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ORIGINAL RESEARCH

CYP3A5 Polymorphism in Circulating Tumor Cells Confers an Increased Disease-Free Survival in DLBCL Patients Treated with R-CHOP

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Purpose: Diffuse Large B-Cell Lymphoma (DLBCL) is a heterogeneous aggressive lymphoid neoplasm. Cases of refractoriness and relapse persist in approximately 40% of patients treated with first-line R-CHOP regimen, thus, the identification of factors associated with disease progression have become a necessity. Diverse polymorphisms in genes encoding proteins involved in the metabolism and elimination of chemotherapeutic drugs have been studied as potential causes of treatment failure. In oncology, liquid biopsies have emerged as a non-invasive method for detecting circulating biomarkers, thereby strengthening both diagnosis and prognosis for patients. Therefore, the purpose of this study was to determine polymorphisms in Circulating Tumor Cells (CTCs) to describe the relevance of liquid biopsy in the clinical outcomes of patients with DLBCL.

Patients and Methods: We analyzed 102 liquid biopsies of peripheral blood from DLBCL patients, of which CTCs were isolated by density gradient and CD20 immunomagnetic antibodies. Allelic discrimination assays were performed to analyze *ABCB1 C3435T, ABCG2 C421A* and *CYP3A5 A6986G* polymorphisms. Overall survival (OS) and disease-free survival (DFS) analysis were performed using Kaplan-Meier curves and risk analysis was performed using Cox regression.

Results: We found that GG genotype of *CYP3A5 A6986G* was associated with a longer DFS (68.6% vs 49%, p=0.019) and lower risk of course with adverse event related to disease (progression, relapse and death) (OR 0.374, CI 0.187–0.745, p=0.011). No significant associations were found between *ABCB1 C3435T* and *ABCG2 C421A* genotype with the clinical outcome.

Conclusion: In this study, we demonstrated that in CTCs derived from liquid biopsies, the GG genotype in the *CYP3A5 A6986G*, which is related to the metabolism and elimination of chemotherapy drugs, impacts in longer DFS. These findings confirm the relevance of circulating biomarkers in non-invasive biological samples for strengthening the prognosis of DLBCL.

Keywords: polymorphism, lymphoma, chemoresistance, CTCs, R-CHOP

Introduction

Diffuse Large B-Cell Lymphoma (DLBCL) is the most common aggressive type of Non-Hodgkin Lymphoma (NHL) in Mexico and worldwide.[1–3] The presence of Circulating Tumor Cells (CTCs) in peripheral blood, which derive from primary tumors, has been demonstrated to cause dissemination and spread of the disease to other organs and tissues. Our research group has identified and molecularly characterized CTCs in liquid biopsies from DLBCL patients.[4] This methodology allows a faster and less invasive study of molecular alterations.

The current standard treatment for DLBCL continues to be the R-CHOP regimen (Rituximab-Cyclophosphamide, Hydroxydaunorubicin (Doxorubicin), Oncovin (Vincristine), and Prednisone), achieving response rates ranging from

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50% to 75%.[5–9] Several molecular alterations can cause treatment failure, such as the presence of certain SNPs (Single Nucleotide Polymorphisms) in genes involved in drug metabolism, for example those of the cytochrome P450 and drug resistance genes (ABC/MDR-1).[10,11]

Cytochrome P450 encoding genes include 18 families, divided into 41 subfamilies, encompassing 57 genes.[12] Members of the cytochrome P450 3A (*CYP3A*) family include *CYP3A4, CYP3A5, CYP3A7*, and *CYP3A43* genes, which are involved in elimination and metabolism of drugs, xenobiotic compounds, and endogenous molecules.[13,14] For the *CYP3A5* subfamily, 34 SNPs have been identified, one of the most studied being the A to G transition (*A6986G*) within intron 3. This SNP alters the correct splicing of mRNA transcription, leading to low levels of protein expression and, consequently, decreased enzymatic activity.[15,16]

On the other hand, ATP-binding cassette (ABC) drug resistance genes are part of seven subfamilies ranging from A to G, with 49 genes identified.[17] The B Subfamily consists of 11 genes, which confer resistance to multiple drugs in cancer.[18] One of the most studied is the *ABCB1* gene, which consists of 28 introns and 28 exons that encode a membrane transporter protein called P-glycoprotein (P-gp).[19] More than 50 SNPs of the *ABCB1* gene have been identified, being *C3435T* the most studied and addressed by our group. This variant is associated with decreased mRNA expression and protein stability. It has been described that this SNP is related to multidrug resistance in cancer and various pathologies such as epilepsy.[20–22] *ABCG2* is another multidrug transporter gene that affects pharmacokinetics and contributes to the resistance of various types of cancers. Originally known as the Breast Cancer Resistance Protein (*BCRP*), it is located on chromosome 4q22 and encodes a 72 kDa membrane protein.[23,24] One of the most studied SNP in this gene is located in exon 5 (*C421A*), causing a substitution of glutamine by lysine, which leads to a decrease in the expression and activity of the protein. It has been demonstrated that CYP3A enzymes and the ABCB1 drug transporter share the same substrates.[14,25]

Despite the development of therapeutic strategies with new molecules, cases of progression and relapse persist. Therefore, the aim of this study was to assess the clinical implications of these SNPs involved in drug metabolism in DLBCL patients. Identifying them at the beginning of treatment could allow their use as potential biomarkers to introduce these SNPs as new prognosis factors that predict the individualized behavior of the chemotherapy response.

Materials and Methods

Study Population

We included 102 liquid biopsies from patients with DLBCL at diagnosis, from the Hospital General de México "Dr. Eduardo Liceaga", from January 2018 to December 2022. The included patients had a complete medical record and signed informed consent. The mean age at diagnosis of the study cohort was 53 years (range 18 to 82), with a predominance of females with 53 patients (52%). Among other relevant clinical parameters, the ECOG (Eastern Cooperative Oncology Group) performance status of 69 patients (67.6%) was between 0 and 1, 40 patients (39.2%) had Lactate Dehydrogenase (LDH) levels above the reference limit (271 U/L), 65 patients (63.7%) were diagnosed in advanced stages of the disease (III and IV), of which 39 patients (38.2%) had extranodal sites. Based on these parameters, 64 patients (62.7%) had an IPI score (International Prognostic Index) between 0 and 2 (Low, Low Intermediate) and 38 (37.3%) had a score between 3 and 5 (Intermediate High - High). Furthermore, according to the Hans algorithm (CD10, BCL6, and MUM1), the predominant cell of origin was non-Germinal Center B-cell (non-GCB) with 53 patients (52%), while 49 (48%) corresponded to Germinal Center B-cell (GCB) (Table 1).

Treatment Response

The first-line treatment regimen used was R-CHOP (Rituximab 375 mg/kg, Cyclophosphamide 750 mg/kg, Doxorubicin 50 mg/kg, Vincristine 1.4 mg/kg, and Prednisone 1 mg/kg) administered in 6 cycles, every 21 days.

During a median follow-up time of 855 days, a total of 60 (58.8%) patients completed their first-line treatment regimen with a complete response (CR), maintaining it throughout this study. On the other hand, 42 (41.2%) patients had an unfavorable clinical outcome, with 17 deaths (16.7%), of which 1 was due to relapse and 6 due to treatment refractoriness, and the rest (n=10) died before completing the treatment. Additionally, there were 7 (6.9%) relapse cases and 18 (17.6%) refractory cases, resulting in overall survival (OS, the percentage of patients that remained alive from DLBCL diagnosis to

Table 1 Clinicopathological Characteristics of DLBCL Patients (n=102)

Parameters	Cases (%)	
Age (years)	Mean (Range)	53 (18–82)
	< 60	66 (65)
	≥60	36 (35)
Gender	Male	49 (48)
	Female	53 (52)
Leucocytes (x10^3 / µL)	Mean (Range)	7.0 (0.5–32.0)
	Low	30 (29.4)
	Normal	58 (56.9)
	High	14 (13.7)
Hb (g/dL)	Mean (Range)	11.8 (4.9–18)
	Anemia	109 (79)
	> 12 g/dL	29 (21)
Platelets (x10^3 / µL)	Mean (Range)	309 (24–919)
	<150	18 (17.6)
	Normal	76 (74.5)
	≥ 450	8 (7.8)
Lactate dehydrogenase (U/L)	Mean (Range)	350 (63–3196)
	<271	62 (60.8)
	≥271	40 (39.2)
Cell of origin	GCB	49 (48)
	Non-GCB	53 (52)
ECOG	0–1	69 (67.6)
	2–4	33 (32.4)
Extranodal sites	No	63 (61.8)
	Yes	39 (38.2)
Clinical stage	I–II	37 (36.3)
	III–IV	65 (63.7)
IPI Score	0–2	64 (62.7)
	3–5	38 (37.3)
R-IPI Score	Very Good	12 (11.8)
	Good	52 (51)
	Poor	38 (37.2)

Abbreviations: ECOG, Eastern Cooperative Oncology Group Performance Status; Hb, Hemoglobin; GCB, Germinal Center B-cell; non-GCB, non-Germinal Center B-cell; IPI, International Prognostic Index; R-IPI, Revised International Prognostic Index.

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death or surveillance length) rate of 83.3% (85/102) and disease-free survival (DFS, the percentage of patients that remained free of disease complications, death, relapse progression through the time of study duration) rate of 58.8% (60/102).

CTCs Enrichment

From each patient, an 8 mL liquid biopsy of peripheral blood was obtained before the administration of the first R-CHOP cycle. The total blood volume was mixed with PBS 1X and Lymphoprep (Axis-Shield, Oslo, Norway), according to the manufacturer's recommendations for isolating mononuclear cells and CTCs by density gradient. Subsequently, B cells from the liquid biopsy were isolated by positive selection using anti-CD20 microbeads (marker present in DLBCL cells), (Miltenyi Biotec, Bergisch Gladbach, Germany), according to the manufacturer's recommendations.

DNA Extraction and Purification

DNA was extracted using DNAzol reagent according to the manufacturer's recommendations (Invitrogen, Life Technologies Carlsbad, CA). Subsequently, DNA purification was performed using the QIAamp DNA Mini Kit (Qiagen, Maryland, US). The genetic material was stored at −20°C until used. The concentration and purity of the DNA were evaluated with a Nanodrop 2000 (ThermoScientific, Wilmington, DE, US).

Detection of C3435T, C421A and A6986G SNPs on CTCs

To detect the polymorphisms rs1045642 (*C3435T*), rs2231142 (*C421A*), and rs776746 (*A6986G*) of the genes *ABCB1, ABCG2*, and *CYP3A5*, respectively, we used SNP Genotyping Assay *ABCB1* (C_7586657_20), *ABCG2* (C_15854163_70), and *CYP3A5* (C_26201809_30) (ThermoScientific, Wilmington, DE, US), TaqMan Genotyping Master Mix, and 10 ng of genomic DNA, in the Step One™ Applied Biosystems instrument.

Statistical Analysis

Pearson correlation analyses were performed to correlate clinical-pathological parameters (co-variables), treatment response/clinical outcome (dependent variables), and the genotypes of the analyzed genes (independent variables). For OS and DFS analysis, the Kaplan-Meier and Log Rank methods were used. Odds Ratio (OR) risk analysis was performed by Cox regression. A p-value less than 0.05 was considered statistically significant. All statistical analysis was performed using SPSS version 25 software (IBM, Armonk, NY, US).

The sample size (N=102) was calculated a priori using G*power 3.1.9.7 software, based on an effect size of 0.8, α value of 0.05, and 95% confidence level.

Ethical Considerations

This trial is a minimum risk investigation and was performed based on the Declaration of Helsinki. For its realization, it was approved by the Ethics and Research Committees of the Hospital General de México "Dr. Eduardo Liceaga" with registration numbers DI/19/103/03/00 and DI/16/103/03/035.

Results

Characteristics of the Cohort Study

The frequency of polymorphisms in DLBCL patients is shown in Table 2.

For the evaluation of clinically relevant prognostic variables, patients were divided into two groups based on clinical response: the first group of patients with a maintained complete response and the second group of patients with progression, relapse, or death.

For the first group (maintained response), there were 60 patients, with 24.5% (25/102) over 60 years old, 30.4% (31/102) were female and 28.4% (29/102) were male, 15.7% (16/102) had an ECOG score higher than 2, and 18.6% (19/102) had involved extranodal sites, 31.4% (32/102) were in stages III and IV vs 27.5% (28/102) in stages I and II, with an IPI score of 3–5 in 19.6% (20/102). From this group, 33.3% (34/102) of patients were classified as non-GCB.

Table 2 ABCB1, ABCG2 and CYP3A5 Genotype Frequencies in DLBCL Patients

SNP	Genotype	N (%)
ABCB1 C3435T	CC	19 (18.63)
	CT	62 (60.78)
	TT	21 (20.59)
ABCG2 C421A	CC	37 (36.27)
	CA	53 (51.96)
	AA	12 (11.76)
CYP3A5 A6986G	AA	12 (11.76)
	AG	39 (38.24)
	GG	51 (50)

The second group consisted of 42 patients with progression, relapse, or death. Of these, 10.8% (11/102) were over 60 years old, 21.6% (22/102) were female and 19.6% (20/102) were male, 18.6% (19/102) had elevated LDH values, 16.7% (17/102) had an ECOG score of 2 or higher, 19.6% (20/102) had extranodal sites, 17.6% (18/102) had an IPI score of 3–5. From this group, 18.6% (19/102) of patients corresponded to non-GCB cases. There were no significant differences between the two groups except for the clinical stage (p=0.009).

The frequency of the *C3435T, C421A*, and *A6988G* genotypes of the *ABCB1, ABCG2*, and *CPY3A5* genes showed that in the group of patients with progression, relapse, and death, the CC/CT genotype was 31.4% (32/102) and the TT genotype was 9.8% (10/102) for the *ABCB1 C3435T*; the CA/CC genotype was 35.3% (36/102) and AA was 5.9% (6/102) for the *ABCG2 C421A*; and the AG/AA genotype was 25.5% (26/102) and GG was 15.7% (16/102) for the *CYP3A5 A6986G*.

In the group of patients with complete response, the CC/CT genotypes of the *ABCB1 C3435T* were 48% (49/102) and the TT genotype was 10.8% (11/102); the CA/CC genotypes of the *ABCG2 C421A* of the gene were 52.9% (54/102) and the AA genotype was 5.9% (6/102); and finally, for the *CYP3A5 A6986G*, the AG/AA genotypes were 24.5% (25/102) and the GG genotype was 34.3% (35/102), obtaining a statistically significant result (p=0.044) (Table 3).

Polymorphisms and Overall/Disease-Free Survival

In an average follow-up time of 855 days, the OS was 83.33% and the DFS was 58.82% (Figure 1). Comparing the polymorphisms with OS, no significant results were found with any of them.

For DFS, the GG genotype of *CYP3A5 A6986G* showed longer survival of 68.6% with 1534 days (CI 1286–1782) vs the AG/AA genotypes, with 49% (1038 days, CI 812–1263) Log Rank p=0.019 (Figure 2A). No significant results were found with the polymorphisms of the *ABCB1* and *ABCG2* genes (Figure 2B and C).

Subsequently, DFS in patients with the GG genotype of *CYP3A5 A6986G* was evaluated by stratifying according to clinical parameters, finding a relationship with good prognostic variables: age equal to or less than 60 years (p=0.001, GG 68.6% vs AG/AA 35.5%); and normal LDH levels (N=62) (p=0.027, GG 74.2% vs AG/AA 51.6%). In the lower IPI scores (Low, Low Intermediate) (N=64), lower DFS was found in patients with the AG/AA genotype (835 days, CI 617–1054) vs those with the GG genotype (1483 days, CI 1224–1742) Log Rank p=0.018 (73.5% vs 50%) (Figure 3A).

On the other hand, the impact of genotypes was analyzed based on the sub-classification of the cell of origin (Han's algorithm), where GCB cases (N=49) presented lower DFS in patients with the AG/AA genotype (882 days, CI 599–1165), compared to those with the GG genotype (1470 days. CI 1118–1822) Log Rank p=0.040 (65.4% vs 39.1%) (Figure 3B).

The analysis was performed in the high-risk IPI group (intermediate-high, high) and in the non-GCB cell subtype. The results did not show statistical significance.

Table 3 Correlation Between Clinical Parameters-Polymorphisms and Clinical Outcome in DLBCL Patients (n=102)

Parameters		Clinical Outcome		p value
		N=102		
		Maintained Response	Progression, Relapse or Death	
		N=60	N=42	
Age	≤60 years	35 (34.3)	31 (30.4)	0.107
	>60 years	25 (24.5)	11 (10.8)	
Gender	Male	29 (28.4)	20 (19.6)	0.943
	Female	31 (30.4)	22 (21.6)	
LDH	Normal	39 (38.2)	23 (22.5)	0.297
	High	21 (20.6)	19 (18.6)	
ECOG	0–1	44 (43.1)	25 (24.5)	0.142
	≥ 2	16 (15.7)	17 (16.7)	
Extranodal sites	No	41 (40.2)	22 (21.6)	0.103
	Yes	19 (18.6)	20 (19.6)	
Clinical stage	I–II	28 (27.5)	9 (8.8)	0.009*
	III–IV	32 (31.4)	33 (32.4)	
IPI score	0–2	40 (39.2)	24 (23.5)	0.328
	3–5	20 (19.6)	18 (17.6)	
Cell of origin	GCB	26 (25.5)	23 (22.5)	0.256
	Non-GCB	34 (33.3)	19 (18.6)	
B Symptoms	No	20 (19.6)	11 (10.8)	0.440
	Yes	40 (39.2)	31 (30.4)	
ABCB1 C3435T	CC / CT	49 (48.0)	32 (31.4)	0.501
	TT	11 (10.8)	10 (9.8)	
ABCG2 C421A	CA / CC	54 (52.9)	36 (35.3)	0.509
	AA	6 (5.9)	6 (5.9)	
CYP3A5 A6986G	AG / AA	25 (24.5)	26 (25.5)	0.044*
	GG	35 (34.3)	16→ 15.7)	

Note: *Significance level $p < 0.05$.
Abbreviations: LDH, Lactate Dehydrogenase; ECOG, Eastern Cooperative Oncology Group Performance Status; Hb, Hemoglobin; GCB, Germinal Center B-cell; non-GCB, non-Germinal Center B-cell; IPI, International Prognostic Index.

A Cox regression was performed to analyze risk of presenting and event related to the disease (progression, relapse, death), thus confirming that the GG genotype of *CYP3A5 A6986G* has been associated to favorable outcomes in terms of maintained complete response after R-CHOP therapy (OR 0.470, CI 0.244–0.903 p=0.023). (Figure 4).

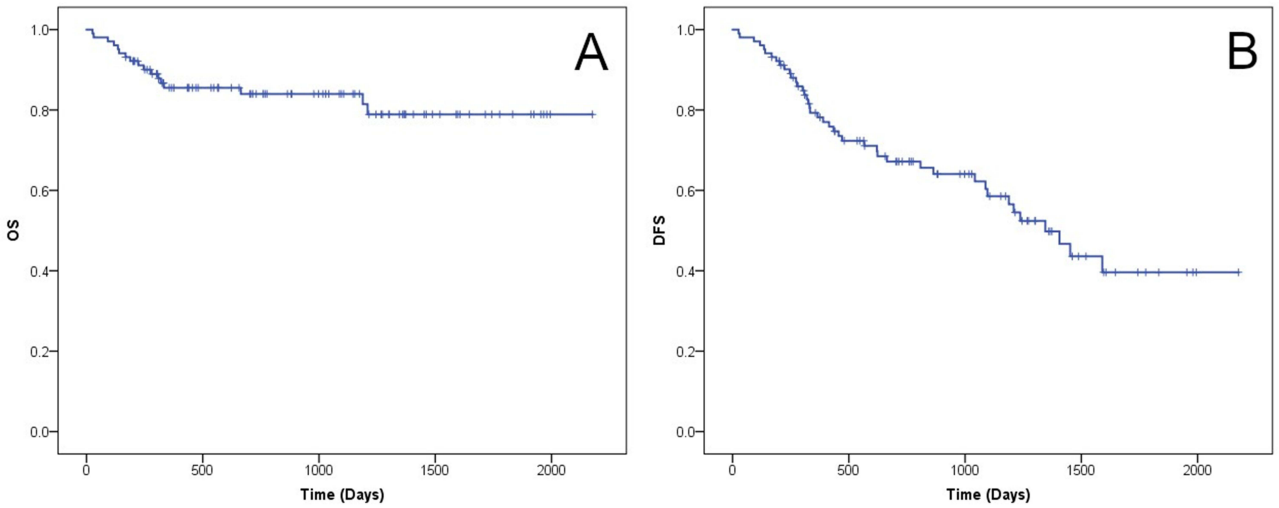

Figure 1 Overall Survival (OS) and Disease-Free Survival (DFS) of DLBCL patients (N=102). (**A**) General OS (83.3%). (**B**) General DFS (58.8%).

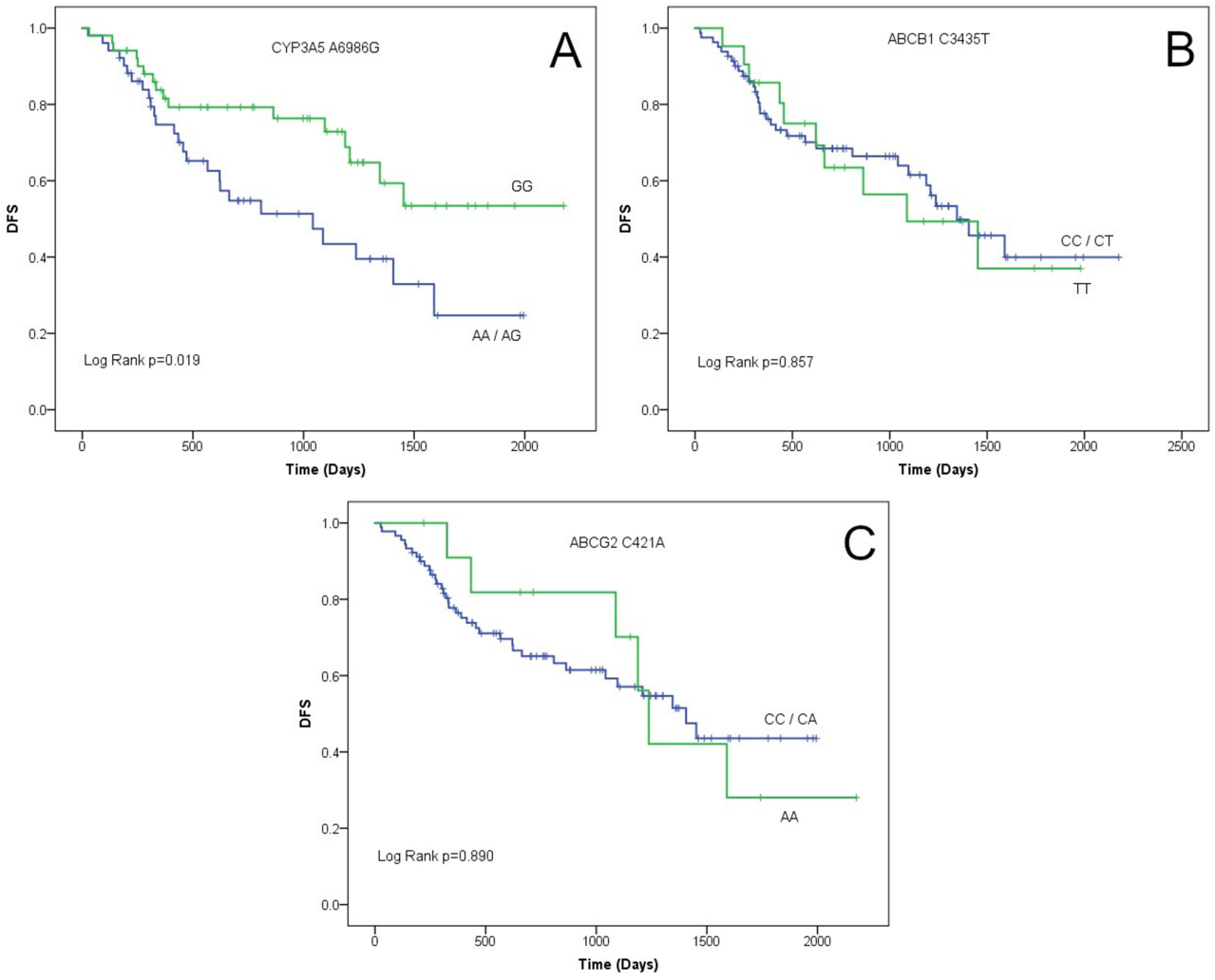

Figure 2 DFS of DLBCL patients (N=102) according to genotype of polymorphisms. (**A**) *CYP3A5 A6986G*. (**B**) *ABCB1 C3435T*. (**C**) *ABCG2 C421A*.

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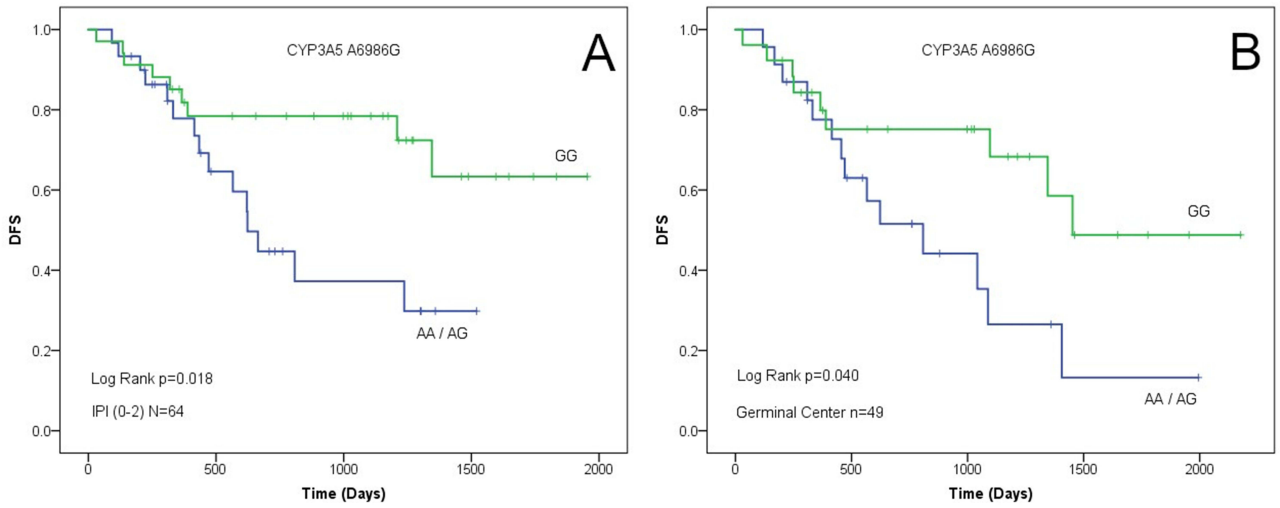

Figure 3 DFS of DLBCL patients according to genotype of *CYP3A5 A6986G* in subgroups. (**A**) IPI score 0–2 (N=64). (**B**) Cell of origin Germinal Center B-cell (N=49).

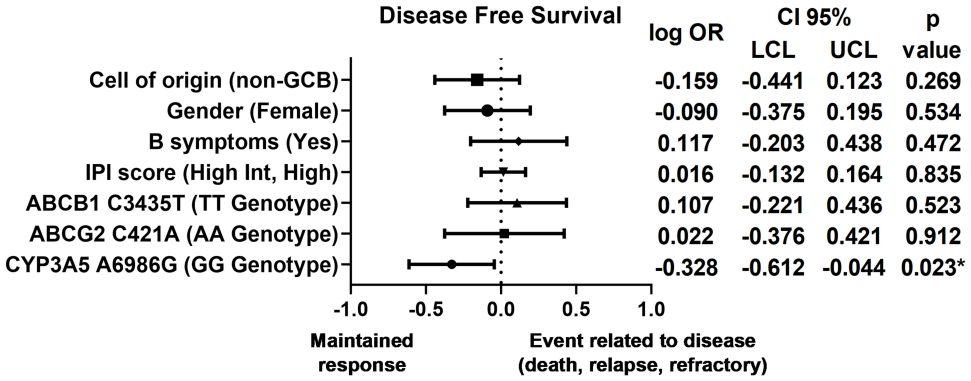

Figure 4 Cox regression analysis between clinicopathological parameters/genotypes and DFS of DLBCL patients (N=102). OR Odds Ratio, LCL Lower Confidence Limit, UCL Upper Confidence Limit. *Significance level $p < 0.05$.

Discussion

Globally, patients with DLBCL treated with R-CHOP usually have a favorable response rate, exceeding 83%; however, 17% exhibit unfavorable clinical behavior.[8] In our study, the OS was 83.3%, similar to the 87% reported by Tilly et al in 2022, for patients treated with R-CHOP in a European population. We observed a DFS of 58.8% over a median follow-up time of 855 days, while in Tilly's study, it was similar (70.2%) over a follow-up of 700 days.[8]

Multiple factors can lead to unfavorable clinical behavior; the most studied by our workgroup are the overexpression of drug resistance genes and the presence of SNPs in genes of the ABC family, as well as drug-metabolizing genes like CYP450.[20,26]

In our study population, the most frequent genotype in *ABCB1 C3435T* was CT, followed by TT and CC (60.78%, 20.59%, and 18.63%, respectively). These results contrast with those reported in Chinese patients with the same pathology, where the most frequent genotype is CT, followed by CC, and the least frequent is TT.[27–29] There are no studies on this polymorphism in DLBCL in which the genotype frequency matches our findings. However, reports in other hematologic neoplasms show similar frequencies, such as Mhaidat et al study in 2011 on Jordanian Hodgkin's lymphoma (HL) patients.[30] In the Mexican onco-hematologic population, Leal-Ugarte et al in 2008 and Olarte et al in 2021, reported similar results to ours: a higher frequency of the CT genotype, followed by TT and, less frequently, CC in pediatric patients with acute lymphoblastic leukemia (ALL) and in adults with acute myeloid leukemia (AML), respectively.[20,31]

For *ABCG2 C421A* SNP, our cohort showed a higher frequency of the CA genotype, followed by CC and AA (51.6%, 36.27%, and 11.76%, respectively). These results are consistent with those reported by Hu et al in 2007 and Kim et al in 2008 in Chinese and Korean DLBCL patients, respectively.[32,33]

Regarding the *CYP3A5 A6986G* polymorphism, our patients showed a higher frequency of the GG genotype, followed by AG, with AA being the least frequent. However, no other reports have addressed this polymorphism in DLBCL patients. In other hematologic malignancies, such as Chronic Myeloid Leukemia (CML), Harivenkatesha et al in 2017 reported a frequency similar to ours.[34] In Mexican population without cancer (including conditions like alopecia,[35] hypertension,[36] respiratory diseases,[37] and mental disorders[38]), various studies have shown similar genotype frequencies for this polymorphism.

Differences in SNP frequencies may be attributed to the geographical location of the studied populations, as well as the methodologies used for detection, such as PCR-ACP, PCR-RFLP, Sanger sequencing, or genotyping by qPCR.

The clinical significance of SNPs in cancer is variable. Several studies have linked genetic variants to a worse prognosis, associated with reduced OS and DFS.[30,39]

In hematologic neoplasms, it has been reported that the *ABCB1 C3435T* SNP in pediatric ALL, multiple myeloma, and lymphoma, the CC genotype is associated with lower OS.[29,40,41] However, in AML patients and breast cancer, the opposite has been reported, the CC genotype was associated with higher OS.[20,42] In lymphoma, our results are consistent with those reported by Hu et al in 2013 and Liu et al in 2020, where no significant association was found between OS and the *ABCB1 C3435T* SNP.[27,29] The analysis showed no significant association between DFS and this SNP. In contrast, Ni et al 2016, reported that the CC genotype was associated with longer DFS in DLBCL.[28] In breast cancer, Li et al in 2017, found that the TT genotype was associated with longer DFS.[43]

For the *ABCG2 C421A* SNP, our results showed no significant association with OS or DFS, which is consistent with reports by Hu et al (2007), Kim et al (2008), and Liu et al (2017) in DLBCL.[32,33,44] In contrast, Perrone et al in 2024 found association between the presence of the AA genotype and shorter DFS and OS in DLBCL.[45] In other cancers, such as prostate and breast cancer, the CC genotype has been associated with better OS and DFS rates, respectively.[39,43] In contrast, Wu et al in 2015, reported higher survival rates in breast cancer patients with the AA genotype.[23] In hematological malignancies such as CML, no association has been found between the genotype and response to imatinib, a substrate of ABCG2.[46,47]

Analysis of the *ABCB1 C3435T* and *ABCG2 C421A* SNPs did not show any impact on OS, DFS, or clinical characteristics. However, other gene families, such as the cytochrome family and their SNPs, are known to deregulate enzymatic activity, which impacts the metabolism of certain drugs, potentially leading to unfavorable clinical outcomes.[48] Several studies have shown that the *C3435T* and *C421A* polymorphisms in the *ABCB1* and *ABCG2* genes can alter mRNA expression levels, protein activity, and substrate specificity. A possible explanation for the absence of a significant association between the *ABCB1* and *ABCG2* SNPs in our study, as reported in other studies, could be the heterogeneity of solid and hematological neoplasms, the diversity of anti-neoplastic drugs administered as part of the treatment, and the genetic variability inherent to each analyzed population and ethnic groups.[18,36,49]

Our results showed that the presence of the *CYP3A5 A6986G* SNP confers longer DFS in patients with DLBCL treated with R-CHOP. In various types of cancer, such as lung and colorectal cancer, the presence of the GG genotype has been described as a favorable prognostic factor for OS and DFS.[50,51] Similarly, in CML, Harivenkatesh et al in 2017, reported a higher rate of treatment failure with imatinib in patients with the AA genotype.[34]

Odds ratio analysis showed that the presence of the GG genotype of the *CYP3A5 A6986G* SNP favors a sustained complete response. In DLBCL, there are no reports on the risk associated with this SNP genotype regarding treatment response. However, in hematological neoplasms such as ALL and Acute Myeloid Leukemia/Myelodysplastic Syndromes (AML/MDS), studies by Borst et al in 2011 and Li et al in 2024, reported no clinical implications of this polymorphism.[52,53] Wegman et al in 2007 and Jiang et al in 2016, reported in breast and lung cancer that the presence of the GG genotype is associated with a lower risk of relapse and better treatment responses, similar to our findings.[51,54]

The *CYP3A5 A6986G* SNP has been reported to cause alterations in the metabolism of drugs like cyclophosphamide, vincristine and prednisone (used in the R-CHOP regimen for DLBCL), and therefore impact chemotherapy responses in both solid and hematological cancers.[55] This could be due to the A>G change in intron 3, which causes a decrease in mRNA expression, leading to a truncated protein with lower catalytic activity, resulting in higher bioavailability of

chemotherapeutic drugs and enhancing tumor cell elimination.[14,56,57] In contrast, the AA genotype leads to increased mRNA and protein expression, as well as higher metabolic activity for drug elimination.[58] Similar findings were reported by Rodríguez-Antona et al in 2007, who found that in T-cell lymphomas, patients with lower expression of other family members, such as CYP3A4, had better survival.[59]

It has been described that, in addition to pharmacological metabolism, *CYP3A5* expression in cellular models inhibits cell adhesion capacity, facilitating tumor metastasis and the presence of CTCs in liquid biopsies.[48,60] Other circulating biomarkers, such as circulating tumor DNA (ctDNA), have gained relevance in cancer prognosis; however, genotyping analysis has been shown to have higher sensitivity in CTCs than in ctDNA.[61]

The use of liquid biopsies will enable the identification and characterization of CTCs from primary tumors in DLBCL patients, serving as a molecular strategy to detect the genotypes of genes involved in drug resistance and metabolism within the R-CHOP regimen. This approach could facilitate the adjustment of personalized treatments based on these circulating biomarkers. Although no genotyping studies have been conducted on drug-metabolism genes in CTCs, an initial study in lung cancer suggests that this molecular technique can identify *EGFR* gene alterations in CTCs, which might be utilized not only as a prognostic factor but also to guide treatment decisions.[61]

Conclusion

In conclusion, our results demonstrated that the GG genotype of *CYP3A5 A6986G* SNP, present in CTCs obtained from liquid biopsies of patients with DLBCL, confers longer DFS. Its detection is of great relevance to establish a prognosis and might be implemented as a molecular tool for the follow-up of DLBCL patients. Expanding knowledge of the drug resistance family and cytochrome will allow for the stratification of patients who may not respond to first-line therapy in this type of cancer and other hematologic malignancies.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Carballo-Zarate A, Garcia-Horton A, Palma-Berre L, et al. Distribution of lymphomas in Mexico: a multicenter descriptive study. *J Hematopathol*. 2018;11(4):99–105. doi:10.1007/s12308-018-0336-0
2. Hernandez-Ruiz E, Alvarado-Ibarra M, Juan Lien-Chang LE, et al. Epidemiology and clinical characteristics of non-Hodgkin lymphoma in Mexico. *World J Oncol*. 2021;12(1):28–33. doi:10.14740/wjon1351
3. Wang SS. Epidemiology and etiology of diffuse large B-cell lymphoma. *Semin Hematol*. 2023;60(5):255–266. doi:10.1053/j.seminhematol.2023.11.004
4. Cerón R, Martínez A, Ramos C, et al. Overexpression of *BCL2, BCL6, VEGFR1* and *TWIST1* in circulating tumor cells derived from patients with DLBCL decreases event-free survival. *Onco Targets Ther*. 2022;15:1583–1595. doi:10.2147/OTT.S386562
5. Farooq U, Maurer MJ, Thompson CA, et al. Clinical heterogeneity of diffuse large B cell lymphoma following failure of front-line immunochemotherapy. *Br J Haematol*. 2017;179(1):50–60. doi:10.1111/bjh.14813
6. Hilton LK, Scott DW, Morin RD. Biological heterogeneity in diffuse large B-cell lymphoma. *Semin Hematol*. 2023;60(5):267–276. doi:10.1053/j.seminhematol.2023.11.006
7. Coiffier B, Lepage E, Briere J, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. *N Engl J Med*. 2002;346(4):235–242. doi:10.1056/NEJMoa011795
8. Tilly H, Morschhauser F, Sehn LH, et al. Polatuzumab vedotin in previously untreated diffuse large B-cell lymphoma. *N Engl J Med*. 2022;386(4):351–363. doi:10.1056/NEJMoa2115304
9. Stegemann M, Denker S, Schmitt CA. DLBCL 1L-what to expect beyond R-CHOP? *Cancers*. 2022;14(6):1453. doi:10.3390/cancers14061453
10. Ankathil R. *ABCB1* genetic variants in leukemias: current insights into treatment outcomes. *Pharmgenomics Pers Med*. 2017;10:169–181. doi:10.2147/PGPM.S105208
11. Huang Z, Wang J, Qian J, et al. Effects of cytochrome P450 family 3 subfamily A member 5 gene polymorphisms on daunorubicin metabolism and adverse reactions in patients with acute leukemia. *Mol Med Rep*. 2017;15(6):3493–3498. doi:10.3892/mmr.2017.6470
12. Scorcella C, Domizi R, Amoroso S, et al. Pharmacogenetics in critical care: association between CYP3A5 rs776746 A/G genotype and acetaminophen response in sepsis and septic shock. *BMC Anesthesiol*. 2023;23(1):55. doi:10.1186/s12871-023-02018-y

13. Li Q, Wang J, Wang ZL, et al. The impacts of CYP3A4 genetic polymorphism and drug interactions on the metabolism of lurasidone. *Biomed Pharmacother*. 2023;168:115833. doi:10.1016/j.biopha.2023.115833
14. Klyushova LS, Perepechaeva ML, Grishanova AY. The role of CYP3A in health and disease. *Biomedicines*. 2022;10(11):2686. doi:10.3390/biomedicines10112686
15. Bellah SF, Salam MA, Billah SMS, Karim MR. Genetic association in *CYP3A4* and *CYP3A5* genes elevate the risk of prostate cancer. *Ann Hum Biol*. 2023;50(1):63–74. doi:10.1080/03014460.2023.2171122
16. Asadov C, Karimova N, Hasanova A, et al. Association of *CYP3A5*3, CYP3A4*18 & CYP2B6*6* polymorphisms with imatinib treatment outcome in Azerbaijani chronic myeloid leukaemia patients. *Ind J Med Res*. 2023;158(2):151–160. doi:10.4103/ijmr.ijmr_1103_22
17. Chufan EE, Sim HM, Ambudkar SV. Molecular basis of the polyspecificity of P-glycoprotein (ABCB1): recent biochemical and structural studies. *Adv Cancer Res*. 2015;125:71–96. doi:10.1016/bs.acr.2014.10.003
18. Turiján-Espinoza E, Ruíz-Rodríguez VM, Uresti-Rivera EE, et al. Clinical utility of ABCB1 and ABCG2 genotyping for assessing the clinical and pathological response to FAC therapy in Mexican breast cancer patients. *Cancer Chemother Pharmacol*. 2021;87(6):843–853. doi:10.1007/s00280-021-04244-y
19. Skinner KT, Palkar AM, Hong AL. Genetics of *ABCB1* in cancer. *Cancers*. 2023;15(17):4236. doi:10.3390/cancers15174236
20. Olarte Carrillo I, García Laguna AI, la Cruz Rosas AD, et al. High expression levels and the C3435T SNP of the ABCB1 gene are associated with lower survival in adult patients with acute myeloblastic leukemia in Mexico City. *BMC Med Genomics*. 2021;14(1):251. doi:10.1186/s12920-021-01101-y
21. Wang D, Johnson AD, Papp AC, et al. Multidrug resistance polypeptide 1 (MDR1, ABCB1) variant 3435C>T affects mRNA stability. *Pharmacogenet Genomics*. 2005;15(10):693–704. doi:10.1097/01.fpc.0000178311.02878.83
22. Wang D, Sadée W. Searching for polymorphisms that affect gene expression and mRNA processing: example ABCB1 (MDR1). *AAPS J*. 2006;8(3):E515–E520. doi:10.1208/aapsj080361
23. Wu H, Liu Y, Kang H, et al. Genetic variations in ABCG2 gene predict breast carcinoma susceptibility and clinical outcomes after treatment with anthracycline-based chemotherapy. *Biomed Res Int*. 2015;2015:279109. doi:10.1155/2015/279109
24. Suominen L, Sjöstedt N, Vellonen KS, et al. In vitro identification of decreased function phenotype ABCG2 variants. *Eur J Pharm Sci*. 2023;188:106527. doi:10.1016/j.ejps.2023.106527
25. Tandia M, Mhiri A, Paule B, et al. Correlation between clinical response to sorafenib in hepatocellular carcinoma treatment and polymorphisms of P-glycoprotein (ABCB1) and of breast cancer resistance protein (ABCG2): monocentric study. *Cancer Chemother Pharmacol*. 2017;79(4):759–766. doi:10.1007/s00280-017-3268-y
26. Nebert DW, Wikvall K, Miller WL. Human cytochromes P450 in health and disease. *Philos Trans R Soc Lond B Biol Sci*. 2013;368(1612):20120431. doi:10.1098/rstb.2012.0431
27. Hu LL, Yu B, Yang J. MDR1 polymorphisms associated with risk and survival in diffuse large B-cell lymphoma. *Leuk Lymphoma*. 2013;54(6):1188–1193. doi:10.3109/10428194.2012.736980
28. Ni Y, Yin G, Xiao Z, et al. MDR1 polymorphisms have an impact on the prognosis of Chinese diffuse large B cell lymphoma patients. *Tumour Biol*. 2016;37(1):1237–1244. doi:10.1007/s13277-015-3930-0
29. Liu W, Li Y, Zhao Z, Li X. Clinical relevance of multi-drug resistance gene C3435T polymorphism in diffuse large B-cell lymphoma in Xinjiang. *Medicine*. 2020;99(35):e21704. doi:10.1097/MD.0000000000021704
30. Mhaidat NM, Alshogran OY, Khabour OF, et al. Multi-drug resistance 1 genetic polymorphism and prediction of chemotherapy response in Hodgkin's Lymphoma. *J Exp Clin Cancer Res*. 2011;30(1):68. doi:10.1186/1756-9966-30-68
31. Leal-Ugarte E, Gutiérrez-Angulo M, Macías-Gómez NM, et al. MDR1 C3435T polymorphism in Mexican children with acute lymphoblastic leukemia and in healthy individuals. *Hum Biol*. 2008;80(4):449–455. doi:10.3378/1534-6617-80.4.449
32. Hu LL, Wang XX, Chen X, et al. BCRP gene polymorphisms are associated with susceptibility and survival of diffuse large B-cell lymphoma. *Carcinogenesis*. 2007;28(8):1740–1744. doi:10.1093/carcin/bgm113
33. Kim IS, Kim HG, Kim DC, et al. ABCG2 Q141K polymorphism is associated with chemotherapy-induced diarrhea in patients with diffuse large B-cell lymphoma who received frontline rituximab plus cyclophosphamide/doxorubicin/vincristine/prednisone chemotherapy. *Cancer Sci*. 2008;99(12):2496–2501. doi:10.1111/j.1349-7006.2008.00985.x
34. Harivenkatesh N, Kumar L, Bakhshi S, et al. Influence of MDR1 and CYP3A5 genetic polymorphisms on trough levels and therapeutic response of imatinib in newly diagnosed patients with chronic myeloid leukemia. *Pharmacol Res*. 2017;120:138–145. doi:10.1016/j.phrs.2017.03.011
35. Martinez-Chapoy D, Cruz-Arroyo FJ, Ancer-Leal FD, et al. Pilot study: genetic distribution of AR, FGF5, SULT1A1 and CYP3A5 polymorphisms in male Mexican population with androgenetic alopecia. *Int J Mol Epidemiol Genet*. 2022;13(3):32–41.
36. Galaviz-Hernández C, Lazalde-Ramos BP, Lares-Assef I, et al. Influence of genetic admixture components on *CYP3A5*3* allele-associated hypertension in amerindian populations from Northwest Mexico. *Front Pharmacol*. 2020;11:638. doi:10.3389/fphar.2020.00638
37. de León M B, León-Cachón RBR, Silva-Ramírez B, et al. Association study of genetic polymorphisms in proteins involved in oseltamivir transport, metabolism, and interactions with adverse reactions in Mexican patients with acute respiratory diseases. *Pharmacogenomics J*. 2020;20(4):613–620. doi:10.1038/s41397-020-0151-8
38. Sagahón-Azúa J, Medellín-Garibay SE, Chávez-Castillo CE, et al. Factors associated with fluoxetine and norfluoxetine plasma concentrations and clinical response in Mexican patients with mental disorders. *Pharmacol Res Perspect*. 2021;9(5):e00864. doi:10.1002/prp2.864
39. Gardner ER, Ahlers CM, Shukla S, et al. Association of the ABCG2 C421A polymorphism with prostate cancer risk and survival. *BJU Int*. 2008;102(11):1694–1699. doi:10.1111/j.1464-410X.2008.07913.x
40. 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. doi:10.1111/j.1600-0609.2004.00228.x
41. Buda G, Maggini V, Galimberti S, et al. MDR1 polymorphism influences the outcome of multiple myeloma patients. *Br J Haematol*. 2007;137(5):454–456. doi:10.1111/j.1365-2141.2007.06605.x
42. Sensorn I, Sirachainan E, Chamnanphon M, et al. Association of CYP3A4/5, ABCB1 and ABCC2 polymorphisms and clinical outcomes of Thai breast cancer patients treated with tamoxifen. *Pharmgenomics Pers Med*. 2013;6:93–98. doi:10.2147/PGPM.S44006
43. Li W, Zhang D, Du F, et al. *ABCB1* 3435TT and *ABCG2* 421CC genotypes were significantly associated with longer progression-free survival in Chinese breast cancer patients. *Oncotarget*. 2017;8(67):111041–111052. doi:10.18632/oncotarget.22201

44. Liu D, Wu N, Sun H, et al. *ABCG2* and *NCF4* polymorphisms are associated with clinical outcomes in diffuse large B-cell lymphoma patients treated with R-CHOP. *Oncotarget*. 2017;8(35):58292–58303. doi:10.18632/oncotarget.16869
45. Perrone G, Rigacci L, Roviello G, et al. Validation of single nucleotide polymorphisms potentially related to R-CHOP resistance in diffuse large B-cell lymphoma patients. *Cancer Drug Resist*. 2024;7:21. doi:10.20517/cdr.2024.10
46. Salimizand H, Amini S, Abdi M, et al. 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. *Tumour Biol*. 2016;37(1):791–798. doi:10.1007/s13277-015-3874-4
47. Nouri N, Mehrzad V, Khalaj Z, et al. Effects of ABCG2 C421A and ABCG2 G34A genetic polymorphisms on clinical outcome and response to imatinib mesylate, in Iranian chronic myeloid leukemia patients. *Egypt J Med Hum Genet*. 2023;24(1). doi:10.1186/s43042-022-00379-6.
48. Jiang F, Chen L, Yang YC, et al. CYP3A5 functions as a tumor suppressor in hepatocellular carcinoma by regulating mTORC2/Akt signaling. *Cancer Res*. 2015;75(7):1470–1481. doi:10.1158/0008-5472.CAN-14-1589
49. Badavi E, Safavi B, Jalali A, et al. Association of CYP3A4 and CYP3A5 polymorphisms with Iranian breast cancer patients. *Egypt J Med Hum Genet*. 2015;16(3):219–225. doi:10.1016/j.ejmhg.2015.03.004
50. Dong N, Meng F, Wu Y, Wang M, Cui Y, Zhang S. Genetic polymorphisms in cytochrome P450 and clinical outcomes of FOLFIRI chemotherapy in patients with metastatic colorectal cancer. *Tumour Biol*. 2015;36(10):7691–7698. doi:10.1007/s13277-015-3492-1
51. Jiang LP, Zhu ZT, He CY. Effects of CYP3A5 genetic polymorphism and smoking on the prognosis of non-small-cell lung cancer. *Onco Targets Ther*. 2016;9:1461–1469. doi:10.2147/OTT.S94144
52. 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. doi:10.1111/j.1600-0609.2011.01608.x
53. Li Y, Wan Q, Wan J, et al. Plasma concentrations of venetoclax and pharmacogenetics correlated with drug efficacy in treatment naive leukemia patients: a retrospective study. *Pharmacogenomics J*. 2024;24(6):37. doi:10.1038/s41397-024-00359-6
54. Wegman P, Elingarami S, Carstensen J, et al. Genetic variants of CYP3A5, CYP2D6, SULT1A1, UGT2B15 and tamoxifen response in postmenopausal patients with breast cancer. *Breast Cancer Res*. 2007;9(1):R7. doi:10.1186/bcr1640
55. Xie HG, Wood AJ, Kim RB, Stein CM, Wilkinson GR. Genetic variability in CYP3A5 and its possible consequences. *Pharmacogenomics*. 2004;5(3):243–272. doi:10.1517/phgs.5.3.243.29833
56. Lolodi O, Wang YM, Wright WC, Chen T. Differential regulation of CYP3A4 and CYP3A5 and its implication in drug discovery. *Curr Drug Metab*. 2017;18(12):1095–1105. doi:10.2174/1389200218666170531112038
57. El Wahab N A, Shafik NF, Shafik RE, et al. Association of CYP3A5*3 and CYP1A1*2C polymorphism with development of acute myeloid leukemia in Egyptian patients. *Asian Pac J Cancer Prev*. 2017;18(3):747–752. doi:10.22034/APJCP.2017.18.3.747
58. Zhenhua L, Tsuchiya N, Narita S, et al. CYP3A5 gene polymorphism and risk of prostate cancer in a Japanese population. *Cancer Lett*. 2005;225(2):237–243. doi:10.1016/j.canlet.2005.03.009
59. Rodríguez-Antona C, Leskelä S, Zajac M, et al. Expression of CYP3A4 as a predictor of response to chemotherapy in peripheral T-cell lymphomas. *Blood*. 2007;110(9):3345–3351. doi:10.1182/blood-2007-02-075036
60. Murray GI. The role of cytochrome P450 in tumour development and progression and its potential in therapy. *J Pathol*. 2000;192(4):419–426. doi:10.1002/1096-9896(2000)9999:9999<::AID-PATH750>3.0.CO;2-0
61. Maheswaran S, Sequist LV, Nagrath S, et al. Detection of mutations in EGFR in circulating lung-cancer cells. *N Engl J Med*. 2008;359(4):366–377. doi:10.1056/NEJMoa0800668

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