast cancer resistance protein (BCRP)/ABCG2 drug pump. *Blood.* 2004;104(9):2940–2942.
84. Sailaja K, Surekha D, Nageswara Rao D, Raghunadha Rao D, Vishnupriya S. ABCB1 (MDR1, P-glycoprotein) C3435T gene polymorphism and its possible association with chronic myeloid leukemia prognosis. *Curr Trends Biotechnol Pharm.* 2008;2:514–522.
85. Dulucq S, Preudhomme C, Guilhot F, Mahon FX. Response: is there really a relationship between Multidrug Resistance Gene (MDR1) polymorphisms and major molecular response to imatinib in chronic myeloid leukemia? *Blood.* 2010;116(26):6145–6146.
86. Angelini S, Soverini S, Ravegnini G, et al. Association between imatinib transporters and metabolizing enzymes genotype and response in newly diagnosed chronic myeloid leukemia patients receiving imatinib therapy. *Haematologica.* 2013;98(2):193–200.
87. Ni LN, Li JY, Miao KR, et al. Multidrug resistance gene (MDR1) polymorphisms correlate with imatinib response in chronic myeloid leukemia. *Medical Oncol.* 2011;28(1):265–269.
88. Maffioli M, Camós M, Gaya A, et al. Correlation between genetic polymorphisms of the hOCT1 and MDR1 genes and the response to imatinib in patients newly diagnosed with chronic-phase chronic myeloid leukemia. *Leuk Res.* 2011;35(8):1014–1019.
89. Au A, Baba AA, Goh AS, et al. Association of genotypes and haplotypes of multi-drug transporter genes ABCB1 and ABCG2 with clinical response to imatinib mesylate in chronic myeloid leukemia patients. *Biomed Pharmacother.* 2014;68(3):343–349.
90. Salimizand H, Amini S, Abdi M, Ghaderi B, Azadi NA. Concurrent effects of ABCB1 C3435T, ABCG2 C421A, and XRCC1 Arg194Trp genetic polymorphisms with risk of cancer, clinical output, and response to treatment with imatinib mesylate in patients with chronic myeloid leukemia. *Tumor Biology.* 2016;37(1):791–798.

91. Dulucq S, Bouchet S, Turcq B, et al. Multidrug resistance gene (MDR1) polymorphisms are associated with major molecular responses to standard-dose imatinib in chronic myeloid leukemia. *Blood.* 2008;112(5):2024–2027.
92. Galimberti S, Cervetti G, Guerrini F, et al. Quantitative molecular monitoring of BCR-ABL and MDR1 transcripts in patients with chronic myeloid leukemia during Imatinib treatment. *Cancer Genet Cytogenet.* 2005;162(1):57–62.
93. Elghannam DM, Ibrahim L, Ebrahim MA, Azmy E, Hakem H. Association of MDR1 gene polymorphism (G2677T) with imatinib response in Egyptian chronic myeloid leukemia patients. *Hematology.* 2014; 19(3):123–128.
94. Deenik W, van der Holt B, Janssen JJ, et al. Polymorphisms in the multidrug resistance gene MDR1 (ABCB1) predict for molecular resistance in patients with newly diagnosed chronic myeloid leukemia receiving high-dose imatinib. *Blood.* 2010;116(26):6144–6145.
95. Zu B, Li Y, Wang X, He D, Huang Z, Feng W. MDR1 gene polymorphisms and imatinib response in chronic myeloid leukemia: a meta-analysis. *Pharmacogenomics.* 2014; 15(5):667–677.
96. Zheng Q, Wu H, Yu Q, et al. ABCB1 polymorphisms predict imatinib response in chronic myeloid leukemia patients: a systematic review and meta-analysis. *Pharmacogenomics J.* 2015;15(2):127–134.
97. Singh O, Chan JY, Lin K, Heng CC, Chowbay B. SLC22A1-ABCB1 haplotype profiles predict imatinib pharmacokinetics in asian patients with chronic myeloid leukemia. *PloS One.* 2012;7(12):e51771.
98. Skoglund K, Moreno SB, Jönsson JI, Gréen H. Functional characterization of ABCG2 polymorphisms and their influence on tyrosine kinase inhibitor effects in chronic myeloid leukemia cells. *Blood.* 2011;118(21):3495–3495.
99. Ali MA, Elsalakawy WA. ABCB1 haplotypes but not individual SNPs predict for optimal response/failure in Egyptian patients with chronic-phase chronic myeloid leukemia receiving imatinib mesylate. *Med Oncol.* 2014;31(11):1–10.
100. Eadie LN, Dang P, Saunders VA, et al. The clinical significance of ABCB1 overexpression in predicting outcome of CML patients undergoing first-line imatinib treatment. *Leukemia.* 2017;31(1):1–8.
101. Esposito N. New Bcr-Abl-independent mechanisms of resistance to imatinib treatment in chronic myelogenous leukaemia patients, Università degli Studi di Napoli Federico II; 2008.
102. Dessilly G, Panin N, Elens L, Haufroid V, Demoulin JB. Impact of ABCB1 1236C> T-2677G> T-3435C> T polymorphisms on the anti-proliferative activity of imatinib, nilotinib, dasatinib and ponatinib. *Sci Rep*. 2016;6:29559.
103. Jamroziak K, Balcerczak E, Smolewski P, et al. MDR1 (ABCB1) gene polymorphism C3435T is associated with P-glycoprotein activity in B-cell chronic lymphocytic leukemia. *Pharmacol Rep.* 2006;58(5): 720.
104. Dong HJ, Miao KR, Qiao C, et al. Polymorphisms and haplotypes in multidrug resistance 1 gene are not associated with chronic lymphocytic leukemia susceptibility and prognostic parameters of chronic lymphocytic leukemia in Chinese population. *Leuk Lymphoma*. 2011;52(6):1003–1009.
105. Penna G, Allegra A, Alonci A, et al. MDR-1 polymorphisms (G2677T and C3435T) in B-chronic lymphocytic leukemia: an imppact on susceptibility and prognosis. *Med Oncol*. 2011;28:1549–1554.
106. Marchetti S, Mazzanti R, Beijnen JH, Schellens JH. Concise review: clinical relevance of drug–drug and herb–drug interactions mediated by the ABC transporter ABCB1 (MDR1, P-glycoprotein). Oncologist. 2007;12(8):927–941.
107. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood.* 2016;127(20):2391–2405.
108. Meyer UA, Zanger UM, Schwab M. Omics and drug response. *Ann Rev Pharmacol*. 2013;53:475–502.
109. Zakim D, Schwab M. Data collection as a barrier to personalized medicine. *Trends Pharmacol Sci*. 2015;36:68–71.