RESEARCH ARTICLE

Editorial Process: Submission:12/14/2023 Acceptance:04/15/2024

Efficacy and Toxicity of Vincristine and *CYP3A5* Genetic Polymorphism in Rhabdomyosarcoma Pediatric Egyptian Patients

Norhan Shalaby[1], Hala F Zaki[1], Osama A Badary[2], Sherif Kamal[3], Mohamed Nagy[4], Dalia Makhlouf[5], Amr Elnashar[6], Enas Elnady[7], Sameh A Abdelshafi[5], Sherif Abouelnaga[7], Mona M Saber[1]*

Abstract

Background: Rhabdomyosarcoma (RMS) is a rare cancer that develops in soft tissue, particularly skeletal muscle tissue and occasionally hollow organs like the bladder or uterus. Vincristine (VCR) is the main therapy used in treatment of RMS, it is an alkaloid produced from vinca and it is one of the most commonly prescribed drugs in pediatric oncology for the treatment of a number of tumors. The *CYP3A5* enzyme is responsible for vincristine metabolism. The effect of *CYP3A5* genetic polymorphism on the efficacy and toxicity of VCR on RMS patients still needs further research. **Methods:** Genotyping for *CYP3A5* SNPs rs776746, rs10264272 and rs41303343 was performed using Taqman Real-Time PCR assays in a retrospective cohort study of 150 RMS pediatric patients treated with vincristine. The relationship between these genotypes and RMS survival was then examined. **Results:** We found that patients with *CYP3A5**3/*3 had the highest incidence of vincristine-induced neuropathy reaching 61.3%. Patients with *CYP3A5**1/*3, *CYP3A5**3/*6 and the normal metabolizers with *CYP3A5**1/*1 had frequencies of 22%, 10.7%, and 4.7%. patients with the lowest frequency of 1.3% were those with the *CYP3A5**1/*6 genotype. There was no correlation between the genotypes of *CYP3A5**3, *CYP3A5**6, *CYP3A5**7, and RMS survival. Initial risk, metastasis, response, convulsions, unsteady gait and hepatotoxicity grade had a significant effect on overall survival with $p<0.05$. **Conclusion:** *CYP3A5**1/*1 have less severe vincristine-induced neuropathy than *CYP3A5* *1/*3, *CYP3A5* *1/*6 and *CYP3A5* *3/*3, *CYP3A5* *3/*6. There is a significant influence of *CYP3A5* mutation on neuropathy grade and assist of ADL as a part of neurotoxicity.

Keywords: *CYP3A5*- SNP- Vincristine- Rhabdomyosarcoma- Neuropathy

Asian Pac J Cancer Prev, **25 (4)**, 1391-1409

Introduction

Rhabdomyosarcoma (RMS) is a rare cancer that develops in soft tissue, particularly skeletal muscle tissue and occasionally hollow organs like the bladder or uterus. RMS can strike anyone at any age, but it is more common in children. Soft tissue sarcoma is responsible for 7% of cancers in children and 1% of cancers in adults [1].

The type of rhabdomyosarcoma, where it develops, tumor size, whether the cancer has progressed, and risk categorization into low, moderate, and high risk, all influence treatment decisions. Physicians generally used Surgery, chemotherapy, and radiation therapy in combination to treat cancer [2]. The typical chemotherapy regimen for treating RMS is a combination of vincristine, actinomycin, and cyclophosphamide (VAC) [3].

Vincristine (VCR), an alkaloid produced from vinca, is frequently used in combination with other chemotherapeutic medications to treat a variety of cancers. It is one of the most prescribed drugs in paediatric oncology for the treatment of several tumors, including RMS. In the United States, Physicians prescribed vincristine in the treatment of more than half of all children with cancer [4]. Vincristine is largely metabolized in the liver by *CYP3A* subtype enzymes, notably *CYP3A4* and *CYP3A5* enzymes, with M1 (4-O-desacetyl-vincristine) being the primary metabolite and M2 (N-deformyl-vincristine) and M4 (4-O-desacetyl-N-deformyl-vincristine) being minor

*[1]Pharmacology and Toxicology Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt. [2]Clinical Pharmacy Department, Faculty of Pharmacy, Misr University for Science and Technology, Egypt. [3]Egypt Healthcare authority, Cairo, Egypt. [4]Pharmaceutical Services Department and Personalized Medication Management Unit, Children's Cancer Hospital Egypt (57357), Cairo, Egypt. [5]Research Department, Children's Cancer Hospital Egypt (57357), Cairo, Egypt. [6]Pediatric Medical Oncology, Beni Suef University and Children's Cancer Hospital Egypt (57357), Cairo, Egypt. [7]Pediatric Oncology Department. National Cancer Institute; Cairo University and Children's Cancer Hospital Egypt 57357, Cairo, Egypt. *For Correspondence: mona.magdy@pharma.cu.edu.eg*

metabolites [5].

VCR's most serious side effect is neurotoxicity, which can manifest as sensory, motor, or autonomic neuropathy. Paresthesia, muscle weakness, discomfort, and reduced sensation are among symptoms of VCR-induced peripheral neuropathy (VIPN). VIPN, which frequently manifests as a symmetric sensory-motor neuropathy, is the predominant adverse effect of vincristine. Foot drop, loss of deep tendon reflexes, unsteadiness, and discomfort or tingling are some of the warning indications [6, 7]. Up to 30% of patients could experience severe VIPN, necessitating either a dose reduction or end to treatment [8, 9].

Sensory neuropathy causes numbness and tingling in the hands and feet, as well as sensory nerve injury. Peripheral sensory numbness, paresthesia, decreased balance, tendon weakening, and gait abnormalities are common symptoms of motor neuropathy [10-15]. Dysuria and paralytic ileus are symptoms of autonomic neuropathy [16]. Patients may further experience autonomic symptoms including constipation or orthostatic hypotension. VCR can also induce cranial neuropathy, which can lead to visual and hearing problems, as well as blindness and deafness [17].

The *CYP3A5* enzyme catalyzes VCR [18], and *CYP3A5* is the functional enzyme that aids the liver in VCR clearance. Genetic variations in *CYP3A5* expression may affect the risk of VIPN and thus inter-individual variability in VCR metabolism. There are a number of single nucleotide polymorphisms (SNPs) in the *CYP3A5* gene that change how the enzyme works [19]. The name of the wild type allele is *CYP3A5**1 and the mutant alleles are *CYP3A5**3 rs776746, *CYP3A5**6 rs1026427, and *CYP3A5**7 rs4130334.

Only People with at least one *CYP3A5**1 allele can express high concentrations of the *CYP3A5* enzyme [20]. *CYP3A5**3 allele on the other hand, results in alternative splicing and prevents the production of proteins, reducing or eliminating the *CYP3A5* enzyme [21, 22].

The term "*CYP3A5* expressers" refers to individuals who have at least one functional allele (*1), whereas "*CYP3A5* non-expressers" are individuals who only have non-functional variant alleles. *CYP3A5**1 allele produces the active enzyme. Mutations in *CYP3A5**3, *CYP3A5**6, and *CYP3A5**7 result in a lack of functioning enzymes or none at all. The *CYP3A5**1 expression type has a 5 times greater intrinsic VCR clearance than the non-expression type [5].

Previous research has indicated that patients with *CYP3A5* *3/*3 genotypes are more likely to be white than black [23, 24]. Black Africans had a far higher likelihood of carriers of *CYP3A5* *1/*1 and *1/*3 displaying full- or partial-expression *CYP3A5* in comparison to white. In contrast, no black African (*CYP3A5* *3/*3) was a *CYP3A5* non-expressor. A homozygous *CYP3A5* non-expressor makes up 88% of the white population, and none of them express CYP3A normally [25].

Understanding the elements that influence VCR metabolism could help to explain why VCR reaction varies so much between people. The capacity to forecast therapy failure might aid in the development of more effective VCR dose regimens. Our hypothesis is that SNPs in *CYP3A5* could affect the enzyme's functionality in VCR metabolism, which could affect treatment response. Based on these considerations, we conducted this study to determine the prevalence of three *CYP3A5* common nonfunctional SNPs in our cohort of Egyptian paediatric RMS patients: rs776746 (C 26201809 30 *CYP3A5**3), rs10264272 (C 30203950 10 *CYP3A5**6), and rs41303343 (C 32287188 10 *CYP3A5**7). Our goal was to see if the *CYP3A5* polymorphism has an effect on VCR therapy toxicity, response, and survival in RMS patients.

Materials and Methods

Patient eligibility and treatment

This retrospective study provides information for pharmacogenetic research of vincristine toxicity and efficacy in a pediatric group receiving vincristine as part of an RMS therapy plan. One hundred fifty RMS pediatric patients under age of 18 were included in this retrospective study. Patients with chronic renal disease or chronic liver disease or patients who have any other types of cancer rather than RMS were excluded from the study.

We selected patients at the Children's Cancer Hospital of Egypt (CCHE) from 2013 until 2017. The inclusion criteria were; newly diagnosed RMS patients, under 18 years of age, and had vincristine in their protocol. The exclusion criteria were; patients with chronic renal or liver disease or had any other type of cancer. They received treatment with a combination therapy which consists of the drugs vincristine; actinomycin and cyclophosphamide (VAC) as the standard treatment protocol for RMS.

The vincristine dose is 0.025 mg/kg/dose (maximum dose 2 mg) IV push with Infants < 1 year, 0.05 mg/kg/dose (maximum dose 2 mg) IV push with infants ≥ 1 year and < 3 years and 1.5 mg/m2/dose (maximum dose 2 mg) IV push with infants ≥ 3 years. This dose is the same in all types of RMS risk (low, intermediate, high).

Group sample sizes of 94 in group one and 56 in group two achieve 97% power to detect an odds ratio in the group proportions of 3.75. The proportion in group one is assumed to be 0.37 under the null hypothesis and 0.6877 under the alternative hypothesis. The proportion in group two is 0.3700. The test statistic used is the two-sided Z test with pooled variance. The significance level of the test was targeted at 0.05. The significance level actually achieved by this design is 0.0497.

Ethics statement

The Children's Cancer Hospital of Egypt 57357 Institutional Review Board (IRB) gave its approval to this retrospective study (Approval number: PT 2610). Before including patients in the study, parents or legal guardians had to sign a consent form.

Blood sampling and DNA extraction

We collected samples reserved in the biobank of the hospital; we took Peripheral blood sample (5 ml) from patients and collected in EDTA vacutainers. We extracted genomic DNA by a minicolumn DNA purification kit (Qiagen, Hilden, Germany) according to manufacturer's

instructions. We measured DNA concentration and purity through using Nanoquant™ spectrophotometer (Infinite M200, TECAN, Switzerland). We stored DNA at –20°C to prepare for pharmacogenetic analysis.

CYP3A5 Genotyping

We performed genotyping for *CYP3A5**3 rs776746, *CYP3A5**6 rs10264272 and *CYP3A5**7 rs41303343 by using Taqman Real-Time polymerase chain reaction assays. A *CYP3A5**1 genotype is assigned by default if testing for other alleles (*3, *6, and *7) is negative. The polymorphism which occurs with *CYP3A5**3 rs776746 is 6981A>G, *CYP3A5**6 rs10264272 is 14685G>A and A/-, Insertion/Deletion is the polymorphism which occur with *CYP3A5**7rs41303343. We carried out *CYP3A5* genotype analyses by The TaqMan® SNP Genotyping Assays which use TaqMan® 5′-nuclease chemistry for amplifying and detecting specific polymorphisms in purified genomic DNA samples. Each assay allows genotyping of individuals for a single nucleotide polymorphism (SNP).

Clinical evaluation criteria

An essential aspect of the clinical evaluation of cancer therapies is the assessment of the change in tumor burden. Based on The guidelines for (version 1.1) of the Response Evaluation Criteria in Solid Tumors (RECIST) [26]. Researchers classify the tumor response in each patient as a complete response (CR), complete response stationary lesion CR (SL), a partial response (PR), stationary disease (StD), or progressive disease (PD), Authors used these classifications to measure the efficacy of vincristine and its correlation with different genotypes and phenotypes.

Statistical Analysis

Nominal variables are presented as frequencies and percentages, continuous variables are described as means with standard deviations or medians with interquartile ranges depending on the normality of distribution. Descriptive statistics, frequency distributions and percentages were calculated for the study participants' baseline characteristics, outcome variables and other covariates of interest. Unpaired comparisons of continuous outcomes were performed with Student's t-test or Mann–Whitney test depending on distribution of data. Unpaired comparisons of categorical outcomes were performed with the Chi-square-test or with Fisher's exact test when any expected value was below five. A two-sided probability of $P < 0.05$ was considered statistically significant.

Kaplan-Meier estimates of both OS and EFS was provided. OS was measured from the date of diagnosis to the date of date of death or date of last contact. EFS was measured from the date of diagnosis to the date of initial failure for patients who fail. Failure includes the traditional endpoints of failure as relapse in any site, and death during treatment or after remission. Statistical analysis was performed using Statistical Package for SPSS, version 20.

Results

Patient characteristics, genotyping, and allele frequencies

Table 1 illustrates the patients' baseline characteristics. A total of 150 pediatric RMS patients were enrolled receiving vincristine as part of their clinical treatment protocol for RMS. The study population had a mean age of 5.72 ± 4.04 years (range, 0.07 to 17.4 years) and included 87 male and 63 female patients. The mean body weight, height, and surface area were 21.5 ± 15.6 kg, 109.7 ± 25.7 cm and 0.96 ± 0.440.86 ± 0.39 m2, respectively. 35 patients (23.3%) had metastasis and there were no reported metastatic lesions in 76% of the patients. Table 1 also shows the distribution of the genotypes. In our cohort, 72% carried rs776746 (10.7% as heterozygous and 61.3% as mutant homozygous), 12% mutant heterozygous had rs10264272, 4.7 % had no mutation and there was no detected mutation with rs41303343.

Figure 1 illustrates the frequencies of *CYP3A5* different genotypes. Highest frequencies was in Patients with *CYP3A5* *3/*3 which represent 61.3%, and the lowest frequencies was in patients with *CYP3A5* *1/*6 (1.3%), others in Figure.

CYP3A5 mutation correlation with neurotoxicity, thrombocytopenia, hepatotoxicity, and their grades

Our results showed a correlation between *CYP3A5* mutation types and different toxicities such neurotoxicity,

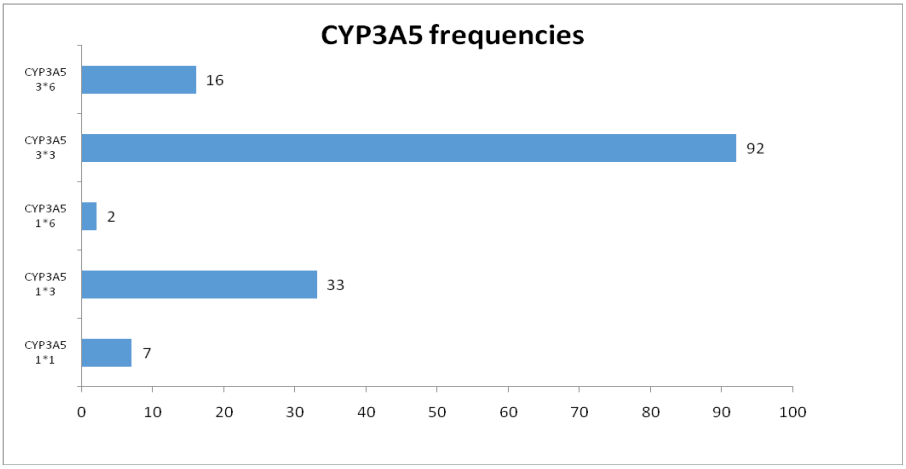

Figure 1. *CYP3A5* Frequencies Showing the Number of Patients who have *CYP3A5 *1/*1 ,CYP3A5 *1/*3,* CYP3A5 **1/*6, CYP3A5 *3/*3,* and CYP3A5 *3/*6.

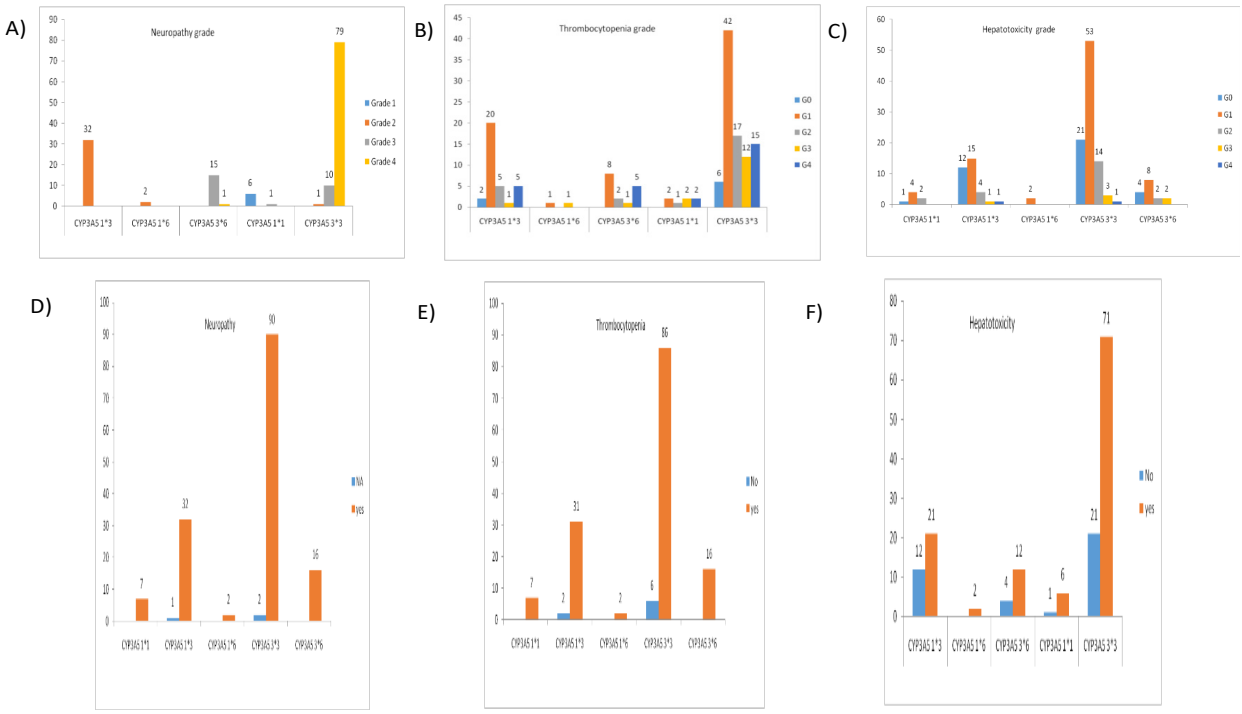

Figure 2. Correlation of *CYP3A5* Mutations with the A) grade of neuropathy, p<0.05, B) grade of thrombocytopenia, p=0.46, C) grade of hepatotoxicity, p=0.9, D) neurotoxicity, E) thrombocytopenia, p=0.79, and F) hepatotoxicity, p=0.46. Significance was measured by CTCAE v5.0 which shows the number of patients with different types of allele mutation and toxicity grade.

hepatotoxicity and thrombocytopenia as shown in Figure 2 and Table 2. Neuropathy of 61.2% occurred in patients had *CYP3A5* 3*3 genotype which is the most frequent and those with *CYP3A5* 1*6 genotype experience 1.4% of neuropathy which is the lowest. Patients with *CYP3A5* *1/*6 genotype experience 1.8% of hepatotoxicity which is the less frequent, while 63.4% of hepatotoxicity occurred in patients had *CYP3A5* *3/*3 genotype which is the most frequent. Patients with *CYP3A5* *3/*3 experienced thrombocytopenia at a rate of 60.6% and those with the least common genotype, *CYP3A5* *1/*6, experienced thrombocytopenia at a rate of 1.4%.

Metabolizing status correlation with neurotoxicity, thrombocytopenia, hepatotoxicity, and their grades

Results showed correlation between metabolizing status and different types of toxicity such neurotoxicity,

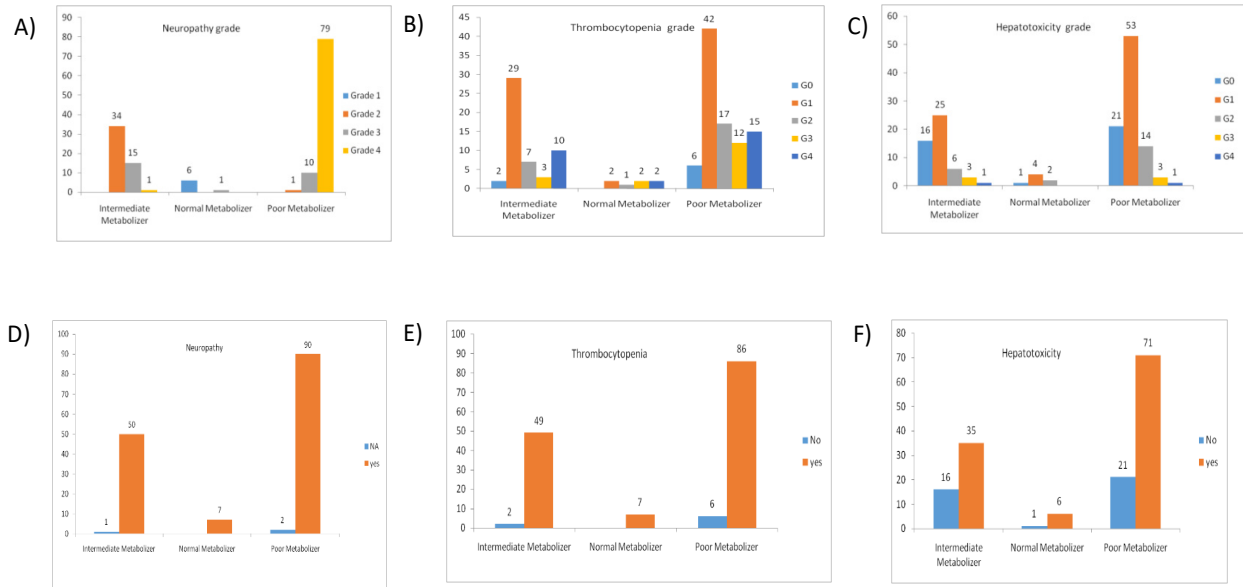

Figure 3. Correlation of Intermediate, Normal and Poor Metabolizers with the A) grade of neuropathy, p=0.0, B) grade of thrombocytopenia, p=0.45, C) grade of hepatotoxicity, p=0.889, D) neurotoxicity, p=0.0, E) thrombocytopenia, p=0.653, and F) hepatotoxicity, p=0.419. Significance was measured by CTCAE v5.0 which shows the number of patients with different types of allele mutation and toxicity grade

Table 1. Patient Demographics and Characteristics

Variables		Total number of patients = 150	
Age	Mean ± SD	5.72 ± 4.04	
Weight	Mean ± SD	21.5 ± 15.6	
Height	Mean ± SD	109.7 ± 25.7	
BSA	Mean ± SD	0.96 ± 0.44	
		Number of patients	Percentage
Gender	Male	87	58%
	Female	63	42%
Primary site	Abdominal Wall	1	0.70%
	Biliary Tract Liver	3	2.00%
	Chest Wall	3	2.00%
	Extremities	16	10.70%
	Genitourinary (Non-Bladder / Non-Prostate)	8	5.30%
	Head & Neck	70	46.70%
	Pelvic	25	16.70%
	Perineal Region	1	0.70%
	Retroperitoneal Pelvic	1	0.70%
	Urinary bladder	21	14.00%
	Missing data	1	0.70%
Stage	Stage I	23	15.30%
	Stage II	11	7.30%
	Stage III	80	53.30%
	Stage IV	35	23.30%
	Missing data	1	0.70%
Type of RMS	Alveolar	18	12.00%
(Histopathology)	Embryonal	132	88.00%
Tumor Site	Favorable	132	88.00%
	Unfavorable	18	12.00%
Initial risk	High Risk	35	23.30%
	Intermediate Risk	106	70.70%
	Low Risk	8	5.30%
	Missing data	1	0.70%
CYP3A5 Interpretation	*CYP3A5 *1/*1*	7	4.70%
	*CYP3A5 *1/*3*	33	22.00%
	*CYP3A5 *1/*6*	2	1.30%
	*CYP3A5 *3/*3*	92	61.30%
	*CYP3A5 *3/*6*	16	10.70%
Metabolizing Status	Normal Metabolizer	7	4.70%
	Intermediate Metabolizer	51	34.00%
	Poor Metabolizer	92	61.30%
Metastasis	No	114	76.00%
	Yes	35	23.30%
	Missing data	1	0.70%
Response	Complete Remission	67	44.70%
	Partial Remission	20	13.30%
	Progressive Disease	51	34.00%
	Stationary Disease	6	4.00%
	Missing data	6	4.00%

Table 1. Continued

Variables		Total number of patients = 150	
		Number of patients	Percentage
Neuropathy	Yes	147	98.00%
	Missing data	3	2.00%
Hepatotoxicity	No	38	25.30%
	Yes	112	74.70%
Thrombocytopenia	No	8	5.30%
	Yes	142	94.70%

thrombocytopenia and hepatotoxicity (Figure 3). Of the normal metabolizers, 4.8% experienced neuropathy, 0.0% thrombocytopenia and 2.6% experienced hepatotoxicity. 34% of intermediate metabolizers experienced neuropathy, 25% thrombocytopenia and 42.1% experienced hepatotoxicity. Poor metabolizers experienced neuropathy with the percentage of 61.2%, 75% experienced thrombocytopenia and 55.3% had hepatotoxicity.

Figure 3 also illustrates the correlation between metabolizing status and the grade of different types of toxicity such neurotoxicity, thrombocytopenia and hepatotoxicity. All the normal metabolizers experienced grade1 neuropathy, while 0.0% experienced grade 2and grade 4neuropathy. Grade 3 thrombocytopenia was experienced by 11.8% of the normal metabolizers, while 2.7% experienced grade1. Normal metabolizers experienced grade 2 hepatotoxicity with the percentage of 9.15% while none experienced grade 3 hepatotoxicity and grade 4 hepatotoxicity. None of the intermediate metabolizers experienced grade 1 neuropathy and 97.1% experienced grade2. The frequency of intermediate metabolizers which experienced grade 1 thrombocytopenia

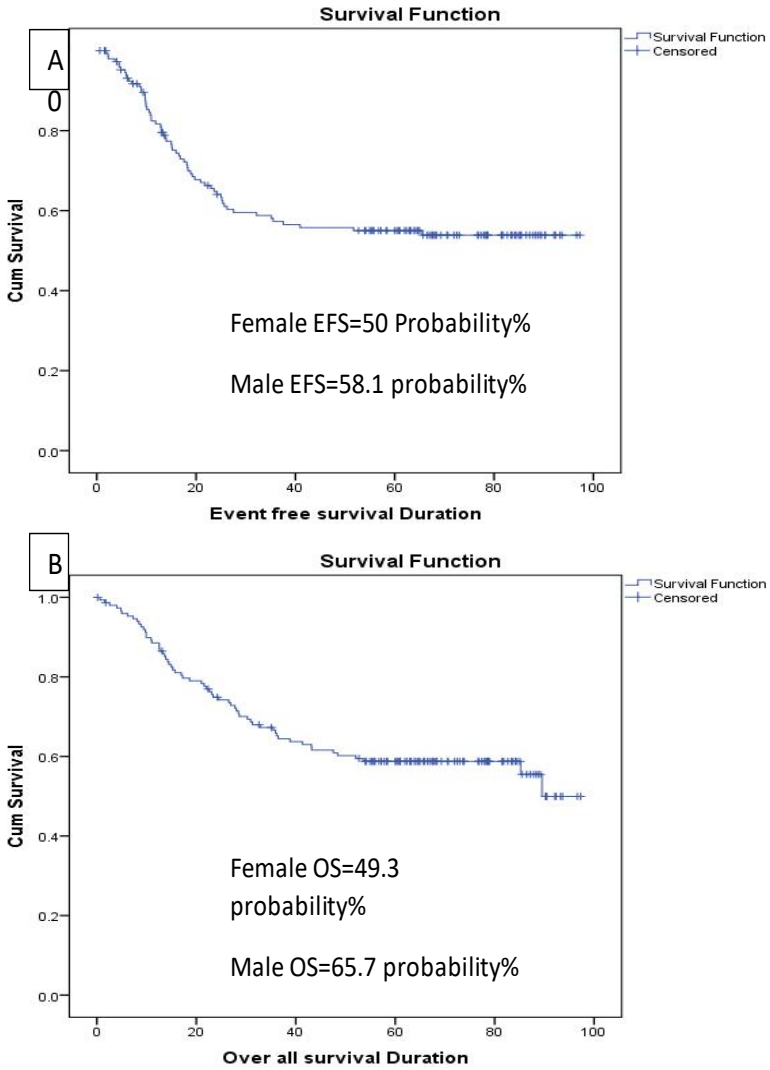

Figure 4. Kaplan Meier Showing OS and EFS of the Cohort Study. A) 5 years overall survival $p < 0.05$*, B) 5 years event free survival $p = 0.51$.

Table 2. *CYP3A5* Interpretation

			*CYP3A5*_Interpretation					Total	P value
			*CYP3A5 *1/*1*	*CYP3A5 *1/*3*	*CYP3A5 *1/*6*	*CYP3A5 *3/*3*	*CYP3A5 *3/*6*		
Gender	Female	Count	4	14	1	35	9	63	0.62
		%	6.30%	22.20%	1.60%	55.60%	14.30%	100%	
	Male	Count	3	19	1	57	7	87	
		%	3.40%	21.80%	1.10%	65.50%	8%	100%	
Primary site	Abdominal Wall	Count	0	0	0	1	0	1	0.8
		%	0%	0%	0%	100%	0%	100%	
	Biliary Tract Liver	Count	0	2	0	1	0	3	
		%	0%	66.70%	0%	33.30%	0%	100%	
	Chest Wall	Count	0	0	0	2	1	3	
		%	0%	0%	0%	66.70%	33.30%	100%	
	Extremities	Count	1	2	0	11	2	16	
		%	6.20%	12.50%	0%	68.80%	12.50%	100%	
	Genitourinary (NonBladder/ NonProstate)	Count	0	4	1	2	1	8	
		%	0%	50%	12.50%	25.00%	12.50%	100%	
	Head & Neck	Count	4	16	0	42	8	70	
		%	5.70%	22.90%	0%	60%	11.40%	100%	
	Pelvic	Count	1	5	0	17	2	25	
		%	4%	20%	0%	68%	8%	100%	
	Perineal Region	Count	0	1	0	0	0	1	
		%	0%	100%	0%	0%	0%	100%	
	Retroperitoneal Pelvic	Count	0	0	0	1	0	1	
		%	0%	0%	0%	100%	0%	100%	
	Urinary bladder	Count	1	3	1	14	2	21	
		%	4.80%	14.30%	4.80%	66.70%	9.50%	100%	
Stage	Stage I	Count	1	7	1	12	2	23	0.92
		%	4.30%	30.40%	4.30%	52.20%	8.70%	100%	
	Stage II	Count	1	3	0	7	0	11	
		%	9.10%	27.30%	0%	63.60%	0%	100%	
	Stage III	Count	4	16	1	49	10	80	
		%	5.00%	20.00%	1.20%	61.20%	12.50%	100.00%	
	Stage IV	Count	1	7	0	23	4	35	
		%	2.90%	20%	0%	65.70%	11.40%	100%	

Table 2. Continued

			*CYP3A5*_Interpretation					Total	P value
			*CYP3A5 *1/*1*	*CYP3A5 *1/*3*	*CYP3A5 *1/*6*	*CYP3A5 *3/*3*	*CYP3A5 *3/*6*		
Type of RMS (Histopathology)	Alveolar	Count	1	2	0	13	2	18	0.77
		%	5.60%	11.10%	0%	72.20%	11.10%	100%	
	Embryonal	Count	6	31	2	79	14	132	
		%	4.50%	23.50%	1.50%	59.80%	10.60%	100%	
Tumor site	Favorable	Count	6	31	2	79	14	132	0.77
		%	4.50%	23.50%	1.50%	59.80%	10.60%	100%	
	Unfavorable	Count	1	2	0	13	2	18	
		%	5.60%	11.10%	0%	72.20%	11.10%	100%	
Initial risk	High Risk	Count	1	7	0	23	4	35	0.93
		%	2.90%	20%	0%	65.70%	11.40%	100%	
	Intermediate Risk	Count	6	23	2	64	11	106	
		%	5.70%	21.70%	1.90%	60.40%	10.40%	100%	
	Low Risk	Count	0	3	0	4	1	8	
		%	0%	37.50%	0%	50.00%	12.50%	100%	
Metabolizing Status	Intermediate Metabolizer	Count	0	33	2	0	16	51	< 0.05*
		%	0%	64.70%	3.90%	0%	31.40%	100%	
	Normal Metabolizer	Count	7	0	0	0	0	7	
		%	100%	0%	0%	0%	0%	100%	
	Poor Metabolizer	Count	0	0	0	92	0	92	
		%	0%	0%	0%	100%	0%	100%	
Metastasis	No	Count	6	26	2	68	12	114	0.87
		%	5.30%	22.80%	1.80%	59.60%	10.50%	100%	
	Yes	Count	1	7	0	23	4	35	
		%	2.90%	20.00%	0%	65.70%	11.40%	100%	
Response	CR	Count	3	17	1	40	6	67	0.74
		%	4.50%	25.40%	1.50%	59.70%	9.00%	100%	
	Partial Remission	Count	1	6	1	10	2	20	
		%	5%	30%	5%	50%	10%	100%	
	Progressive Disease	Count	2	9	0	35	5	51	
		%	3.90%	17.60%	0%	68.60%	9.80%	100%	
	Stationary Disease	Count	0	1	0	3	2	6	
		%	0%	16.70%	0%	50%	33.30%	100%	

Table 2. Continued

			*CYP3A5*_Interpretation					Total	P value
			*CYP3A5 *1/*1*	*CYP3A5 *1/*3*	*CYP3A5 *1/*6*	*CYP3A5 *3/*3*	*CYP3A5 *3/*6*		
Neuropathy	Yes	Count	7	32	2	90	16	147	-
		%	4.80%	21.80%	1.40%	61.20%	10.90%	100%	
Grade	Grade 1	Count	6	0	0	0	0	6	< 0.05*
		%	100%	0%	0%	0%	0%	100%	
	Grade 2	Count	0	32	2	1	0	35	
		%	0%	91.40%	5.70%	2.90%	0%	100%	
	Grade 3	Count	1	0	0	10	15	26	
		%	3.80%	0%	0%	38.50%	57.70%	100%	
	Grade 4	Count	0	0	0	79	1	80	
		%	0.00%	0.00%	0.00%	98.80%	1.20%	100%	
Fonatal description	No	Count	6	32	2	89	16	145	0.051
		%	4.10%	22.10%	1.40%	61.40%	11%	100%	
	Yes	Count	1	0	0	1	0	2	
		%	50%	0%	0%	50%	0%	100%	
Ptosis	No	Count	7	31	2	83	15	138	0.82
		%	5.10%	22.50%	1.40%	60.10%	10.90%	100%	
	Yes	Count	0	1	0	7	1	9	
		%	0%	11.10%	0%	77.80%	11.10%	100%	
Convulsions	No	Count	7	32	2	88	16	145	0.86
		%	4.80%	22.10%	1.40%	60.70%	11.00%	100%	
	Yes	Count	0	0	0	2	0	2	
		%	0%	0%	0%	100%	0%	100%	
Optic atrophy	No	Count	7	32	2	88	16	145	0.86
		%	4.80%	22.10%	1.40%	60.70%	11%	100%	
	Yes	Count	0	0	0	2	0	2	
		%	0%	0%	0%	100%	0%	100%	
Limited sensory perception	No	Count	5	26	1	57	12	101	0.38
		%	5%	25.70%	1%	56.40%	11.90%	100%	
	Yes	Count	2	6	1	33	4	46	
		%	4.30%	13%	2.20%	71.70%	8.70%	100%	

Table 2. Continued

			*CYP3A5*_Interpretation					Total	P value
			*CYP3A5 *1/*1*	*CYP3A5 *1/*3*	*CYP3A5 *1/*6*	*CYP3A5 *3/*3*	*CYP3A5 *3/*6*		
Unsteady gait	No	Count	6	25	2	72	14	119	0.87
		%	5%	21%	1.70%	60.50%	11.80%	100%	
	Yes	Count	1	7	0	18	2	28	
		%	3.60%	25%	0%	64.30%	7.10%	100%	
Assist of ADL	No	Count	3	30	2	83	13	131	< 0.05*
		%	2.30%	22.90%	1.50%	63.40%	9.90%	100%	
	Yes	Count	4	2	0	7	3	16	
		%	25%	12.50%	0%	43.80%	18.80%	100%	
Limited mobility	No	Count	4	6	0	19	5	34	0.17
		%	11.80%	17.60%	0%	55.90%	14.70%	100%	
	Yes	Count	3	26	2	71	11	113	
		%	2.70%	23%	1.80%	62.80%	9.70%	100%	
Constipation	No	Count	6	29	2	84	15	136	0.92
		%	4.40%	21.30%	1.50%	61.80%	11%	100%	
	Yes	Count	1	3	0	6	1	11	
		%	9.10%	27.30%	0%	54.50%	9.10%	100%	
Hepatotoxicity	No	Count	1	12	0	21	4	38	0.46
		%	2.60%	31.60%	0.00%	55.30%	10.50%	100%	
	Yes	Count	6	21	2	71	12	112	
		%	5.40%	18.80%	1.80%	63.40%	10.70%	100%	
Grade	G1	Count	4	15	2	53	8	82	0.9
		%	4.90%	18.30%	2.40%	64.60%	9.80%	100%	
	G2	Count	2	4	0	14	2	22	
		%	9.10%	18.20%	0.00%	63.60%	9.10%	100%	
	G3	Count	0	1	0	3	2	6	
		%	0%	16.70%	0%	50%	33.30%	100.00%	
	G4	Count	0	1	0	1	0	2	
		%	0.00%	50.00%	0.00%	50.00%	0.00%	100%	

Table 2. Continued

			*CYP3A5*_Interpretation					Total	P value
			*CYP3A5 *1/*1*	*CYP3A5 *1/*3*	*CYP3A5 *1/*6*	*CYP3A5 *3/*3*	*CYP3A5 *3/*6*		
Total bilirubin	G1	Count	4	15	2	53	8	82	0.9
		%	4.90%	18.30%	2.40%	64.60%	9.80%	100%	
	G2	Count	2	4	0	14	2	22	
		%	9.10%	18.20%	0%	63.60%	9.10%	100%	
	G3	Count	0	1	0	3	2	6	
		%	0%	16.70%	0%	50%	33.30%	100%	
	G4	Count	0	1	0	1	0	2	
		%	0%	50%	0%	50%	0%	100%	
AST Grade	G1	Count	4	15	2	53	8	82	0.9
		%	4.90%	18.30%	2.40%	64.60%	9.80%	100%	
	G2	Count	2	4	0	14	2	22	
		%	9.10%	18.20%	0.00%	63.60%	9.10%	100%	
	G3	Count	0	1	0	3	2	6	
		%	0%	16.70%	0%	50%	33.30%	100%	
	G4	Count	0	1	0	1	0	2	
		%	0%	50%	0%	50%	0%	100%	
ALT Grade	G1	Count	4	15	2	53	8	82	0.9
		%	4.90%	18.30%	2.40%	64.60%	9.80%	100%	
	G2	Count	2	4	0	14	2	22	
		%	9.10%	18.20%	0%	63.60%	9.10%	100%	
	G3	Count	0	1	0	3	2	6	
		%	0%	16.70%	0%	50%	33.30%	100%	
	G4	Count	0	1	0	1	0	2	
		%	0%	50%	0%	50%	0%	100%	
Thrombocytopenia	No	Count	0	2	0	6	0	8	0.79
		%	0%	25%	0%	75%	0%	100%	
	Yes	Count	7	31	2	86	16	142	
		%	4.90%	21.80%	1.40%	60.60%	11.30%	100%	

Table 2. Continued

			CYP3A5_Interpretation					Total	P value
			*CYP3A5 *1/*1*	*CYP3A5 *1/*3*	*CYP3A5 *1/*6*	*CYP3A5 *3/*3*	*CYP3A5 *3/*6*		
Grade	G1	Count	2	20	1	42	8	73	0.46
		%	2.70%	27.40%	1.40%	57.50%	11%	100%	
	G2	Count	1	5	0	17	2	25	
		%	4%	20%	0%	68%	8%	100%	
	G3	Count	2	1	1	12	1	17	
		%	11.80%	5.90%	5.90%	70.60%	5.90%	100%	
	G4	Count	2	5	0	15	5	27	
		%	7.40%	18.50%	0%	55.60%	18.50%	100%	

was 39.7% which was the highest and 17.6% of intermediate metabolizers experienced grade 3 which was the lowest frequent., 50% of intermediate metabolizers experienced grade 3 and grade 4 hepatotoxicity, while 27.3% experienced grade 2 hepatotoxicity.

Regarding poor metabolizers, 2.9% of the cases had grade 2 neuropathy and 98% grade 4 neuropathy. Grade 3 thrombocytopenia was detected in 70.6% of the poor metabolizers and 55.6% experienced grade 4 thrombocytopenia. 64.6% of poor metabolizers experience grade 1 hepatotoxicity and 50% of cases experienced grade 3 and grade 4 hepatotoxicity.

Response and survival function

We employed Overall survival (OS), event free survival (EFS), and clinical response as the endpoints in this retrospective cross-sectional study to measure the clinical outcomes (Figure 4). The mean follow-up duration 4.7 ± 28.7 months. the mean duration of OS was 4.7 ± 28.7 months, mean survival duration for 95% CI (48.078_67.384) for female and (62.669¬_78.301) for male, 5 years overall survival p value < 0.05*, the median duration of OS was 56.3 ± 28.7 months.

The mean duration EFS was 43.5 ± 31.3 months, 5 years EFS mean survival duration for 95% CI (46.060_67.617) for female and (53.848_71.270) for male, the 5 years Event free survival p value = 0.51, the median duration EFS was 40.9 ± 31.3 months. Our results represent the frequencies of clinical response for patients with complete remission 44.7 % and 4.0 % for Stationary Disease. Our results showed a significant association between gender and overall survival, there is no significant correlation between *CYP3A5* interpretation and OS.

Table 3 illustrated factors influencing overall survival and showed that the number of patients who are still alive are 31 female and 57 male, number of patients at which the event occur (dead) are 32 females and 30 males. The probability of 5 years overall survival is 49.3% with female and 65.7% with male. The probability of 5 years overall survival in patients with metastasis is 21.6% and the probability of 5 years overall survival in patients without metastasis 67.9% which showed a significant correlation between metastasis and OS with p value < 0.05. The probability of 5 years overall survival in patients with complete response is 92.4% and 13.8% in patients with progressive disease which show a significant effect of response on overall survival with a p value < 0.05.

As shown in Table 3 there was a significant association of convulsions, unsteady gait and hepatotoxicity grade with OS. Table 4 illustrated factors influencing Event free survival (progression of disease) and showed that the number of patients without event are 36 female and 50 male, number of patients at which the event occur (progression of disease) are 27 female and 36 males. The probability of 5 years event free survival is 50% with female and 58.1% with male. Our results showed non-significant correlation between gender and event free survival, there is no significant association between *CYP3A5* interpretation and EFS. The probability of 5 years event free survival in patients with metastasis is 14.7% and the probability of 5 years overall survival

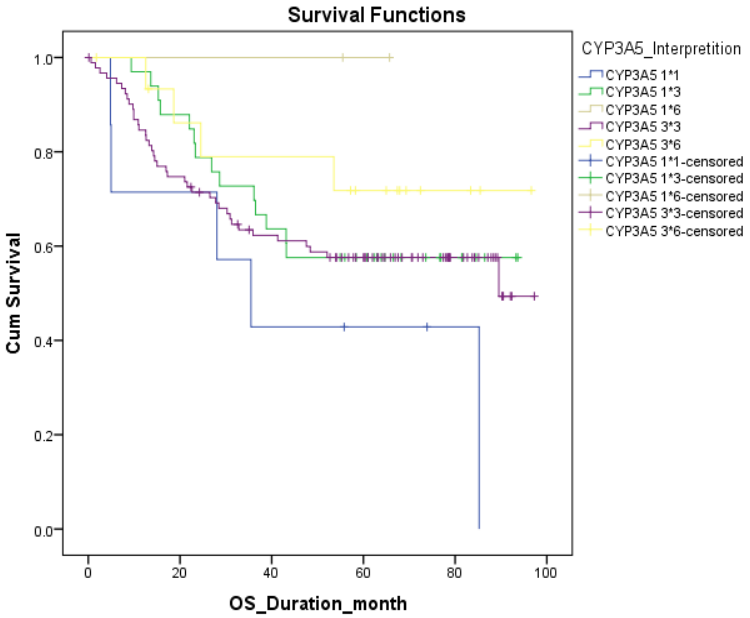

Figure 5. Kaplan-Meier Curve Correlation between *CYP3A5* Interpretation and Overall Survival.

in patients without metastasis 66.8% which showed a significant association between metastasis and EFS with p value < 0.05.The probability of 5 years overall survival in patients with complete response is 90.8%, 64.7% and 0% in patients with progressive disease and 80% in patients with stationary disease which show a significant effect of response on overall survival with a P value < 0.05. As shown in Table 4 there was a significant association of neuropathy grade and hepatotoxicity grade with EFS.

CYP3A5 interpretation and the 5 years overall survival

Our study reported the association between 5 years overall survival and *CYP3A5* interpretation which reported that patients with *CYP3A5* *1/*1 who were still alive are 2 and who were dead 5, the probability of 5 years overall survival in patients with *CYP3A5* *1/*1 is 42.9% and 100% in patients with *CYP3A5* *1/*6. There was no significant effect of *CYP3A5* interpretation on overall survival with p value 0.28 (Figure 5).

Factors influencing the 5 years Event free survival

Figure 6 shows the association between 5 years event free survival and *CYP3A5* Interpretation which clarifies that patients with *CYP3A5* *1/*1 who were still alive are

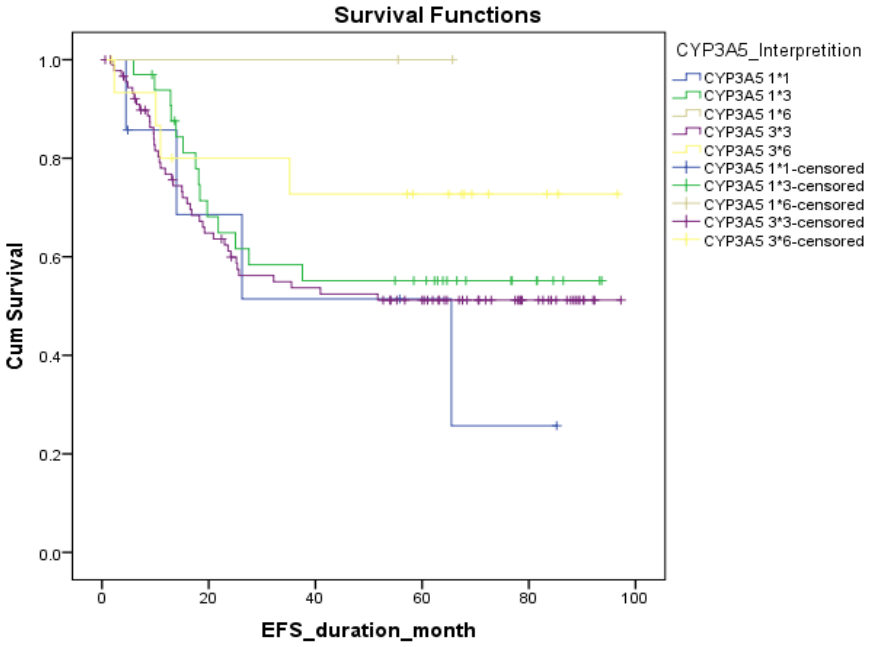

Figure 6. Kaplan-Meier Curve Correlation between *CYP3A5* Interpretation and Event Fee Survival

Table 3. Factors Influencing the 5 Years OS

Factors influencing the OS		Case Processing Summary		OS (5 years)	95% CI		P value
					Mean survival duration		
		Event number	Censored Number	Probability %	Lower	Upper	
Gender	Female	32	31	49.3	48.078	67.384	< 0.05*
	Male	30	57	65.7	62.669	78.301	
Primary site	Abdominal Wall	0	1	100	-	-	0.079
	Biliary Tract Liver	2	1	33.3	-	-	
	Chest Wall	1	2	66.7	-	-	
	Extremities	8	8	50	-	-	
	Genitourinary	1	7	87.5	-	-	
	Head & Neck	27	43	62.9	-	-	
	Pelvic	16	9	35.2	-	-	
	Perineal Region	1	0	100	-	-	
	Retroperitoneal	1	0	100	-	-	
	Urinary bladder	5	16	57.6	-	-	
Stage	Stage I	7	16	73.2	62.176	85.644	< 0.05*
	Stage II	3	8	70.7	61.622	97.396	
	Stage III	26	54	68.4	64.587	80.205	
	Stage IV	26	9	21.6	22.5	42.007	
Type of RMS	Alveolar	8	10	55.6	38.315	73.251	0.06
(Histopathology)	Embryonal	54	78	59.1	59.468	72.48	
Tumor site	Favorable	54	78	59.1	59.468	72.48	0.06
	Unfavorable	8	10	55.6	38.315	73.251	
Initial risk	High Risk	26	9	21.6	22.5	42.007	< 0.05*
	Intermediate Risk	35	71	68.3	66.429	79.814	
	Low Risk	1	7	87.5	68.156	99.098	
CYP3A5 Interpretation	CYP3A5 *1/*1	5	2	42.9	-	-	0.28
	CYP3A5 *1/*3	14	19	57.6	-	-	
	CYP3A5 *1/*6	0	2	100	-	-	
	CYP3A5 *3/*3	39	53	57.6	-	-	
	CYP3A5 *3/*6	4	12	71.8	-	-	
	Intermediate Metabolizer	18	33	63.3	61.47	80.689	0.15
Metabolizing Status	Normal Metabolizer	5	2	0	18.279	75.744	
	Poor Metabolizer	39	53	57.6	55.5	71.762	
Metastasis	No	36	78	67.9	67.839	80.578	< 0.05*
	Yes	26	9	21.6	22.5	42.007	
Response	CR	7	60	92.4	85.902	95.355	< 0.05*
	Partial Remission	7	13	65	44.533	65.598	
	Progressive Disease	42	9	13.8	24.705	39.66	
	Stationary Disease	2	4	66.7	33.605	85.521	
Neuropathy	Grade 1	4	2	50	23.992	84.095	0.08
	Grade 2	15	20	57.1	52.723	75.839	
	Grade 3	5	21	80.3	70.371	93.845	
	Grade 4	38	42	52.2	51.04	68.575	
Fonatal description	No	61	84	57.7	58.519	71.134	0.98
	Yes	1	1	0	85.246	85.246	
Ptosis	No	60	78	56.1	57.204	70.204	0.17
	Yes	2	7	88.9	67.434	96.77	
Convulsions	No	60	85	59	59.188	71.727	< 0.05*
	Yes	2	0	0	5.951	38.147	
Optic atrophy	No	61	84	58.5	58.721	71.28	0.59
	Yes	1	1	0	27.77	27.77	

Table 3. Continued

Factors influencing the OS		Case Processing Summary		OS (5 years)	95% CI		P value
					Mean survival duration		
		Event number	Censored	Probability %	Lower	Upper	
Limited sensory perception	No	40	61	61.4	59.191	74.213	0.34
	Yes	22	24	51.3	47.505	67.376	
Unsteady gait	No	46	73	61.6	59.899	72.604	< 0.05*
	Yes	16	12	44.1	35.572	66.32	
Assist of ADL	No	56	75	57.7	57.701	70.978	0.66
	Yes	6	10	62.5	42.779	67.942	
Limited mobility	No	12	22	64.1	56.717	82.052	0.42
	Yes	50	63	56.4	56.412	70.641	
Constipation	No	57	79	58.5	58.638	71.58	0.78
	Yes	5	6	54.5	35.844	75.697	
Hepatotoxicity	No	16	22	60.9	51.074	74.783	0.75
	Yes	46	66	58.2	58.897	73.082	
Grade	G1	28	54	65.1	63.627	79.452	< 0.05*
	G2	14	8	36.4	30.94	60.383	
	G3	2	4	66.7	33.836	85.705	
	G4	2	0	0	14.413	43.652	
Total bilirubin	G1	28	54	65.1	63.627	79.452	< 0.05*
	G2	14	8	36.4	30.94	60.383	
	G3	2	4	66.7	33.836	85.705	
	G4	2	0	0	14.413	43.652	
AST Grade	G1	28	54	65.1	63.627	79.452	< 0.05*
	G2	14	8	36.4	30.94	60.383	
	G3	2	4	66.7	33.836	85.705	
	G4	2	0	0	14.413	43.652	
ALT Grade	G1	28	54	65.1	63.627	79.452	< 0.05*
	G2	14	8	36.4	30.94	60.383	
	G3	2	4	66.7	33.836	85.705	
	G4	2	0	0	14.413	43.652	
Thrombocytopenia	No	0	8	100	-	-	< 0.05*
	Yes	62	80	56.8	-	-	
Grade	G1	25	48	65.5	62.659	79.223	0.79
	G2	12	13	52	43.06	72.156	
	G3	9	8	52.9	42.529	77.556	
	G4	16	11	40.7	33.334	61.004	

3and who were dead 4, The probability of 5 years event free survival in patients with *CYP3A5* *1/*6 was 100% and 51.2% in patients with *CYP3A5* *3/*3. There was no significant effect of *CYP3A5* Interpretation on event free survival with p value 0.41.

Discussion

This study investigated the effect of *CYP3A5* genetic polymorphism on peripheral neuropathy induced by vincristine. We found that *CYP3A5**1/*1 (normal metabolizer) have less severe vincristine-induced neuropathy when compared to *CYP3A5* *1/*3, *CYP3A5* *1/*6 (intermediate metabolizers) and *CYP3A5* *3/*3, *CYP3A5* *3/*6 (poor metabolizers). Patients who have *CYP3A5* expresser genotypes *1/*1 and *1/*3, experience high metabolic and high clearance rate of vincristine, while people who have the *CYP3A5* non-expresser genotypes, *3/*3 and *CYP3A5* *3/*6, experience low metabolic and low clearance rate of vincristine thus are at high risk of developing vincristine induced peripheral neuropathy (VIPN).

In our study we found that 98% of the patients enrolled in this retrospective cross-sectional study had signs of neuropathy. Convulsions and optic atrophy were the most common neuropathies which occurred in 96% in the patients. Limited sensory perception occurred in 67.3% of the cases, unsteady gait in 79.3%, assist of activities of daily living (ADL) in 87.3%, constipation in 90.7%, and ptosis in 92% of the patients. Limited mobility was less frequent, and occurred in 22.7% of the patients.

In a study of 18 lymphoma pateints, researchers closely monitored patients for the onset of peripheral neuropathy, they discovered that after three months of vincristine

Table 4. Factors Influencing the 5 Years Event Free Survival

Factors influencing the EFS		Case Processing Summary		EFS (5 years)	95% CI		P value
					Mean survival duration		
		Event number	Censored Number	Probability %	Lower	Upper	
Gender	Female	27	36	50	46.06	67.617	0.51
	Male	36	50	58.1	53.848	71.27	
Primary site	Abdominal Wall	0	1	100	-	-	< 0.05*
	Biliary Tract Liver	1	2	50	-	-	
	Chest Wall	1	2	66.7	-	-	
	Extremities	9	7	34.9	-	-	
	Genitourinary	1	7	87.5	-	-	
	Head & Neck	25	45	59.9	-	-	
	Pelvic	17	8	29.3	-	-	
	Perineal Region	1	0	0	-	-	
	Retroperitoneal	1	0	0	-	-	
	Urinary bladder	7	14	66.3	-	-	
Stage	Stage I	6	17	72.7	57.962	85.716	< 0.05*
	Stage II	3	8	68.6	50.099	95.97	
	Stage III	27	53	64.6	60.172	77.266	
	Stage IV	27	8	14.7	13.935	32.381	
Type of RMS	Alveolar	9	9	50.7	32.628	68.682	0.38
(Histopathology)	Embryonal	54	77	55.6	54.28	68.724	
Tumor site	Favorable	54	77	55.6	54.28	68.724	0.38
	Unfavorable	9	9	50.7	32.628	68.682	
Initial risk	High Risk	27	8	14.7	13.935	32.381	< 0.05*
	Intermediate Risk	35	71	65	62.153	77.068	
	Low Risk	1	7	87.5	63.035	100.605	
CYP3A5 Interpretation	CYP3A5 1*1	4	3	51.4	-	-	0.41
	CYP3A5 1*3	14	19	55.1	-	-	
	CYP3A5 1*6	0	2	100	-	-	
	CYP3A5 3*3	41	50	51.2	-	-	
	CYP3A5 3*6	4	12	72.7	-	-	
	Intermediate Metabolizer	18	33	62.2	55.785	77.772	0.28
Metabolizing Status	Normal Metabolizer	4	3	51.4	21.171	71.436	
	Poor Metabolizer	41	50	51.2	48.743	66.442	
Metastasis	No	36	78	66.8	63.785	77.996	< 0.05*
	Yes	27	8	14.7	13.935	32.381	
Response	CR	7	60	90.8	84.855	95.268	< 0.05*
	Partial Remission	6	14	64.7	43.848	66	
	Progressive Disease	49	2	0	11.718	16.49	
	Stationary Disease	1	5	80	47.174	91.317	
Neuropathy	Grade 1	4	2	50	19.5	70.784	< 0.05*
	Grade 2	14	21	56.5	47.958	73.978	
	Grade 3	4	22	83.9	71.626	95.414	
	Grade 4	41	39	43.7	42.08	61.01	
Fonatal description	No	62	83	54.4	53.078	66.918	0.93
	Yes	1	1	50	12.338	97.039	
Ptosis	No	60	78	53.5	52.199	66.416	0.44
	Yes	3	6	66.7	42.068	87.703	
Convulsions	No	61	84	55.2	53.726	67.538	0.13
	Yes	2	0	0	7.52	31.169	
Optic atrophy	No	62	83	54.5	53.263	67.058	0.66
	Yes	1	1	50	11.285	24.19	
Limited sensory perception	No	39	62	59.3	55.909	72.168	0.08
	Yes	24	22	43.3	36.584	58.825	

Table 4. Continued

Factors influencing the EFS		Case Processing Summary		EFS (5 years)	95% CI		P value
					Mean survival duration		
		Event number	Censored Number	Probability %	Lower	Upper	
Unsteady gait	No	49	70	56.9	53.371	67.566	0.94
	Yes	14	14	43.5	32.272	65.929	
Assist of ADL	No	56	75	53.2	52.21	66.872	0.73
	Yes	7	9	62.5	36.861	64.542	
Limited mobility	No	12	22	65.3	53.726	80.624	0.24
	Yes	51	62	51	49.843	65.619	
Constipation	No	59	77	54.2	52.426	66.718	0.66
	Yes	4	7	56.3	37.914	78.935	
Hepatotoxicity	No	15	22	55.4	45.292	71.908	0.98
	Yes	48	64	54.9	52.704	68.264	
Grade	G1	32	50	58.3	55.302	73.085	< 0.05*
	G2	13	9	40.8	28.174	60.198	
	G3	1	5	83.3	48.41	92.388	
	G4	2	0	0	3.868	23.018	
Total bilirubin	G1	32	50	58.3	55.302	73.085	< 0.05*
	G2	13	9	40.8	28.174	60.198	
	G3	1	5	83.3	48.41	92.388	
	G4	2	0	0	3.868	23.018	
AST Grade	G1	32	50	58.3	55.302	73.085	< 0.05*
	G2	13	9	40.8	28.174	60.198	
	G3	1	5	83.3	48.41	92.388	
	G4	2	0	0	3.868	23.018	
ALT Grade	G1	32	50	58.3	55.302	73.085	< 0.05*
	G2	13	9	40.8	28.174	60.198	
	G3	1	5	83.3	48.41	92.388	
	G4	2	0	0	3.868	23.018	
Thrombocytopenia	No	2	5	71.4	45.385	87.28	0.32
	Yes	61	81	54.1	52.732	66.747	
Grade	G1	73	27	58	55.46	74.078	0.054
	G2	25	11	56.9	40.143	72.077	
	G3	17	6	62.2	45.494	85.833	
	G4	27	17	37	26.486	55.574	

therapy, all individuals had missing ankle reflexes and 75% had sensory signs or symptoms, diminished vibration feeling being the most common (62.5%). Constipation (62.5%) was the only autonomic indication, while motor abnormalities were substantially less frequent (18.7%). They detected denervation (46.7%) by concentrated needle electromyography, particularly in the hand's tiny muscles [27]. The study of Egbelakin et al. reported that vincristine-induced neuropathy is less severe in active *CYP3A5* expressers than in non-expressers. *CYP3A5* expresser and non-expresser groups had significantly different rates of vincristine-induced neuropathy [18].

The study illustrates the frequencies of *CYP3A5* different genotypes, patients who have *CYP3A5* *3/*3 represent 61.3%, *CYP3A5* *1/*3 represents 22%, *CYP3A5* *3/*6 represents 10.7%, *CYP3A5* *1/*6 represents 1.3% and 4.7% for patients who have *CYP3A5* *1/*1. To adjust dose for specific patients, it is necessary to assess the severity of the side effects, specifically neurotoxicity, between the different genotype groups. Of the enrolled patients, 92% with poor metabolizing status had *CYP3A5* *3/*3 genotype, 100% of patients with normal metabolizing status had *CYP3A5* *1/*1 genotype and we found that 64.7% of patients with intermediate metabolizing status had *CYP3A5* *1/*3 genotype, 3.9% of patients with intermediate metabolizing status had *CYP3A5* *1/*6 genotype and 31.4% of patients with intermediate metabolizing status had *CYP3A5* *3/*6 genotype. Similar to another study demonstrated *CYP3A5* genetic polymorphisms in different ethnic populations and reported that *CYP3A5**3, *CYP3A5**6, and *CYP3A5**7 allele distribution was shown to be significantly different between white and Black populations. Around 93% of white Canadians have the *CYP3A5**3 allele, compared to 77.6% of Zimbabweans (p 0.001); this difference in frequency is significant. *CYP3A5**6 and *CYP3A5**7 alleles, on the other hand, are uncommon in white people (p 0.001) but very common in African people (10–20%

each). These variations may be the results of local environmental conditions in geographically separate places [23].

We found that the patients who had *CYP3A5* *3/*3 genotype are poor metabolizers thus directly affecting vincristine concentration in blood leading to high neurotoxicity rates. 61.2% of neuropathy occurred in patients had *CYP3A5* *3/*3 genotype which is the highest percentage in comparison with other genotypes.

These ratios will lead us to the fact that there is a significant variation in vincristine metabolism depending on *CYP3A5* genotype, which made a difference in metabolic ratio and primary metabolite production between *CYP3A5* expressers and non-expressers. Due to that reason, *CYP3A5* expressers experience less vincristine-induced peripheral neuropathy (VIPN) than do non-expressers. The percentage of neuropathy occurring in patients with *CYP3A5* *1/*1 genotype was 4.8%, 21.8% of neuropathy in patients with *CYP3A5* *1/*3 genotype, 10.9% of neuropathy in patients with *CYP3A5* *3/*6 genotype and 1.4% of neuropathy in patients with *CYP3A5* *1/*6 genotype which is the less frequent, while 61.2% of neuropathy occurred in patients with *CYP3A5* *3/*3 genotype which is the most frequent. Another toxicity which the patients suffered from is hepatotoxicity with percentage of 74.4% and 94.7% experienced thrombocytopenia.

Our results showed that one of the parameters which have a significant influence on vincristine efficacy and toxicity is single nucleotide polymorphisms (SNPs) in the *CYP3A5* gene, which may influence the amount of enzyme produced. In our study, *CYP3A5* different mutations have an association with the reduction of metabolizing status which by extension affect gene expression, our cohort study showed that *CYP3A5* mutations have a significant effect on metabolizing status of vincristine for every individual with p value < 0.05.There was a significant influence of *CYP3A5* mutation on neuropathy grade and assist of ADL as a part of neurotoxicity with p value < 0.05, and there was a significant effect of metabolizing status on neuropathy grade with p value 0.000.

Parameters which have a significant influence on event free survival in our study are stage, initial risk, metastasis, response, neuropathy grade and hepatotoxicity grade with p value < 0.05. Stage, initial risk, metastasis, response, convulsions, unsteady gait and hepatotoxicity grade have a significant effect on overall survival with p value < 0.05.

In conclusions, *CYP3A5* expressors (normal, intermediate metabolizers) have less severe vincristine-induced neuropathy than non-expressors (poor metabolizers). There is a significant influence of *CYP3A5* mutation on neuropathy grade and assist of ADL as a part of neurotoxicity with p value < 0.05.

Author Contribution Statement

Norhan Shalaby; Writing-Original draft preparation, Investigation. Hala F. Zaki; Conceptulization, Validation, Writing-Review and Editing. Osama A. Badary; Conceptulization, Methodology. Sherif kamal; Data Curation. Mohamed Nagy; Formal analysis. Dalia Makhlouf; Investigation. Amr Elnashar; Formal analysis. Inas elnady; Resources. Sameh A. Abdelshafi; Project administration. Sherif Abou El Naga; Supervision. Mona M. Saber; Writing-Review and Editing, Formal analysis.

Acknowledgements

The authors acknowledge the support of the Pharmacology and Toxicology department, Faculty of Pharmacy, Cairo University and the Children's Cancer Hospital Egypt (57357).

Data availability statement

All data supporting the findings of this study are available within the paper.

Ethics statement

The Children's Cancer Hospital of Egypt 57357 Institutional Review Board (IRB) gave its approval to this retrospective study (Approval number: PT 2610). Before including patients in the study, parents or legal guardians had to sign a consent form.

Conflict of interest

The authors declare no conflict of interest.

References

1. Hawkins DS, Spunt SL, Skapek SX. Children's oncology group's 2013 blueprint for research: Soft tissue sarcomas. Pediatr Blood Cancer. 2013;60(6):1001-8. https://doi.org/10.1002/pbc.24435.
2. Hayes-Jordan A, Andrassy R. Rhabdomyosarcoma in children. Curr Opin Pediatr. 2009;21(3):373-8. https://doi.org/10.1097/MOP.0b013e32832b4171.
3. Crist WM, Anderson JR, Meza JL, Fryer C, Raney RB, Ruymann FB, et al. Intergroup rhabdomyosarcoma study-iv: Results for patients with nonmetastatic disease. J Clin Oncol. 2001;19(12):3091-102. https://doi.org/10.1200/jco.2001.19.12.3091.
4. McCune JS, Lindley C. Appropriateness of maximum-dose guidelines for vincristine. Am J Health Syst Pharm. 1997;54(15):1755-8. https://doi.org/10.1093/ajhp/54.15.1755.
5. Dennison JB, Jones DR, Renbarger JL, Hall SD. Effect of cyp3a5 expression on vincristine metabolism with human liver microsomes. J Pharmacol Exp Ther. 2007;321(2):553-63. https://doi.org/10.1124/jpet.106.118471.
6. Smith EML, Kuisell C, Cho Y, Kanzawa-Lee GA, Gilchrist LS, Park SB, et al. Characteristics and patterns of pediatric chemotherapy-induced peripheral neuropathy: A systematic review. Cancer Treat Res Commun. 2021;28:100420. https://doi.org/10.1016/j.ctarc.2021.100420.
7. Mora E, Smith EM, Donohoe C, Hertz DL. Vincristine-induced peripheral neuropathy in pediatric cancer patients. Am J Cancer Res. 2016;6(11):2416-30.
8. van de Velde ME, Kaspers GL, Abbink FCH, Wilhelm AJ, Ket JCF, van den Berg MH. Vincristine-induced peripheral neuropathy in children with cancer: A systematic review. Crit Rev Oncol Hematol. 2017;114:114-30. https://doi.org/10.1016/j.critrevonc.2017.04.004.
9. Lavoie Smith EM, Li L, Chiang C, Thomas K, Hutchinson RJ, Wells EM, et al. Patterns and severity of vincristine-induced peripheral neuropathy in children with acute lymphoblastic

leukemia. J Peripher Nerv Syst. 2015;20(1):37-46. https://doi.org/10.1111/jns.12114.
10. Anghelescu DL, Faughnan LG, Jeha S, Relling MV, Hinds PS, Sandlund JT, et al. Neuropathic pain during treatment for childhood acute lymphoblastic leukemia. Pediatr Blood Cancer. 2011;57(7):1147-53. https://doi.org/10.1002/pbc.23039.
11. Gutierrez-Camino A, Martin-Guerrero I, Lopez-Lopez E, Echebarria-Barona A, Zabalza I, Ruiz I, et al. Lack of association of the cep72 rs924607 tt genotype with vincristine-related peripheral neuropathy during the early phase of pediatric acute lymphoblastic leukemia treatment in a spanish population. Pharmacogenet Genomics. 2016;26(2):100-2. https://doi.org/10.1097/fpc.0000000000000191.
12. Gilchrist LS, Marais L, Tanner L. Comparison of two chemotherapy-induced peripheral neuropathy measurement approaches in children. Support Care Cancer. 2014;22(2):359-66. https://doi.org/10.1007/s00520-013-1981-6.
13. Gutiérrez-Gutiérrez G, Sereno M, Miralles A, Casado-Sáenz E, Gutiérrez-Rivas E. Chemotherapy-induced peripheral neuropathy: Clinical features, diagnosis, prevention and treatment strategies. Clin Transl Oncol. 2010;12(2):81-91. https://doi.org/10.1007/S12094-010-0474-z.
14. Gomber S, Dewan P, Chhonker D. Vincristine induced neurotoxicity in cancer patients. Indian J Pediatr. 2010;77(1):97-100. https://doi.org/10.1007/s12098-009-0254-3.
15. Windebank AJ, Grisold W. Chemotherapy-induced neuropathy. J Peripher Nerv Syst. 2008;13(1):27-46. https://doi.org/10.1111/j.1529-8027.2008.00156.x.
16. Balayssac D, Ferrier J, Descoeur J, Ling B, Pezet D, Eschalier A, et al. Chemotherapy-induced peripheral neuropathies: From clinical relevance to preclinical evidence. Expert Opin Drug Saf. 2011;10(3):407-17. https://doi.org/10.1517/14740338.2011.543417.
17. Nazir HF, AlFutaisi A, Zacharia M, Elshinawy M, Mevada ST, Alrawas A, et al. Vincristine-induced neuropathy in pediatric patients with acute lymphoblastic leukemia in oman: Frequent autonomic and more severe cranial nerve involvement. Pediatr Blood Cancer. 2017;64(12). https://doi.org/10.1002/pbc.26677.
18. Egbelakin A, Ferguson MJ, MacGill EA, Lehmann AS, Topletz AR, Quinney SK, et al. Increased risk of vincristine neurotoxicity associated with low cyp3a5 expression genotype in children with acute lymphoblastic leukemia. Pediatr Blood Cancer. 2011;56(3):361-7. https://doi.org/10.1002/pbc.22845.
19. Tsuchiya N, Satoh S, Tada H, Li Z, Ohyama C, Sato K, et al. Influence of cyp3a5 and mdr1 (abcb1) polymorphisms on the pharmacokinetics of tacrolimus in renal transplant recipients. Transplantation. 2004;78(8):1182-7. https://doi.org/10.1097/01.tp.0000137789.58694.b4.
20. Hustert E, Haberl M, Burk O, Wolbold R, He YQ, Klein K, et al. The genetic determinants of the cyp3a5 polymorphism. Pharmacogenetics. 2001;11(9):773-9. https://doi.org/10.1097/00008571-200112000-00005.
21. Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, et al. Sequence diversity in cyp3a promoters and characterization of the genetic basis of polymorphic cyp3a5 expression. Nat Genet. 2001;27(4):383-91. https://doi.org/10.1038/86882.
22. Ho H, Pinto A, Hall SD, Flockhart DA, Li L, Skaar TC, et al. Association between the cyp3a5 genotype and blood pressure. Hypertension. 2005;45(2):294-8. https://doi.org/10.1161/01.Hyp.0000151361.31736.96.
23. Roy JN, Lajoie J, Zijenah LS, Barama A, Poirier C, Ward BJ, et al. Cyp3a5 genetic polymorphisms in different ethnic populations. Drug Metab Dispos. 2005;33(7):884-7. https://doi.org/10.1124/dmd.105.003822.
24. Shuker N, Bouamar R, van Schaik RH, Clahsen-van Groningen MC, Damman J, Baan CC, et al. A randomized controlled trial comparing the efficacy of cyp3a5 genotype-based with body-weight-based tacrolimus dosing after living donor kidney transplantation. Am J Transplant. 2016;16(7):2085-96. https://doi.org/10.1111/ajt.13691.
25. Macphee IA, Fredericks S, Tai T, Syrris P, Carter ND, Johnston A, et al. Tacrolimus pharmacogenetics: Polymorphisms associated with expression of cytochrome p4503a5 and p-glycoprotein correlate with dose requirement. Transplantation. 2002;74(11):1486-9. https://doi.org/10.1097/00007890-200212150-00002.
26. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: Revised recist guideline (version 1.1). Eur J Cancer. 2009;45(2):228-47. https://doi.org/10.1016/j.ejca.2008.10.026.
27. Pal PK. Clinical and electrophysiological studies in vincristine induced neuropathy. Electromyogr Clin Neurophysiol. 1999;39(6):323-30.

This work is licensed under a Creative Commons Attribution-Non Commercial 4.0 International License.