SNP analysis in ATP-binding cassette, Solute carrier transporters and Thymidylate synthase genes in North Indian lung cancer patients

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in the partial fulfilment of the requirements for the award of degree of

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May, 2023

DEDICATED TO MY FATHER . . .

CERTIFICATE

Certified that the thesis entitled "**SNP analysis in ATP-binding cassette, Solute carrier transporters and Thymidylate synthase genes in North Indian lung cancer patients**" submitted by Ms. Parul Sharma, Reg. no. 901900001 in the partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy in the Department of Biotechnology, Thapar Institute of Engineering & Technology, Patiala, Punjab is a record of candidate's own independent and original research work carried out by herself under my supervision and guidance. The material embodied in this thesis has not been submitted in part or full to any other University or institute for the award of any degree.

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CANDIDATE'S DECLARATION

I hereby declare that the work being presented in the thesis entitled **"SNP analysis in ATP-binding cassette, Solute carrier transporters and Thymidylate synthase genes in North Indian lung cancer patients"** in partial fulfilment of the requirements for the award of degree of Doctor of Philosophy, Department of Biotechnology, Thapar Institute of Engineering & Technology, Patiala, Punjab is an authentic record of my work during the period from July 2019 to May 2023, under the conception and supervision of Dr. Siddharth Sharma, Associate Professor, Department of Biotechnology, Thapar Institute of Engineering & Technology, Patiala, Punjab. This report has not been submitted for the award of any other degree or certificate in this or any other university.

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6) **Sharma, P.,** Singh, N., & Sharma, S. (2021). ATP binding cassette transporters and cancer: Revisiting their controversial role. *Pharmacogenomics*, *22*(18), 1211-1235. Impact factor: 2.63
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9) Walia, H., **Sharma, P**., Singh, N., & Sharma, S. (2022). Predictive role of polymorphic variants of phase II drug metabolising enzyme in modulating toxicity in North Indian lung cancer patients undergoing chemotherapy. *Xenobiotica*, *52*(3), 322-331. Impact factor: 1.99.

CONFERENCES

1) Poster presentation in the "5th Edition of International Cancer Conference" Online Conference (September 16-17, 2022).
2) Oral presentation in the 8th International conference on selenium at Thapar Institute of engineering and technology, Patiala, Punjab (November, 2019).

Abstract:

Background: Lung carcinoma is the second most common type of cancer (accounting for 13% of all occurrences) and the leading cause of carcinoma fatalities. Drug transporters are well-known mediators of drug disposition that facilitate drug influx and efflux from cells. Polymorphisms in transporter genes and drug targets alters intracellular concentrations of drugs that determine the efficacy, toxicity, and clinical outcomes in lung cancer patients.

Objective: To evaluate the sequence variants of ABC (*ABCB1, ABCC1, ABCC2*, and *ABCG2*) and SLC (*SLC19A1, SLCO1B1,* and *SLCO1B3*) drug transporters and *Thymidylate synthase* gene (chemotherapeutic target) in North Indian population.

Methodology: Lung cancer patients undergoing platinum-based chemotherapy were recruited in the current study. Genotyping of *ABCB1 ($C^{1236}T$, $C^{3435}T$, and $G^{2677}T/A$), ABCC1 ($G^{317}3A$* and *$G^{2168}A$), ABCC2 ($G^{4544}A$), ABCG2 ($C^{421}A$), SLC19A1 ($G^{80}A$), SLCO1B1 ($A^{388}G$, $T^{521}C$), SLCO1B3 ($A^{1683\text{-}5676}G$), TS TSER (2R/3R),* and *TS 1494del6* polymorphisms were evaluated using polymerase chain reaction-restriction fragment length polymorphism analysis (PCR-RFLP). The univariate Kaplan-Meier method was used to assess overall survival in lung cancer patients. Cox regression analysis was employed to retrieve the adjusted hazard ratio. Stratified analysis was carried out to estimate risk for subgroups based on different clinicopathological parameters, clinical outcome, and toxicity by odds ratios (ORs) and 95% confidence intervals (CIs). In all analyses, a p-value of <0.05 was chosen to be significant.

Results: Poor survival outcomes were noted in patients carrying heterozygous genotype *(CT)* for *ABCB1 $C^{1236}T$* polymorphism as compared to the wild-type genotype *(CC)* (p=**0.04**)**.** Patients with ADCC harboring heterozygous (*CT)* genotype for *ABCB1 $C^{1236}T$* had lower survival as compared to the patients having wild type (*CC)* genotype (p=**0.03**). The patients administered with cisplatin/carboplatin and docetaxel and having both copies of the mutant allele (*TT)* for *ABCB1 $C^{1236}T$* polymorphism showed higher survival as compared to the wild-type genotype (*CC)* (p=**0.04).** The lung cancer subjects harboring heterozygous genotype (*CT)* for *ABCB1 $C^{1236}T$* and treated with the pemetrexed and cisplatin/carboplatin had substantially poor survival as compared to the patients with wild type *(CC)* genotype (p=**0.02).** The patients harboring heterozygous

genotype *(CT)* for *ABCB1 $C^{1236}T$* polymorphism had lower survival than those with wild-type genotype *(CC)* in stage IV lung cancer patients (p=**0.002**). For *ABCB1 $C^{3435}T$*, a drastic decrease in survival was observed in the ADCC patients having heterozygous genotype (*CT*) as compared to the wild-type genotype (*CC*) (p=**0.04**). For *ABCB1 $C^{3435}T$*, individuals who carried heterozygous *(CT)* (p=**0.03**) and mutant *(TT)* genotypes (p=**0.04**) had poor survival as compared to the wild-type *(CC)* genotype in patients treated with pemetrexed and cisplatin. SCLC patients with *ABCB1 $G^{2677}A$* polymorphism having heterozygous genotype (*GA)* showed poor survival as compared to the wild-type genotype *(GG)* (p=**0.03**).The patients administered with cisplatin and irinotecan and having mutant alleles *(AA)* for *ABCB1 $G^{2677}A$* polymorphism showed lower survival as compared to the individuals carrying wild-type alleles *(GG)* (p=**0.007**). The mutant genotype *(AA)* for *ABCC1 $G^{3173}A$* exhibited a lower survival time as compared to the reference genotype *(GG)* (p=**0.01**). For *ABCC1 $G^{3173}A$*, ADCC patients having mutant genotype *(AA)* had reduced survival as compared to the wild-type *(GG)* genotype (p=**0.001**). Lower survival was observed in individuals carrying heterozygous genotype *(GA)* for *ABCC2 $G^{4544}A$* polymorphism as compared to the wild-type genotype *(GG)* (p=**0.04**). Patients having SCLC and carrying heterozygous genotype (*GA)* for *ABCC2 $G^{4544}A$* polymorphism showed a poor survival time as compared to the wild-type genotype (*GG)* (p=**0.03**). The patients administered with cisplatin/carboplatin and pemetrexed and harboring heterozygous genotype for the *ABCC2 $G^{4544}A$* variant exhibited poorer survival than those carrying the wild-type genotype (p=**0.03).** ADCC patients harboring mutant genotype (*AA*) showed better survival outcomes as compared to the ADCC subjects with wild-type genotype (*GG*) for *SLC19A1 $G^{80}A$* (p=**0.04**). SCLC patients' with *SLC19A1 $G^{80}A$* polymorphism showed increased survival in patients with the mutant genotype (p=**0.04**). Patients with mutant genotype (*GG)* for *SLCO1B1 $A^{388}G$* polymorphism depicted lower survival as compared to the wild-type genotype (*AA)* (p=**0.008**). In *SLCO1B3 $A^{1683\text{-}5676}G$,* patients carrying heterozygous alleles and administered with platinum and docetaxel showed inferior survival (p=**0.006**). *TSER* polymorphism was associated with poor overall survival in lung cancer patients (p=**0.04**). Stage IV patients having mutant genotype (*3R3R*) for *TSER* polymorphism showed reduced survival as compared to the combined wild and heterozygous genotype (*2R2R+3R3R*) (p=**0.02**). Analysis of combined genotypes of *TS* polymorphism showed a better prognosis in subjects carrying both the wild and heterozygous alleles [(*2R3R)* + (*+6/-6)* and (*2R2R)* + (*+6/+6)]* as compared to patients having the homozygous variant alleles for both the genotypes (*3R3R+ -6/-6 bp*) (**p=0.04).**

Additionally, a substantial improvement was observed in patients' survival time with the genotypic combination of *2R2R* &-6/-6 (low TS expression) as compared to patients harboring the *3R3R* & *+6/+6bp* genotype (p=**0.04**). Patients harboring heterozygous *(GA)* genotype for *ABCC1* $G^{3173}A$ had an increased risk of developing anemia (AOR=2.70, p=**0.03**). For *ABCC2* $G^{4544}A$ polymorphism, patients harboring one copy of the mutant *(GA)* allele showed an increased risk of developing anemia (p=**0.03**). The *ABCB1* $G^{2677}A$ (OR=0.37, p=**0.008**) and *ABCC1* $G^{3173}A$ (OR=0.54, p=**0.04**) polymorphism showed a lower incidence of developing nephrotoxicity. For *ABCC1* $G^{3173}A$ polymorphism, a lower incidence of anorexia was observed in patients with one copy of the mutant allele (*GA)* as compared to patients having both copies of wild-type alleles (*GG)* (p=**0.04).** There was an increased risk of developing diarrhea in the patients with heterozygous genotype *(GA)* for *ABCC1* $G^{3173}A$ polymorphism (AOR=2.78, p=**0.04**). Also, patients harboring heterozygous (*CT)* (p=**0.015)** and mutant genotype (*AA)* (p=**0.02)** for *ABCB1* $C^{1236}T$ variant were associated with a reduced risk of having nausea/vomiting as compared to the wild type (*CC)* genotype. An increased risk of liver injury was noted in patients having heterozygous genotype for *ABCB1* $C^{1236}T$ as compared to the wild-type (*CC)* genotype *(*p=**0.04**). Patients with *SLCO1B1* $A^{388}G$ polymorphism harboring a single copy of mutant allele (*AG*) were associated with a lower incidence of thrombocytopenia when compared to the patients carrying wild-type alleles (*AA*) (p=**0.02**). Patients with *SLCO1B3* $A^{1683\text{-}5676}G$ variants showed a lower incidence of nephrotoxicity. For *SLCO1B1* $A^{388}G$, lung cancer patients with heterozygous genotype *(AG)* revealed a 3.87-fold increased risk of having constipation (p=**0.04**) as compared to the patients with wild-type genotype (*AA*). Patients with the mutant (*-6/-6)* genotype showed a 2.57-fold elevated risk of developing leukopenia (p=**0.03**). Lung cancer patients with mutant genotype (*-6/-6bp)* for *1494 ins/del6* polymorphism had a roughly 2-fold risk of developing anemia as compared to patients with wild genotype (*+6/+6bp)* (p=**0.04**). A substantial risk of 4.76-fold constipation was found in the individuals having heterozygous genotype *(2R3R)* for *TSER* polymorphism (p=**0.0016**). The combined mutant and heterozygous genotype (*3R3R+2R3R*) revealed a 4.09-fold elevated risk of having constipation (**p=0.02).** An increased risk of nausea/vomiting was observed in patients with mutant genotype *(-6/-6bp)* for *1494del6* polymorphism compared to patients with wild-type genotype *(+6/+6bp)* (p=**0.03**).

Conclusion: Survival analysis revealed that three variants (*$C^{1236}T$, $C^{3435}T$*, and *$G^{2677}A$*) of the *ABCB1* gene predicts survival of lung cancer patient. ADCC patients having polymorphism of *ABCC1 $G^{3173}A$* appear to be a potential prognostic marker as mutant genotype *(AA)* was associated with poor survival outcomes. Pemetrexed cisplatin/pemetrexed carboplatin therapy is associated with poor prognosis for lung cancer patients having *ABCC2 $G^{4544}A$* polymorphism.

The *SLC19A1 $G^{80}A$* polymorphism appears to be a potential prognostic factor for OS of SCLC and ADCC patients. *SLCO1B1 $A^{388}G$* was associated with poor survival outcomes in lung cancer patients. *SLCO1B3 $A^{1683\text{-}5676}G$* independently affects the OS of patients treated with docetaxel and cisplatin/carboplatin regimen. *TSER* polymorphism was associated with poor overall survival in lung cancer patients. Our results showed a strong association between *ABCC1 $G^{3173}A$* and *ABCG2 $G^{4544}A$* in causing anemia in lung cancer patients who underwent platinum-based doublet chemotherapy. We also report a correlation between *ABCB1 $C^{1236}T$, $C^{3435}T$,* and *ABCC1 $G^{3173}A$* polymorphism in predicting gastrointestinal toxicity.

Additionally, *ABCB1 $C^{1236}T$* polymorphism had a strong association in causing hepatic toxicity in lung cancer patients undergoing platinum-based doublet chemotherapy. *SLCO1B1 $A^{388}G$* and *SLCO1B3 $A^{1683\text{-}5676}G$* polymorphisms modify the associated adverse events. Patients with *TS* polymorphisms might impact the development of platinum-related toxicities such as hematological and gastrointestinal toxicity. This study in the future can facilitate the assessment of genetic variations of *ABC/SLC/TS* and help patients to make therapeutic decisions for individualized therapy in advanced lung cancer patients.

TABLE OF CONTENTS

Chapters	Title	Page No.
	Acknowledgment	***iii-v***
	List of publications ***List of conferences attended***	***vi-viii***
	Abstract	***ix-xii***
	Table of contents	
	List of figures	
	List of Tables	
	List of abbreviations	
	List of symbols	
1.	**Introduction**	1-8
2.	**Review of Literature**	9
2.1	Cancer	9
2.2	Lung and its anatomy	10-11
2.3	Lung carcinoma	
2.3.1	Global epidemiology of lung cancer	12-13
2.3.2	Indian epidemiology of lung cancer	14-15
2.4	Etiology of lung cancer	16
2.4.1	Tobacco Smoking	16
2.4.2	Tobacco Smoke Carcinogens	16
2.4.3	Beedi smoke carcinogens	17
2.4.4	Passive Smoking	18
2.4.5	Alcohol and Diet	19
2.4.6	Air pollution	19
2.4.7	Occupational exposure	19-20
2.5	Histopathology of lung cancer	20
2.5.1	Large cell carcinoma (LCC)	21
2.5.2	Adenocarcinoma (ADCC)	22
2.5.3	Squamous cell carcinoma (SQCC)	23
2.5.4	Small cell lung carcinoma (SCLC)	23

2.6	TNM classification	24-26
2.7	Molecular pathogenesis of lung cancer	27-41
2.8	Lung carcinoma diagnosis	41-44
2.9	Treatment of lung cancer	44-49
2.9.1	Platinum-based doublet chemotherapy	44
2.9.1.1	Pemetrexed along with cisplatin/carboplatin	44-45
2.9.1.2	Irinotecan along with cisplatin/carboplatin	45
2.9.1.3	Docetaxel along with cisplatin/carboplatin	46-47
2.9.2	Targeted therapy and immunotherapy	47
2.9.2.1	Immune Checkpoint inhibitors in carcinoma treatment	48-49
2.10	Drug transporters	49
2.11	ATP-Binding Cassette transporters	50-53
2.11.1	Location, expression, and functional role of ABC transporters	54-56
2.12	ATP-binding cassette sub-family B member 1 (ABCB1)	56
2.12.1	Genomic organization	56
2.12.2	Structure of ABCB1 protein	57-58
2.12.3	Expression of *ABCB1* gene	58
2.12.4	Gene regulation of *ABCB1* gene	59
2.12.5	Substrate specificity of *ABCB1* gene	60-61
2.13	ATP-binding cassette sub-family C members 1 and 2 (ABCC1, ABCC2)	62
2.13.1	Genomic organization	62-63
2.13.2	Structure of MRP1 and MRP2 protein	63-65
2.13.3	Localization and expression of *ABCC1* and *ABCC2* gene	65-66
2.13.4	Gene regulation of ABCC1 and ABCC2 gene	66-67
2.13.5	Substrate specificity of *ABCC1* and *ABCC2* gene	67-68
2.14	ATP-binding cassette sub-family G member 2	69
2.14.1	Genomic organization	69
2.14.2	Structure of BCRP protein	70-71
2.14.3	Localization and expression of *ABCG2* gene	71-72
2.14.4	Gene regulation of *ABCG2* gene	72-73
2.14.5	Substrate specificity of ABCG2 gene	73
2.15	Influence of ABC transporters on the intracellular distribution of drug	74
2.15.1	Drug absorption	74

2.15.2	Drug metabolism	75
2.15.3	Drug excretion	75-76
2.16	Role of ABC transporters in cancer	76-81
2.17	Impact of single nucleotide polymorphisms in the ABC genes on lung cancer	82-83
2.17.1	Association of ABC gene (*ABCB1, ABCC1/C2,* and *ABCG2*) polymorphism with prognosis in lung cancer patients	83-86
2.17.2	Association of ABC gene (*ABCB1, ABCC1/C2, and ABCG2*) polymorphism with clinical outcomes and response towards chemotherapy in lung cancer patients	86-88
2.17.3	Association of *ABC* gene polymorphism and toxicity	88-93
2.18	Solute Carrier Transporters	94-96
2.19	Location, expression, and functional role of SLC transporters	97
2.19.1	Solute carrier family 19, member 1	97
2.19.2	Genomic organization	97
2.19.3	Structure of RFC protein	98-99
2.19.4	Expression of *SLC19A1* gene	99
2.19.5	Gene regulation of *SLC19A1* gene	99-100
2.19.6	Substrate specificity of SLC19A1	100
2.20	Solute Carrier Organic Anion Transporter family members 1B1 and 1B3	101
2.20.1	Genomic Organization	102
2.20.2	Structure of OATP1B1 and OATP1B3	103-104
2.20.3	Gene regulation of *SLCO1B1* and *SLCO1B3*	104-105
2.20.4	Substrate specificity of SLCO1B1 and SLCO1B3	105-107
2.21	Role of SLC (*SLC19A1, SLCO1B1,* and *SLCO1B3*) transporters in cancer	108-110
2.22	Impact of single nucleotide polymorphisms in the *SLC19A1, SLCO1B1,* and *SLCO1B3* genes on lung cancer	111
2.22.1	Association of *SLC* genes *(SLC19A1, SLCO1B1,* and *SLCO1B3)* polymorphism with prognosis and clinical outcomes in lung cancer patients	112-114
2.22.2	Association of *SLC19A1, SLCO1B1, and SLCO1B3* gene polymorphism and toxicity in lung cancer	114-117
2.23	*Thymidylate synthase* gene	118
2.23.1	Genomic organization	119
2.23.2	Structure of TS	119-120
2.24	Role of Thymidylate synthase in cancer	121-122

2.25	Impact of single nucleotide polymorphisms in the *TS* gene on lung cancer	123-124
3	**Gaps in the study and objectives**	125
4	**Material and methods**	126-138
4.1	Study design	126-127
4.1.1	Chemotherapy regimen	127
4.1.2	Follow-up and tumor response assessment	128
4.2	DNA Extraction	
4.2.1	Genomic DNA extraction	128-129
4.2.2	Qualitative and quantitative estimation of genomic DNA	129
4.3	Genotyping of polymorphic sites of *TS, ABC,* and *SLC* genes	130-134
4.3.1	Genotyping of TS polymorphism by PCR-RFLP	130
4.3.2	Polyacrylamide gel electrophoresis (PAGE)	130
4.3.3	Ethidium bromide (EtBr) staining	131-133
4.3.4	Genotyping of *ABCB1, ABCC1, ABCC2,* and *ABCG2* polymorphism by PCR-RFLP	131-133
4.3.5	Genotyping of *SLC19A1, SLCO1B1, and SLCO1B3* polymorphism by PCR-RFLP	133-134
4.4	Statistical analysis	
4.4.1	Demographic analysis	134
4.4.2	Genotypic and allelic Frequency analysis	135
4.4.3	Overall survival (OS) and Hazard ratio analysis	135
4.4.4	Stratified analysis	135
4.4.5	Adverse events (AEs) analysis	136-137
4.4.6	Survival analysis trees analysis	138
5	**Results**	
5.1	Genomic DNA Isolation	139
5.2	PCR Amplification	139-140
5.3	Genotyping of *Thymidylate synthase (TS)* transporter gene	
5.3.1	Genotyping of *TS* variants	140-141
5.4	Statistical analysis	

5.4.1	Demographic characteristics of the study group for *TS* gene	141-143
5.4.2	Clinical features of lung carcinoma patients assessed for prognostic significance of *TS* gene	144-145
5.5	Epidemiological statistics of Northern Indian lung carcinoma patients	146
5.5.1	Regional distribution of lung carcinoma patients	146-147
5.5.2	Regional distribution of lung carcinoma patients	148-149
5.5.3	Distribution of lung carcinoma patients based on age	149-150
5.5.4	Distribution of lung carcinoma patients based on TNM staging	150-151
5.5.5	Distribution of lung carcinoma patients based on the tumor histology	151-152
5.5.6	Distribution of lung carcinoma patients based on performance status	152-153
5.5.7	Distribution of lung carcinoma patients based on platinum-based doublet chemotherapeutic regimen	153-154
5.6	Statistical analysis of *TS* gene	
5.6.1	Genotype Frequencies of *TS* Variants	154-155
5.7	Elucidation of overall survival (OS) of lung carcinoma patients with TS polymorphism	
5.7.1	Overall survival analysis for *TSER* and *1494 ins/del6* polymorphism in lung cancer patients	155-158
5.7.2	Association of *TS* polymorphism with histology and OS	158-162
5.7.3	Association of *TS* polymorphism with chemotherapeutic regimen and OS	163-165
5.7.4	Association of *TS* polymorphism with gender and OS	166-167
5.7.5	Association of *TS* polymorphism with OS based on smoking habits	168-170
5.7.6	Association of *TS* polymorphism with OS based on tumor stage	170-172
5.7.7	Association of *TS* polymorphism with OS based on performance status	172-174
5.7.8	Association of combined genotypes in *TS* polymorphism with OS	175-177
5.8	Impact of *TS* polymorphisms on clinical outcomes in lung cancer patients	
5.8.1	Association of *TS* polymorphism and chemotherapy response	178
5.8.2	Association of clinicopathological features *TS* polymorphic variants	179-180
5.9	Association between *TS* genotypes and toxicity	
5.9.1	Association between *TS* genotypes and hematological toxicity	181-184
5.9.2	Association between *TS* genotypes and nephrological toxicity	185-186
5.9.3	Association between *TS* genotypes and gastrointestinal toxicity	187-189
5.10	ATP-binding cassette (*ABC*) transporter genes	

5.10.1	Illustration of PCR Amplification	190
5.10.2	Genotyping of *ABCB1* variants	190-192
5.10.3	Genotyping of *ABCC1* variants	193
5.10.4	Genotyping of *ABCC2* variants	194
5.10.5	Genotyping of *ABCG2* variants	194-195
5.11	Statistical analysis of *ABC* genes	
5.11.1	Demographic characteristics of the study group for *ABCB1, ABCC1, ABCC2,* and *ABCG2* gene	195-197
5.11.2	Clinical features of lung carcinoma patients assessed for prognostic significance of different *ABC* genes	197-198
5.12	Statistical analysis of *ABCB1 gene*	
5.12.1	Genotypic and allelic frequencies of *ABCB1* polymorphic variants	199-200
5.13	Elucidation of overall survival (OS) of lung carcinoma patients with *ABCB1* polymorphism	
5.13.1	Elucidation of overall survival (OS) of lung carcinoma patients with *ABCB1* polymorphism	201-205
5.13.2	Association of *ABCB1* polymorphism with OS in lung cancer histological subtypes	206-212
5.13.3	Prognosis of lung cancer patients treated with various chemotherapy regimens	
5.13.3.1	Association of *ABCB1* polymorphisms on OS according to cisplatin/carboplatin and docetaxel regimen	212-214
5.13.3.2	Association of *ABCB1* polymorphisms on OS according to cisplatin/carboplatin and paclitaxel regimen	214-215
5.13.3.3	Association of *ABCB1* polymorphisms on OS according to cisplatin/carboplatin and pemetrexed regimen	216-218
5.13.3.4	Association of *ABCB1* polymorphisms on OS according to cisplatin/carboplatin and irinotecan regimen	218-220
5.13.4	Association of *ABCB1* polymorphism with gender and OS	221-223
5.13.5	Association of *ABCB1* polymorphism with OS based on smoking status	224-226
5.13.6	Association of *ABCB1* polymorphism with OS based on tumor stage	227-230
5.14	Impact of *ABCB1* polymorphisms on clinical outcomes in lung cancer patients	
5.14.1	Association of *ABCB1* polymorphisms with clinicopathological parameters	231-234
5.14.2	Correlation of *ABCB1* polymorphisms and their response toward chemotherapy	235-236
5.15	Statistical analysis for polymorphic sites of *ABCC1* gene	237
5.15.1	Genotypic and allelic frequencies of *ABCC1* polymorphic variants	237

5.16	Impact of *ABCC1* polymorphism on overall survival of lung carcinoma patients undergoing platinum-based doublet chemotherapy	
5.16.1	Overall survival analysis for *ABCC1* gene polymorphism	238-240
5.16.2	Association of *ABCC1* polymorphism with OS in lung cancer histological subtypes	240-243
5.16.3	Prognosis of lung cancer patients having *ABCC1* polymorphism and treated with various chemotherapy regimens	244-245
5.16.4	Association of *ABCC1* polymorphism and overall survival based on gender	246
5.16.5	Association of *ABCC1* polymorphism and overall survival based on tumor stage	247
5.16.6	Association of *ABCC1* polymorphism and overall survival based on smoking status	248
5.17	Role of *ABCC1* polymorphisms on clinical outcomes in lung cancer patients	
5.17.1	Correlation of *ABCC1* polymorphisms with clinicopathological parameters	249-250
5.17.2	Correlation of *ABCC1* polymorphisms with chemotherapeutic response	251
5.18	Statistical analysis of *ABCC2* gene	
5.18.1	Genotypic and allelic frequencies of *ABCC2* polymorphic variants	252
5.18.2	Elucidation of overall survival (OS) of lung carcinoma patients with *ABCC2* polymorphism	
5.18.2.1	Association of *ABCC2* polymorphism with a prognosis based on different parameters such as histology and regimen	252-256
5.18.2.2	Association of *ABCC2* polymorphism with a prognosis based on gender, smoking habits, and tumor stage	256-258
5.18.3	Role of *ABCC2* polymorphism on clinical outcomes in lung cancer patients	259-260
5.19	Statistical analysis of *ABCG2* gene	
5.19.1	Genotypic and allelic frequencies of *ABCG2* polymorphic variants	261
5.19.2	Elucidation of overall survival of lung carcinoma patients with *ABCG2* variation	
5.19.2.1	Association of *ABCG2* polymorphism with a prognosis based on different parameters	261-266
5.19.3	Role of ABCG2 polymorphism on clinical outcomes in lung cancer patients	266-268
5.20	Association of ABC transporter genes (*ABCB1, ABCC1, ABCC2, and ABCG2*) and toxicity	
5.20.1	Association between *ABC* polymorphisms and hematological toxicity	269-279
5.20.2	Association between *ABC* polymorphisms and nephrological toxicity	280-282
5.20.3	Association between *ABC* polymorphisms and gastrointestinal toxicity	283-287

5.20.4	Association between *ABC* polymorphisms and hepatotoxicity	288-290
5.21	Solute carrier (SLC) transporter genes	
5.21.1	Genotyping of *SLC19A1* gene	291
5.21.2	Genotyping of *SLCO1B1* gene	291-292
5.21.3	Genotyping of *SLCO1B3* gene	293
5.22	Patients characteristics and clinical predictors for SLC gene	293-295
5.23	Statistical analysis of *SLC19A1* gene	
5.23.1	Genotypic and genetic equilibrium for *SLC19A1* variants	296
5.24	Elucidation of overall survival analysis of *SLC19A1* variants	
5.24.1	Association of *SLC19A1* $G^{80}A$ polymorphism in predicting prognosis in LC patients	296-298
5.24.2	Association of *SLC19A1* polymorphism based on histological examination	299-301
5.24.3	Correlation of *SLC19A1* polymorphism with chemotherapy regimens and overall survival	302-303
5.24.4	Relationship of *SLC19A1* polymorphism with prognosis based on smoking habits	304
5.24.5	Relationship of *SLC19A1* polymorphism with prognosis based on tumor size	305
5.24.6	Relationship of *SLC19A1* polymorphism with prognosis based on ECOG performance status	306
5.24.7	Association of *SLC19A1* polymorphism and clinic-pathological parameters	307-308
5.25	Statistical analysis of *SLCO1B1* variants	
5.25.1	Genotypic and allelic distribution of *SLCO1B1* variants	309
5.26	Elucidation of *SLCO1B1* polymorphism in predicting overall survival in LC patients	
5.26.1	Correlation of *SLCO1B1* polymorphisms with prognosis	310-311
5.26.2	Association of *SLCO1B1* polymorphism with histology and overall survival	311-314
5.26.3	Association of *SLCO1B1* variants with OS in LC patients treated with different chemotherapeutic regimens	314-317
5.26.4	Association of *SLCO1B1* polymorphisms with gender and OS	317-319
5.26.5	Association of *SLCO1B1* polymorphisms with a prognosis based on tumor stage	319-322
5.26.6	Association of *SLCO1B1* polymorphisms with a prognosis based on smoking habits	323-324
5.26.7	Association of *SLCO1B1* genotype with tumor characteristics	325-326
5.26.8	Association of *SLCO1B1* polymorphism and chemotherapy response	327

5.27	Statistical analysis for the polymorphic site of the *SLCO1B3 gene*	
5.27.1	Genotypic and allelic frequencies of *SLCO1B3* polymorphic variant	328
5.28	Association of *SLCO1B3* polymorphism in predicting prognosis based on different parameters	328-332
5.28.1	Association of *SLCO1B3* polymorphism with clinical outcomes in lung carcinoma patients	333-334
5.29	Elucidation of different genotypic combinations of *SLC19A1, SLCO1B1, and SLCO1B3* genes with the prognosis of lung carcinoma patients	335-338
5.30	Association of *SLC* transporter genes (*SLC19A1, SLCO1B1, and SLCO1B3)* and toxicity	
5.30.1	Correlation of *SLC* gene polymorphisms and hematological toxicity	339-347
5.30.2	Association of *SLC* polymorphism with nephrotoxicity	348-349
5.30.3	Association of *SLC* polymorphisms and gastrointestinal toxicity	350-352
6	**Discussion**	
6.1	Thymidylate synthase	353
6.1.1	Prognostic implications of *TS* gene polymorphisms in patients with lung cancer	353-354
6.1.2	Mechanistic explanation of the impact of *TS* polymorphism on survival	354-355
6.1.3	Impact of combined genotypes in *TS* polymorphism with OS	355-356
6.1.4	Prognostic impact of *TS* polymorphism based on histology and regimen	356-357
6.2	Impact of *TS* polymorphisms in predicting toxicity in LC patients undergoing platinum-based doublet chemotherapy	
6.2.1	Association of *TS* polymorphisms with hematological toxicity	357-358
6.2.2	Association of *TS* polymorphisms with gastrointestinal toxicity	359
6.3	Prognostic implications of *ABC* gene polymorphisms in patients with lung cancer	
6.3.1	Impact of *ABCB1 ($C^{1236}T$, $C^{3435}T$, and $G^{2677}A/T$)* polymorphisms in predicting overall survival	360-361
6.32	Impact of *ABCC1 ($G^{3173}A$ and $G^{2168}A$)* polymorphisms in predicting overall survival	361
6.3.3	Association of *ABCC2 $G^{4544}A$* polymorphisms in predicting overall survival	361-362
6.3.4	Association of *ABCG2 $G^{4544}A$* polymorphisms in predicting overall survival	362
6.3.5	Correlation of *ABC* gene polymorphism and prognosis based on histology and regimen	362-364
6.4	Genetic polymorphisms of *ABC* gene and the toxicity of chemotherapy treatment in lung cancer patients	

6.4.1	*ABC* gene polymorphisms as a determinant of hematological toxicity	364-365
6.4.2	Correlation of *ABC* gene polymorphisms in predicting nephrotoxicity	365-366
6.4.3	Correlation of *ABC* gene polymorphism with gastrointestinal toxicity of platinum-based chemotherapy in lung cancer patients	366-367
6.4.4	Association of *ABC* gene polymorphism with drug induced-hepatic toxicity	367
6.5	Prognostic implications of *SLC* gene polymorphisms in patients with lung cancer	
6.5.1	Impact of *SLC19A1* $G^{80}A$ polymorphisms in predicting overall survival	368
6.5.2	Impact of *SLCO1B1* gene polymorphisms in predicting overall survival	368-369
6.5.3	Correlation of *SLC* polymorphism in predicting survival based on histology	370
6.5.4	Correlation of *SLC* polymorphism in predicting survival based on regimen stratification	370-371
6.6	Role of *SLC* polymorphism in predicting toxicity in LC patients undergoing platinum-based doublet chemotherapy	
6.6.1	Correlation of *SLC* polymorphisms with hematological toxicity	371
6.6.2	Correlation of *SLC* polymorphisms with nephrological toxicity	371-372
6.6.3	Correlation of *SLC* polymorphisms with gastrointestinal toxicity	372
7	**Conclusion**	373-378
8	**Bibliography**	379-422
	Appendix-I	423-425
	Appendix-II	426-428
	Appendix-III	429-431
	Appendix-IV	432

LIST OF FIGURES

Figure No.	Title	Page No.
Review of Literature		
2.1	Schematic representation of tumour development stages and metastatic mechanisms	9
2.2	A labelled representation of the human lung	11
2.3	Statistical representation of new Cancer Incidence and Mortality rates – Estimates for 2021	13
2.4	Age-standardized prevalence and fatality rates of lung carcinoma in India compared to surrounding nations	15
2.5	An illustration depicting the risk factors associated with causing lung cancer.	18
2.6	Histological representation of NSCLC and SCLC	20
2.7	A histological representation of NSCLC subtypes (a) SQCC (b) ADCC (c) LCC	21
2.8	Microscopic depiction of various histological subtypes of lung tumour, (A): Large cell carcinoma; (B) Adenocarcinoma; (C) Squamous cell carcinoma; (D) Small cell lung cancer	24
2.9	A depiction summarizing molecular pathogenesis of lung cancer	31
2.10	Basic molecular alterations involved in the aetiology of lung cancer	36
2.11	Illustration depicting the expression of ABC and SLC transporters in various human organs	50
2.12	Models of secondary structures of ABC efflux transporters. NBD: nucleotide-binding domain; TMD: transmembrane domain. The cytoplasmic and extracellular compartments are "in" and "out," respectively. (B) The fundamental elements of NBD are arranged in a linear pattern with residues	52
2.13	Role of ATP-binding cassette transporter in substrate transport mechanism (A) Ligand binding to the TMD pocket changes the configuration of the NBD region. (B) The two ATP molecules bind in the NBD area. (C) Energy is released by the formation of a closed NBD dimer, which causes a conformational shift in the TMD region. (D) ATP hydrolysis causes the closed NBD dimer to dissociate, causing a conformational shift in the TMD region. Ultimately, ADP and phosphate are released, allowing the ABC transporter to reopen the NBD dimer and continue the cycle	53
2.14	Chromosome 7 depicting the localization of the *ABCB1* gene on it	57
2.15	The structure of human P-gp/ABCB1 (outward-facing conformation, ATP-bound). (A) The interaction of Q loops in the NBD dimer (indicated in blue) with Mg^{2+}/ATP (shown in magenta and yellow) and the TMDs via the two intracellular helices IH2 and IH4 (shown in orange) (B) Molecular connections for each ATPase site, along with Mg^{2+}-ATP density (depicted in green mesh)	58
2.16	Chromosome 16 depicting the localization of *ABCC1* gene	62

2.17	Chromosome 10 depicting the localization of *ABCC2* gene on it	63
2.18	An illustration showing a 2-dimensional depiction of the domain structure of MRP1 (B): Depiction of a homology model of the core human MRP1 protein	64
2.19	Structure of MRP2 protein generated by *in silico* methods	65
2.20	Mechanism illustrating the uptake of endogenous substances, drugs, or carcinogens, biotransformation, and exportation through MRP	68
2.21	Chromosome 4 depicting the localization of the ABCG2 gene on it	69
2.22	An illustration depicting the structure of ABCG2 (A) A ribbon diagram of *ABCG2* complexed with 5D3-Fab. The monomers in the ABCG2 structure are demarcated as G2 and G2'. (B) Depiction of the topology of ABCG2; the intra and intermolecular disulfide bonds are formed by cysteines (C592, C603, &C608) so as is the N-glycosylated residue	71
2.23	Diagrammatic Illustration showing SLC and non-SLC transporters in the plasma membrane or within intracellular compartments	94
2.24	Localization of *SLC19A1* on chromosome 21	97
2.25	Human RFC model; (A) A topological representation of human RFC protein consisting of 12 transmembrane domains, linker domain (joining TMD 6 and 7, and internal N- & C- termini. Yellow circles represent a conserved stretch of the amino acids necessary for translocation activity. Red circles represent functionally significant amino acids. (B) A side-view of a 3-dimensional homology model of the human RFC	98
2.26	Localization of (A) SLCO1B1 on chromosome (B) SLCO1B3 on chromosome 12	102
2.27	Structural modelling of (A) OATP1B1 protein and (B) OATP1B3 protein	104
2.28	Anticancer drugs as substrates for OATP1B1 and OATP1B3	106
2.29	An illustration of the Thymidylate synthase pathway	118
2.30	Localization of Thymidylate synthase gene on chromosome 18	119
2.31	The structure of human Thymidylate synthase in active form (Active state of human TS is shown in cyan, with loops 181–198 (blue) and loops 107–128 (green). The inactive form of human TS is depicted in pink color with loops 181–197 (red)	120

Results

5.1	0.8% agarose gel illustrating the isolated genomic DNA from the blood samples of patients with lung cancer	139
5.2	Illustration demonstrating agarose gel electrophoresis of the PCR amplification product	140
5.3	*TS* variants polymorphisms (A) 6.0% Native-PAGE of PCR products for *TSER* polymorphism Wild genotype (*2R2R*): 215 bp; Mutant genotype (*3R3R*): 243 bp; Heterozygous genotype (*2R3R*): 215 bp, 243bp. (B) 6.0% Native-PAGE of PCR products after digestion with *DraI* for TS *1494del6* polymorphism Wild genotype (+6/+6bp):80,70bp; Mutant genotype (-6/-6bp): 152 bp; Heterozygous genotype (+6/-6bp): 158bp, 88bp, and 70bp	141

5.4	The regional distribution of Northern Indian lung carcinoma patients	146
5.5	Regional distribution of Northern Indian Lung carcinoma patients based on gender (a) Males (b) Females	147
5.6	Distribution of Northern Indian Lung carcinoma patients based on smoking status	148
5.7	Graph illustrating the distribution of male and female study subjects based on smoking status	149
5.8	Graph illustrating the distribution of lung cancer patients based on age	150
5.9	Graph illustrating the distribution of lung cancer patients based on tumor stage in North Indians	151
5.10	Distribution of Northern Indian Lung carcinoma patients based on histology	152
5.11	Distribution of Northern Indian Lung carcinoma patients based on ECOG performance status	152
5.12	Distribution of Northern Indian lung carcinoma patients based on KPS score	153
5.13	Distribution of Northern Indian lung carcinoma patients based on the chemotherapeutic regimen	154
5.14	Kaplan Meier curves illustrating the association between overall survival in different genotypes of *TSER* polymorphism in lung cancer patients.	156
5.15	Kaplan Meier curves illustrating the association between overall survival in different genotypes of *TSER* polymorphism in males with lung cancer	166
5.16	Kaplan Meier curves illustrating the association between overall survival in different genotypes of *TSER* polymorphism in Stage IV lung cancer patients.	172
5.17	Kaplan Meier curves illustrating the association between overall survival in different genotypes of *TSER* polymorphism in ECOG-PS lung cancer patients.	173
5.18	Kaplan Meier curves illustrating the combinatorial analysis of the *TS* SNPs on overall survival (recessive model) in lung cancer patients.	175
5.19	Kaplan Meier curves illustrating the combinatorial analysis of the *TS* SNPs on overall survival (recessive model) in lung cancer patients.	176
5.20	Illustration demonstrating agarose gel electrophoresis of the PCR amplification product	190
5.21	Restriction digestion pattern of *ABCB1* variants (A) $C^{1236}T$ polymorphism Wild type (*CC*): 269,97bp; Mutant genotype (*TT)*: 269,32, 35bp (the 35bp was too small to resolve accurately); Heterozygous genotype (*CT)*: 269,97,62,35 bp. (B) *C3435T* polymorphism Wild type (*CC)*: 130,76bp; Mutant genotype (*TT)*: 206 bp; Heterozygous genotype (*CT)*: 206,10,76 bp. (C) $G^{2677}T$ polymorphism Mutant genotype (*TT)*: 224 bp; Heterozygous genotype (*GT)*: 224,198 bp. (D) $G^{2677}A$ polymorphism Wild genotype (*GG*): 220 bp; Heterozygous genotype (*GA*): 220,206 bp.	192
5.22	Restriction digestion pattern of *ABCC1* variants (A) $G^{3173}A$ polymorphism Wild type (*GG*): 300,44bp (the 44 bp was too small to resolve accurately); Mutant genotype (*AA)*: 344bp; Heterozygous genotype (*GA)*: 344, 300, 44 bp. B) $G^{2168}A$ polymorphism Wild type (*GG*): 132, 25bp (the 25 bp was too small to resolve	193

accurately); Mutant genotype (*AA)*: 157 bp; Heterozygous genotype (*GA)*: 157, 132, 25 bp.

5.23 Restriction digestion pattern of *ABCC2* $G^{4544}A$ polymorphism Wild type (*GG*): 307,71, 22 bp (the 22bp was too small to resolve accurately); Mutant genotype (*AA)*: 196, 111, 71, 22bp; Heterozygous genotype (*GA)*: 307, 196, 111, 71, 22 bp. 194

5.24 Restriction digestion pattern of *ABCG2* $C^{421}A$ polymorphism Wild type (*CC*): 320bp (generated from reverse primer 1 in one tube); Mutant genotype (*AA)*:320bp (generated from reverse primer 2 in one tube); Heterozygous genotype (*CA)*: 320 bp (generated from both reverse primer 1 and 2 in two distinct tubes). 195

5.25 Kaplan Meier curves illustrating the association between overall survival in different genotypes of (a) *ABCB1 C1236T* polymorphism in lung cancer patients and (b) *ABCB1 C3435T* polymorphism in lung cancer patients 204

5.26 Kaplan Meier curves illustrating the association between overall survival in different genotypes of (a) *ABCB1* $G^{2677}A$ polymorphism in lung cancer patients and (b) *ABCB1* $G^{2677}T$ polymorphism in lung cancer patients 205

5.27 Kaplan Meier curves illustrating the association between overall survival in different genotypes of (A) *ABCB1 C1236T* polymorphism in ADCC patients and (B) *ABCB1 C3435T* polymorphism in ADCC patients 207

5.28 Kaplan Meier curves illustrating the association between overall survival in different genotypes of *ABCB1 G2677A* polymorphism in SCLC patients 212

5.29 Kaplan Meier curves illustrating the association between overall survival in different genotypes of *ABCB1* $C^{1236}T$ polymorphism in lung cancer patients undergoing cisplatin/ carboplatin and docetaxel chemotherapy 213

5.30 (A) Kaplan Meier curves illustrating the association between overall survival in different genotypes of *ABCB1* $C^{1236}T$ polymorphism in lung cancer patients undergoing cisplatin/ carboplatin and pemetrexed chemotherapy 216

5.30 (B) Kaplan Meier curves illustrating the association between overall survival in different genotypes of *ABCB1* $C^{3435}T$ polymorphism in lung cancer patients undergoing cisplatin/carboplatin and pemetrexed chemotherapy. 217

5.31 Kaplan Meier curves illustrating the association between overall survival in different genotypes of *ABCB1* $G^{2677}A$ polymorphism in lung cancer patients undergoing cisplatin/ carboplatin and irinotecan chemotherapy 219

5.32 Kaplan Meier curves illustrating the association between overall survival in different genotypes of *ABCB1* $C^{3435}T$ polymorphism in females having lung cancer 221

5.33 Kaplan Meier curves illustrating the association between overall survival in different genotypes of *ABCB1* $G^{2677}A$ polymorphism in stage III lung cancer patients 227

5.34 Kaplan Meier curves illustrating the association between overall survival in different genotypes of *ABCB1* $C^{1236}T$ polymorphism in stage IV lung cancer patients 228

5.35 Kaplan Meier curves illustrating the association between overall survival in different genotypes of *ABCC1* $G^{3173}A$ polymorphism in lung cancer patients 238

5.36	Kaplan Meier curves illustrating the association between overall survival in different genotypes of *ABCC1* $G^{3173}A$ polymorphism in ADCC patients	241
5.37	Kaplan Meier curves illustrating the association between overall survival in different genotypes of *ABCC2 G4544A* polymorphism in lung cancer patients	254
5.38	Kaplan Meier curves illustrating the association between overall survival in different genotypes of *ABCC2 G4544A* polymorphism in lung cancer patients undergoing cisplatin/carboplatin and pemetrexed therapy	256
5.39	Restriction digestion pattern of *SLC19A1* $G^{80}A$ polymorphism, Wild type (*GG*): 125,68, 37 bp (the 37bp was too small to resolve accurately); Mutant genotype (*AA)*: 162, 68 bp; Heterozygous genotype (*GA)*: 162,125,68,37 bp	291
5.40	Restriction digestion pattern of *SLCO1B1* polymorphisms (A) 6% Native-PAGE of PCR products for $A^{388}G$ polymorphism, Wild type (*AA*): 151, 63 bp; Mutant genotype (*GG)*: 128, 63, 23 bp (the 23 bp was too small to resolve accurately); Heterozygous genotype (*AG)*: 151,128,63,23 bp. (B) 6% Native-PAGE of PCR products for $T^{521}C$ polymorphism, Wild type (*TT*): 209 bp; Mutant genotype (*CC)*: 189 and 20 bp (the 20bp was too small to resolve accurately); Heterozygous genotype (*TC)*: 209, 189, and 20 bp.	292
5.41	Restriction digestion pattern of *SLCO1B3* $A^{1683\text{-}5676}G$ polymorphism, Wild type (*AA*): 262 bp; Mutant genotype (*GG)*: 155, 107, 24 bp (the 24 bp was too small to resolve accurately); Heterozygous genotype (*AG)*: 262,155,107,24 bp.	293
5.42	Kaplan Meier curves illustrating the association between overall survival in different genotypes of *SLC19A1* ($G^{80}A$) polymorphism in ADCC patients	299
5.43	Kaplan Meier curves illustrating the association between overall survival in different genotypes of *SLC19A1* ($G^{80}A$) polymorphism in SCLC patients	300
5.44	Kaplan Meier curves illustrating the association between overall survival in different genotypes of *SLCO1B1* ($A^{388}G$) polymorphism in lung cancer patients	310
5.45	Kaplan Meier curves illustrating the association between overall survival in different genotypes of *SLCO1B1* ($A^{388}G$) polymorphism in male lung cancer patients	318
5.46	Kaplan Meier curves illustrating the association between overall survival in different genotypes of *SLCO1B1* ($A^{388}G$) polymorphism in stage III lung cancer patients	320
5.47	Kaplan Meier curves illustrating the association between overall survival in different genotypes of *SLCO1B1* ($A^{388}G$) polymorphism in stage IV lung cancer patients	321
5.48	Kaplan Meier curves illustrating the association between overall survival in different genotypes of *SLCO1B3* ($A^{1683\text{-}5676}G$) polymorphism in patients administered with docetaxel cisplatin/carboplatin	330
5.49	Recursive partitioning method (STREE) explaining the correlation of *SLC* SNPs with overall survival in lung carcinoma patients	336
5.50	Survival according to different nodes for SLC genes identified by survival tree analysis	337

Discussion		
6.1	Mechanism of the *Thymidylate synthase* inhibition by chemotherapeutic drugs and its impact on *TS* polymorphisms	355
6.2	Impact of SLC transporter polymorphisms in predicting overall survival	369

LIST OF TABLES

Table No.	Tables	Page No.
Review of Literature		
2.1	TNM stage classification (8th edition)	25
2.2	Definition of tumour stage, lymph node invasion, and metastasis	26
2.3	Chromosome localization and associated physiological functions of the members of ABC transporters	55
2.4	List of various substrates, inhibitors, and inducers of P-gp	61
2.5	A list of the primary ABC transporters expressed in various cancers	81
2.6	Features of the study included in the meta-analysis	86
2.7	Highlighting critical studies on various *ABCB1, ABCC1/C2,* and *ABCG2* polymorphisms in lung cancer patients	91-93
2.8	A summary of all SLC genes that are currently known	95-96
2.9	Overview of selected substrates of SLCO1B1 and SLCO1B3	107
2.10	List of relevant SLC transporters in different tumour types	110
2.11	Salient features of the critical study from different publications chosen for meta-analysis	116
2.12	Highlighting critical studies on TS expression in different cancer types	122
2.13	Summarizing critical studies on TS polymorphism in lung cancer patients	124
Materials and Methods		
4.1	List of primers and restriction enzymes used for *TS* genetic variants	131
4.2	List of primers and restriction enzymes used for *ABCB1, ABCC1, ABCC2, and ABCG2* genetic variants	133
4.3	List of primers and restriction enzymes used for *SLC19A1, SLCO1B1, and SLCO1B3* genetic variants	134
4.4	Summarizing adverse events grading for hematological, gastrointestinal, and nephrotoxicity	137
Results		
5.1	Demographic characteristics among case group	143
5.2	Patients characteristics and clinical features with overall survival	145
5.3	Genotypic and allelic frequencies of *TS* polymorphism in lung carcinoma patients	155
5.4	Univariate and Multivariate analysis for *TS* variants	157
5.5	Cox proportional hazards regression model analysis of overall survival in lung cancer subjects	158
5.6	Association of *TS* SNPs on overall survival according to histology	160-162
5.7	Association of *TS* SNPs on overall survival based on the regimen	164-165

5.8	Association of *TS* SNPs on overall survival based on gender	167
5.9	Association of *TS* SNPs on overall survival based on smoking status	169-170
5.10	Association of *TS* SNPs on overall survival based on tumor stage	171
5.11	Association of *TS* SNPs on overall survival based on performance status	174
5.12	Combinatorial analysis of the *TS* SNPs on overall survival	177
5.13	Association of *TS* SNPs and chemotherapeutic response	178
5.14	Relationship of different *TS* polymorphic genotypes with clinic-pathological parameters	180
5.15	Association of haematological toxicity (grade 0 vs. 1-4, grade 0-1 vs. 2-4, and grade 0-2 vs. 3-4) with *TSER* and *1494del6* polymorphism	183-184
5.16	Association of TS polymorphism with nephrotoxicity toxicity	186
5.17	Association of gastrointestinal toxicity (grade 0 vs. 1-4, grade 0-1 vs. 2-4, and grade 0-2 vs. 3-4) with *TSER* and *1494del6* polymorphism	188-189
5.18	Demographic characteristics among case group recruited to study ABC polymorphisms	196
5.19	Demographic characteristics and clinical features with overall survival	198
5.20	Genotypic and allelic distribution of *ABCB*1 genetic polymorphism in lung cancer patients	200
5.21	Univariate and Multivariate analysis for *ABCB1* variants	202
5.22	Cox proportional hazards regression model analysis of overall survival in lung cancer subjects	203
5.23 (a)	Association of *ABCB1* SNPs on overall survival according to ADCC histology	208
5.23 (b)	Association of *ABCB1* SNPs on overall survival according to SQCC histology	210
5.23 (c)	Association of *ABCB1* SNPs on overall survival according to SCLC histology	211
5.24 (a)	Association of *ABCB1* SNPs on overall survival according to Cisplatin/carboplatin + docetaxel regimen	214
5.24 (b)	Association of *ABCB1* SNPs on overall survival according to Cisplatin/carboplatin + paclitaxel regimen	215
5.24 (c)	Association of *ABCB1* SNPs on overall survival according to Cisplatin/carboplatin + pemetrexed regimen	218
5.24 (d)	Association of *ABCB1* SNPs on overall survival according to Cisplatin/carboplatin + Irinotecan regimen	220
5.25	Association of *ABCB1* SNPs on overall survival according to gender	222-223
5.26	Association of *ABCB1* SNPs on overall survival according to smoking habits	225-226
5.27	Association of *ABCB1 SNPs* on overall survival according to tumor stage	229-230
5.28	Relationship of different ABCB1 polymorphic genotypes with clinic-pathological parameters	233-234
5.29	Association of *ABCB1* SNPs and chemotherapeutic response	235

5.30	Genotypic and allelic distribution of *ABCC1* genetic polymorphism in lung cancer patients	237
5.31	Association of *ABCC1* polymorphism in predicting overall survival in lung cancer patients	239
5.32	Cox proportional hazards regression model analysis of overall survival in lung cancer subjects	240
5.33	Association of *ABCC1* SNPs on overall survival according to histology	242-243
5.34	Association of *ABCC1* SNPs on overall survival according to the regimen	245
5.35	Association of *ABCC1* SNPs on overall survival according to gender	246
5.36	Association of *ABCC1* polymorphism in predicting overall survival in lung cancer patients based on tumor stage	247
5.37	Association of *ABCC1* polymorphism in predicting overall survival in lung cancer patients based on smoking habits	248
5.38	Relationship of different *ABCC1* polymorphic genotypes with clinic-pathological parameters	250
5.39	Association of *ABCC1* SNPs and chemotherapeutic response	251
5.40	Genotypic and allelic distribution of *ABCC2* genetic polymorphism in lung cancer patients	252
5.41	Association of *ABCC2* genetic polymorphism in predicting prognosis in lung cancer patients when stratified based on different parameters	253
5.42	Cox proportional hazards regression model analysis of overall survival in lung cancer subjects	255
5.43	Association of *ABCC2* genetic polymorphism in predicting prognosis in lung cancer patients when stratified based on gender, smoking habits, and tumor stage	258
5.44	Association of *ABCC2 SNPs* and clinical outcomes	260
5.45	Genotypic and allelic distribution of *ABCG2* genetic polymorphism in lung cancer patients	261
5.46	Association of *ABCG2* genetic polymorphism in predicting prognosis in lung cancer patients when stratified based on different parameters	262-264
5.47	Association of *ABCG2 SNPs* and clinical outcomes	268
5.48	Hematological and nephrotoxicity with CTC grades in lung cancer patients	269
5.49 a)	Leukopenia-Haematological toxicity (grade 0 vs. 1-4, grade 0-1 vs. 2-4, and grade 0-2 vs. 3-4) associated with *ABCB1, ABCC1, ABCC2,* and *ABCG2* polymorphism	271-272
5.49 b)	Anemia-Haematological toxicity(grade 0 vs. 1-4, grade 0-1 vs. 2-4, and grade 0-2 vs. 3-4) associated with *ABCB1, ABCC1, ABCC2,* and *ABCG2* polymorphism	274-276
5.49 c)	Neutropenia-Haematological toxicity (grade 0 vs. 1-4, grade 0-1 vs. 2-4) associated with *ABCB1, ABCC1, ABCC2,* and *ABCG2* polymorphism	277-278
5.49 d)	Thrombocytopenia-Haematological toxicity (grade 0 vs. 1-4) associated with *ABCB1, ABCC1, ABCC2,* and *ABCG2* polymorphism	279

5.50	Nephrological toxicity (grade 0 vs. 1-4 and grade 0-1 vs. 2-4) associated with *ABCB1, ABCC1, ABCC2*, and *ABCG2* polymorphism	281-282
5.51	Gastrointestinal toxicity with CTC grades in lung cancer patients	283
5.52	Gastrointestinal toxicity (grade 0 vs. 1-4 and grade 0-1 vs. 2-4) associated with *ABCB1, ABCC1, ABCC2*, and *ABCG2* polymorphism	285-287
5.53	Hepatotoxicity with CTC grades in lung cancer patients	288
5.54	Hepato-toxicity (grade 0 vs. 1-4 and grade 0-1 vs. 2-4) associated with *ABCB1, ABCC1, ABCC2*, and *ABCG2* polymorphism	289-290
5.55	Patient characteristics among case group recruited to study SLC polymorphisms	295
5.56	Genotypic and allelic distribution of *SLC19A1* genetic polymorphism in lung cancer patients	296
5.57	Correlation of SLC*19A1* polymorphism in predicting overall survival in lung cancer patients	298
5.58	Correlation of *SLC19A1* polymorphism based on histological examination	301
5.59	Association of *SLC19A1* SNP on overall survival according to Chemotherapeutic regimen	303
5.60	Association of *SLC19A1* SNP on overall survival according to smoking habits, tumor stage, and performance status	304
5.61	Association of *SLC19A1* polymorphism with overall survival based on tumor size	305
5.62	Association of *SLC19A1* polymorphism with overall survival based on ECOG-PS	306
5.63	Association of *SLC19A1* SNPs and chemotherapeutic response	308
5.64	Genotypic and allelic distribution of *SLCO1B1* variants	309
5.65	Association of *SLCO1B1* polymorphism and overall survival in lung cancer patients	311
5.66	Association of *SLCO1B1* polymorphisms and prognosis in lung cancer patients based on histological subtypes	313-314
5.67	Association of *SLCO1B1* SNPs on overall survival according to chemotherapeutic regimen	316-317
5.68	Association of *SLCO1B1* SNPs on overall survival based on gender	319
5.69	Association of *SLCO1B1* SNPs on overall survival based on tumor stage	322
5.70	Association of *SLCO1B1* SNPs on overall survival based on smoking habits	324
5.71	Association of *SLCO1B1* polymorphism based on clinicopathological parameters	326
5.72	Association of *SLCO1B1* SNPs and chemotherapeutic response	327
5.73	Genotypic and allelic distribution of *SLCO1B3* variants	328
5.74	Association of *SLCO1B3* polymorphism in predicting prognosis based on various parameters	331-332
5.75	Association of SLCO1B3 polymorphism based on clinical outcomes	334
5.76	Overall survival in *SLC* SNPs by applying STREE	338
5.77	Hematological with CTC grades in lung cancer patients	339

5.78 (a)	Leukopenia-haematological toxicity (grades 0 vs, 1-4; grades 0-1 vs 2-4) associated with *SLC* polymorphisms	341
5.78 (b)	Anemia-Haematological toxicity (grades 0 vs, 1-4; grades 0-1 vs 2-4, and grades 0-2 vs. 3-4) associated with *SLC* polymorphisms	343-344
5.78 (c)	Neutropenia-haematological toxicity (grades 0 vs. 1-4; grades 0-1 vs. 2-4) associated with *SLC* polymorphisms	345-346
5.78 (d)	Thrombocytopenia-haematological toxicity (grades 0 vs. 1-4; grades 0-1 vs. 2-4) associated with *SLC* polymorphisms	347
5.79	Nephrological toxicity (grades 0 vs, 1-4; grades 0-1 vs 2-4) associated with *SLC* polymorphisms	348-349
5.80	Gastrointestinal toxicity with CTC grades in lung cancer patients	350
5.81	Gastrointestinal toxicity (grades 1 vs. 2-4; grades 1-2 vs. 3-4) associated with *SLC* polymorphisms	351-352

List of abbreviations

Abbreviated form	Full form
ABC	ATP Binding cassette
SLC	Solute carrier transporter
TS	Thymidylate synthase
MDR	Multidrug resistance
P-gp	P-glycoprotein
MRP	Multidrug resistance-associated protein
BCRP	Breast cancer resistance protein
NBDs	Nucleotide binding domains
TMDs	Transmembrane domains
NSCLC	Non-Small Cell Lung Cancer
SNP	Single Nucleotide Polymorphism
UTR	Untranslated region
dUMP	Deoxyuridine monophosphate
dTMP	Deoxythymidine monophosphate
IASLC	International Association for the Study of Lung Cancer
DNA	Deoxyribonucleic acid
PET	Positron Emission Tomography
CT	Computed Tomography
TNM	Tumour Node and Metastasis
IARC	International Agency For Research On Cancer
ICMR	Indian Council Of Medical Research
PBCR	Population Based Cancer Registry
PAH	Polycyclic Aromatic Hydrocarbon
SCLC	Small Cell Lung Cancer
LCC	Large Cell Carcinoma
ADCC	Adenocarcinoma
SQCC	Squamous Cell Carcinoma
Rb	Retinoblastoma
EGFR	Epidermal Growth Factor Receptor
PIK3CA	Phosphatidylinositol 3-Kinase, Catalytic, Alpha
mTOR	Mammalian target of rapamycin

RAF	Rapidly Accelerated Fibrosarcoma
MAPK	Mitogen-Activated Protein Kinase
ATP	Adenosine Triphosphate
TKI	Tyrosine Kinase Inhibitor
PTEN	Phosphatase Tensin
TGF-β	Transforming Growth Factor
E2F	Elongation factor
BCL-2	B-Cell Lymphoma-2
PIP3	Phosphatidylinositol (3,4,5)-Trisphosphate
CR	Complete Response
PR	Partial Response
SD	Stable Disease
PD	Progressive Disease
EDTA	Ethylenediaminetetraacetic acid
TBE	Tris/Borate/EDTA
TE	Tris-EDTA buffer
RBC	Red Blood Cells
HR’	Adjusted Hazards Ratio
HR	Hazards Ratio
OR	Odds Ratio
AOR	Adjusted Odds Ratio
C.I	Confidence Interval
PGIMER	Post Graduate Institute of Medical Education and Research
PCR	Polymerase Chain Reaction
RFLP	Restriction Fragment Length Polymorphism
OD	Optical Density
dNTPs	Deoxyribose Nucleoside triphosphates
BSA	Bovine Serum Albumin
NEB	New England biolab
PAGE	Polyacrylamide Gel Electrophoresis
APS	Ammonium Persulfate
TEMED	N,N,N’.N’ Tetramethylethylene-1,2-Diamine
HWE	Hardy Weinberg equilivrium
MST	Median survival time

OS	Overall Survival
STREE	Survival tree
WHO	World Health Organization
W	Wild
H	Heterozygous
M	Mutant
Pro	Proline
Ser	Serine
Arg	Arginine
His	Histidine
Tyr	Tyrosine
Leu	Leucine
Gln	Glutamine
Lys	Lysine
Gly	Glycine
Ala	Alanine
Val	Valine
Glu	Glutamate
Ile	Isoleucine
AEs	Adverse events
ANC	Absolute neutrophil count
MAF	Minor Allele Frequency
RECIST	Response Evaluation Criteria In Solid Tumors
KPS	Karnofsky Performance Status
ECOG	Eastern Cooperative Oncology Group
WBC	White blood cells

List of Symbols

Symbol	
%	Percentage
<	Less than
≥	Equals to or greater than
°	Degree
°C	Degree Celsius
µg	Microgram
µm	Micrometer
cm	Centimeter
g	Gram
h	Hour
rpm	Revolutions per minute
mg/m^2	Milligram per square meter
L	Litre
M	Molar
χ^2	Chi-square
mg	Milligram
min	Minute
ml	Milliliter
mm	Millimeter
mM	Millimolar
Mg	Magnesium
nm	Nanometer
df	Degree of freedom
s	Second
U	Unit (activity)
v	Volume
Cl	Chloride
D	Coefficient of linkage disequilibrium
r^2	Extent of linkage disequilibrium
µg/ml	Microgram per milliliter
µM	Micromolar

EtBr	Ethidium Bromide
HCl	Hydrochloride
ng/µl	Nanograms per microliter
bp	Base pair
α	Alpha
β	Beta
γ	Gamma
±	Plus minus
NaCl	Sodium chloride
A	Adenine
T	Thymine
G	Guanine
C	Cytosine

CHAPTER 1

INTRODUCTION

Introduction

Lung cancer is a malignant tumour growth characterized by the uncontrolled growth of cells in the lung tissue. Globally, it is the second most prevalent diagnosed disease with high morbidity and mortality rates, with an estimated 2.2 million new cases and 1.8 million fatalities in 2020 (Sung *et al*., 2021). According to the International Agency for Research on Cancer (IARC), under-developed nations account for more than half of lung cancer cases (Bray & Soerjomataram, 2015). In India, oral/mouth and lung cancers are the most prevalent cancers in men. According to the statistics, 1 in 68 men and 1 in 201 women could contract lung cancer in their lifespan (Mathur *et al*., 2020). The heterogeneity of lung cancer underlines and demarcates the significance of not just one but many determinants influencing the prevalence of the disease, which is the subject of voluminous literature, research, and scientific advancements.

Smoking tobacco is a leading risk factor for lung cancer, accounting for 90% of the total cases. Smoking alone contributes to 80% of lung cancer deaths, but not everyone who smokes gets lung cancer (Ankathil, 2010). Lifelong never-smokers account for 15–25% of all lung cancer cases worldwide. Simultaneously, the number of cigarettes and duration of smoking in a lifetime also raise the hazard of lung cancer (Hori *et al.*, 2020). There is a 20 to 50-fold increase in the risk of lung cancer for continuous smokers as compared to never smokers. Tobacco smoking is usually done in cigarettes, bidis, and hookah (Bade & Cruz, 2020). Connectively, alternate disparate risk factors for lung cancer are asbestos, radon, arsenic compounds, and sniff chemicals. Other than smoking, various genetic and environmental factors are also accountable for causing lung cancer (Moini *et al*., 2022).

Lung cancer is primarily divided into non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC is further branched into large cell carcinoma, adenocarcinoma, and squamous cell carcinoma, while SCLC is the most destructive subtype of lung cancer divided into small cells and combined small cells. Approximately, 85% of lung cancer cases are graded as NSCLC, and 15% of total diagnoses are demarcated as SCLC (Zappa & Mousa, 2016). Traditional diagnostic approaches include computer tomography (CT), cytology, biopsy, and bronchoscopy; advanced and modified diagnostic techniques include biosensors, biomarkers, radiogenomics, liquid biopsy, and several other diagnostic devices (Prabhakar *et al*., 2018). Radiation treatment and surgery influence the cancer cure, but the treatment scenario has changed after the arrival of

chemotherapy. Even in the realm of personalized medicine, chemotherapy remains effective. The standard treatment of SCLC and NSCLC involves the use of platinum-based compounds (cisplatin or carboplatin) co-administrated either with the irinotecan, pemetrexed, or taxane drugs (paclitaxel and docetaxel) (Baxevanos & Mountzios, 2018).

It has been well reported that platinum-based chemotherapeutic agents are indispensable for lung cancer treatment (Zhang *et al*., 2022). Intriguingly, it is worth understanding how an anticancer drug enters or exits a tumour cell. This process is mediated by membrane transporters that play a crucial role in translocating chemotherapeutic drugs across the cancer cell membrane, thus influencing chemotherapy efficiency and toxicity. Retention of the chemotherapeutic drugs in tumour cells is an outcome of the net function of the influx and efflux transporters (Li & Shu, 2014). The ATP binding cassette (ABC) and solute carrier (SLC) transporters are the primary drug transporters that mediate the outflow and inflow of the drugs according to the direction of the movement of substrates (Lin *et al*., 2015).

The ABC transporters are one of the most prominent ubiquitous families of membrane proteins essential for the translocation of numerous substrates across the cell membrane. ABC transporters rely on ATP to export various substances. Hydrolysis of ATP is critical for facilitating the transportation of various substrates against a concentration gradient through intracellular to extracellular regions. This vast ABC transporter family plays an essential part in various cellular activities. ABC proteins not only play a role in pumping drugs (xenobiotics) out, but it also transports sugars, metal ions, amino acids, variety of hydrophobic compounds, and peptides across the membrane (Wilkens, 2015). It has been reported that ABC transporters are widely localized in the kidney, brain, small intestine, and liver, where they play a crucial role in absorption, distribution, and metabolism excretion (ADME). Apart from these organs, the efflux transporters are also expressed in the heart, trachea, lung, adrenal gland, placenta, and uterus (Sharma *et al*., 2021). In humans, 49 ABC transporters subtypes are divided into seven subfamilies from A to G. The most common genes studied in the *ABC* family are *ABCB1*, *ABCC1*, *ABCC2*, and *ABCG2*. It is observed that variation in these genes can alter the phenotype and modulate toxicity, disease susceptibility, and drug treatment response in patients (Bruckmueller & Cascorbi, 2021)

ATP-binding cassette sub-family B member 1 *(ABCB1)* gene is responsible for cellular homeostasis and defense against xenobiotics. *ABCB1* gene encodes P-glycoprotein (P-gp) and is positioned on chromosome 7q21.12 (Battista *et al.*, 2020). It consists of 29 translated exons and 28 introns. The expression of P-gp is reported in the plasma membrane of the cells, and excretory organs, as it provides defensive and eliminatory functions (Choudhuri & Klaassen, 2018). In the lung, the P-gp expression has also been reported on the apical side of ciliated epithelial cells & in bronchial glands (Van der Deen *et al.*, 2005). Many anti-cancerous agents include actinomycin D, daunorubicin, doxorubicin, docetaxel, paclitaxel, etoposide, and irinotecan act as substrates of P-gp (Sharma *et al.*, 2021). Studies have shown that single nucleotide polymorphisms (SNPs) in the *ABCB1* gene can impact drug efflux, substrate specificity, mRNA stability, and expression (Tulsyan *et al.*, 2016). Among the numerous polymorphisms identified in the *ABCB1* gene; $C^{1236}T$ (exon 12), $G^{2677}T/A$ (exon 21), and $C^{3435}T$ (exon 26) are the most common and relevant studied SNPs in the exon region of the *ABCB1* and thereby affect the functionality of the transporter (Ganoci *et al.*, 2021). A recent meta-analysis assessed the prognostic value of *ABCB1* $C^{1236}T$ and $C^{3435}T$ polymorphism in patients treated with platinum-based taxane chemotherapy (Chen *et al.*, 2021). Knez *et al.* reported the prognostic value of $C^{3435}T$ polymorphism in Caucasian SCLC patients undergoing cisplatin-based chemotherapy (Knez *et al.*, 2012). Studies have also claimed that in $C^{3435}T$ and $C^{1236}T$, the *T* -allele (Caronia *et al.*, 2011) and homozygous *C* allele (Schaich *et al.*, 2009) is significantly correlated with improved outcomes after chemotherapy. A study reported no association between $C^{3435}T$ and $G^{2677}T/A$ polymorphism and adverse events caused by the cisplatin-based regimen in NSCLC patients (Pan *et al.*, 2008). On the contrary, a recent study by Rierra *et al.* showed a significant association between $C^{1236}T$ polymorphism with grade 3-4 gastrointestinal toxicity in patients undergoing irinotecan-based chemotherapy. Additionally, patients with $C^{3435}T$ polymorphism in the *ABCB1* gene are reported to develop grade 3 diarrhea when treated with cisplatin and irinotecan (Sekine *et al.*, 2008). Various other studies have reported the impact of *ABCB1* polymorphism in causing adverse events in lung cancer patients, and the results are conflicting (Han *et al.*, 2007; Isla *et al.*, 2004; Kobayashi *et al.*, 2015; Han *et al.*, 2009).

ATP-binding cassette sub-family C member 1 *(ABCC1)* gene encodes multidrug resistance protein 1 (MRP1) and is located on chromosome 16p13.1. This gene is expressed ubiquitously, but its expression is higher in the intestine, oral mucosa, choroid plexus, and lung (Jiye & Jianting,

2011). ABCC1 transporter has garnered maximum interest because of its dysregulation in numerous carcinomas such as NSCLC, glioma, acute lymphocytic leukemia, and acute myeloid leukemia (Bandariavičiūtė *et al.*, 2015; Hanssen *et al.*, 2021; Rudas *et al.*, 2003; Shen *et al.*, 2011). The two common non-synonymous *ABCC1* polymorphisms in the Asian population are $G^{2168}A$ and $G^{3173}A$. Both the polymorphism is an arginine to glutamine substitution at amino acid positions 723 and 1058, respectively. *In silico* analysis has reported that $G^{3173}A$ polymorphism is deleterious using sorting intolerant from tolerant (SIFT) analysis (Yin *et al.*, 2009). Additionally, studies have reported an association between $G^{2168}A$ mutation and chemotherapy response in ovarian cancer patients (Obata *et al*., 2006). However, Yin *et al.* reported no association between patients with $G^{2168}A$ SNP and chemotherapeutic response in lung cancer patients (Yin *et al*., 2011). These results are also ambiguous and need to be further confirmed.

Another essential member of the ABCC subfamily is the *ABCC2* gene that encodes the multidrug resistance-associated protein 2 (MRP2) protein. The *ABCC2* gene is positioned on chromosome 10q24.2 (Wu *et al*., 2018). MRP2 is exclusively localized to the apical membrane of the polarized cells. It is seen that the *ABCC2* gene is generally expressed in normal lungs, but a high expression is observed in lung cancer patients. In the past, MRP2 over-expression has been correlated with cisplatin resistance (Borst *et al*., 2006). A non-synonymous *ABCC2* $G^{4544}A$ mutation is a cysteine into tyrosine substitution that decreases ATPase activity, thereby significantly impacting cellular drug disposal (Elens *et al.*, 2011). Numerous studies have suggested that this mutation can cause variations in pharmacological effects, intracellular substrate levels, and toxicity (Wojnowski *et al.*, 2005; Ni *et al.*, 2010; Elens *et al.*, 2009). A report in the past has shown a strong association between $G^{4544}A$ polymorphism and overall survival in NSCLC patients (Cuffe *et al.*, 2016). It has been published that *ABCC2* expression is an independent prognostic factor in lung cancer patients. However, no correlation was reported between *ABCC2* $G^{4544}A$ polymorphism and overall survival in a cohort of platinum-treated NSCLC patients. Further, a lack of substantial correlation was reported between $G^{4544}A$ polymorphism and platinum drugs causing nephrotoxicity in lung cancer patients (Sprowl *et al.*, 2012).

ATP-binding cassette sub-family G member 2 (*ABCG2*) gene encodes a breast-cancer resistant protein (BCRP). The *ABCG2* is positioned at chromosome 4q22.1. There are 15 introns and 16 exons in the human *ABCG2* gene (Shah *et al*., 2021). The flux arbitrated by *ABCG2* is unidirectional and transit its substrates from the cytoplasm to the outside of the cell. The most common SNP of this gene is $C^{421}A$ and is lysine to glutamine substitution, which often leads to folding errors in the BCRP protein and decreases the protein expression, thereby reducing the transportation activity (Sarankó *et al*., 2013). The meta-analysis found no significant correlation between *ABCG2* $C^{421}A$ polymorphism in predicting prognosis or other clinical outcomes in lung cancer patients undergoing chemotherapy (Fu *et al.*, 2020). Chen *et al.* did not show any substantial correlation between $C^{421}A$ variation and prognosis in Chinese advanced NSCLC patients (Chen *et al.*, 2015). Qiao *et al*., 2016 found that *ABCG2 421 A/A* was associated with a higher response rate towards paclitaxel and platinum-based chemotherapy. A study on the Asian population (Indian, Malaysians and Chinese) concluded a non-significant influence of $C^{421}A$ polymorphism on SN-38 and the severity of neutropenia (Jada *et al.*, 2007). Another study by Cusatis *et al.* pointed out that $C^{421}A$ variation is responsible for the reduced activity of ABCG2 and depicted the role of this variation in the elevated risk of developing diarrhea in NSCLC patients (Cusatis *et al.*, 2006).

The other family of crucial transmembrane transporters are solute carrier (SLC) proteins that mediate the inflow of small molecules across plasma and intracellular membranes (Hediger *et al.*, 2004). These transporters do not rely on ATP to translocate the substances. SLC transporters are essential for several physiological functions, such as nutrition intake and disposal, neurotransmission, and metabolic waste excretion (Lin *et al*., 2015). They also participate in the disposal of numerous therapeutically relevant drugs, including antidiuretics and chemotherapeutics. Remarkably, SLC transporters are generally co-expressed in the groups and patterns in various normal tissues, indicating that they might contribute to a concerted regulatory circuit that mediates the functions of normal tissues. However, the expression profiles of SLC transporters undergo significant alterations in cancer (Nyquist *et al*., 2017). These modifications in the expression of SLC transporters might influence the efficacy of chemotherapeutics (resistance or sensitivity) by modifying the efficiency of drug absorption into cells (Li & Shu, 2014). Transporters like *SLC19A1, SLCO1B1,* and *SLCO1B3* are mentioned and studied in some

reports as they modulate the expression of SLC transporters and thereby alter the efficacy of the chemotherapy in various tumour types.

SLC19A1 gene encodes reduced Folate Carrier protein (RFC) and is located on chromosome 21 (21q22.3) (Hou & Matherly, 2014). SLC19A1 is liable for translocating pemetrexed drugs inside the cell and may affect drug transport. Besides this, it plays a significant role in folate metabolism. It is highly polymorphic in humans, and a non-synonymous polymorphism of exon 2, $G^{80}A$ (*Arg27His*), is accountable for modifying the transporter function, thus altering drug bioavailability and influencing the therapeutic outcomes. Adjei *et al*. concluded that *SLC19A1* polymorphism predicts survival differences in pemetrexed-treated NSCLC (Adjei *et al*., 2010). On the contrary, a lack of association was reported between $G^{80}A$ polymorphism, overall survival, and response rate toward pemetrexed-based chemotherapy in NSCLC patients (Tiseo *et al.,* 2012). Increased risk of gastrointestinal toxicity was observed in variant genotype *(AA)* of *SLC19A1* $G^{80}A$ polymorphism compared to the other genotypes. Besides, $G^{80}A$ polymorphism did not reveal any impact on nephrotoxicity and haematological toxicity (Pérez-Ramírez *et al.,* 2016). Further, Smit *et al*. reported no association between the *SLC19A1* $G^{80}A$ SNP and chemotherapy-related toxicity for patients on pemetrexed-carboplatin versus pemetrexed (Smit *et al.*, 2009). There are conflicting reports regarding the role of this polymorphism in influencing clinical outcomes; thus, more studies are to be done to corroborate further.

The *SLCO1B1* gene encodes organic anion transport polypeptide 1B1 (OATP1B1), a transmembrane protein that translocate various anionic drugs. The *SLCO1B1* gene is positioned on chromosome 12 (12p12.2). The expression of *SLCO1B1* has been well-reported in the basolateral membrane of the hepatic cells (Svoboda *et al.*, 2011). Anticancer drugs, such as docetaxel, methotrexate, and irinotecan metabolite SN-38, are translocated by OATP1B1 (Buxhofer-Ausch *et al*., 2013). Various genetic polymorphisms are described in the *SLCO1B1* gene. $A^{388}G$ (*Asn130Asp*) and $T^{521}C$ $(Val^{174}Ala)$ polymorphisms are located in exons 4 and 5, respectively. $A^{388}G$ and $T^{521}C$ have been studied regarding drug transport, efficacy, and toxicity, as *SLCO1B1* mediates the uptake of endogenous substrates, drugs, and other xenobiotics (Schulte *et al.,* 2020; Han *et al.,* 2009). Variation in $T^{521}C$ polymorphism and $A^{388}G$ polymorphism was associated with predicting prognosis in breast cancer patients. (Zhang *et al*., 2015). A study

reported an association between *SLCO1B1* $A^{388}G$ polymorphism and response rate to chemotherapy in colorectal cancer (Huang *et al.*, 2013). A meta-analysis study showed that $T^{521}C$ was correlated with an elevated risk of irinotecan-induced neutropenia, and lower excretion of irinotecan and $A^{388}G$ polymorphism was correlated with a reduced risk of haematological and gastrointestinal toxicity (Zair & Singer, 2016).

The *SLCO1B3* gene encodes OATP1B3 protein and is localized on chromosome 12. The expression of *SLCO1B3* is reported in the hepatocytes closer to the central vein of the liver (Svoboda *et al.*, 2011). Studies have reported various anti-cancerous drugs such as docetaxel, paclitaxel, methotrexate, and SN-38 as substrates of OATP1B3 (Nie *et al.*, 2019). A non-synonymous variant of *SLCO1B3* $A^{1683\text{-}5676}G$ has been reported at intron 12 (Song *et al*., 2021). Polymorphism in *SLCO1B3* is associated with altered *OATP* activity, leading to frequent drug toxicity and associated adverse effects (Kiyotani *et al*., 2008). Studies have shown that $A^{1683\text{-}5676}G$ variation is associated with reduced OATP1B3 activity (Shitara *et al*., 2013). A study reported a lack of association between patients with *SLCO1B3* $A^{1683\text{-}5676}G$ polymorphism and tumour response (Choi *et al.*, 2015). A substantial difference between *SLCO1B3* $A^{1683\text{-}5676}G$ polymorphism and docetaxel-induced toxicity has been reported (Choi *et al.*, 2015; Kiyotani *et al.*, 2008).

Besides drug transporters, the role of the *Thymidylate synthase (TS)* gene in modulating clinical outcomes in lung cancer patients has also been evaluated. The gene is localized on the short arm of chromosome number 18p11.32 (Marcuello *et al.*, 2004). The *TS* gene is the main enzyme in the folate metabolism cycle that mediates the catalyzes of deoxyuridylate (dUMP) and converts it into thymidylate (dTMP), which is used in DNA biosynthesis and repair (Zhao *et al.*, 2021). The level of *TS* expression may influence carcinogenesis as a dNTP pool is required for tumour cell proliferation. Studies have widely reported that the *TS* gene is a significant target for chemotherapeutic drugs (pemetrexed or 5-fluorouracil) and other platinum-based therapies (Ooyama *et al*., 2007; Dong *et al*., 2015). Inhibition of *TS* causes dTMP depletion, which contributes to uracil incorporation into DNA and chromosomal instability (Lima *et al*., 2014). Reports have suggested two well-studied *TS* gene polymorphisms such as TS enhancer region *(TSER) 2R/3R* and TS *1494del6* in the 5'- and 3'-untranslated regions (UTRs) (Sharma *et al.*, 2022). Triple 28-bp tandem repeats (*3R*) are associated with enhanced mRNA stability and

increased transcriptional and translational efficiency, thereby elevating TS mRNA levels, and thus are associated with poor clinical outcomes in cancer patients (Ishida *et al.*, 2002). The presence of homozygous 6bp deletion in 3'UTR of *TS* reduces the mRNA stability and is correlated with low TS expression, which is associated with enhanced treatment efficacy. Thus, it has been proposed that *TS* polymorphisms may influence the effectiveness of chemotherapy treatment and can be vital in determining the overall survival of NSCLC patients (Lima *et al.*, 2014).

The primary motive of this study was to assess the sequence variants of ABC (*ABCB1*, *ABCC1*, *ABCC2*, and *ABCG2*) and SLC (*SLC19A1*, *SLCO1B1*, and *SLCO1B3*) drug transporters, and *Thymidylate synthase* gene (chemotherapeutic target) in North Indian population. No reports have examined role of drug transporters and folate metabolism pathway genes in the Indian population. The study aimed to evaluate the impact of these polymorphisms in predicting prognosis in the subgroups of the sample population via stratifying the patients based on histology, chemotherapy regimen, age, gender, and smoking habits. In estimating high-risk subgroups in the sample population, patients were stratified based on parameters such as tumour stage, lymph node invasion, primary tumour extension, and metastasis. The polymorphisms of the genes mentioned above and their correlation with the clinical response towards platinum-based doublet chemotherapy in lung cancer patients have also been elaborated. Furthermore, the recursive partitioning strategy was used to study high-order gene interactions via survival tree (STREE) analysis. Furthermore, the impact of these polymorphisms was evaluated in modulating haematological, nephrological, gastrointestinal, and hepatotoxicity in North Indian lung cancer patients receiving platinum-based doublet.

CHAPTER 2
REVIEW OF LITERATURE

2.1 Cancer

Cancer is a diverse group of diseases with as many symptoms as the human body's tissues and cell types, involving many exogenous and endogenous environmental agents and various etiological pathways. All these pathological conditions share certain essential features of the cancer cells, such as uncontrolled (clonal) cellular proliferation, altered cellular differentiation, invasiveness, or metastatic potential (Bukhtoyarov & Samarin, 2015). Cancer, in its most basic form, is generally recognized as a genetic abnormality, or more precisely, a disease of aberrant gene expression, as shown in **figure 2.1.**

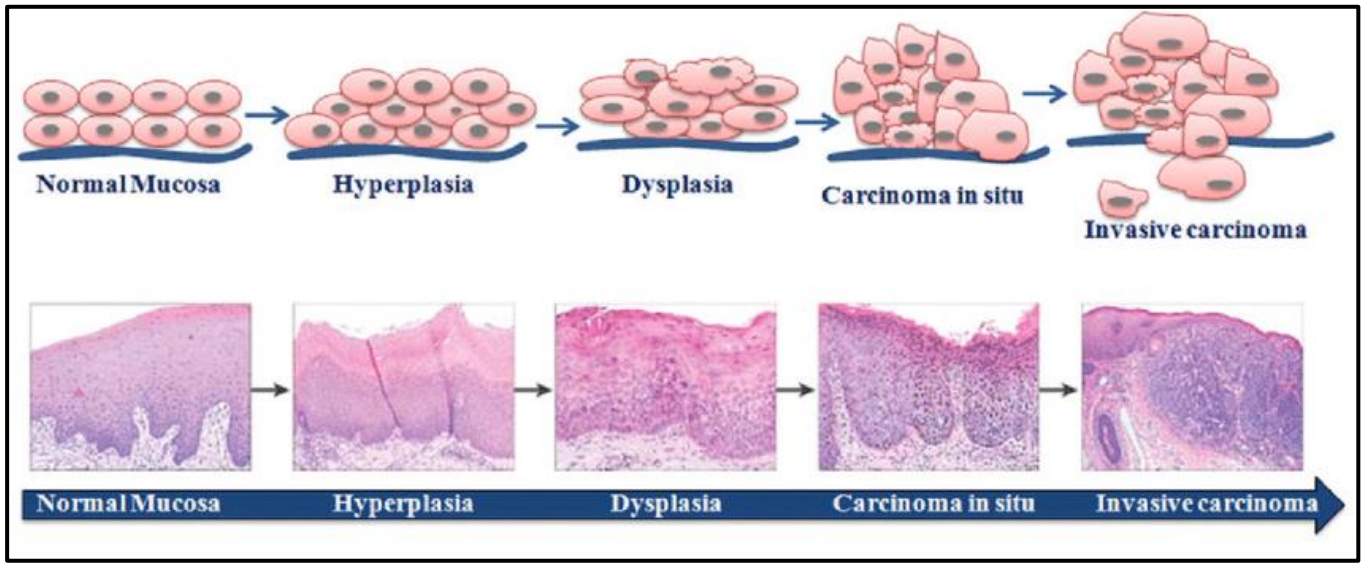


Figure 2.1: Schematic representation of tumour development stages and metastatic mechanisms (Shah *et al.*, 2011)

Note: Tumor development is divided into several stages: (A) Hyperplasia is a stage in which genetically altered or aberrant cells proliferate rapidly and aggressively. (B) Dysplasia is a stage of tumour growth in which overgrowing cells revert to their original shape. It has a higher proportion of immature cells compared to mature cells. (C) *In situ* cancer is a neoplastic lesion in which cells do not undergo maturation, have lost their tissue identity, and are overgrown. (D) Overgrowing cells in a malignant tumour rupture the basal membrane, allowing them to invade other sites. Cancer cells spread to distant body parts via the lymphatic system and blood circulation, causing metastases.

Research efforts have shown that different forms of carcinoma share common molecular mechanisms driving uncontrolled cell proliferation, including mutation, loss, or dysregulation of the genes that negatively and positively regulate cellular proliferation, differentiation, and migration (commonly classified as proto-oncogenes and tumour suppressor genes). Understanding the distribution of cancer among world populations, considering specific risk factors and etiologic agents involved in disease aetiology, is essential to studying the molecular mechanisms that regulate disease pathogenesis for specific cancers. For cancer aetiology research, long-term patterns in cancer incidence and death in diverse populations must be monitored. Given the time it takes for a clinically detectable tumour to emerge after the carcinogenic process (exposure to a carcinogenic agent), present cancer incidence trends are most likely a result of exposures that occurred many years (and possibly decades) ago (Coleman & Tsongalis, 2017). Hence, as the human population's life expectancy rises due to fewer causes of early mortality (such as infectious and cardiovascular diseases), the average chance of developing cancer rises.

2.2 Lung and its anatomy

The lung's function is to supply oxygen to the blood, which is done by alveoli. The respiratory system has two parts, mainly airways and lung parenchyma. A labelled representation of the human lung is depicted in **figure 2.2.** The airways contain the bronchus, which splits from the trachea and breaks into bronchioles and eventually into alveoli. The parenchyma is crucial for exchanging gases and contains the alveoli, bronchioles, and alveolar ducts. The texture of the lungs is spongy, and the color is pinkish-grey. According to anatomical descriptions, they also have an apex, three surfaces, and three boundaries. They are further divided into lobes and segments. A pleura also covers the lung parenchyma (Chaudhry & Bordoni, 2022).

The lung does have an apex, three surfaces, and three borders anatomically. Above the first rib is the apex. The anterior, inferior, and posterior boundaries are the three borders. Among these borders, the anterior border of the lung aligns with the pleura reflection and forms a cardiac notch in the left lung. The cardiac notch is a protrusion on the left lung's surface that provides space for the heart. The posterior border is thick and stretches from cervical vertebrae 7 to thoracic vertebrae 10. The sharp and thin inferior border separates the base of the lung from the mediastinal and costal surface (Patwa & Shah, 2015). The anatomy of the left and right lungs is identical yet

asymmetrical. The left lung is divided into the left lower lobe and upper lobe, whereas the right lung is categorized into the right lower lobe, middle lobe, and upper lobe. An oblique fissure separates the lower and upper lobes of the left lobe, while an oblique and horizontal fissure splits the right lobe, with the horizontal fissure separating the middle and upper lobes and the oblique fissure separating the lower and middle lobes (Peng *et al.*, 2021).

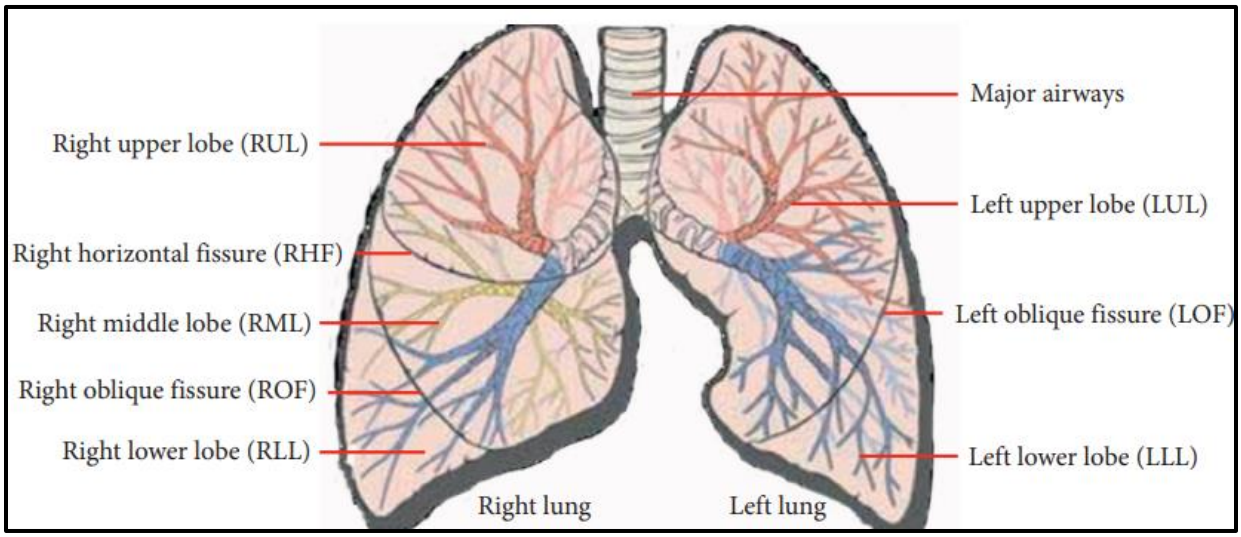


Figure 2.2: A labelled representation of the human lung (Peng *et al.*, 2021)

The lobes are further subdivided into segments, each of which corresponds to a segment of the bronchi. The third-order branches (segmental bronchi) are divided into the main bronchus' and second-order branches (lobar bronchi). Depending on how the lobe is divided, there are 8 to 9 segments on the left lung. The Upper left lobe has four segments (inferior, anterior, superior lingula, and apicoposterior), whereas the left lower lobe comprises 4-5 segments (superior, lateral, posterior, and anteromedial) (Oizumi *et al.*, 2014). The hilum (root) is a depressed area at the center of the medial surface. It comprises mainly bronchi, pulmonary vasculature, phrenic nerve, nodes, lymphatics, and bronchial vessels. The right and left hilum consist of a pulmonary vein (inferior and superior), a pulmonary artery, and bronchial arteries. The vein, artery, and then bronchus are in order from anterior to posterior in the hilum (Chaudhry & Bordoni, 2022).

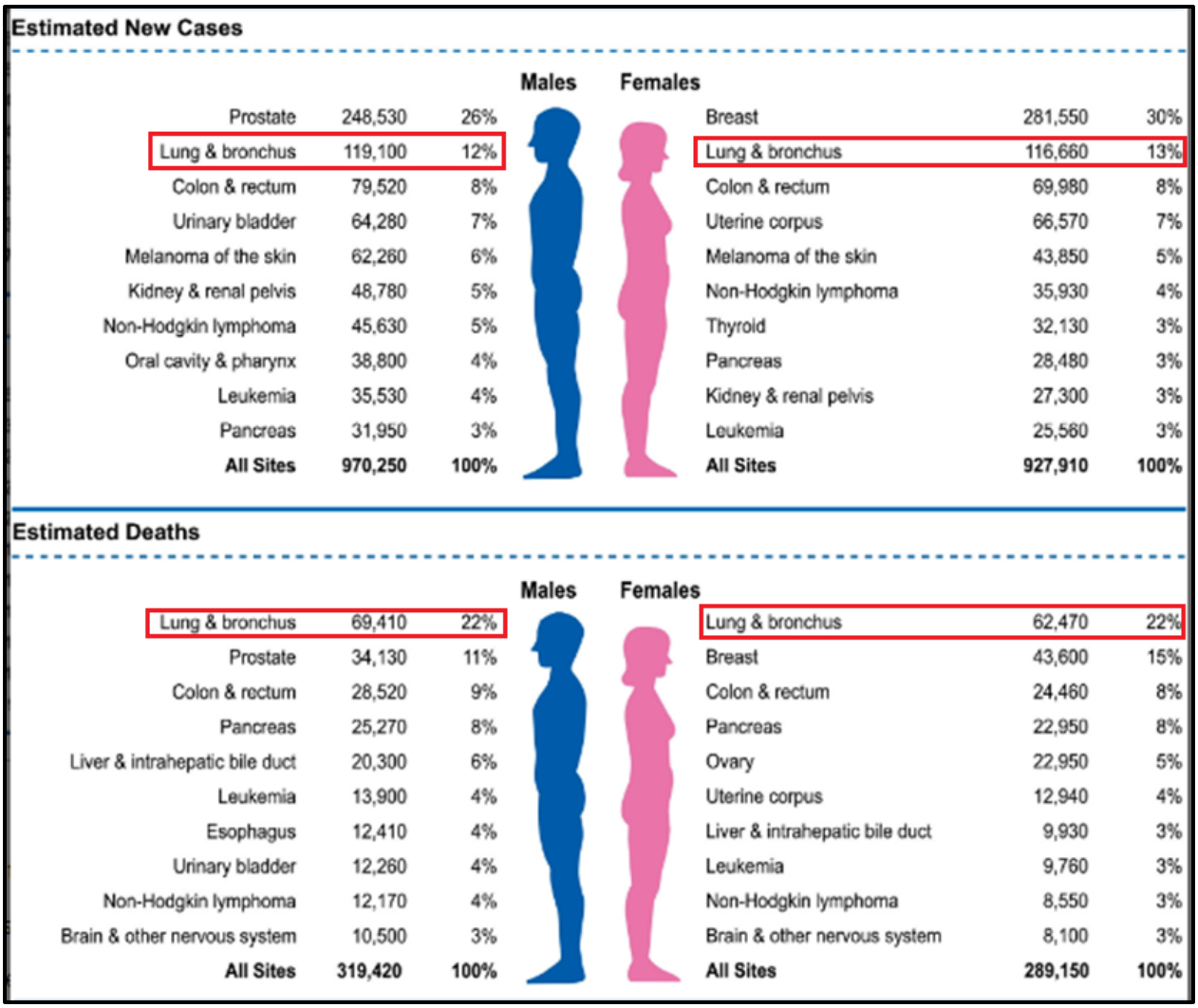
2.3 Lung carcinoma

Lung carcinoma occurs due to abnormal cell behavior, resulting in mass expansion of the lung tissue and other pathological conditions. The ability of abnormal neoplastic cells to invade neighboring tissue leads to cellular metastasis, spreading to other body parts (Jiang *et al*., 2015). Mutations or errors in the cell's genome can significantly cause cell-cycle dysregulation, and these mutations can cause cell growth disruption and cell death, resulting in a tumour as a mass. These tumour masses are divided into two types: "benign" and "malignant". Benign tumour do not spread to other parts of the body and can be easily removed, whereas malignant tumour infiltrate other tissues and proliferate to other body parts through the blood or lymphatic system. Tumour malignancy causes mucous formation, necrosis, and fibrosis in the tissues of the lungs. Coughing, bronchitis, dysphonia, asthenia, shortness of breath, change in voice, harsh breathing sounds, loss of appetite, pleural effusion, weakness, and hemoptysis are all symptoms of lung carcinoma (Shi *et al.,* 2015).

Smoking, second-hand smoking, radiation therapy, exposure to radon gas, asbestos, other carcinogens, arsenic in drinking water, and a family history of lung cancer are all risk factors. Epithelial cells are responsible for 90-95 % of lung cancers. When some cell lines cover the larger and smaller airways, *i.e.*, bronchi and bronchioles, lung cancer is also known as bronchogenic. Cancer in the lungs can also arise from the pleura, mesothelioma, or, less frequently, from supporting tissue, such as blood vessels found inside the lungs (Tamura *et al.,* 2015)

2.3.1 Global epidemiology of lung cancer

Lung cancer is the primary cause of cancer cases and fatality rates in both men and women worldwide. It is responsible for approximately 2 million diagnoses and 1.8 million deaths globally, per 2020 reports (Sung *et al.*, 2021). It is the second most common carcinoma in both genders, after prostate and breast cancer, as shown in **figure 2.3**. Increased tobacco and industrial development access in developing countries have significantly increased lung cancer incidences. The average prognosis for this cancer is 70 years old (Thandra *et al.*,2021).



Estimated New Cases

	Males			Females		
Prostate	248,530	26%		Breast	281,550	30%
Lung & bronchus	119,100	12%		Lung & bronchus	116,660	13%
Colon & rectum	79,520	8%		Colon & rectum	69,980	8%
Urinary bladder	64,280	7%		Uterine corpus	66,570	7%
Melanoma of the skin	62,260	6%		Melanoma of the skin	43,850	5%
Kidney & renal pelvis	48,780	5%		Non-Hodgkin lymphoma	35,930	4%
Non-Hodgkin lymphoma	45,630	5%		Thyroid	32,130	3%
Oral cavity & pharynx	38,800	4%		Pancreas	28,480	3%
Leukemia	35,530	4%		Kidney & renal pelvis	27,300	3%
Pancreas	31,950	3%		Leukemia	25,560	3%
All Sites	**970,250**	**100%**		**All Sites**	**927,910**	**100%**

Estimated Deaths

	Males			Females		
Lung & bronchus	69,410	22%		Lung & bronchus	62,470	22%
Prostate	34,130	11%		Breast	43,600	15%
Colon & rectum	28,520	9%		Colon & rectum	24,460	8%
Pancreas	25,270	8%		Pancreas	22,950	8%
Liver & intrahepatic bile duct	20,300	6%		Ovary	22,950	5%
Leukemia	13,900	4%		Uterine corpus	12,940	4%
Esophagus	12,410	4%		Liver & intrahepatic bile duct	9,930	3%
Urinary bladder	12,260	4%		Leukemia	9,760	3%
Non-Hodgkin lymphoma	12,170	4%		Non-Hodgkin lymphoma	8,550	3%
Brain & other nervous system	10,500	3%		Brain & other nervous system	8,100	3%
All Sites	**319,420**	**100%**		**All Sites**	**289,150**	**100%**

Figure 2.3: Statistical representation of new Cancer Incidence and Mortality rates – Estimates for 2021 (Siegel *et al.*, 2021)

Since the 1990s, lung carcinoma and death rates have decreased in the United Kingdom and the United States of America (USA). Conversely, developing countries such as Brazil, Russia, India, China, and South Africa (BRICS) continue rising cigarette smoking rates for both genders (Barta *et al.*, 2019). A report in 2021 showed that males have around two times more incidence and fatality rates than females, while the men to women ratio vary significantly by region, spanning from 1.2 in Northern America to 5.6 in Northern Africa (Sung *et al.*, 2021).

Lung carcinoma is most common in developing countries where smoking is prevalent, with a 20-fold variation in incidence between areas. Lung cancer is prevalent in 37 nations, including China, Russia, and a large portion of Eastern Europe, Southeast Asia, and the Middle East (World

Health Organization, 2019). Women are more prone to developing lung carcinoma in North Korea than in other countries. Micronesia/Polynesia has the highest incidence of lung carcinoma globally, with 52.2/100,000 cases among men, while Hungary has the highest occurrence, with 77.4/100,000 cases among males. Northern and Western Europe, as well as North America, have the highest prevalence among females worldwide. Men and women are less likely to develop lung cancer in Western, Eastern, and Central Africa (Bray *et al*., 2018).

Lung carcinoma is still the second most common cancer and the leading cause of cancer death in the United States. By the Surveillance, Epidemiology, and End Results (SEER) programme, lung carcinoma accounts for approximately 12.9 % of carcinoma cases, and 538,243 people in the United States were estimated to be living with lung cancer in 2016. (Bade *et.al.*, 2020). Furthermore, an estimated 229,000 new lung carcinoma cases were diagnosed in the United States in 2020, accounting for 12.7% of all carcinoma diagnoses. Lung carcinoma is the leading cause of cancer mortality globally, among both genders and males and females. Lung cancer was responsible for an estimated 1,761,000 deaths in 2018, accounting for 18.4 % of carcinoma mortality worldwide. Lung cancer was the biggest cause of mortality among males in 93 countries, including the United States, China, and Russia, accounting for nearly 1,185,000 deaths in the same year. With 576,000 deaths in 2018, lung cancer was less than half as common in women as in men. It was the major cause of female fatality in 28 countries, including China and the United States. Unlike men, female lung cancer incidence and mortality continue to rise in the United States and Europe. With 14.6 deaths per 100,000 females in 2017, lung cancer surpassed breast cancer as Europe's leading cause of cancer fatality (Thandra *et al.*, 2021).

2.3.2 Indian epidemiology of lung cancer

According to an Indian Council of Medical Research (ICMR) report, by 2020, India would record 17.3 lakh new carcinoma cases and 8.8 lakh cancer-related deaths, putting it second on the ranking. The ICMR's Indian epidemiological surveys article for 2016, there were 1.14 lakh new lung carcinoma cases, with 83,000 men and 31,000 women diagnosed (New cancer causes in India by 2020 by 2020,2015). As per GLOBOCON 2012, lung cancer was the third biggest killer in India, accounting for 63,759 deaths. Moreover, men died (48,697) more than women (15,062). According to the ICMR, between 1982 and 2005, lung carcinoma per one lakh increased by approximately 100 % in Bengaluru, 160 % in Chennai, and 40 % in Delhi, while they decreased

by 60 % in Mumbai. The estimated number of lung and bronchus cancer cases in India in 2020 was 98278 in both males and females (Mathur *et al.*, 2020). Statistics show that almost 80% of people with lung cancer are smokers.

Figure 2.4 shows the age-standardized prevalence and fatality rates of lung carcinoma in India compared to surrounding nations. Lung cancer rates increased in both men and women in Kamrup urban, Delhi, Bengaluru, and Chennai between 2012 and 2016. Lung cancer was the most frequent cause in eleven states, ranging from 21.5 % in Sikkim's population-based cancer registries (PBCR) to 48.2 % in Manipur. Lung carcinoma was the most prevalent type in females in eight registries, varying from 24.1 % in the Chennai PBCR to 72.2 % in the Manipur PBCR. Furthermore, it has been ascertained that the prevalence rate of lung cancer in females is significantly lower, estimated at around 3%. (Three Year Report of Population-Based Cancer Registries 2012-2014, 2016).

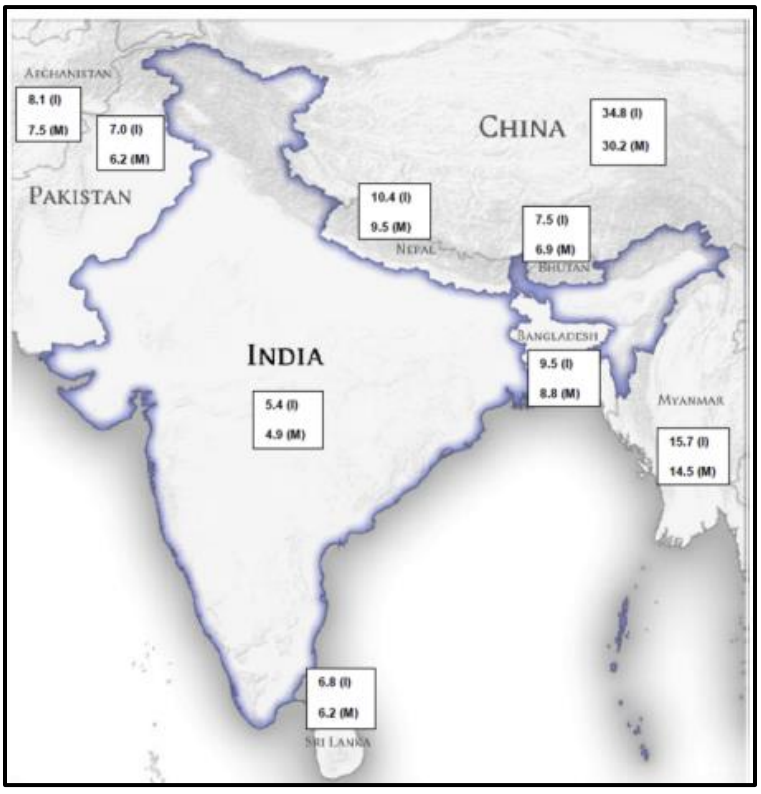


Figure 2.4: Age-standardized prevalence and fatality rates of lung carcinoma in India compared to surrounding nations [(Singh & Agrawal, 2021)Data Source: GLOBOCAN 2020]

Lung carcinoma was relatively rare in India at the turn of the 19th century. The history of data published on cancer was influenced by the powerful impact of industrialization and the Indian

population's proclivity to smoke. In the early 1950s, 27.4 per million cases were reported, which had risen to 78.6 per million worldwide by 1959. On the global level, 1.8 million lung carcinoma cases were confirmed, with lung cancer accounting for 6.9% of all new carcinoma cases and 9.3% of all cancer-related fatalities in both sexes in India (Behera and Balamugesh, 2012). Aizwal has the highest incidence of males (38.8 per 100,000) and females (37.9 per 100,000). (Malik and Raina, 2015).

2.4 Etiology of lung cancer

2.4.1 Tobacco Smoking

The most critical risk factor for lung carcinoma is tobacco smoke. Smokers are more likely to develop lung carcinoma than non-smokers. The severity of the risk increases with the number of pack-years smoked. Smoking is responsible for 90 % of male lung cancer deaths and 75-80 % of female lung cancer deaths worldwide (Ankathil, 2010). Numerous epidemiological studies have supported the idea that smoking cigarettes cause carcinoma. Squamous cell cancer is the most frequent among smokers. Ex-smokers have a 19% higher risk of lung carcinoma when compared to non-smokers. Bidis are popular in India; these are the tobacco flakes wrapped in tendu plant leaves. These are the most widely known tobacco products, alongside hookah and cigarettes. Bidi is consumed by 73 % of India's smokers compared to cigarettes. Bidis constitute more nicotine than cigarettes (21.2 mg/g *vs* 13.5 mg/g), raising carcinoma risk (Mishra *et al.*,2012). **Figure 2.5** illustrates the associated risk factors of lung cancer.

2.4.2 Tobacco Smoke Carcinogens

Tobacco smoke is a composite aerosol made up of gaseous and solid components. The smoke is comprised of both mainstream and side stream elements. The primary source of cigarette smoke for the smoker is mainstream smoke, which is produced by inhaling air from the cigarette. Mainstream smoke comprises many possible carcinogens such as aromatic amines, polycyclic aromatic hydrocarbons (PAH), N-nitrosamines, and various inorganic (mainly arsenic, chromium, vinyl chloride) and organic compounds (Bade & Dela Cruz, 2020). Burning the cigarette between puffs produces side stream smoke, which is the leading cause of environmental tobacco smoke. Nicotine is the most crucial element in tobacco addiction, while tar is the whole particle matter of cigarette smoke once nicotine & water have been purged. Exposure to tar over time appears to be

a substantial factor in lung cancer risk. Cigarette smoke has around 4000 chemical compounds. The components of specific concern in lung carcinoma are the N-nitrosamines formed by the nitrosation of nicotine during tobacco manufacturing and smoking. Among the N-nitrosamines, the 4-(methylnitrosamino)-1(3-pyridyl)-1-butanone is the most potent stimulator of lung carcinoma. The primary cause of tumorigenesis from tobacco is the accumulation of DNA adducts, their metabolites, and free radical cell damage (Akopyan & Bonavida, 2006).

2.4.3 Beedi smoke carcinogens

Bidis are hand-rolled, slender, tubular cigarettes native to South Asian countries. Bidis are famous in low- and middle-income countries and are supplied from India to more than 122 countries worldwide. Bidi smoking has been linked to many ailments, including lung and head and neck cancers, according to Indian research. Compared to non-smokers, heavy bidi smokers have a higher prevalence of lower ventilatory capability and cardiorespiratory signs such as coughing, wheezing, dyspnea, breathlessness, and chest pain (Oladipupo *et al.*, 2019). As a result, heavy bidi smoking increases baseline respiratory illness and death risk, particularly among poor socioeconomic and aged smokers.

Due to the apparent unusual components used in bidi manufacture, bidi smoke may contain additional substances than mainstream cigarette smoke. Bidis comprise 0.2 g of darkish, sun-dried, or processed tobacco granules wrapped on tendu leaves (Diospyros elanoxylon). Although bidis contain about 0.2 g of tobacco per piece, their nicotine level is exceptionally high (21.1 mg nicotine/g tobacco filler). The amount of tar and carbon monoxide released during bidi smoking is hugely significant due to the use of non-porous tendu leaves that restricts circulation and the lack of a filter portion in the bidis. The proportion of tar delivered is reduced by 66% when the tendu leaf is replaced with porous cigarette wrappings. The primary ingredient of bidi smoke, nicotine, was found in much higher concentrations in the granular size fraction of <0.25 μm. This means nicotine can quickly accumulate and assimilate into human lung tissues (Malson *et al.*, 2001).

2.4.4 Passive Smoking

Passive smoking is also considered dangerous, as research shows that nonsmokers have an elevated risk of lung cancer than smokers. Tobacco smoke inhalation by nonsmokers who live or work with smokers is also a renowned risk factor for developing lung carcinoma (McCarthy *et al.*, 2012). A meta-analysis study assessed the cancer risk correlated with environmental tobacco smoke in all cancers and found an elevated risk of lung carcinoma (odds ratio:1.24) never relating smokers with the exposure of tobacco smoke as compared to never smokers deprived of such exposure with correlation strongest in the women (Kim *et al.*, 2018). Non-smokers exposed to side stream smoke from cigarette machines exhibited detectable carcinogenic compounds in their urine. Almost 88% of non-tobacco consumers exhibited measurable amounts of serum cotinine, a nicotine metabolite, implying environmental tobacco smoke exposure and widespread prevalence. As per research, nonsmokers who reside with smokers have a 24% higher risk of developing lung carcinoma than nonsmokers who do not reside with people who smoke. Non-smokers are responsible for 10-15% of all lung carcinoma cases (Noronha *et al.*, 2016).

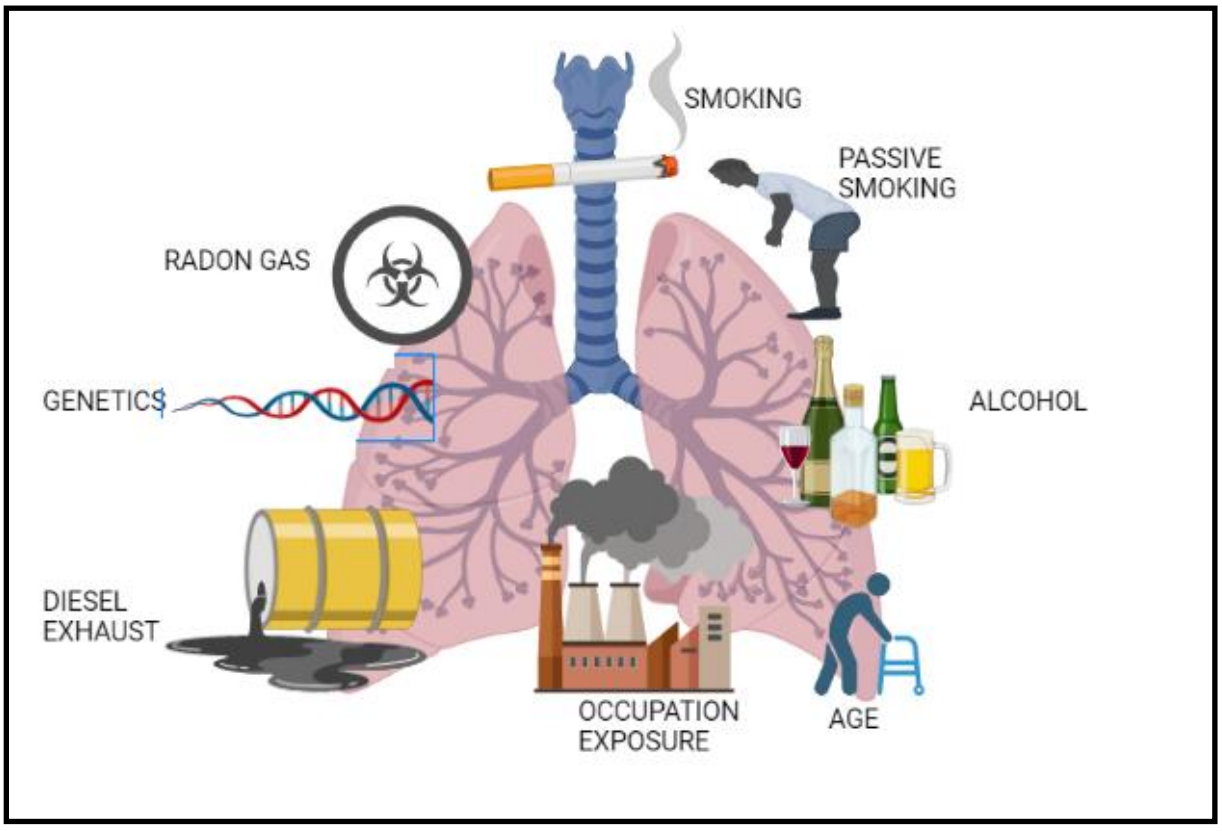


Figure 2.5: An illustration depicting the risk factors associated with causing lung cancer

2.4.5 Alcohol and Diet

Smokers who consume more alcohol are more likely to experience lung cancer. Over time, people's eating habits shifted from home-cooked meals to processed foods laden with spices and oils. Our health has suffered a lot due to changes in our eating habits. Diet rich in vegetables, fruits, and cruciferous vegetables contain isothiocyanates, which are vital in lowering the risk of lung cancer (Malhotra *et al.*, 2016).

2.4.6 Air pollution

Epidemiological reports indicate the concern regarding air pollution, especially particulate matter exposure and its correlation with lung carcinoma susceptibility and morbidity irrespective of cigarette smoking. Lung cancer fatality was 8 % higher when a fine particle and sulfur oxide pollution were predominant. Gaseous components of diesel exhaust present in air pollution, like formaldehyde, benzene, or 1,3-butadiene, have been linked to cancer. There is considerable evidence that occupational exposure to vehicular emissions, particularly in the trucking business, is linked to a high risk of lung cancer (Bade & Dela Cruz, 2020).

2.4.7 Occupational exposure

2.4.7.1 Asbestos: Asbestos fibres are silicate fibres found in lung tissue for the rest of one's life. They are common in the workplace. They were previously widely used for both thermal and acoustic insulation. Asbestos fibres become lodged in the lungs' lining and have been associated with the lung cancer mesothelioma. Exposure to asbestos and tobacco smoking synergistically impact lung cancer development. Compared with the general population, smokers who work with asbestos are 45 times more prone to lung cancer (Mustafa *et al.*, 2016). If asbestos-exposed workers smoke, they are at an increased risk of getting asbestos-related lung carcinoma. Non-smoking asbestos laborers are five times more likely to have lung cancer than nonsmokers (Reddy *et al.*, 2004).

2.4.7.2 Radon gas: Radon gas is an ionizing radiation-emitting naturally occurring radioactive gas. It is a natural byproduct of uranium decay. Radon gas causes lung cancer and is liable for 12% of lung cancer fatalities worldwide. Radon gas accounts for nearly 21,000 lung cancer deaths in the United States annually. It is the second major cause of cancer after smoking. Radon gas can

enter residences via cracks in the foundation, pipes, drains, and other openings. Radon gas harms the lungs' lining and emits radiation that can cause lung cancer (Kapala *et al.*, 2017).

2.4.7.3 Metals and Mixed Occupation Exposures: People exposed to arsenic, found in drinking water, are more prone to develop lung carcinoma. Workers in jobs; involving silica, such as brickmaking, stone cutting, poultry, and earth mining, have a higher risk of lung cancer, with scientific proof of an exposure-response association in the high exposure range (Steenland *et al.*, 2001)

2.4.7.3 PAH and Diesel exhaust: Polycyclic aromatic hydrocarbons (PAH) are generated when organic matter is combusted. Aluminum, coke manufacture, coal gasification, roofing, and tar distillation all produce PAH, which has been related to a higher risk of lung cancer in people exposed to elevated levels of PAH. Motor vehicle exhaust, a high-risk factor, causes lung cancer (Rota *et al.*, 2014).

2.5 Histopathology of lung cancer

Lung cancer is categorized based on the histological features of lung tumour cells, as it is a highly heterogeneous malignancy. It is characterized by two histological subgroups, mainly NSCLC (non-small cell lung carcinoma) and small-cell-lung cancer (SCLC). Almost 85% of all tumour cases are categorized as NSCLC, and 15% of lung cancer cases contribute to SCLC. The figure depicting the histology of NSCLC and SCLC is represented in **figure 2.6.**

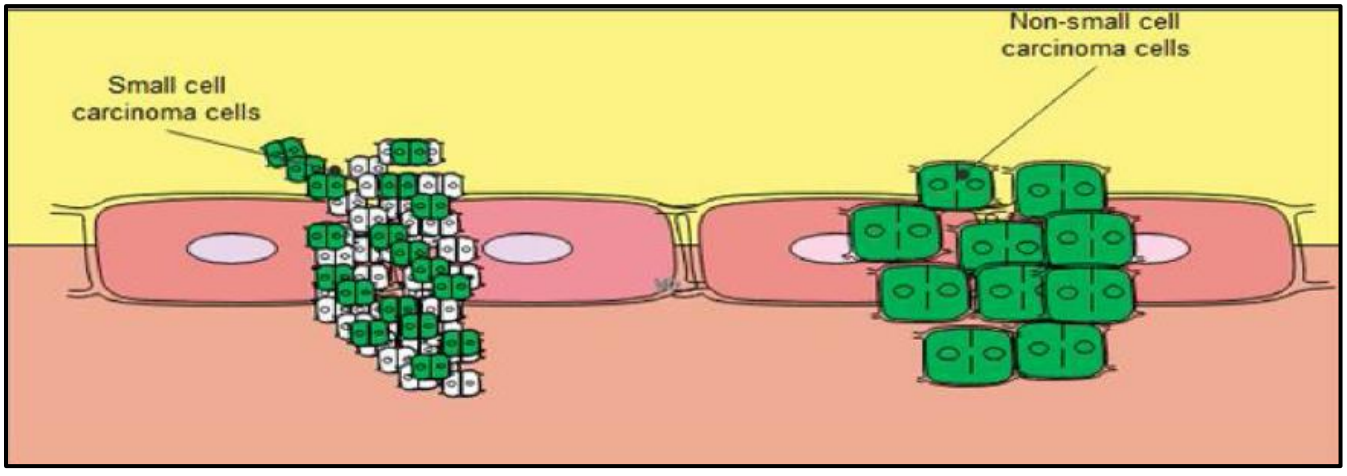


Figure 2.6: Histological representation of NSCLC and SCLC (Prabhakar *et al.*, 2018)

Furthermore, NSCLC is divided into three main subtypes, mainly adenocarcinoma (ADCC), squamous cell cancer (SQCC), and large cell carcinoma (LCC). The figure depicting the histological subtypes of NSCLC is represented in **figure 2.7.**

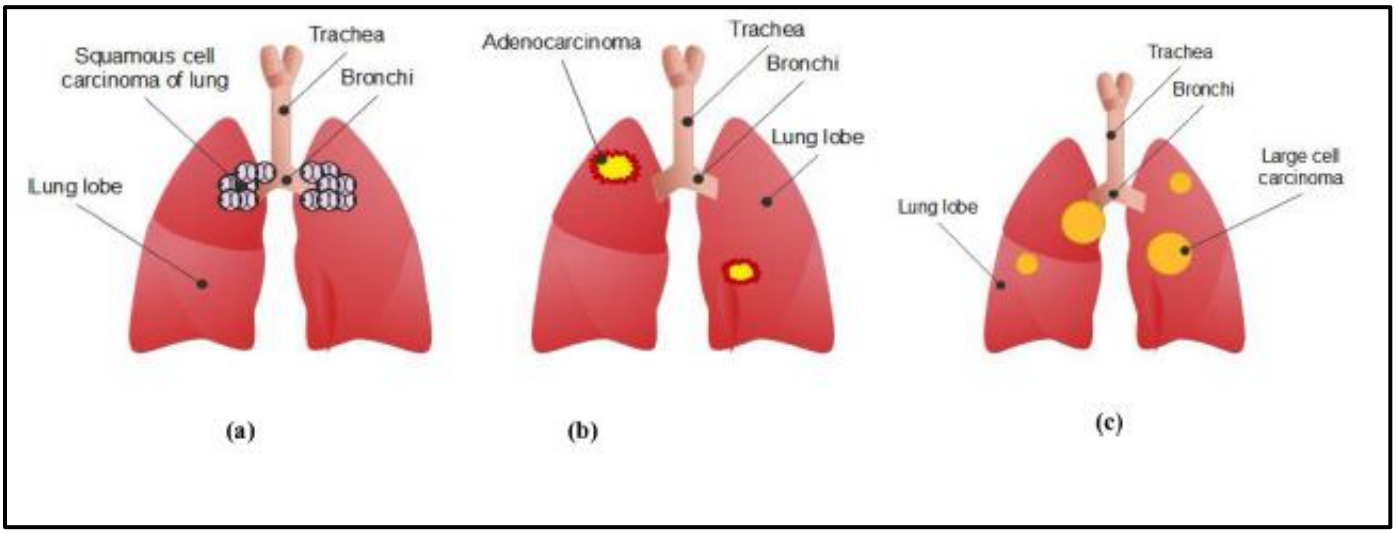


Figure 2.7: A histological representation of NSCLC subtypes (a) SQCC (b) ADCC (c) LCC (Prabhakar *et al.*, 2018)

2.5.1 Large cell carcinoma (LCC)

Large cell cancer is another subtype of NSCLC, and it does not display an architectural and cytological characterization of small cell cancer and squamous or glandular carcinoma. In most studies, large cell cancer accounts for roughly 9% of all cases. There are no cytological characteristics that distinguish this form of tumour. Since the cell boundaries are not delineated, the syncytium development lacks a distinct pattern. The nucleus' shape is similarly difficult to define, varying from circular to irregular. The distribution of chromatin is also disorganized, although a prominent nucleolus is visible. The cytoplasm is scarce and basophilic and has a higher nucleus-to-cytoplasm (N/C) ratio (Travis *et al.*, 2013). **Figure 2.8 (A)** depicts the microscopic images of LCC. The chest radiographs diagnose LCC, and these tumour cells are recognized by the substantial peripheral mass that may penetrate some of the segments or large bronchi.

The tumor's invasiveness is evident in the viscera, pleural wall, and surrounding tissues. Necrosis, cavitation, and hemorrhage are all common symptoms of this tumour. To distinguish

LCC tumours from others, immune histochemical markers such as synaptophysin, chromogranin, or N-CAM (CD56) are utilized. Large cell cancer is an aneuploid neoplasm with the most chromosomes and DNA content of any lung cancer subtype. Like other NSCLCs, large cell carcinoma shows a mutation in p53 mutations, K-ras, Rb alterations, and cyclin D1 overexpression (Kitamura *et al.*, 2008).

2.5.2 Adenocarcinoma (ADCC)

Adenocarcinoma is a subtype of NSCLC in which epithelial cells undergo extensive glandular differentiation and generate mucin to form different development patterns contributing to papillary, acinar, and bronchioalveolar. ADCC is the most common among people who do not smoke or are just exposed to a small amount of smoke. This cancer is generally localized around peripheral nodules instead of the central hilar or other perihilar nodes. Pleural membrane or chest wall invasion is seen in 15% of all cases and is the maximum among all histopathological subtypes. Cytological determination of ADCC cells depends on the architectural arrangement of cell clusters as well as the morphological appearance of the cells. The cells are structured in sheets, or 3D morulae, acini, & papillae, as well as fibrovascular cores. **Figure 2.8 (B)** depicts the microscopic images of ADCC. The cytoplasm in the cells varies, although it is frequently abundant and quite translucent compared to SQCC (Kondo *et al.*, 2002). The cellular borders of these cells are also demarcated, which is a common characteristic. These cells have eccentric, single, and oval-shaped nuclei.

The tumorigenicity of ADCC is revealed by fibrosis, accompanied by necrosis, hemorrhagic area, and cavitation. Expressing epithelial markers like AE1/AE3, CAM 5.2, carcinoembryonic antigen (CEA), and epithelial membrane antigen (EMA) reveals typical immuno-histochemical properties of these cells. To differentiate primary ADCC from metastatic ADCC, the immunophenotype of cytokeratin (CK) 20 and CK7 is done. The immuno-phenotype of lung adenocarcinoma tumours is characterized by the presence of CK7 and the absence of CK20. The level of difference in the morphological frame of cancer cells from healthy lung tissue is used to classify ADCC into different grades. Grade 1 is strongly differentiated, grade 2 is moderately differentiated, and grade 3 is poorly differentiated (Rubin *et al.*, 2001).

2.5.3 Squamous cell carcinoma (SQCC)

SQCC is another classification of NSCLC that affects epithelial cells and is marked by keratinization and the formation of intercellular connections between bronchial epithelial cells. Cigarette smokers are responsible for 90 % of squamous cell carcinoma occurrences (Chu & Weiss, 2002). Arsenic is another key occupational agent considered the second most crucial aetiology of SQCC. Large tumour cells with a hyperchromatic nucleus and an abundance of cytoplasm are notable cytological features. The cells are either formed like a spindle or like a tadpole. **Figure 2.8 (C)** depicts the microscopic images of SQCC. They also have solid aggregates, and the tumour cells' appearance varies from white to grey, suggesting lung tissue fibrosis. Immunohistochemical characteristics reveal the presence of cytokeratins 5/6, keratin (34ßE12), and CEA in SQCC cells (Chu & Weiss, 2002). This type of tumour cell is usually found in the central portion of the bronchial system, where it enters the bronchus wall and invades nearby tissues, causing the bronchial tract to stop producing secretions and, as a result, bronchial dilatation, atelectasis, infective bronchopneumonia, and obstructive lipoid pneumonia may develop (Funai *et al.*, 2003). The Tumor-Node-Metastasis (TNM) system is used to stage SQCC patients. SQCC has a lower rate of metastasis when compared to other histological subtypes.

2.5.4 Small cell lung carcinoma (SCLC)

The most common pulmonary neuroendocrine neoplasm is small cell lung carcinoma, a tumour of small epithelial lung cells with sparse cytoplasm, irregular cellular boundaries, granular chromatin, and no conspicuous nucleoli. The cells are round and spindle-shaped, with extensive necrosis and a high division rate (Kitamura *et al.*, 2008). It is the most aggressive and resilient type of cancer, with the highest proliferation rate. Certain cytological features, such as neoplastic cells arranged in loose irregular clusters with a high nuclear/cytoplasmic ratio, as shown in **figure 2.8 (D),** help identify SCLC specimen samples. In 90% of cases, immunohistochemical predictors of SCLC cells include neuroendocrine markers like CD56, chromogranin, synaptophysin, and TTF-1 (Nicholson *et al.*, 2002). The intrusiveness of this solid tumour is unmatched, with 60-70 % of cases having metastasis and a poor prognosis. There is also no widely used prognostic or predictive biomarker. Tobacco consumption is responsible for 95 % of SCLC cases. SCLC is usually detected in patients over the age of 60. Patients with SCLC have the highest risk of relapse. Weight loss, fatigue, shortness of breath (anorexia), cough, chest pain, dyspnea, and hemoptysis are prevalent

clinical symptoms seen at the time of diagnosis. Another hallmark clinical implication of SCLC is vocal cord paralysis. Extrapulmonary tissues show specific indications in advanced and metastatic stages of SCLC, such as jaundice in the liver, seizures in the brain, pruritus, and bone pain. Specific changes in mental status and ataxia are also seen during brain metastasis (Carter *et al.*, 2014).

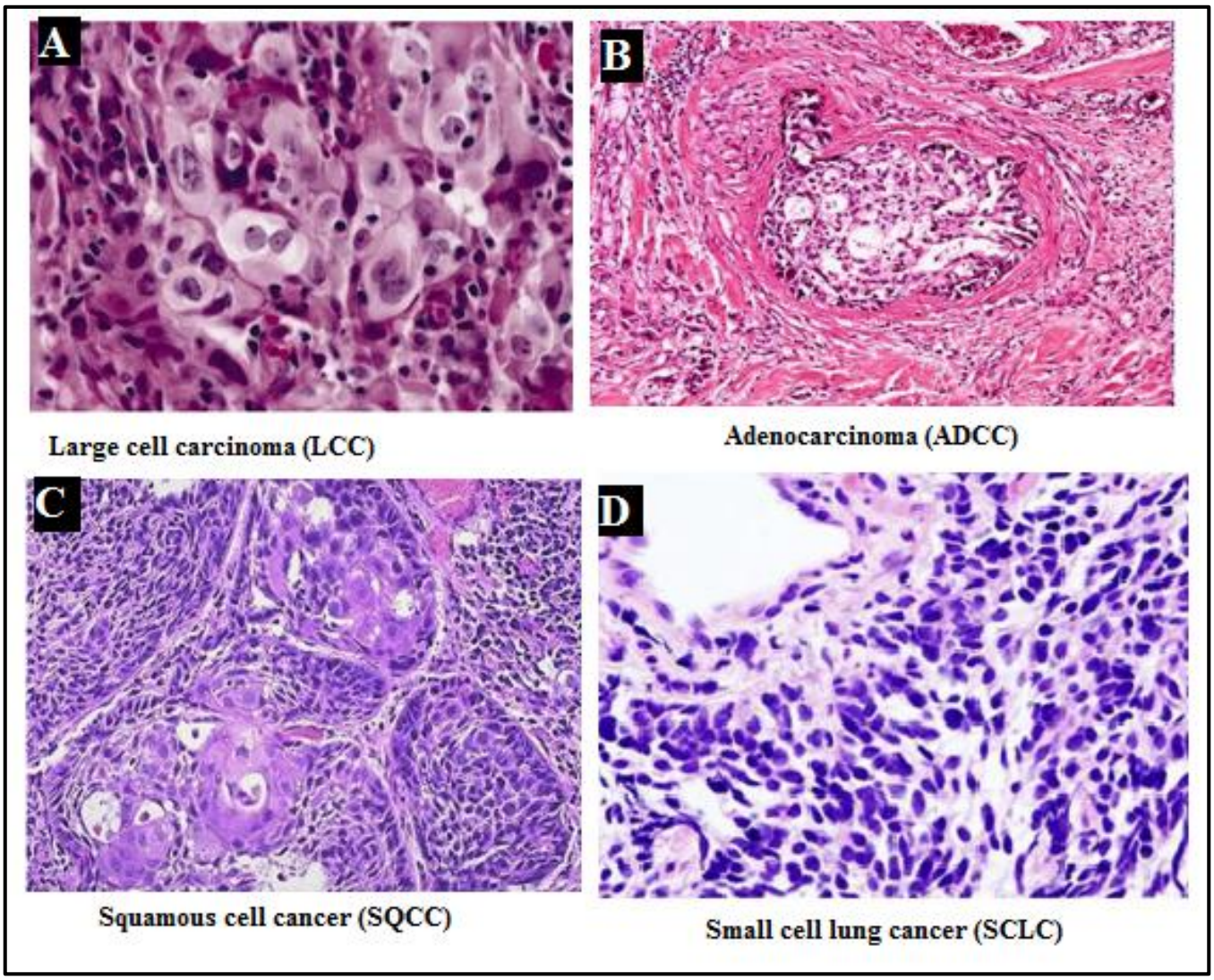


Figure 2.8: Microscopic depiction of various histological subtypes of lung tumour, (A): Large cell carcinoma; (B) Adenocarcinoma; (C) Squamous cell carcinoma; (D) Small cell lung cancer (Weiss, 2010)

2.6 TNM classification

The Tumor-Node-Metastasis (TNM) staging method reveals the anatomic spread of cancer. The International Association for the Study of Lung Cancer has published the 8th version of the

TNM categorization of lung tumours (Lababede & Meziane, 2018). Lung cancers are identified at late stages due to the asymptomatic condition. For determining the stage of a lung tumour, both pathological & clinical parameters are essential. The TNM stage groups are illustrated in **table 2.1**.

Table 2.1: TNM stage classification (8th edition) (Lababede & Meziane, 2018)

Stage	Tx
Stage 0	TisN0M0
Stage IA1	T1aN0M0/ (T1(mi)N0M0)
Stage IA2	T1bN0M0
Stage IA3	T1cN0M0
Stage IB	T2aN0M0
Stage IIA	T2bN0M0
Stage IIB	T(1-2) N1M0/(T3N0M0
Stage IIIA	T (1-2) N2M0/ (T3N1M0)/ T4 N(0-1)M0
Stage IIIB	(T(1-2) N3M0)/T(3-4)N2M0
Stage IIIC	T (3-4) N3M0
Stage IVA	Any T and N, M1a,b
Stage IVB	Any T and N, M1c

The outcomes of physical exams, imaging tests, and biopsies are used to evaluate pathological and clinical stages after surgery. Once the diagnosis is completed, staging is done utilizing microscopic tissue detection by immunohistochemistry, Positron emission tomography, fibre optic bronchoscopy, computed tomography, and fine-needle aspiration cytology (FNAC). This method differentiates between surgically eligible individuals, those who have a loco-regional disease, and those who possess the metastatic disease. The better the patient's prognosis, the lower the tumour's stage (Rami-Porta *et al.*, 2009). The summary of TNM staging of lung carcinoma is depicted in **Table 2.2.**

Table 2.2: Definition of tumour stage, lymph node invasion, and metastasis (Lababede & Meziane, 2018)

Primary tumour (T)	
Tx	The tumour is histopathologically proven; however, it cannot be assessed bronchoscopically or radiologically
T0	The primary tumour is not evident in imaging
Tis	In situ cancer: In situ SQCC In situ ADCC (having lepidic pattern with ≤3cm in the most significant dimension)
T1	Tumour having a size of ≤ 3cm with local invasion and not associating main bronchi
T1a(mi)	The tumour is minimally invasive
T1a	Tumour with a most substantial dimension of 1 cm or less
T1b	Tumours more prominent than 1 cm, however ≤ 2 cm in the most significant dimension
T1c	Tumours more prominent than 2 cm, however ≤ 3 cm in the most significant dimension
T2	Tumours more prominent than 3 cm however ≤ 5 cm in the most significant dimension, involvement of main bronchi, irrespective of the distance to the carina, is linked to obstructive pneumonitis or else atelectasis; invasion of visceral pleura (PL1/PL2); also correlated with the pneumonitis which then extends to the hilar region;
T2a	Tumours more prominent than 3 cm, however ≤ 4 cm in the most significant dimension or else may not be determined
T2b	Tumours more prominent than 4 cm, however, ≤ 5 cm in the most significant dimension
T3	Tumours more prominent than 5 cm however ≤ 7 cm in the most significant dimension; direct involvement of parietal pleura (PL3), chest wall (encompassing superior sulcus tumour), parietal pericardium or phrenic nerve; Primary tumour may have distinguished tumour nodules in the same lobe
T4	Tumour more prominent than 7 cm in the most significant dimension; involvement of carina or trachea; any tumour invasion to heart, diaphragm, mediastinum, great vessels, recurrent laryngeal nerve, vertebral body, or oesophagus; A primary tumour having distinguishes tumour nodule in a different ipsilateral lobe
Regional lymph nodes (N)	
Nx	The lymph nodes in the region cannot be examined.
N0	There are no lymph nodes in the region that are involved.
N1	Ipsilateral hilar node(s) or ipsilateral peri-bronchial along with intrapulmonary nodes, as well as a direct extension to intrapulmonary nodes
N2	Subcarinal nodes or Ipsilateral mediastinal
N3	Any of the lymph nodes groups such as contralateral hilar, contralateral scalene, ipsilateral, contralateral mediastinal, or supraclavicular nodes
Distant metastasis (M)	
Mo	Absence of metastasis
M1	Distant metastasis
M1a	Separate tumour nodule(s) present in a contralateral lobe along with pleural tumour or primary tumour or malignant effusion, or pericardial nodules
M1b	Metastasis in the single extrathoracic region
M1c	Metastasis in the multiple extrathoracic regions (one or multiple organs)

2.7 Molecular pathogenesis of lung cancer

The accumulation of various alterations at both the molecular and genetic levels has contributed to the pathogenicity of lung cancer. The ability to proliferate while not responding to regulatory factors, escaping the apoptotic pathway, overexpression of the factors responsible for vascular angiogenesis, unregulated replication due to telomerase hyperactivity, and finally, the ability to invade adjacent tissues and distant sites is the most crucial attribute attained by cells undergoing the path of hyperplasia, dysplasia, and malignant neoplasia (Hanahan and Weinberg, 2000). These functional distinctions are ascribed to variations in the expression of genes and proteins that regulate various homeostasis processes.

Another noteworthy feature is that these heterogeneous lung tissue cells develop from precursor cancer stem cells from various lineages, which dedifferentiate and form these vigorously proliferating cells. This causes the pathogenesis of different histological subtypes of lung cancer. Recent studies have demonstrated that epigenetic changes play a pivotal role in dysregulating the expression of genes that control cellular proliferation and differentiation. Even though promoter methylation directly or indirectly checks the genome's integrity the methylation profile of all these target genes could be critical in unveiling the pathogenicity of lung tissue (Zöchbauer-Müller *et al.*, 2002). Various kinds of molecular alterations that happen in cells as a result of carcinogenesis entail abnormal regulation of tumour suppressor genes, loss of heterozygosity (LOH), activation of a proto-oncogene to an oncogene under the influence of any environmental carcinogen, and occurrence of genomic instability due to gain or loss of whole chromosomes or the fragments (Massion and Carbone, 2003).

Several studies have documented the specific genetic changes that occur during the development and progression of lung cancer. There are few differences in the molecular aberrations discovered between SCLC and NSCLC. Allelic loss of various chromosomal loci containing proto-oncogenes, such as 8q21-23, 9p21, 3p14-23, 13q, 17q, 22p, and 18q, is one of the common genetic deciding factors seen in all histological subtypes of lung cancer (Panov, 2005). The following section goes into depth about significant molecular abnormalities in the pathogenesis of lung cancer **(Figure 2.9 and 2.10).**

2.7.1 Growth factors and their receptors

As an outcome of the expression of various growth factors, regulatory peptides, and their associated receptors by either adjacent normal or cancerous cells, paracrine and autocrine growth stimulatory loops are standard in lung carcinoma.

2.7.1.1 Gastrin-releasing peptide (GRP)/bombesin (BN) autocrine loop: Various growth factors and their corresponding receptors have been identified as expressing on lung carcinoma cells or neighboring normal cells, resulting in different autocrine and paracrine growth stimulatory loops. Gastrin-releasing peptides (GRP) and bombesin (BN)-like peptides are examples of autocrine system components. BN-like peptides promote bronchial epithelial cell growth in normal mammalian tissues and play an essential role in the regulation of human lung development (Gonzalez *et al.*, 2008). The effects of GRP are mediated by the gastrin-releasing peptide receptor (GRPR), the bombesin receptor subtype 3 (BRS-3), and the neuromedin B receptor (NMBR). GRPR and BRS-3 expression are uncommon in NSCLC, whereas NMBR expression is more prevalent (Sancho *et al.*, 2011). Ligands for these receptors are frequently expressed in lung carcinoma, suggesting the possibility of autocrine (self-stimulatory) loops. The NMB gene is expressed in 100% NSCLCs and SCLCs. GRP expression is less expressed in NSCLCs, whereas GRP expression has been reported in approximately 20-60% of SCLCs (Zochbauer-Muller *et al.*, 2002).

Furthermore, the presence of two copies (expressed) of the X-linked GRPR gene in females may be a factor in increased susceptibility to tobacco-stimulated cancer. Inhibiting this system with receptor antagonists or antibodies can inhibit tumour growth in model systems; this idea is being researched as a novel therapeutic opportunity (Zochbauer-Muller *et al.*, 2002). Insulin-like growth factors I and II are two other peptides that may play an autocrine growth role in lung carcinoma. It has been observed that the accumulation of genetic deformation depends on a person's intrinsic carcinogen sensitivity and various humoral factors such as insulin-like growth factors. In patients with poorly or advanced differentiated carcinoma, mean blood insulin-like growth factor-I levels and the molar ratio of insulin-like growth factor-I/insulin growth factor binding protein-3 was higher than in patients with well-differentiated or early carcinoma (Pavelić *et al.*, 2002).

***2.7.1.2 ERBB family*:** The ERBB1 proto-oncogene encodes EGFR, which regulates epithelial proliferation and differentiation and is activated in lung carcinoma cells by overexpression (Schramm *et al.*, 2022). Increased expression of EGFR has been noted in 13% of NSCLC patients, and expression of EGFR at the protein level by tumours appears to be a risk factor for poor diagnosis in NSCLC patients. The ERBB2 (HER2/neu) oncogene is a significant proto-oncogene implicated in the development of lung carcinoma. HER2/neu is a transmembrane glycoprotein with a molecular weight of 185KDa homologous to EGFR. Numerous studies have discussed the overexpression of ERBB2 in 1-4 % of NSCLC cases, particularly adenocarcinoma. Upon ligand binding, ERBB receptors either homodimerize or heterodimerize to induce intrinsic kinase activities, initiating an intracellular signal transduction cascade that includes the MAP kinase pathway. The transfection experiments suggest that ERBB2 overexpression contributes to tumorigenicity in immortal human bronchial epithelial cells (Sithanandam *et al.*, 2003). Anti-ERBB2 monoclonal antibody (mAb) inhibited the growth of ERBB2 expressing NSCLC cell lines in vitro. Elevated ERBB2 levels were associated with multiple drug resistance phenotypes and increased metastatic potential in NSCLC. This could explain why some researchers attribute poor clinical outcomes to ERBB2 overexpression (Hirsch *et al.*, 2003).

2.7.1.3 MET: Hepatocyte growth factor (HGF) promotes epithelial cell division, movement, and complex differentiation programs. HGF functions as a mesenchyme-derived morphogenic factor during fetal lung development. In response to lung injury, constitutively low levels of HGF increase (Chen *et al.*, 2019). In vitro, HGF stimulates mitogenesis in human bronchial cells, alveolar type II cells, and SCLC cells. The MET proto-oncogene is an HGF receptor commonly expressed in the normal lung and NSCLC and SCLC. As a result, these cancer cells possess both the receptor and the ligand for this growth factor. However, HGF is primarily expressed in NSCLCs, implying an NSCLC-specific autocrine loop. Clinically, increased HGF was associated with a poor outcome in patients with respectable NSCLC (Ujiie *et al.*, 2012).

2.7.2 Activation of dominant oncogenes

Proto-oncogenes, also known as dominant oncogenes, play an essential role in regulating average cell growth and development, and their physiological expression is tightly regulated (Zochbauer-

Muller *et al.*, 2002). Different genetic mechanisms, such as DNA translocation, amplification, and point mutations, can stimulate these genes.

2.7.2.1 RAS activation

HRAS, KRAS, and NRAS are human RAS genes that play a vital role in signal transduction pathways by coding for guanosine triphosphate (GTP)-binding proteins. RAS-GTP generates a growth-promoting signal, whereas the hydrolysis of RAS-bound GTP inactivates the RAS signal to guanosine diphosphate (GDP). Oncogenic RAS mutations are widespread in the development of lung cancer. GTP cannot hydrolyze to GDP in the presence of RAS mutations, resulting in constitutive active RAS-GTP growth-promoting activity. RAS oncogenes gain their transforming ability through point mutations, found in approximately 20-30% of adenocarcinoma cases and 15-20% of NSCLCs at the KRAS codons 61, 13, or 12 (Silvers *et al.*, 2002).

On the other hand, RAS mutations have not been found in SCLCs. Mutations occur preferentially in KRAS and account for approximately 90% of oncogenic RAS mutations in adenocarcinomas. Almost all KRAS mutations have been reported to affect codon 12, and most of the mutations are G-T transversions of the type that have been linked to cigarette smoking carcinogens. Previous studies have shown a correlation between oncogenic RAS mutations and a poor prognosis in NSCLC patients. KRAS mutations, however, were not associated with in vitro resistance to a variety of chemotherapeutic agents in NSCLC cell lines (Friedlaender *et al.*, 2020).

2.7.2.2 EGFR alterations

EGFR mutations are linked to a wide range of NSCLC tumour pathogenesis. The EGFR gene encodes a transmembrane tyrosine kinase with an intracellular tyrosine kinase domain and an extracellular ligand-binding domain. The interaction of the ligand EGF to its receptor causes dimerization with the other members of the EGFR family, activating the tyrosine kinase domain. EGFR stimulates signal transduction via the JAK/STAT, PI3K/AKT/mTOR, and RAS/RAF/ MAPK signaling pathways (Scagliotti *et al.*, 2004). EGFR regulates various oncogenic functions, including cell division, survival, differentiation, invasion, and metastasis. EGFR mutation can cause constitutive tyrosine kinase activation and oncogenic transformation of lung epithelial cells in vitro. A previous study discovered activating mutations at exon 18 in an Australian population,

accounting for 14% of EGFR mutations in the initial stage of lung carcinoma patients and Lue858Arg mutations for 29% of EGFR mutations in this group (Yip *et al.*, 2013). Although it may also be seen in adenosquamous cancer, nearly all EGFR mutations occur in ADCC in lung carcinoma (Marchetti *et al.*, 2013).

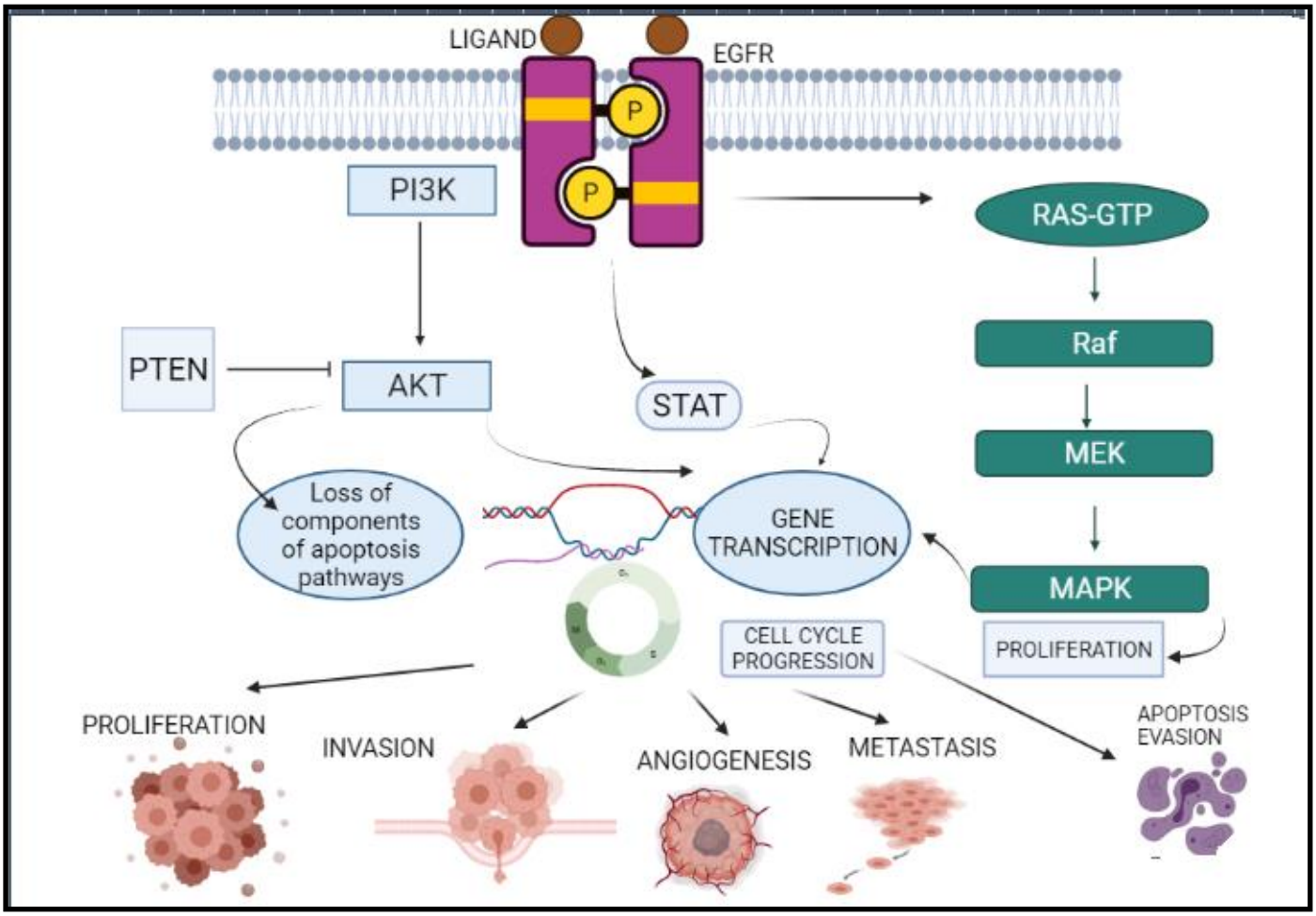


Figure 2.9: A depiction summarizing molecular pathogenesis of lung cancer

2.7.2.3 BRAF mutations

BRAF is a Ser-Thr protein kinase that functions as a downstream effector of the KRAS protein, activating the MAPK signaling pathway that governs cell proliferation and survival. When activated, BRAF phosphorylates the downstream mediators MEK 2 and MEK 1, which activate ERK1 and ERK2 (Extracellular signal-regulated kinase 1/2). Growth-regulating proteins such as

c-JUN and ELK1 are regulated by ERK1 and ERK2 (Downward, 2003). Activating mutations in the BRAF gene increase kinase activity, exhibiting transforming activity in vitro.

BRAF mutations were first identified in melanomas and are now found in approximately 3% of NSCLCs, most of which are ADCC. In ADCC, around half of the mutations are of $Val^{600}Glu$ residue in exon 15, followed by $Asp^{594}gly$ in exon 15 and $Gly^{469}Ala$ in exon 11. (Paik *et al.*, 2011). In NSCLC, few BRAF mutations occur in the kinase domain, such as $Val^{600}Glu$, $Asp^{594}Gly$, and $Leu^{596}Arg$, whereas $Gly^{468}Ala$ and $Gly^{465}V$ occur in the activation domain, which is located in the gene's G-loop. As KRAS and BRAF genes are essential components of EGFR-regulated signaling pathways, it is not surprising that mutations in these genes are almost always mutually exclusive, keeping with the general downstream pathway to transformation.

2.7.2.4 ALK Fusion genes

Anaplastic lymphoma kinase (ALK), a receptor tyrosine kinase, is a human enzyme encoded by the ALK gene. ALK rearrangements have been identified in 5% of NSCLCs (Kris *et al.*, 2014). ALK is fused to the echinoderm microtubule-associated protein-like 4 (EML4) in 95% of ALK-rearranged NSCLCs. Biologically, EML4-ALK fusion causes protein oligomerization and constitutive kinase activation. In vitro studies have shown that these fusions result in function gain. In vivo, mice expressing a variant of EML4-ALK under the promoter of lung epithelial cells developed multiple lung ADCC tumours susceptible to pharmacologic ALK (Soda *et al.*, 2008). A short inversion causes the rearrangement of chromosome 2p; however, the fusion of intron 13 of EML4 to intron 19 of ALK has been reported in the most common variant. Because of the varying lengths of the EML4, many variants of the EML4-ALK fusions have been identified; the most prevalent type is exon 1-13 of EML4 fused to exon 20-29 of ALK. TFG (TRK-fused gene), KIF5B (kinesin family member 5b), and KLC-1 (kinesin light chain1) have been discovered in a small subset of ALK rearrangements (approximately <1% of cases) (Rikova *et al.*, 2007).

2.7.2.5 ROS1

ROS1, a proto-oncogene located on chromosome 6q22.1, encodes a receptor tyrosine kinase with an N-terminal extracellular domain, a C-terminal intracellular tyrosine kinase domain, and a hydrophobic single-pass transmembrane region (Acquaviva *et al.*, 2009). ROS1 activation causes

signaling through various pathways, including RAS/MAPK/ERK, PI3K/AKT/mTOR, and STAT3. ROS1 fusions were first discovered in a human glioblastoma cell line. ROS1 fusions have been observed in ovarian cancer (Birch *et al.*, 2011), colorectal cancer (Aisner *et al.*, 2014), NSCLC (Zhu *et al.*, 2016), gastric cancer, and angiosarcoma (Giacomini *et al.*, 2013). NSCLC is one of the solid tumours recognized using ROS1 arrangements. In the past, an investigation with more than 150 NSCLC tumours and 41 NSCLC cell lines identified ROS1 as a highly activated kinase in one tumour cell and one cell line (Rikova *et al.*, 2007). ROS1 sends signals through multiple pathways to induce cell growth and survival (Davies *et al.*, 2012).

2.7.2.6 RET rearrangements

The RET proto-oncogene gene is located on chromosome 10q11.2 and encodes a tyrosine kinase receptor found on the cell surface. The intracellular kinase domain shares homology with the ALK kinase domain. RET signaling is essential for developing and maintaining the enteric nervous system and kidneys' normal cell function (Takahashi *et al*., 2022). Loss of function RET mutations can cause Hirschsprung disease, congenital urinary tract, and kidney deformities (Wenskus *et al.*, 2021). RET is activated when its ligands, glial-derived neurotrophic factor (GDNF), bind to GDNF family receptor (GFR) co-receptors. This binding causes RET to homodimerize and auto phosphorylate within its intracellular tyrosine kinase domains (Wang *et al*., 2013). This, in turn, activates downstream signaling pathways required for normal cell division and proliferation, primarily the MAPK, RAS, JAK-STAT, and PI3K pathways (Qian *et al.*, 2014).

RET activation via chromosomal rearrangements has recently been observed in a small percentage of lung cancers (Wang *et al.*, 2013; Qian *et al.*, 2014). Oncogenic activation of RET occurs in NSCLC via chromosomal rearrangements, which fuse the 3'coding region for the RET kinase domain on chromosome 10 with the 5' upstream partner gene. This fusion promotes ligand-independent dimerization, constitutive RET activation, and oncogenesis. Intra-chromosomal rearrangements are usual, with partner genes such as *NCOA4*, *CCDC6*, and *KIF5B* originating on chromosome 10. (Drilon *et al.*, 2019). Patients with RET fusions are more likely to be never smokers, younger, and have ADCC more frequently than other histological types. It is important

to note that these fusions have also been mentioned, albeit less frequently, in older patients with a significant smoking history and ADCC histology.

2.7.2.7 MYC overexpression

RAS signaling is involved in the activation of nuclear proto-oncogenes like MYC. MYC transcriptionally stimulates downstream genes upon heterodimerization, thereby promoting cell growth. The MYC proto-oncogene gene family includes MYC, MYCN, and MYCL, which encode nuclear phosphoproteins and belong to the helix-loop-helix leucine-zipper transcription factor class. MYC is primarily activated in NSCLC and SCLC, whereas MYCL and MYCN anomalies are common in SCLC. MYC proteins mediate average cell growth by directly activating genes involved in DNA synthesis, RNA metabolism, and cell-cycle progression (Grandori *et al.*, 2000). Overexpressed MYC proteins have the potential to promote cell proliferation while also repressing terminal differentiation, which is consistent with the fact that cancers of various types show genetic changes in the MYC family genes. The primary mechanism of MYC activation in lung carcinoma is either gene amplification or transcriptional dysregulation resulting in overexpression. MYC amplification occurs in 5-10% of NSCLCs and 15-30% of SCLCs (Sekido *et al.*, 2001). MYC amplification genes have been considered to harm survival in SCLC patients. Amplification has been observed as 20-115 copies of one of the genes/cells. Richardson *et al.* examined 17 studies and discovered that 18-31 % of SCLC cell lines had gene amplification of at least one member of the MYC family. Nevertheless, only 85-20% of NSCLC cell lines were found to have MYC amplification. Amplification of the MYC gene appears more common in chemotherapy patients, and the "variant" subtype of SCLC may be related to poor survival (Mollaoglu *et al.*, 2017).

2.7.2.8 BCL-2 Overexpression

BCL-2, a proto-oncogene on chromosome 18q21, is overexpressed in several lung cancers. The anti-apoptotic protein produced by the BCL-2 gene is negatively regulated by p53. Cancerous cells may overexpress BCL-2 to overcome apoptotic signals from RAS and MYC expression. BCL-2 upregulation has been confirmed in 75 to 95% of SCLC cases, compared to 10 to 35 % of NSCLC cases (Mitsuuchi & Testa, 2002). In NSCLC, an inverse relationship has been observed between mutant p53 and BCL-2 overexpression, indicating that either alteration may play an essential role in disease pathogenesis. Immunohistochemical studies have reported that those BCL-2 proteins

are commonly expressed in NSCLCs and SCLCs, but their expression is higher in SQCC than in ADCC (Zöchbauer-Müller *et al.*, 2002). Surprisingly, much research has shown that people with BCL-2 positive tumours have a higher chance of survival, although others have not validated the finding, implying that BCL-2 expression indicates a higher susceptibility to cytotoxic treatment (Adams & Cory, 2018). As BCL-2 plays an essential part in inhibiting apoptosis and can potentially impair patients' response to conventional chemotherapies, it is of great interest to develop antisense BCL-2 therapeutics, which are currently in clinical trials (Knight *et al.*, 2019).

2.7.3 Loss of function of tumour suppressor genes

Tumour suppressor genes (TSGs) control cell proliferation and repair DNA damage. The loss of function of anti-proliferative genes is prevalent in cancer. TSG dysfunction is attributed to losing a specific allele at a chromosome locus, genetic transformation, or epigenetic alterations such as hypermethylation of these genes' promoter regions (Wistuba *et al.*, 2000).

2.7.3.1 p53 gene

Changes in the p53 gene, which plays an essential role in ensuring the integrity of the human genome, are the most widely known redundant mutation found in almost all histological subtypes. The p53 gene, found on chromosome 17p13.1, encodes a nuclear protein of 53 kDa. p53 acts as a transcription factor, activating the DNA deterioration response program's expression, stopping cell progression through the cell cycle in the late G1 phase, and inducing apoptosis (Sherr *et al.*, 2000). DNA damage is a significant indication for p53 phosphorylation, catalyzed by kinase. P53 phosphorylation functions as a specific DNA-binding transcription factor for several genes, including BAX, GADD45, MDM2, p21/WAF1/CIP1, and cyclin G. (Sekido *et al.*, 2003). Apoptosis, DNA repair, and cell cycle arrest are all caused by stimulating these genes. Mutations in the p53 gene are among the most common genetic changes associated with cancer, and they result in the loss of TSG function and the inability to induce cell apoptosis. A GC to TA transversion is the most prevalent type of point mutation that causes missense mutations. This type of point mutation has been linked to benzo (a) pyrene-induced cigarette smoking deterioration (Pfeifer *et al.*, 2009). Smokers had a significantly higher risk of p53 mutations when compared to nonsmokers (Husgafvel-Pursiainen *et al.*, 2000). Missense p53 mutations cause an increase in protein half-life, resulting in higher levels of p53 protein that can be easily identified by

immunohistochemistry. Immunohistochemistry revealed that p53 expression was abnormal in 40-60% of NSCLCs and 40-70% of SCLCs. Numerous reports have been examined for the correlation of p53 abnormalities with NSCLC patient prognosis; however, the results have been contradictory as to whether or not p53 abnormalities influence diagnosis. A meta-analysis was conducted by inspecting 43 published articles to determine whether p53 alterations have a detrimental effect on the survival of NSCLC patients (Mitsudomi *et al.*, 2000). The negative prognostic effect of p53 mutations was highly significant in patients with adenocarcinoma but not in patients with squamous cell carcinoma. p53 mutations were discovered in the DNA studies, while over-expression was discovered in the protein studies. The incidence of p53 alterations in DNA studies was lower than in protein studies, and the occurrence of p53 over-expression and mutations in ADCC was significantly lower than in SQCC.

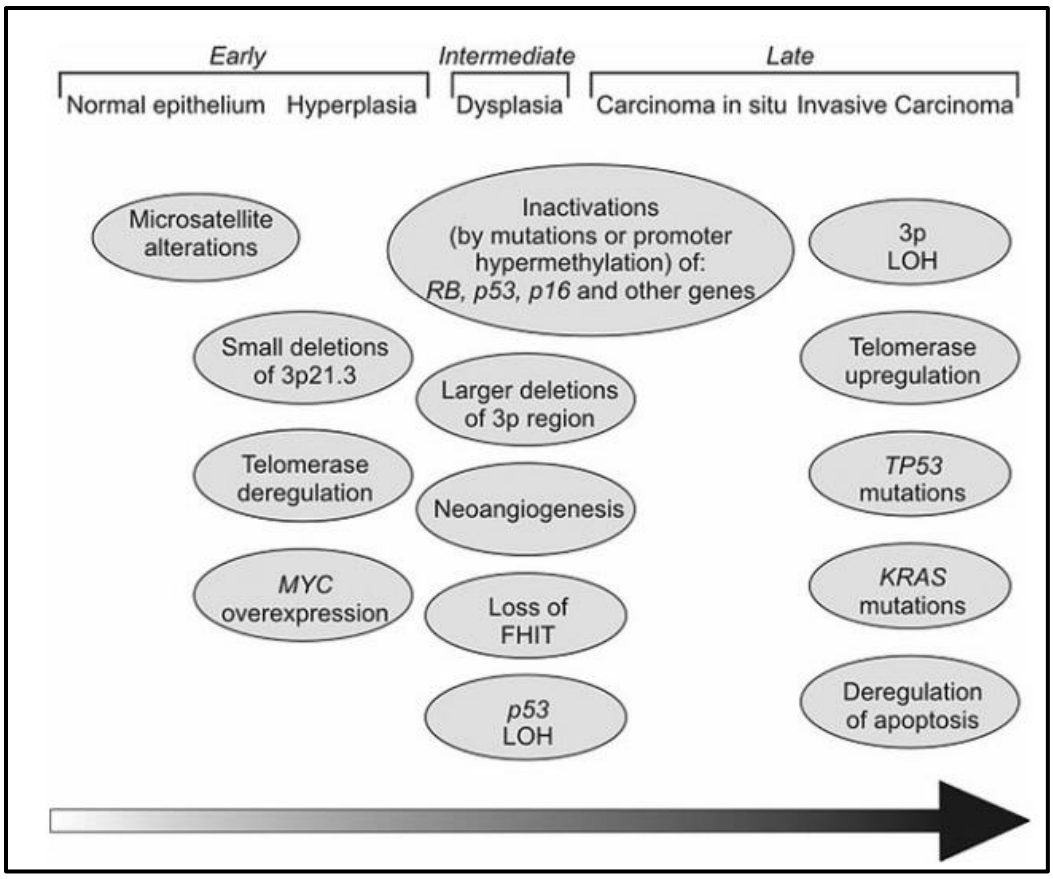


Figure 2.10: Basic molecular alterations involved in the aetiology of lung cancer (Panov, 2005)

2.7.3.2 Other candidate TSGs

On NSCLCs and SCLCs, a genome-wide high-resolution LOH search was performed to identify new loci that may harbor tumour suppressor genes (Girard *et al.*, 2000). Twenty-two diverse regions with more than 60 % LOH were identified. Seven regions showed a preference for NSCLC, thirteen indicated a preference for SCLC, and two regions influenced both NSCLC and SCLC. 9p (p16), 9q, 13q (Rb), 17p (p53), 1p, 3p, 4q, 4p, 5q, 8p, 10q, 10p, 15q, 18q, 190, Xq, and Xp are among the chromosomal regions with common LOH. Candidate TSGs in other regions include WT1 at 11p13, APC at 5q21, and NF2 at 22q12. NSCLC and SCLC had different regions of common LOH, with SCLC having fewer of these regions than NSCLC, denoting that NSCLC and SCLC both undergo various genetic changes. Until now, 3p allele loss was the most common molecular change in lung cancer (Wistuba *et al.*, 2000). Markers involved in LOH on 3p are found in approximately 90% of SCLCs and 80% of NSCLCs (Zochbauer-Muller *et al.*, 2002). Different 3p regions, including 3p12, 3p14, 3p21.3-22, and 3p25-26, have demonstrated common allele loss, implying that various TSGs are localized on chromosome 3p (Wistuba *et al.*,2000). Furthermore, other independent homozygous deletions at 3p12, 3p14.2, and 3p21.3 have been reported in lung cancer, providing strong evidence for the presence of TSGs (Zochbauer-Muller *et al.*, 2002). Ras Association Domain Family Member 1 (RASSF1) is a protein expressed by the RASSF1 gene in humans. The gene encodes several crucial transcripts generated by alternative mRNA splicing and alternative promoter selection. In lung carcinoma, the expression of one of the transcripts, RASSF1A, is lost. The major region for RASSF1A inactivation is the unique methylation of its associated promoter region, which is responsible for turning off its mRNA expression, though mutational inactivation of this gene is extremely rare (Burbee *et al.*, 2001).

FHIT (**Fragile Histidine Triad**) is another TSG candidate found on 3p14.2 and undergoes common hemizygous and infrequent homozygous deletion in lung carcinoma cells. It encodes the enzyme dinucleoside hydrolase. A 40-80% lung carcinoma cell expresses FHIT mRNA transcripts but almost always expresses wild-type FHIT transcripts (Virmani *et al.*, 2001). FHIT point mutations are infrequent, and unusual FHIT can also be observed in normal lung tissue (Zöchbauer-Müller *et al.*, 2000). FHIT transcripts were identified as abnormal in 40% of NSCLC and 80% of SCLC specimens (Mitsuuchi & Testa *et al.*, 2002), and no FHIT protein expression was found in an estimated 50% of all lung carcinomas. LOH at the FHIT gene locus was more

common in smokers than nonsmokers, suggesting that this gene is a molecular target of carcinogens in tobacco smoke (Pylkkanen *et al.*, 2002).

2.7.3.3 PTEN

This gene on chromosome 10q23 encodes a protein phosphatase and a lipid that inhibits the PI3K/AKT/mTOR pathway by dephosphorylating PI-3,4,5-trisphosphate. PTEN's TSG function is inactivated, resulting in Akt/PKB (Protein kinase B) activation independent of ligand binding (Cully *et al.*, 2006). PTEN mutations are rare in NSCLC (approximately 5%), more common in SQCC than ADCC (10.2 % versus 1.7 %), and are associated with a smoking history. On the contrary, reduced protein expression has been reported in approximately 75% of NSCLC (Jin *et al.*, 2010).

2.7.4 Aberrant Promoter Methylation

Transcriptional inactivation of TSG has been associated with abnormal methylation of typically unmethylated CpG-rich regions, commonly referred to as CpG islands, which are found in or near the promoter region of many genes (Deaton *et al.*, 2011). Methylation can be used as an alternative to genetic deletion or mutation, resulting in a TSG function loss. Several genes, such as adenomatous polyposis coli (APC), retinoic acid receptor-2 (RAR), CDH13 (H-cadherin), fragile histidine triad (FHIT), RASSF1A, tissue inhibitor of metalloproteinase-3 (TIMP-3), p16, and death-associated protein kinase, are frequently methylated in primary lung tumours (Zochbauer Muleer *et al.*, 2002). Grawenda *et al.* discovered that patients whose tumours were methylated for RASSF1A had lower overall survival and a significantly shorter disease-free survival than patients whose tumours were methylated for DAPK (Grawenda *et al.*, 2015). Methylated DNA sequences have been discovered in primary lung cancers, lung cancer patients' serum DNA, sputum samples before the onset of invasive lung cancer, and lung carcinoma precursor lesions. These results indicate that abnormal methylation can happen during the preneoplastic process and that it might be used as a diagnostic marker for early monitoring and detection of lung cancer. The methylation status of specific genes in bronchial biopsies, bronchiole alveolar washings, and sputum samples from high-risk people, such as heavy smokers, is being studied as a risk assessment marker for lung cancer. In vitro, methylation inhibitors such as 5-aza-20-deoxycytidine can correct aberrant

methylation, resulting in gene re-expression and tumour growth suppression (Bensberg *et al.*, 2021).

2.7.5 Activation of tumour-stimulated angiogenesis for tumour growth

A highly complicated tumour microvasculature network provides nutrients, oxygen supply, and efficient metabolite drainage in tumours (Kabiraj *et al.*, 2018). Angiogenesis is required for tumour growth and cell dissemination in neoplastic progression metastatic regions. Angiogenic factors influence vasculature development, growth patterns, and vascular permeability, which regulates tumour invasion, metastasis, and prognosis. They also affect tumour invasion, metastasis, and prognosis by modulating the host response. Hypoxia and oncogene mutations, such as p53 and RAS, stimulate angiogenic cell development (Muz *et al.*, 2015). VEGF (vascular endothelial growth factor) is important in lung cancer metastasis and is usually expressed in lung tumours. These growth factors and their receptors regulate both physiological and pathological angiogenesis. The VEGF receptors explicitly expressed on endothelial cells have been identified, and antibodies to block their interaction with VEGF have been produced (Shibuya *et al.*, 2011). It is crucial because dendritic cells play an essential part in antigen presentation, and their impaired function may contribute to tumour pathogenesis (Gardener *et al.*, 2020).

NSCLC patients had high levels of VEGF expression and a poor prognosis for new vessel formation. Xu *et al.* discovered that the activated microvessel density was higher in the invasion front of the tumour and within the normal lung adjacent to the tumour than in the normal lung distal to the tumour or inside the tumour in observation of early operable NSCLC. These results suggest that activated microvessel density is an independent predictor of NSCLC in patients. Upregulation of platelet-derived endothelial cell growth factors may be associated with a poor prognosis in patients with node-negative cancer (Xu *et al.*, 2018). Salgia *et al.* discovered that the microvessel count was a pretty significant adverse predictor of both overall and disease-free survival in NSCLC patients, implying that assessing tumour angiogenesis may be helpful in the postsurgical staging of NSCLC patients to identify subsets of patients who may benefit from adjuvant treatment studies (Salgia *et al.*, 2011). New therapy options targeting angiogenic factors or their receptors are being investigated in lung cancer clinical trials.

2.7.6 Expression of telomerase activity and cellular immortality

Telomerase is an enzyme that provides additional guanine-rich repeating sequences to telomeres to prevent them from shortening. Telomerase activity is found in gametes, stem cells, and tumour cells. Human somatic cells have a limited proliferative capacity, and senescence occurs after 50-70 cell divisions. Most tumour cells, on the other side, have an infinite capacity for multiplication (Srinivas *et al.*, 2020). TTAGGG hexameric tandem repeats are found at the ends of human chromosomes (telomeres). The loss of telomerase activity during normal cell division is linked to increased telomere shortening, which causes cell senescence and natural cell death (Jafri *et al.*, 2016).

In contrast, germ cells, some stem cells, and most cancer cells have telomerase activity, which causes the hexameric repeats to be replaced, potentially leading to cellular immortality (Fernandes *et al.*, 2020). Most SCLCs and more than 80% of NSCLCs have telomerase activity, associated with increased cell proliferation and advanced stage in NSCLCs (Valles *et al.*, 2012). As a result, anti-telomerase treatments are being investigated as a potential novel therapeutic for various cancers, including lung cancer. Telomerase components are activated in the latent preneoplastic stages of lung cancer. The mechanism supporting the re-expression of telomerase's catalytic component hTERT (Telomerase reverse transcriptase in humans) or the RNA components in cancer is unclear. The combined effect of hTERT, SV40 T antigen, and a mutant RAS gene can immortalize and transform normal epithelial cells into cancerous cells. (Sato *et al.*, 2020)

2.7.7 Loss of components of apoptosis pathways

Apoptosis has long been acknowledged as the most critical metabolic mechanism for mitigating the effects of carcinogens, DNA damage, and necrotic cells. The natural process of cells going through the apoptotic pathway maintains the human body's homeostasis. These biological pathways are disrupted when the tumour develops (Tang *et al.*, 2019). The BCL-2 protein family is engaged in the development, tissue homeostasis, and immunology because it regulates whether a cell commits to apoptosis. As a result, interfering with this control can either promote cancer and autoimmune diseases (due to insufficient apoptosis), aggravate ischemia, and contribute to degenerative diseases (too much apoptosis). There are interactions of three BCL-2 family members, namely the pro-survival subfamily (for example, BCL-2, BCL-XL, and MCL1) and two

pro-apoptotic factions. The BH3-only proteins (for example, BIM, PUMA, and BID) impart various cytotoxic signals, and the death effectors BAX and BAK, which can transform into homo-oligomers, determine whether a cell lives or dies (Czabotar *et al.*, 2014). Tobacco and other pollutants cause the loss of the 3p21.3 allele in different respiratory system epithelial cells.

Moreover, the tumour suppressor gene at 3p21.3 is deleted, rendering it a haploid insufficient. The following sequence of events is the inactivation of growth-regulating genes such as Rb, p53, or p16 due to mutation or epigenetic silencing. This enables the clonal multiplication of modified cells. It is proposed that during the pathogenesis of SCLC, neoplastic cells arise directly from normal or hyperplastic epithelial cells without passing through preneoplastic intermediate clinical stages. The pathogenesis of NSCLC, on the other hand, is followed by several morphological changes (Kashima *et al.*, 2019). Some oncogenic driver mutations have obtained clinical significance, and they are now being used in the treatment regimens of ADCC patients alongside their ongoing chemotherapy, which mainly comprises platinum-based prescription drugs. These potential targets have been critical in increasing the average survival time of lung carcinoma patients, especially those with ADCC (Sholl *et al.*, 2016).

2.8 Lung carcinoma diagnosis

Lung carcinoma is a multifactorial disease with many causes, tumours, and symptoms, making early detection critical for the best prognosis. Lung cancer diagnostic methods are divided primarily into conventional, advanced, and modified methods. Computerized tomography (CT), cytology, biopsy, and bronchoscopy are examples of traditional diagnostic methods, whereas advanced and modified diagnostic methods include biosensors, biomarkers, radiogenomics, liquid biopsy, and various other diagnostic tools (Prabhakar *et al.*, 2018).

CT scans are computer-generated cross-sectional images of any body part using X-rays. Compared to traditional X-rays, tomographic images contain comprehensive information about a specific area in cross-section, eliminating image superimposition, which gives them a substantial advantage over plain films (Patel *et al.*, 2022). However, CT scan has two drawbacks: poor patient compliance and less accurate detection. Positron emission tomography (PET), Magnetic resonance imaging (MRI) are also imaging tests that help in the diagnosis of the severity of cancer. A biopsy

is a procedure that involves using a needle and a small incision to remove a portion of a tissue or lump or whole nodule. It is used to diagnose a specific disease in the lungs, breast, colon, stomach, and other body parts (Prabhakar *et al.*, 2018). It is a highly invasive method that results in low patient compliance. The two types of cytological diagnostic methods for lung carcinoma detection are sputum and pleural fluid cytology. Sputum cytology assesses sputum induced by artificial means or collected naturally. It is commonly used to detect lung cancer in its early stages. This test is non-invasive and highly sensitive for centrally located lung carcinoma (Patriquin *et al.*, 2015). In pleural fluid cytology, cancer can be detected by malignant cells in the pleural fluid that lines the lungs. The pleural fluid examination has approximately 60%–70% sensitivity. According to the latest report, patients with malignant lung tumours had disproportionately high tumour markers in their pleural fluid (Kaur *et al.*, 2017).

Advanced and modified diagnostic techniques are widely used and have much potential for replacing traditional diagnostic methods. Biomarkers are naturally occurring substances, genes, or biological entities that undergo specific changes during disease and can thus be used to detect anomalous cell functions in people living with cancer. Biomarkers are classified based on various factors, including their applications and characteristics. The two types of biomarkers based on characteristics are imaging biomarkers (used in conjunction with LDCT-low dose CT scan and PET) and molecular biomarkers (gene-based biomarkers such as miRNA, proteins, and peptides) (Huss *et al.*, 2015). Based on their applications, biomarkers are categorized as diagnostic, prognostic, or predictive (Soriede *et al.*, 2009). BRAF, PTEN, EGFR, KRAS, ROS1, and ALK are some genomic biomarkers to detect lung carcinoma. A biosensor is a device that transforms chemical signals into an electronic response (Strimbu *et al.*, 2010). It is essentially a device that detects and quantifies biomarkers. Biosensors are designed based on the nature of the molecules to be detected. The biosensors contain binding sites for the markers being studied.

To detect specific biomarkers in lung carcinoma and other cancers such as leukemia, aptasensors and highly specific optical biosensors are used (Monosik *et al.*, 2012). Liquid biopsy is a new technique for detecting lung carcinoma that uses circulating tumour DNA (ctDNA) in the blood. It is critical because it can be done quickly and easily at a blood-testing centre. It is a non-invasive procedure that improves patient compliance. The USFDA approved the cobas EGFR mutation test v1 in 2013 as the first blood test for detecting NSCLC (Brown 2016). Radiogenomics entails the computer-assisted creation of radiological images from which tumour data can be

retrieved. The shape and volume of the tumour and the intensity and texture are usually targeted (Han *et al.*, 2015). The four steps in the RG tumour detection process are (a) recovery and restoration of images, (b) disporting of the targeted region, (c) feature extraction and result amplification, and (d) model building. The radiographic images are transformed into 3D structures on the computer, then screened for the desired site and segmented by the practitioner using a computer-aided method (Thawani *et al.*, 2018). Quantum dots, nanorobots, photo-thermal therapy (PTT), voice frequency and other modern advanced techniques such as artificial intelligence are all used to diagnose various lung carcinomas (Prabhakar *et al.*, 2018).

Oncopanel

Oncopanel is a next-generation sequencing assay that provides patients with various solid tumours with predictive, prognostic, and diagnostic information. The oncopanel is a clinical assay developed to ascertain the genotype status of a combination of genes defined in advance. KRAS, EGFR, BRAF, NRAS, HRAS, PIK3CA Signal Transduction Pathway Genes, RAS-RAF-MEK-MAPK Pathway, HER2, IDH1 and IDH2, ALK, TP53, c-KIT, STAT1&3, and PDGFRA are among the clinically significant genes and exons included in the oncopanel. The tumour material will be assessed for specific gene variants related to adverse events or responses to therapy. The following tumour types can be tested with an oncopanel: Lung cancer (non-neuroendocrine adenocarcinoma, Stage IIIB/IV non-small cell), Colorectal cancer (metastatic), Melanoma (metastatic, non-resectable), Gastrointestinal stromal tumour (GIST), low-grade glioma and FT/Ovarian/Peritoneal (High-grade serous) (The oncopanel pilot study). Oncopanel could be used in various clinical applications, including identifying drivers and potential mutations. Variants can be preferred based on their biological significance compared to targeted therapies (Gong *et al.*,2021). Microsatellite instability (Cordova *et al.*, 2019), homologous recombination defect status (Heeke *et al.*, 2018), copy number alterations (Kacew *et al.*,2019), and gene rearrangements (Hiley *et al.*, 2016) are all assessed using oncopanels. A growing body of evidence suggests that a substantial proportion of patients with low-frequency alleles of these clinically actionable mutations respond to targeted therapies (Martin *et al.*,2016). Even though crucial therapeutic decisions, such as molecular-targeted therapy selection and outcome prediction, are based on these results, it is pivotal that mutation detection has high sensitivity and low false positive (FP) rate and that reports to clinicians are correct (Groopman *et al.*, 2018). This type of testing has added

advantages compared to multiple individual gene mutation tests. It is less burdensome to patients because it eliminates the need for repeated biopsies and saves time.

2.9 Treatment of lung cancer

Local treatments to remove or eliminate the entire tumour are effective for cancers that are localized to a small area and have not progressed further. Radiation and surgery are included in the local treatment. Although surgery is more beneficial than radiation in eradicating tumours, not all patients can undergo surgery successfully (Baskar *et al.*, 2012). Chemotherapy might be given pre- or post-local treatments based on whether the cancerous cells have spread to other organs. The chemotherapeutic treatment is chosen after the surgery, termed adjuvant therapy. It has intended to help eliminate cancer cells that have not yet been eradicated by surgery. Also, in some cases, chemotherapy treatment is given before the surgery and is referred to as neoadjuvant therapy. Chemotherapeutic treatment can also be used as a particular therapy. NSCLC patients undergoing chemotherapy usually contain a platinum-based drug such as carboplatin or cisplatin accompanied by another drug such as docetaxel, gemcitabine, paclitaxel, and vinorelbine. Following first-line chemotherapy, a few more medications, including pemetrexed, have been authorized for second-line chemotherapy for NSCLC. The most often utilized regimen for SCLC patients is carboplatin, irinotecan, or cisplatin in combination with irinotecan. Patients with limited-stage cancer, *i.e.*, tumour confined to the chest, are frequently treated with chest radiation therapy. Also, brain radiation therapy is commonly utilized in limited-stage patients to avoid brain metastases (Walia *et al.*, 2022).

2.9.1 Platinum-based doublet chemotherapy

2.9.1.1 Pemetrexed along with cisplatin/carboplatin

Pemetrexed is a multi-targeted anti-metabolite that targets the folic acid metabolic pathway and its associated enzymes, such as Thymidylate synthase. It is mainly used to treat NSCLC and pleural mesothelioma (Li *et al.*, 2014). As these drugs are potent inducers of toxicity, supplements of folic acid, vitamin B12, and corticosteroids are given to reduce the adverse events. Tiredness, nausea, diarrhea, mucositis, and rashes are the most prevalent adverse effects. Pemetrexed is an authorized second-line treatment for NSCLC. To treat NSCLC, pemetrexed is frequently administered with the platinum-based agent, *i.e.*, carboplatin or cisplatin.

Mechanism of action

Pemetrexed is a chemotherapeutic agent that is chemically identical to folic acid & belongs to the folate antimetabolites class of drugs. Pemetrexed inhibits enzymes that are involved in pyrimidine and purine synthesis, such as *Ribonucleotide formyltransferase* (*GRAFT*), *Dihydrofolate reductase* (*DHFR*), and *Thymidylate synthase* (*TS*). The formation of pyrimidine and purine nucleotides is inhibited; also, pemetrexed limits the formation of DNA and RNA that is commonly required for the survival of malignant and normal cells (Wilson *et al.,* 2014)

2.9.1.2 Irinotecan along with cisplatin/carboplatin

Irinotecan, a semisynthetic camptothecin-derivative compound, is extracted from *Camptothecin acuminate*. Its active compound SN-38 forms a DNA stabilizing complex after interacting with topoisomerase-I. The enzyme Topoisomerase I regulates the topology of DNA and helps facilitate various processes such as DNA repair, recombination, and replication (Cuya *et al.,* 2017). Topoisomerase I lower torsional strain in the DNA in these processes by generating reversible single-strand breaks that allow single strands of DNA to pass through. The 3'-DNA terminal of the fragmented DNA strands forms a catalytic intermediate known as a cleavable complex when it binds covalently with the topoisomerase enzyme. DNA topoisomerase then reattaches the broken strands of DNA to generate chemically unmodified topoisomers that permit transcription to occur after the DNA has been adequately relaxed, and thus the strand passage reaction has been completed. Irinotecan and SN-38 interact with topoisomerase I-DNA complex, preventing single-strand breaks from re-ligating (Pillot *et al.,* 2006).

Mechanism of action

Within the cell, irinotecan, a prodrug, is converted into SN-38 (the active form). Efflux transporters such as *ABCB1* facilitate the outflow of SN-38 and irinotecan. Multidrug transporters like ABCB1 can transport irinotecan and SN-38 out of the cell (Kciuk *et al.,* 2020). Irinotecan is converted into its active compound SN-38 with the help of human carboxylesterases. Nonetheless, CYP3A5 and CYP3A4 can also convert it to the inactive metabolite, and also UGT1A1 can inactivate SN-38 by its glucuronidation to SN-38G (de Man *et al.,* 2018). The inactivation may inhibit DNA replication as well as transcription. It is an authenticated drug in the U.S for metastatic colon carcinoma treatment. The drug is injected with fluorouracil or alone to treat colon cancer. In addition, it is

used in combination with cisplatin to treat SCLC. The response rate in NSCLC was 15%-34% when the single agent was considered (Fujita *et al.,* 2015).

2.9.1.3 Docetaxel along with cisplatin/carboplatin

Docetaxel is an anti-neoplastic, anti-mitotic chemotherapeutic drug prominently used to treat ovarian, breast, and NSCLC tumours (Ojima *et al.,* 2016). It has a strong affinity for microtubules and binds to them reversibly. Docetaxel is a semi-synthetic taxoid produced from 10-deacetyl bacatin III that leads to microtubule assembly formation and suppression of tubulin depolymerization, thereby resulting in microtubule stabilization (Darshan *et al.,* 2011). This chemotherapeutic drug had reported a treatment success rate of 23-33% and an objective response rate of a 21% in NSCLC sufferers and new tumour cases. Cisplatin has a different mechanism than docetaxel and is frequently used to treat NSCLC patients. According to research, cisplatin-associated chemotherapy has a better prognosis and response rate in NSCLC than non-cisplatin-based chemotherapy. Therefore, cisplatin usage is a reliable predictor of improved survival (Cosaert & Quoix, 2002).

Mechanism of action

Cisplatin inhibits DNA replication and also halts the cell proliferation of cancer cells. One of the two chloride ligands is replaced by water upon administration, which results in the formation of aquocomplex cis- $[PtCl(NH_3)_2(H_2O)]^+$. This process is called an aquation. The N-heterocyclic bases present on DNA replace the aqua ligand present in the cis- $[PtCl(NH_3)_2(H_2O)]^+$. Following that, Guanine predominantly bonds, forming $[PtCl(Guanine\text{-}DNA)\ (NH_3)_2]^+$, which hinders the process of mitosis by DNA crosslinking. The damaged DNA stimulates the mechanism of DNA repair, which leads to apoptosis whenever the damages cannot be repaired. The cisplatin is usually regarded as an alkylating agent, but due to the absence of the alkyl group, it is incapable of performing alkylating reactions (Martinho *et al.,* 2019). Cisplatin, combined with other chemotherapeutic agents, has proven to be an effective treatment for malignancies. Although platinum responsiveness is significant at first, many cancerous people likely develop resistance to Cisplatin. Resistance can be shown by various mechanisms such as elevated drug detoxification, drug efflux, modulation in cellular uptake, enhanced DNA repair mechanism, and separation of apoptosis (Dasari & Tchounwou, 2014).

Docetaxel plays a prominent role in stabilizing and promoting microtubule assembly as it inhibits microtubule depolymerization and stabilizes microtubule assembly without GTP. This reduces free tubulin, essential for microtubule production, thereby inhibiting the mitotic cell division between metaphase and anaphase, preventing cancerous cell propagation. Although microtubules do not disassemble when docetaxel is present, they start accumulating in the cell, causing apoptosis. Apoptosis has also been assisted by inhibition of apoptosis- suppressing oncoprotein BCL-2. Various in vivo and in vitro studies have shown that docetaxel exhibits the antineoplastic ability against numerous tumour cells, complies with some other antineoplastic drugs, and has increased toxicity (Pienta, 2001).

2.9.2 Targeted therapy and immunotherapy

The targeted therapy is a tumour treatment that specially targets the proteins expressed on the surface of the tumour cells. The most common targeted therapies are monoclonal antibodies or small-molecule drugs. The therapy works by either inhibiting or destroying these targets. Anti-angiogenesis therapy, commonly known as anti-VEGF (vascular endothelial growth factor), stops tumours from growing and eventually kills them by cutting off their blood supply (Wilkes, 2018). The vascular endothelial growth factor is responsible for tumour angiogenesis. Cancer cells produce VEGF and act as a growth factor that regulates the permeability of vascular endothelial cells. Also, cell survival is aided by VEGF, which also inhibits apoptosis. Anti-angiogenic drugs are expected to improve therapy efficacy or restore normal blood flow within the tumour. The FDA has authorized bevacizumab as a VEGF inhibitor for colon cancer treatment. Also, EGFR inhibitors such as Afatinib, Gefitinib, and Erlotinib impede EGFR-positive cancer cells' proliferation (Cabanero *et al.*, 2017).

EGFR stimulates malignant cells to proliferate and develop (Wee & Wang, 2017). It was recently approved for the treatment of advanced NSCLC when used with carboplatin/paclitaxel, indicating a two-month increase in prognosis over chemotherapy alone (Wu *et al.*, 2012). The side effects of targeted treatment therapy differ from those of standard or traditional chemotherapy. Some targeted drugs have minor adverse effects, while others might cause significant complications. It depends on the targeted therapy a patient receives or the body's reaction to it, determining the adverse effects.

2.9.2.1 Immune Checkpoint inhibitors in carcinoma treatment

Immunotherapy by immune-checkpoint modulators has revolutionized the field of oncology in ways that go well beyond their clinical usefulness in some patients. The use of immunotherapy as a first-line treatment is currently recommended. Immune checkpoint inhibitors are drugs that impede the immunosuppressive processes of cancerous cells. These checkpoint inhibitors exert their anticancer effect by utilizing host autoimmune functions, which is an entirely different mechanism than chemotherapeutic drugs (Robert, 2020). Chemotherapy drugs target the cancerous cells with various molecular targeted drugs that bind specifically to genetic variant sites and, inhibit proliferative signals, suppress the cell cycle; however, checkpoint immunotherapy utilizes humanized monoclonal antibodies targeted against checkpoint signals like programmed cell death receptors & ligands (Desnoyer *et al.*, 2020). The inhibition of these receptors and signal can reactivate the immune system to combat the tumour. Immunotherapy for the advanced NSCLC has resulted in a new treatment paradigm, with higher survival and response rates and a somewhat less harmful but distinct adverse profile than chemotherapy. Checkpoint inhibition, *i.e.*, the antibodies that target the adaptive immune suppression pathways mainly programmed death-ligand 1 (PD-L1), Programmed cell death-1 (PD-1), and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), is a potential anti-tumour therapy that seems to be therapeutically relevant across a wide range of tumour types (Walia *et al.*, 2022).

Additionally, memory T-cell responses can give long-term immunity due to their prolonged clinical response. For the treatment of SCLC and advanced NSCLC, the regulatory agencies have recently authorized the programmed death ligand-receptor inhibitor atezolizumab and the programmed cell death ligand inhibitor nivolumab (Xu *et al.*, 2019). Many biomarkers can also predict whether individuals with NSCLC or SCLC respond to anti-PD-L1 inhibitors. Based on better knowledge of the biological pathways underpinning carcinoma-immune interactions, immunotherapeutic procedures targeting activation and inhibition of T-cell receptors, adoptive cell therapies (ACT), including tumour-infiltrating lymphocytes (TIL), chimeric antigen receptors (CARs), and various specific antibodies are under investigation in clinical trials (Gastman *et al.*, 2020). The first immune checkpoint inhibitor drug is Nivolumab which was authorized in 2014. Since then, numerous immune checkpoint inhibitors have been brought into clinical medicine, including atezolizumab, durvalumab, and pembrolizumab, and they have shown extraordinary efficacy. Previously, clinical trials of immune checkpoint inhibitors have been carried out for the

advanced stage of NSCLC (Onoi *et al.*, 2020). These trials are Checkmate 017 and Checkmate 057, in which nivolumab has been used in stage III/IV SQCC and non-squamous NSCLC patients. Both trials have shown that nivolumab was associated with prolonged survival in NSCLC patients (Brahmer *et al.*, 2015). Also, other clinical trials of immune checkpoint inhibitors for advanced-stage NSCLC included various treatment regimens such as atezolimumab, Pembrolizumab monotherapy, a combination of chemotherapy ± Pembrolizumab, the conjunction of chemotherapy + Bevacizumab ± Atezolizumab has also been reported in various trials in the past and were summarized in an article by (Onoi *et al.*, 2020). Recently, an IMpower133 study was reported, which was administered in conjunction with platinum-based chemotherapy with atezolizumab as first-line therapy for SCLC, and the outcomes showed prolonged overall survival and progression-free survival in the treatment group (Socinski *et al.*, 2018). Although immunotherapy has been quite successful for lung carcinoma treatment, it is expected that the advent of combination treatments with targeted or cellular therapies and new combinations of immunotherapies will improve the individualized therapy.

2.10 Drug transporters

Transporters comprise the most prominent family of membrane proteins in the human body. Based on the driving force, the drug transporters are categorized into two superfamilies: the ATP binding cassette (ABC) and solute carrier (SLC) transporters. These transporters have also been termed the efflux and influx transporters based on the direction of various substrate movements. Membrane transporters are involved in the movement of various exogenous and endogenous molecules across organelle and cellular membranes, including digested peptides, sugars, nucleotides, and amino acids (Liang *et al.*, 2015). Other than these substrates, the protein transporters also regulate the transport of numerous drugs across the cell membrane. Many gene families encode these transporters, which account for about 4% of the genes in the human genome. The expression of ABC and SLC transporters has been reported selectively in kidney, lung, intestine, brain, testis, liver, and placenta, as shown in **figure 2.11** (Nishimura & Naito, 2005)

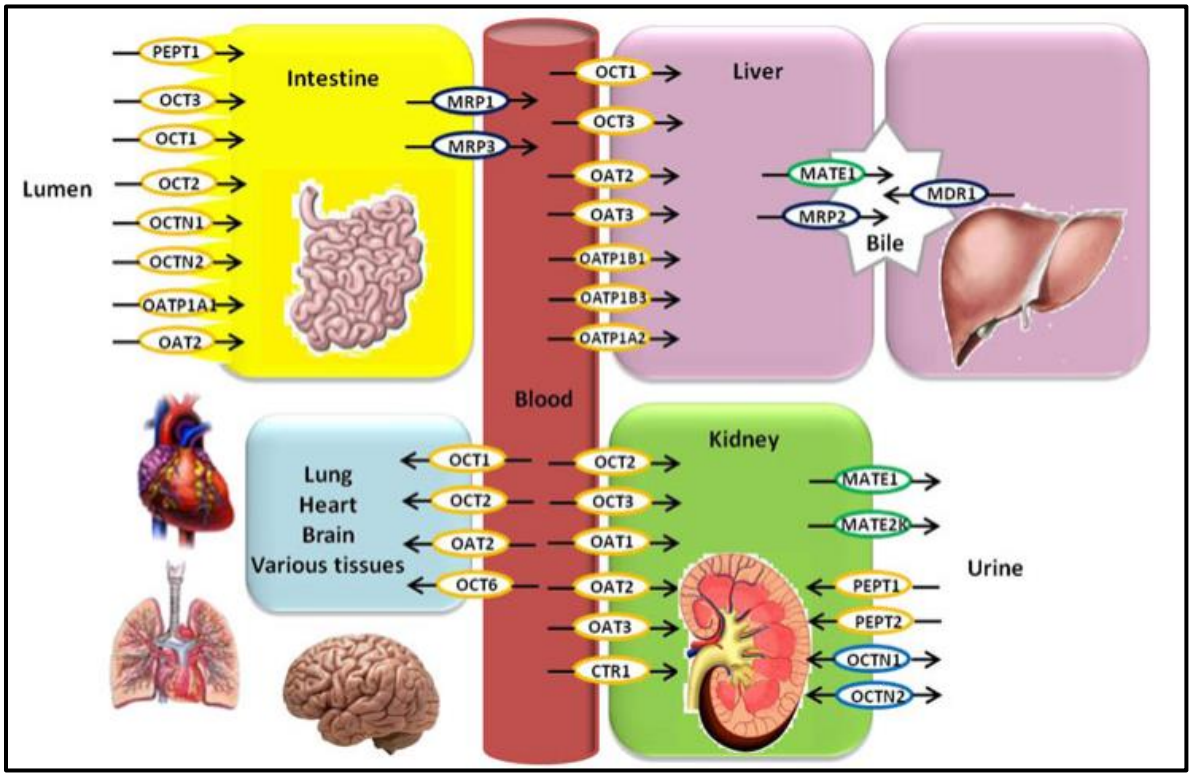


Figure 2.11: Illustration depicting the expression of ABC and SLC transporters in various human organs (Li & Shu, 2014)

Additionally, these membrane proteins have been identified as potential drug transporter within the last two decades. ABC and SLC transporters influence the drug's effectiveness, pharmacokinetics, and side effects. Also, these transporters are essential regulators of drug accumulation in cells, and their activities are frequently linked to therapeutic efficacy, drug-drug interactions, and drug toxicity. Likewise, both of these transporters are reliable candidates for drug delivery. Transport capacity and protein levels of each SLC and ABC transporter must be assessed to select the specific membrane transporter implicated in drug absorption, distribution, and elimination (Nakanishi, 2007). The recent decade's growing data shows drug transporters are critical in human physiology and pathology.

2.11 ATP-Binding Cassette transporters

ATP-binding cassette transporters comprise a ubiquitous family of membrane proteins essential for ATP-dependent translocation of numerous substrates across the cell membrane. ABC proteins

are the most prominent family of transmembrane transporters (Rees *et al*., 2009). The expression of these proteins is known to be very diverse. Since ABC transporters rely on ATP to export various substances, ATP hydrolysis is critical for facilitating the transportation of various substrates against a concentration gradient through the intracellular to extracellular regions. This vast ABC transporter family plays an essential part in various cellular activities. The most prevalent role of ABC transporters is to transport various molecules, mainly carbohydrates, ions, amino acids, toxic metabolites, peptides, drugs, heterotrophic substances, and toxins (Liu, 2019).

The architecture of a functional ABC protein is made up of four specific domains, *i.e.*, two transmembrane domains (TMDs) with two nucleotide-binding domains (NBDs), as shown in **figure 2.12**. TMD domain has six transmembrane helices that are responsible for substrate specificity. The ABC transporters can be of two types: half transporters (TMD-NBD) or full transporters (TMD1-NBD1-TMD2-NBD2). To be a functioning transporter, the half transporter must form either a homodimer or heterodimer. NBD domain contains a few motifs, including walker A and B, D-loop, A-loop, H-loop, Q-loop, and a consensus sequence LSSGQ (Schinkel & Jonker, 2012). NBD is made up of two subdomains: a catalytic core as well as an α helical domain. The catalytic core comprises walker A and walker B. Walker A is a conserved glycine motif interacting with the phosphate group of the nucleotide, whereas walker B is implicated in the binding and hydrolysis of ATP. The consensus sequence LSGGQ motif (C-loop), also referred to as the ABC signature motif, is found in the α helical domain and is crucial in nucleotide binding. NBDs form the location for ATP binding and hydrolysis once they dimerize by interacting with the D loop (Thomas & Tampé, 2020). The ATP binding site is formed by one subunit of the walker A & walker B motifs and another subunit of the Q-loop to facilitate the binding and hydrolysis of two ATP molecules. In the absence of nucleotides, NBDs are separated, but when ATP is bound, they come closer together; nonetheless, hydrolysis disrupts the dimer interface and then releases inorganic phosphate and ADP (Beis, 2015). The intracellular area of TMDs is connected to NBDs, establishing a tight and functional relationship between the two domains. The NBD binds to ATP, hydrolyzed to generate energy for substrate transport. The distinct NBD-TMD interaction acts as a transmission gear for torsional changes made by substrate binding and ATP hydrolysis (Liu, 2019).

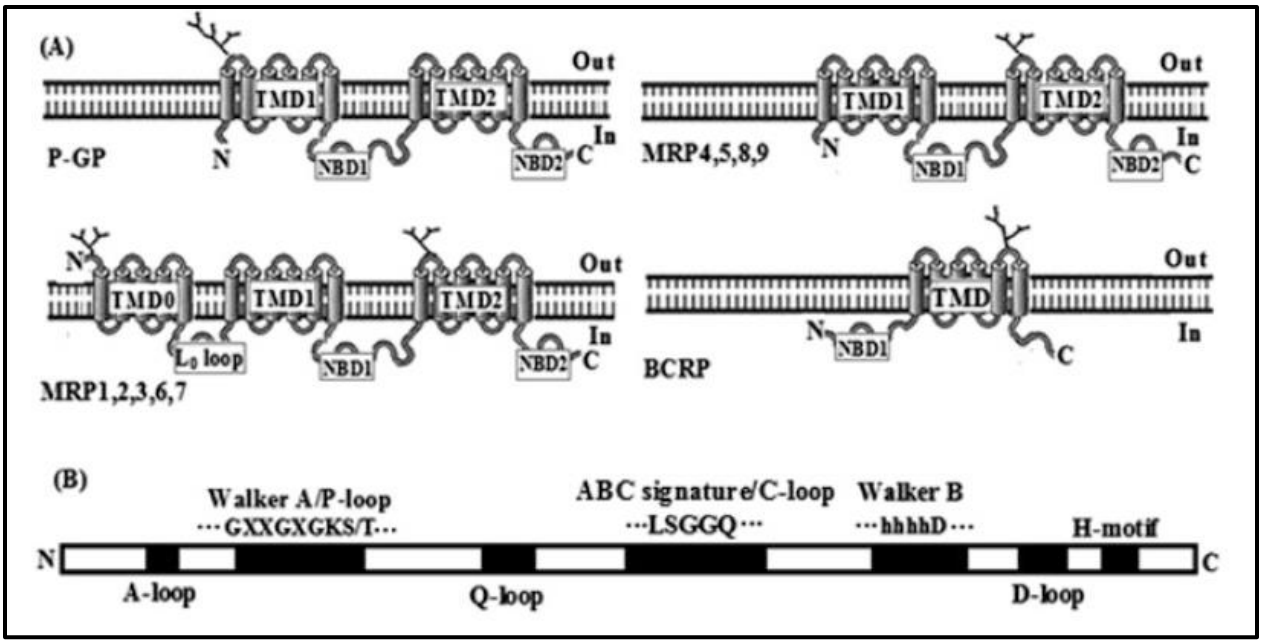


Figure 2.12: (A) Models of secondary structures of ABC efflux transporters. NBD: nucleotide-binding domain; TMD: transmembrane domain. The cytoplasmic and extracellular compartments are "in" and "out," respectively. (B) The fundamental elements of NBD are arranged in a linear pattern with residues. Any hydrophobic residue is denoted by the letter "h" (Liu, 2019)

The mechanism of the ABC outflow transporter-associated substrate translocation is explained by the "ATP switch" model (Schinkel & Jonker, 2012). The detailed mechanism of the ABC transporters is depicted in **figure 2.13.** Three elements are consistent throughout the model. Firstly, the transport cycle begins when the substrate binds to the TMDs of the apo form in the inward-facing conformation (high affinity). The binding of ATP stimulates the generation of a closed nucleotide sandwich structure. At the junction of the two NBDs, ATP functions as a molecular adhesive that keeps the two NBDs together, generating two nucleotide-binding sites (NBDs) (López-Marqués *et al.*, 2015).

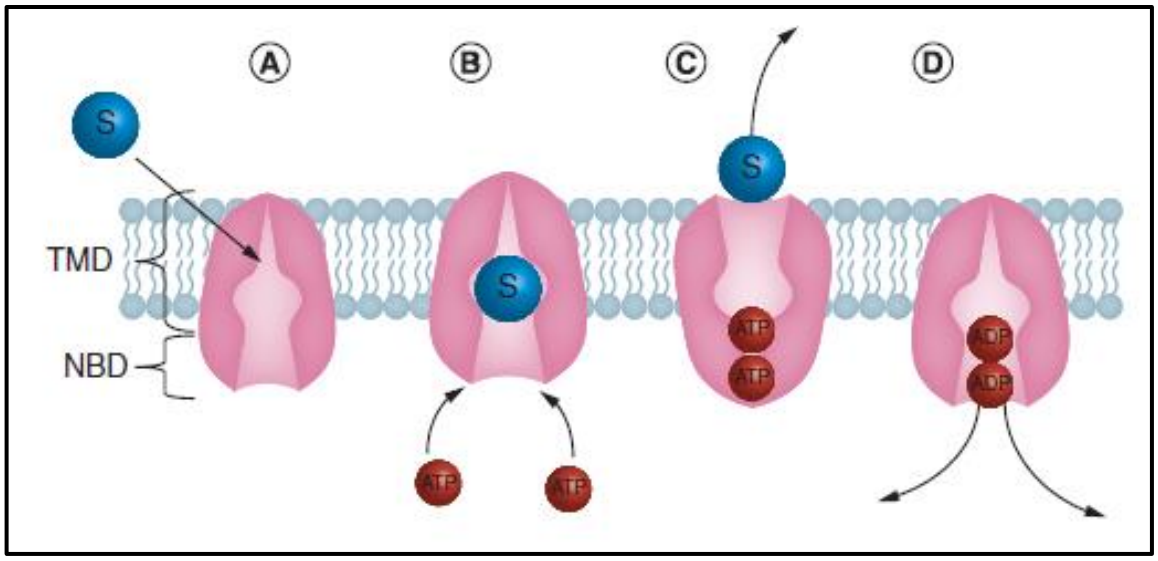


Figure 2.13: Role of ATP-binding cassette transporter in substrate transport mechanism (A) Ligand binding to the TMD pocket changes the configuration of the NBD region. (B) The two ATP molecules bind in the NBD area. (C) Energy is released by the formation of a closed NBD dimer, which causes a conformational shift in the TMD region. (D) ATP hydrolysis causes the closed NBD dimer to dissociate, causing a conformational shift in the TMD region. Ultimately, ADP and phosphate are released, allowing the ABC transporter to reopen the NBD dimer and continue the cycle.

The binding energy retrieved via NBDs/NBSs is conveyed to the TMDs on the other side of the membrane, shifting their availability to the outward-facing configuration (low affinity). The gate to the inner is now closed, whereas the gate to the outer membrane is open. The affinities of transported substances shift from strong (low chemical potential) to low affinity (high chemical potential of the substrate). The ATP-binding process can be regarded as a power stroke that modifies the chemical potential of the transported substance (Liu, 2019). Thirdly, ATP hydrolysis opens the closed nucleotide sandwich arrangement by producing an additional negative charge. Once this structure opens, there is ADP dissociation and release of Pi, which resets the access gates and TMDs to the high-affinity configuration on the initial side of the membrane (George & Jones, 2012).

2.11.1 Location, expression, and functional role of ABC transporters

In humans, 49 ABC transporters subtypes and one pseudogene have been identified. These transporters are further bifurcated based on amino acid sequence, gene structure, phylogenetic analysis, and domain structure (Dean *et al*., 2001). The subfamily classification is from A to G, in which ABCA consists of 12 genes, ABCB contains 11 genes, ABCC comprises 12 genes, ABCD includes four genes, ABCE a single gene, while ABCF and ABCG consist of 3 and 5 genes, respectively. Human ABC transporters are primarily found on the plasma membrane, where they translocate many metabolites, drug conjugates, and drugs out of cells. It has been reported that ABC transporters are widely localized in the kidney, brain, small intestine, and liver, where they play a crucial role in absorption, distribution, and metabolism excretion (ADME). Apart from these organs, the efflux transporters are also expressed in the heart, trachea, lung, adrenal gland, placenta, and uterus (Sharma *et al*., 2021).

ABC membrane proteins are found in the cells of all living organisms and utilize the energy of ATP hydrolysis to transport a broad spectrum of molecules across the cell membrane unidirectionally. Specific ABC transporters are also engaged in many cellular processes, mainly antigen processing, maintaining osmotic homeostasis, immunity, cell division, lipid trafficking, and cholesterol, among other cellular activities (Sharma *et al.*, 2021). However, one of the most prominent transporter families, ABCB, ABCC, and ABCG2, are widely studied and clinically claimed to be most relevant in the oncological field. Additionally, members of subfamily *ABCB* are termed as “MDR-efflux transporters.” The acronym MDR is signified by multiple drug resistance. Also, representatives of the *ABCC* subfamily are characterized as "MRP-ABC transporters," where MRP stands for multidrug resistance-associated protein. Lastly, the *ABCG2* subfamily is called BCRP, which stands for breast cancer resistance protein (Choudhuri & Klaassen, 2006). The location, family, and physiological function of ABC transporters are depicted in **Table 2.3.**

Table 2.3: Chromosome localization and associated physiological functions of the members of ABC transporters

Gene	Alias	Number of genes	Subfamily	Chromosome location	Function
ABCA	ABC1	12	ABCA1	9q31.1	Efflux of cholesterol
			ABCA2	9q34.3	Drug resistance
			ABCA3	16p13.3	Surfactant secretion
			ABCA4	1p21.3	Efflux
			ABCA5	17q24.3	Cholesterol translocation
			ABCA6	17q24.3	
			ABCA7	19p13.3	Phospholipids and cholesterol release
			ABCA8	17q24.3	
			ABCA9	17q24.3	
			ABCA10	17q24.3	
			ABCA11	2q34	
			ABCA12	9q31.1	
ABCB	MDR	11	ABCB1	7q21.12	Multidrug resistance
			ABCB2	6p21.3	Peptide transport
			ABCB3	6p21.3	
			ABCB4	7q21.12	Phosphatidylcholine transport
			ABCB5	7p21.1	
			ABCB6	2q35	Transport of Iron
			ABCB7	Xq21-q22	Fe/S cluster transport
			ABCB8	7q36.1	
			ABCB9	12q24.31	
			ABCB10	1q42.13	
			ABCB11	2q24.3	Transportation of bile salt
ABCC	MRP	12	ABCC1	16p13.12	Multidrug Resistance
			ABCC2	10q24.2	Efflux
			ABCC3	17q21.33	Drug resistance
			ABCC4	13q32.1	Nucleoside transport
			ABCC5	3q27.1	
			ABCC6	16p13.12	
			ABCC7	7q31.31	Ion channel
			ABCC8	11p15.1	Sulfonylurea receptor
			ABCC9	12p12.1	ATP channel regulation
			ABCC10	6p21.1	
			ABCC11	16q12.1	
			ABCC12	16q12.1	
ABCD	ALD	4	ABCD1	Xq28	Cholesterol transport regulation

			ABCD2	12q11	
			ABCD3	1p22.1	
			ABCD4	14q24.3	
ABCE	OABP	1	ABCE1	4q31.31	
ABCF	GGN20	3	ABCF1	6p21.1	
			ABCF2	7q36.1	
			ABCF3	3q27.1	
ABCG	White	5	ABCG1	21q22.3	Cholesterol transport
			ABCG2	4q22	Toxin efflux, Resistance, and urine transporter
			ABCG4	11q23	Sterol trafficking
			ABCG5	2p21	Sterol transport
			ABCG8	2p21	

2.12 ATP-binding cassette sub-family B member 1 (ABCB1)

2.12.1 Genomic organization

ATP-binding cassette sub-family B member 1 *(ABCB1)* gene encodes P-glycoprotein (P-GP), which is responsible for cellular homeostasis and defense against xenobiotics. The *ABCB1 (MDR1/PGY*1*)* gene is positioned on chromosome 7q21 (Battista *et al.*, 2020). **Figure 2.14** depicts the localization of *ABCB1* on chromosome 7**.** P-gp is the most well-characterized member of the *ABCB1* subfamily. The human *ABCB1* gene consists of 29 exons numbered from -1 to 28. The exon numbering indicates that the *ABCB1* gene can be transcribed by two separate promoters, *i.e.,* an upstream and downstream promoter. The upstream promoter can be detected at the start of -exon 1, while the downstream promoter can be found within exon 1. Exon 1 is flanked into two parts, exon 1a and exon 1b. The part of exon 1 positioned 5' to the downstream promoter is referred to as exon 1a, while another part of exon 1 localized 3' to the downstream promoter is exon b (Choudhuri & Klaassen, 2018). Two transcriptional start sites are present: exon 1 has a proximal promoter & intron one is essential for constitutive expression, and also an active crystal distal promoter for overexpression of protein product in samples of cancer patients & drug selected cell lines. Exon 2 contains the ATG translation start codon. Therefore, the protein-coding region of the *ABCB1* gene contains 27 exons, of which 14 code for the first half while the other 13 code for the second half of protein. There are 28 introns, 26 of which interfere with the protein-coding sequence (Choudhuri & Klaassen, 2006).

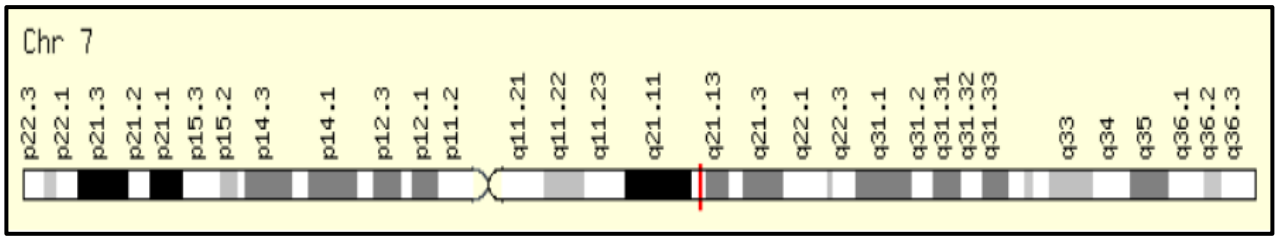


Figure 2.14: Chromosome 7 depicting the localization of the *ABCB1* gene on it (Source: https://www.genecards.org/)

2.12.2 Structure of ABCB1 *protein*

The length of mRNA is 4872bp, with the 5' untranslated region (NM_000927.3), that in turn gives rise to 1280 amino acids protein, named P-gp. The 2^{o} structure of the protein shows that P-gp has two homologous halves, each with six TMDs and NBD domains. Therefore, the protein consists of 12 α-helices (transmembrane segments) and is positioned in the apical region of the plasma membrane. Computer-assisted techniques have shown that two TMDs of the P-gp protein folds differently and are linked together by a linker region of approximately 78 amino acids (Choudhuri & Klaassen, 2006). As mentioned earlier, NBDs contain three motifs, of which walker A and B regions are parted by 90-120 amino acid length, forming hydrogen bonds with bound ATP. The signature motif of NBD1 is localized across the walker A sequence of NBD2, forming a sandwich architecture. Hence, the ATPs are in the sandwich position between the ABC signature motif and walker A (Zhou, 2008).

A human P-gp structure in an outward-facing conformation is represented in **figure 2.15,** which was determined by electron cryo-microscopy at the 3.4-Å resolution. The structure represents the two ATP molecules occluded by the two NBD domains, forming a closed dimer. As the structure is outward-facing, the drug binding cavity is re-oriented to the extracellular space and constricted to prohibit substrate binding. This finding suggests that ATP binding, rather than hydrolysis, stimulates substrate release (Kim & Chen, 2018). The structure suggests a concept in which P- glycoprotein's dynamic activity allows it to translocate a wide range of substrates.

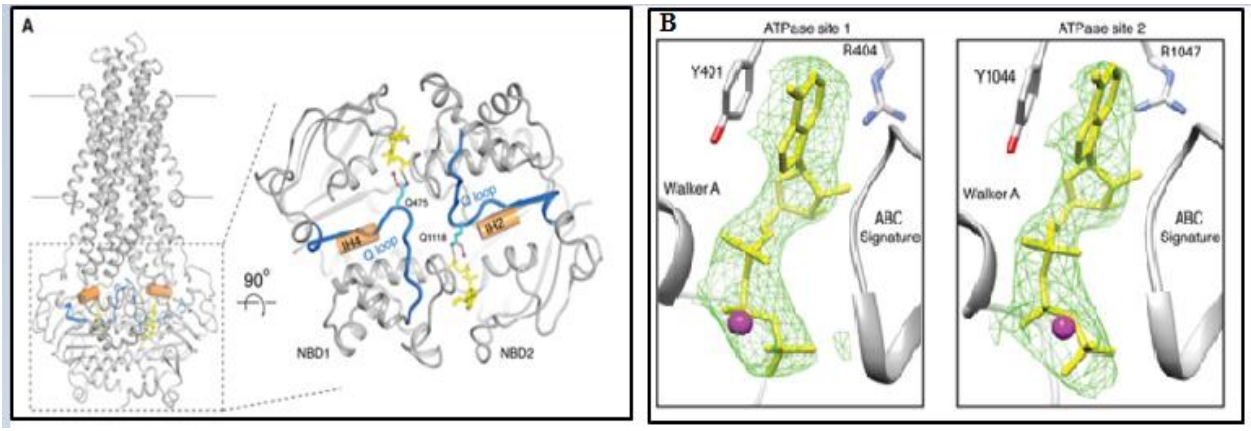


Figure 2.15: The structure of human P-gp/ABCB1 (outward-facing conformation, ATP-bound). (A) The interaction of Q loops in the NBD dimer (indicated in blue) with Mg^{2+}/ATP (shown in magenta and yellow) and the TMDs via the two intracellular helices IH2 and IH4 (shown in orange) (B) Molecular connections for each ATPase site, along with Mg^{2+}-ATP density (depicted in green mesh) (Kim & Chen, 2018)

2.12.3 Expression of ABCB1 gene

The expression of P-gp is reported in the plasma membrane of the cells, and excretory organs, as it provides defensive and eliminatory functions (Brinkmann & Eichelbaum, 2001). It aids the first-pass clearance of orally taken drugs, thereby limiting their bioavailability by the outflow of the drugs from the small intestine and colon, as well as the bile canaliculi of the liver. It removes the substrates from systemic circulation positioned at the urine-facing side of brush-border microvilli of proximal renal tubules and again by biliary excretion. P-gp is expressed in the adrenal cortex, which is considered to have a role in hormone transport, homeostasis, and glucocorticoid resistance (Meijer *et al*., 2003). Other than this, the *ABCB1* gene is widely expressed in the brain. In the blood-brain barrier, P-gp expression is reported in the capillary endothelial cells, allowing it to translocate diverse substrates back into the blood while preventing their access to the brain (Choudhuri & Klaassen, 2006). The P-gp expression has been reported in the lung at the apical side of ciliated epithelial cells or collecting ducts & in bronchial glands. P-gp is also found in the lateral membranes of healthy nasal respiratory mucosa. Briefly, the *ABCB1* gene is widely expressed in numerous normal cells, though its high levels are well reported in the large and small intestine, pancreatic duct, and liver, while the lower levels are found in NK cells, placenta, testis, brain, and prostate. Additionally, P-gp is overexpressed in many tissues altered by cancer, which is relevant to the clinical challenge of multidrug resistance (Kantharidis *et al.*, 2000).

2.12.4 Gene regulation of ABCB1 gene

The expression of *ABCB1* is primarily governed by transcriptional regulation. The transcriptional machinery of eukaryotes is a massive multi-protein complex that includes (co-)activators, polymerases, (co-)repressors, and other components. The transcriptional machinery's ability to bind to target DNA sequences and activate or inhibits gene expression is also influenced by DNA accessibility and responsiveness (Lee & Frank, 2021).

There are multiple sites reported for stimulating transcription factors, such as Sp1, Myc, AP-1, TCF (Scotto, 2003), nuclear factor kappa B (NF-B), Sp3 (Gromnicova, Romero, & Male, 2012), PITX2, and octamer-binding transcription factor 4 (OCT-4)/POU5F1 (Lettnin *et al.*, 2019), can be found in the promoter region of *ABCB1*. DNA methylation, binding of oncogenic chimeric proteins, or an amalgamation of transcription factors, such as c-Fos and NF- κB, can suppress ABCB1 expression. Moreover, new reports in drug-resistant ovarian and breast cancers revealed multiple anomalous ABCB1 transcriptional fusions, putting it under the command of alternative promoter regions from genes on the same chromosome as *ABCB1* (Christie *et al.*, 2019). These findings point to non-canonical transcriptional regulation mechanisms that greatly expand the range of *ABCB1* regulators, increasing the disposition to the progression of multidrug resistance. Upstream signaling pathways' full impact on regulating *ABCB1* transcriptional fusions is still unknown. The high ABCB1 levels can be controlled by increased activator activity, chromatin remodeling, decreased repressor activity, transcriptional fusions, and DNA priming (Moro *et al.*, 2020). During a stress response, *ABCB1* is highly vulnerable to upregulation because many of the previously stated transcription factors are significantly changed by stressors. Additionally, ABCB1 gene expression can be stimulated by various stimuli such as sodium butyrate, chemotherapeutic agents, retinoic acid, ultraviolet, and phorbol esters. Scotto *et al.* have reported that all these divergent stimuli intersect on the promoter site of MDR1, and the researchers have referred to it as “enhanceosome,” which spans across the CCAAT box, GC- rich elements, and also includes binding sites for various transcription factors (Scotto, 2003).

The expression of *ABCB1* can be modulated by various polymorphisms that also play a vital role in cancer therapy, gene rearrangements, epigenetic regulation, and post-translational mechanisms. Reports have made it evident that P- gp is upregulated in the tumour progression.

Reports have shown that mutant *ras* and *p53* enhance the *ABCB1* promoter activity. Also, there was exciting speculation that the tumour suppressor gene (TSG) *p53* is commonly mutated in several cancers (Kantharidis *et al.*, 2000).

2.12.5 Substrate specificity of ABCB1 gene

ABCB1/P-gp exports a wide range of drugs, including immunosuppressive agents, HIV protease inhibitors, chemotherapeutic drugs, antihistamines, analgesics, pesticides, antibiotics, and calcium channel blockers (Bugde *et al.*, 2017). The anti-cancerous agents, which are substrates of P-gp, include actinomycin D, daunorubicin, doxorubicin, docetaxel, paclitaxel, etoposide, irinotecan, mitoxantrone, mitomycin C, teniposide, vincristine, vinblastine, topotecan and Imatinib. A detailed list of substrates, inhibitors, and inducers of ABC transporters is summarized in **Table 2.4.** All P-gp substrates are amphipathic, and hypothesized that P-gp substrates get inserted in the inner leaflet before being flipped to the outer leaflet of the cell membrane. Only the amphipathic molecules have appropriate membrane insertion properties. Reversal agents inhibit the transportation of P-gp mediated substrates, often termed P-gp inhibitors. They inhibit P-gp function by disturbing the ATP hydrolysis, competing for the binding site, or altering the expression of P-gp. Most of the inhibitors, for example, quinidine, verapamil, and cyclosporine A are P-gp substrates; this suggests that they act as competitive inhibitors (Sharma *et al.*, 2021).

Table 2.4: List of various substrates, inhibitors, and inducers of P-gp (Sharma *et al.*, 2021)

Substrates	**Anticancer agent**	Actinomycin D, daunorubicin, doxorubicin, docetaxel, paclitaxel, etoposide, irinotecan, Mitoxantrone, Mitomycin C, Teniposide, Vincristine, Vinblastine, Topotecan, imatinib
	Antihypertensive agents	Celiprolol, Diltiazem, Losartan, Talinolol
	Anti-arrhythmics	Digoxin, Quinidine, Verapamil
	Antidepressants	Amitriptyline
	Anti-human immunodeficiency virus (HIV) agents	Amprenavir, Indinavir, Nelfinavir, Ritonavir, Saquinavir, Anticonvulsants,Phenobarbital Phenytoin
	Anti-emetics	Domperidon Ondansetron
	H2-antagonists	Cimetidine Ranitidine
	Immunosuppressants	Cyclosporine Sirolimus Tacrolimus Valspodar
	Steroid hormones	Aldosterone Cortisol Dexamethasone Methylprednisolone
	Opioids	Loperamide Morphine Pentazocine
	Others	Digoxin Ivermectin Terfenadine Vecuronium
Inducers		Amiodarone, Amprenavir, Bromocriptine, Cisplatin, Colchicine, Clotrimazole, Cyclosporine, Daunorubicin, Dexamethasone, Diltiazem, Doxorubicin, Etoposide, Fluorouracil, Hydroxyurea, Insulin, Indinavir, Methotrexate, Midazolam, Mitoxantrone, Nelfinavir, Morphine, Nicardipine, Phenobarbital, Phenytoin, Phenothiazine, Probenecid, Retinoid acid, Reserpine, Rifampicin, Ritonavir, Tamoxifen, Verapamil, Vinblastine, Vincristine, Yohimbine
Inhibitor		Amiodarone, Astemizole, Bepridil, Atorvastatin, Biricodar, Carvedilol, Bromocriptine, Chlorpromazine, Clarithromycin, Cortisol, Diltiazem, Cyclosporine, Dipyridamole, Disulfiram, Elacridar (GF120918), Erythromycin, Felodipine, Fluoxetine, Itraconazole, Ketoconazole, LY335979, Midazolam, Nicardipine, Nitrendipine, OC144-093, Paroxetine, Progesterone, Propafenone, Quinidine, Quinine, R101933, Reserpine, Ritonavir, Sertraline¸ Tacrolimus, Tamoxifen, Terfenadine, Tetrabenazine, Valinomycin, Valspodar (PSC833), Verapamil

2.13 ATP-binding cassette sub-family C members 1 and 2 (ABCC1, ABCC2)

ABCC subfamily encodes multidrug resistance proteins (MRPs), and among all these MRPs, ABCC1/MRP1 was the first identified form the drug-resistant lung carcinoma cells. The family of multidrug resistance proteins is quite large, and the expression of two members of this subfamily, *i.e., ABCC1* and *ABCC2,* is reported in the tissues that are very crucial for the absorption (such as gut and lung), metabolism, and excretion (kidney and liver) (Leslie *et al*., 2005). So, the current section emphasizes discussing these genes in the following section.

2.13.1 Genomic organization

The *ABCC1/MRP1* gene is localized in chromosome 16p.13.1 and is around 200 kb in length. **Figure 2.16** depicts the location of *ABCC1* on chromosome 16. The human *ABCC1* gene consists of 30 introns and 31 exons, and 1531 amino acids (Jiye & Jianting, 2011). The first 48 bp of the coding sequence and the 5'UTR are found in exon 1, while exon 31 has a 109-bp coding region, and the remaining is 3′-UTR. All 30 introns follow the GT... AG rule (Chambon's rule). Three exons encode each NBDs, such as exons 16-18 for the NBD1 while exons 28-30 for encodes for NBD2. The *ABCC1* gene's proximal promoter is GC-rich (>70%) in humans, rats, and mice. The initial 100 nucleotides in the human are significantly conserved; they have overlapping GC-boxes capable of binding transcription factors Sp1 and Sp3. The human and rodents' genomes diverge significantly after the first 100 nucleotides. There is no CAAT and TATA element in the human MRP1 gene promoters (Muredda *et al*., 2003).

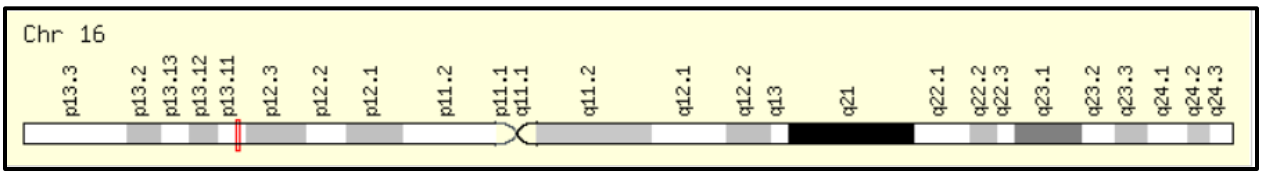


Figure 2.16: Chromosome 16 depicting the localization of *ABCC1* gene (Source: https://www.genecards.org/)

The other important member of MRPs is the *ABCC2* gene. A canalicular multispecific organic anion transporter (cMOAT) encodes the multidrug resistance-associated protein 2 (MRP2) protein. Among several MRPs, only MRP2 is exclusively localized to the apical membrane of the polarized cells. The *MRP2* gene is positioned on chromosome 10q24.2 and has a length of approximately 65 kbp. **Figure 2.17** depicts the location of *ABCC2* on chromosome 16. Human *ABCC2* gene contains

32 exons and 31 introns with a high section of class O introns (Wu *et al.*, 2018). Exon 1 consists of the first 33bp of the coding sequence and the 5' UTR, while exon 32 compromises a coding sequence of 130 bp, and the remainder is 3'UTR. Alike the MRP1 introns, the 31 introns of MRP2 also obey GT…AG rule. Three exons encode each NBDs, such as exons 16-18 for NBD1, whereas exons 29-31 for NBD2. The MRP2 protein consists of 1,545 amino acids and has a molecular mass of 190 kDa (Izzedine *et al.*, 2006).

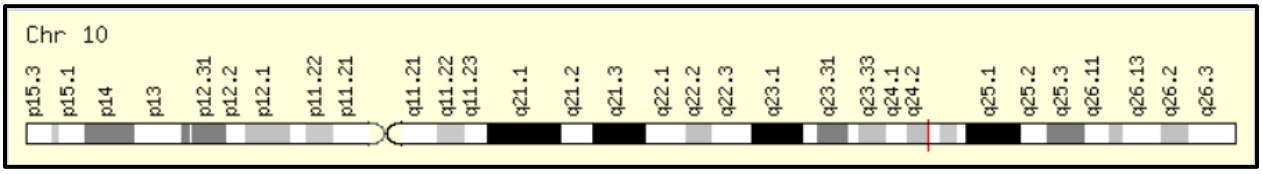


Figure 2.17: Chromosome 10 depicting the localization of *ABCC2* gene on it (Source: https://www.genecards.org/)

2.13.2 Structure of MRP1 and MRP2 protein

The structure of MRP1 is unique compared to the other ABC protein family as the topology of TMDs is a bit peculiar. MRP1 protein consists of 5 domains such as TMD0 (comprising five transmembrane helices) that follows a "core" structure of TMD1 and TMD2 (both of them consisting of six transmembrane α helices), which is further attached to NBD1 and NBD2, respectively by a stretch of 40-50 amino acids (commonly known as "connecting regions" of the transporter (Smith *et al.*, 2021). The configuration is structured as TMD0-TMD1-CR1-NBD1-TMD2-CR2-NBD2, as shown in the topological diagram of **figure 2.18 (A)**. Though the TMD0 domain is a distinctive feature of many members of the ABCC subfamily, this domain was discovered to be dispensable for MRP1's function (Bakos & Homolya, 2007). A study in the past has shown evidence in a truncated mutant that did not have a TMD0 domain but was having intact transporter activity just like a native protein. Therefore, a depiction of a homology model of human MRP1 lacking TMD0 was made based on the cryo-EM structure of bovine MRP1 **(Figure 2.18 (B)** Conseil *et al.*, 2019)**.** Other than this, a group of researchers used a diverse range of drugs, inhibitors, and site-directed mutagenesis and indicated that human MRP1protein has a minimum of 4 pharmacologically different binding sites (Maeno *et al.*, 2009).

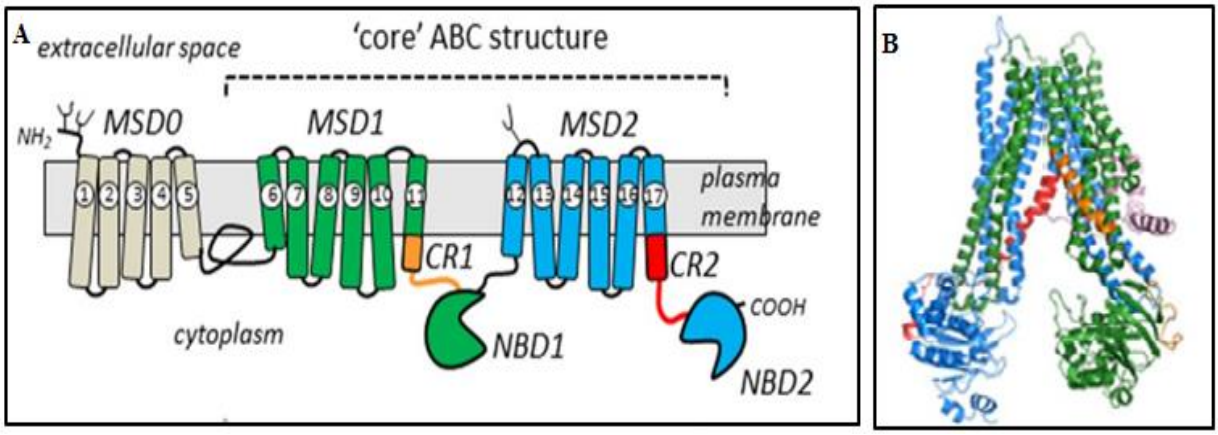


Figure 2.18 (A): An illustration showing a 2-dimensional depiction of the domain structure of MRP1 (B): Depiction of a homology model of the core human MRP1 protein [Note: The CR1 region is depicted in orange color while the red color depicts the CR2 region. The other domain colors are similar to that of figure 2.18 (A)] (Smith *et al.*, 2021;Conseil *et al*., 2019)

The structure of MRP2 is quite similar to that of MRP1. MRP2 protein shares almost 48% sequence similarity with MRP1 and the same topology, indicating the structural and functional resemblance (J.-Q. Wang *et al*., 2021). Briefly, the structure of MRP2 consists of TMD0, two TMDs (TMD1 and TMD 2), two NBDs (NBD1 and NBD2), and a linker segment. As mentioned earlier, the MRP2 protein has 1545 amino acids with approximately 200 amino acids in the NH2 terminal of the TMD0 region. The TMD0 domain of MRP2 protein helps stabilize the ABCC2 transporter in the apical membrane. Based on the vesicle transport study, there are two similar but unidentical binding sites in the MRP2 protein. To date, the high-resolution 3-dimensional structure of MRP2 is not available, but the *in-silico* models have been generated to understand this transporter's structural aspects, as shown in **figure 2.19** (Williamson *et al.*, 2007)**.** As reported in the past, human MRP2 protein with transmembrane helices 6,9,17, and 16 consists of basic residues that are possibly involved in the interaction of substrate and protein, thus forming substrate binding sites. The affinity for substrates is regulated by one site, while the other appears to be directly engaged in transport (Alrefai & Gill, 2007).

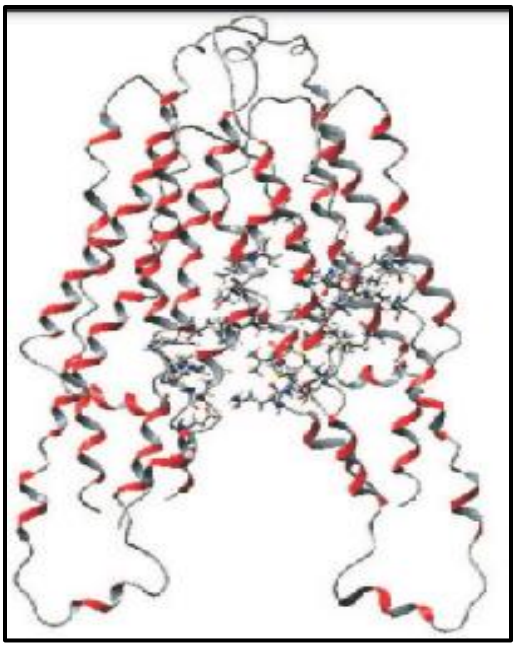


Figure 2.19: Structure of MRP2 protein generated by *in silico* methods(Williamson *et al.*, 2007)

2.13.3 Localization and Expression of ABCC1 and ABCC2 gene

ABCC1 gene is found on the basolateral membrane of epithelial cells and is expressed in most tissues except in brain capillary endothelial cells and placental syncytiotrophoblasts, located on the plasma membrane (Borst *et al*., 2000). Human *ABCC1* is highly expressed in the lung, kidney, testis, placenta, cardiac and skeletal muscles. However, reduced *MRP1* expression is found in the brain, colon, mononuclear cells, and small intestine. Fascinatingly, *ABCC1* is scarcely detected in the normal human liver, while its high expression is reported in the hepatocytes (post tissue damage) and the liver carcinoma cell lines (Klaassen & Aleksunes, 2010). Generally, *MRP1* is expressed highly in the cells that are involved in the barrier function (syncytiotrophoblasts in the placenta and choroid plexus in the brain), as well as in the proliferating cells (such as dividing hepatocytes, hyperplastic type II pneumocytes of the lung, and dividing paneth cells in the colon). *ABCC1* gene is expressed in humans' basolateral membranes of proximal tubular cells. As a result, instead of excreting its substrates in the bile, gut, or urine, it pumps them into the interstitial space (Jiye & Jianting, 2011). MRP1 may aid the tubular resorption of organic anions back into the capillary blood. The location of MRP1 has also been reported to transport xenobiotics from the cerebrospinal fluid (CSF) to the bloodstream (Choudhuri & Klaassen, 2006).

The apical location of ABCC2 not only assists in the drug outflow and detoxification of various xenobiotics and endogenous organic anions, but it also plays a crucial role in the resistance of numerous anti-cancerous drugs, *i.e.*, etoposide, cisplatin, methotrexate, and doxorubicin (Borst *et al.*, 2000). Interestingly, translocation by ABCC2 can also lead to toxicity. MRP2 is primarily expressed in the intestine, liver, and kidney and is found in the apical membranes of these tissues (Klaassen & Aleksunes, 2010). Also, this gene is present in the bronchi, placenta, and gall bladder. Patients with Dubin-Johnson syndrome emphasize the importance of MRP2 (Sharma & Sharma, 2022). Even though MRP2 and MRP1 have a similar range of substrates, MRP2 has distinct roles in the body due to subcellular polarity and differential expression. By secreting metabolites into bile, MRP2 serves a crucial role in detoxification.

2.13.4 Gene regulation of ABCC1 and ABCC2 gene

Various regulatory mechanisms modulate the expression of the *ABCC1* gene (Kunická & Souček, 2014). The 5′-end part of the *MRP1* gene was cloned for the first time, and a study of this region revealed that MRP1 contains a TATA-less promoter, like other human multidrug transporters. In the TATA-less promoters, CCAAT elements and GC-rich regions are the common RNA polymerase II promoter elements. The basal promoter activity was found in a GC-rich region of the *MRP1* gene between nucleotides -91 and +103, where the interaction of Sp1 takes place. It has been reported that the binding of Sp1 to the GC-rich region is important for the optimal transcriptional activity of MRP1. Additionally, the MRP1 promoter region comprise an antioxidant response element/AP-1 binding site that shows interaction with complex *jun* D and c-*jun* oncogenes that might show a role in the ABCC1 expression (Kurz *et al.*, 2001). In the beginning, even cancers emerging from tissues with low levels of MRP1 expression display high levels of MRP1 expression. The overexpression of elements implicated in malignant transformation, like tumour suppressor proteins and oncogenes, is most likely the cause of this increase in MRP1 expression (Bakos & Homolya, 2007). The native-type p53 (tumour suppressor gene) can substantially suppress transcription of MRP1 by inactivating promoter-bound Sp1, while the variant p53 abolishes the repression of MRP1 (Scotto, 2003). So, the absence of p53 function or elevation in Sp1 activity in cancer cells can upregulate the *ABCC1* gene, as confirmed in many tumour types such as NSCLC and prostate cancer. The *MYCN* oncogene can upregulate *MRP1* expression in neuroblastoma. Being a member of the *MYC* family, *MYCN* dimerizes with MAX.

The MRP1 mRNA and protein levels increase, followed by elevated drug resistance. Various oxidative stress-responsive elements regulate the gene expression of *MRP1*. The antioxidant-responsive element-driven genes, such as nuclear factor-E2 p45-related factor 2 (Nrf_2), correlate with the MRP1 expression (Manohar *et al.*, 2004). Other than these factors, there are very scanty reports regarding the post-translation regulation of the *ABCC1* gene. A study in the past has shown that casein kinase 2a is involved in the regulation of ABCC1 via direct phosphorylating of the threonine semi-conserved residue (Stolarczyk *et al.*, 2012).

ABCC2 expression is regulated at transcriptional and post-transcriptional levels concerning numerous xenobiotics and endogenous substances and various disease states. Multiple consensus binding sites for both liver-specific & ubiquitous transcription factors were found during the cloning and characterization of the rat ABCC2 and human ABCC2 promoters. Human *ABCC2* expression depends on the region from nucleotides -517 and -197 upstream of the translation starting codon (Stöckel *et al.*, 2000), with the transcriptional start-site located at nucleotide -247. Additionally, when the 5'-flanking region of the rat *MRP2* gene was characterized, it was identified that this region has binding sites for constitutive androstane receptor (CAR), farnesoid X receptor (FXR), and pregnane X receptor (PXR) (Kast *et al.*, 2002). It was reported that all these three nuclear factors could play a role in the activation of *MRP2* gene expression by a response element (ER-8) in the proximal promoter. Insights of the response element ER-8 in rat *ABCC2* promoter might now clarify the studies that used to explain the induction of ABCC2 mRNA as well as ABCC2 protein in the primary cultures of rat hepatocytes by various xenobiotics, such as anticancer drug cisplatin, carcinogen 2-acetylaminofluorene, the antibiotic dexamethasone, cycloheximide, the antifungal agent clotrimazole, and some other chemotherapeutic agents. The antibiotic rifampin stimulates CYP3A4 and P-gp as well as MRP2 mRNA and protein expression in duodenal enterocytes of humans (Nies & Keppler, 2007).

2.13.5 Substrate specificity of ABCC1 and ABCC2 gene

ABCC1 translocate a diverse spectrum of substrates from anti-cancerous drugs to fluorescent dyes. Anti-cancerous drugs such as vinca alkaloids, anthracyclines, camptothecins, mitoxantrone, epipodophyllotoxins, and methotrexate are the well-known substrates of the ABCC1 transporter (Sharma *et al.*, 2021). Also, the upregulation of ABCC1 leads to multidrug resistance in the case

of carcinoma chemotherapy. MRP1 confers resistance to antineoplastic drugs including vincristine, etoposide, teniposide, vinblastine, daunorubicin, doxorubicin, irinotecan (SN-38). Also, many endogenous substrates, mainly leukotrienes C4, sphingosine-1-phosphate, and hormones like prostaglandins and estrogens, are substrates of ABCC1. Additionally, antivirals such as saquinavir and ritonavir, toxicants including aflatoxin, chlorpropham, and methoxychlor, and heavy metals such as arsenite and trivalent antimony also are the MRP1 substrates. Amphipathic organic anions conjugated with glucuronide, sulfate, or glutathione (GSH) are also transported by MRP1 (He *et al.*, 2011). **Figure 2.20** illustrates the mechanism of the uptake of endogenous substances and other compounds.

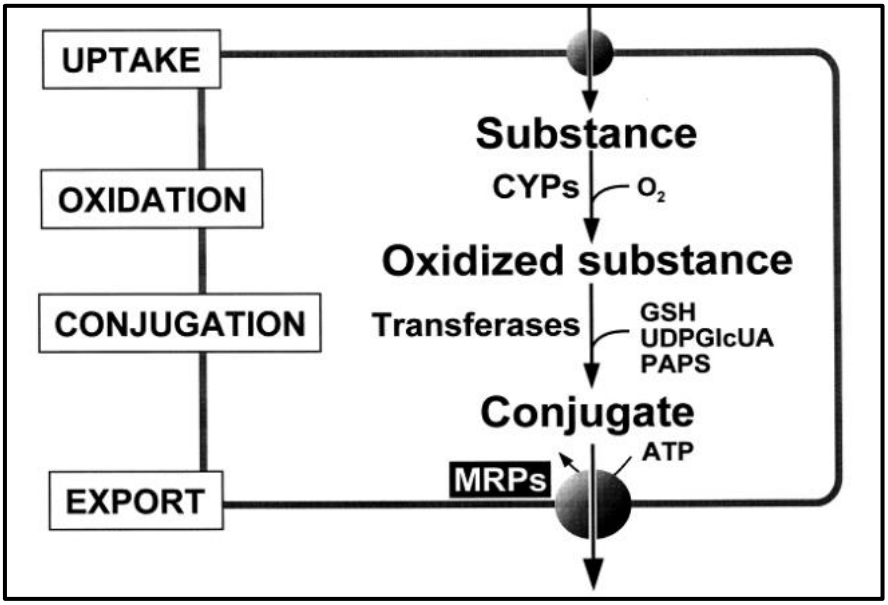


Figure 2.20: Mechanism illustrating the uptake of endogenous substances, drugs, or carcinogens, biotransformation, and exportation through MRP (Source: König *et al.*, 1999)

ABCC2 facilitates the transportation of many organic anions such as GSH conjugates, sulfate, and glucuronate. Many MRP2 substrates overlap with the substrates of MRP1. Drug-like cisplatin, methotrexate, camptothecin, vinca alkaloids, and anthracyclines are substrates of ABCC2 (Schinkel & Jonker, 2012). Other than these drugs, many endogenous substrates are transported through MRP2, such as acidic bile salts, GSSG, bilirubin glucuronide, and estradiol-17beta-d-glucuronide ($E_2$17βG). Also, the over-expression of MRP2 is associated with cisplatin resistance (Guminski *et al.*, 2006). MRP2 substrates are competitive inhibitors utilizing inside-out membrane vesicles such as MK571, LTC4, Fluorescein, and methotrexate. Nonetheless, these compounds do not penetrate the normal cells satisfactorily to attain inhibition (Jain *et al.*, 2008).

2.14 ATP-binding cassette sub-family G member 2

The gene *ABCG2* is also referred to as the breast cancer resistance protein (BCRP). ABCG2 is a half transporter as it contains only one TMD and NBD domain, and it has half the size of full transporter. It also plays a crucial function in physiological detoxification via tissue barriers, such as the blood-brain barrier, placenta, and mammary epithelium (Kuchler, 2011). Additionally, ABCG2 collaborates with members of other ABC families, such as ABCB1 and ABCC1, in causing resistance phenomena in different types of cancer. Besides this, ABCG2 is widely expressed and distributed in normal human tissues; this implicates its crucial role in protecting an organism as it acts as a first-line defence against various environmental insults (Mo & Zhang, 2012).

2.14.1 Genomic organization

The genomic organization of the human *ABCG2* gene and the characterization of its promotor was first described in 2001 (Bailey-Dell, & Ross, 2001). Resequencing has since revised some data, including the nucleotide locations and the gene's length. The human *ABCG2* gene spans almost 141.2 kb and is positioned at chromosome 4 (4q22.1), as shown in **figure 2.21**. There are 15 introns and 16 exons in the human *ABCG2* gene. All the spice junctions follow the GT...AG rule (Chambon's rule). Exon 1 has 474 nucleotides and is fully non-coding. In exon 2, the first 19 nucleotides are also non-coding; the coding region is found after that. Also, exon 2 contains the translation start site (Shah *et al*., 2021). The 5'UTR region of human *ABCG2* mRNA is 493 nucleotides long. The most extended exon is exon 16, with 2118 nucleotides, and the first 145 nucleotides are coding regions before the stop codon. The remaining exon 16 comprises the 3'UTR mRNA with a length of 1970 nucleotides. The smallest exon 3 is of 60bp (Choudhuri & Klaassen, 2018). Additionally, the Walker A motif is encoded by exon3 and Walker B motifs are encoded by exon 6, and the ABC motif (ALSGGQ) is encoded by exon 6 (Choudhuri & Klaassen, 2006).

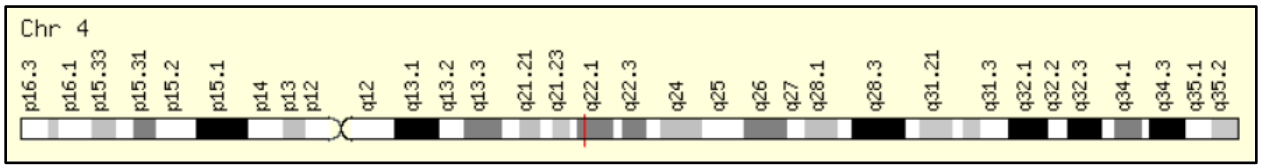


Figure 2.21: Chromosome 4 depicting the localization of the ABCG2 gene on it (Source: https://www.genecards.org/)

2.14.2 Structure of BCRP protein

ABCG2 is a 'half-transporter' with a single NBD, and TMD (TMD-NBD) coupled to a polypeptide chain. The TMD domain structure contains six transmembrane helices. To be functional, the homo-dimerization of ABCG2 takes place, and the molecular mass is approximately 144kDa (Mo & Zhang, 2012). The transmembrane helix and the intracellular loops of BCRP are smaller than the ABCB subfamily transporters. The TM5a and TM2 make the TMD interface of opposite *ABCG2* monomers. It has been postulated that two motifs (406-GXXXG-410) are involved in the dimerization process and positioned on the opposing ends of the ABCG2 transporter where there is no point of contact between them (Polgar *et al.*, 2004). In the past few years, a low-resolution structure of *ABCG2* was proposed, but in 2017, the first high-resolution human ABCG2 with single-particle cryo-EM analysis led to the 3D structure with a resolution of 3.8Å as shown in **figure 2.22** (Taylor *et al.*, 2017).

Also, being a member of the same family, the ABCG2 structure has architectural similarities with ABCG2 and ABCG5/8. A study in 2017 pointed out some breakthrough differences in the structure. There is a backbone shift in both TM5a and TM2 monomers because ABCG2 forms an inward-facing cavity, which is not a feature of ABCG5/G8. Secondly, there is an external loop called EL3, a distinctive feature between the two structures (Taylor *et al.*, 2017). Remarkably, the NBDs of BCRP continue to be in close contact irrespective of the absence of bound nucleotide. The NBD region of the ABCG2 monomer is associated with TM1a. The ABCG2 has a C2 motif, which is crucial for the binding of ATP and is positioned on the α-helix at the bottommost of NBDs (Macalou *et al.*, 2016).

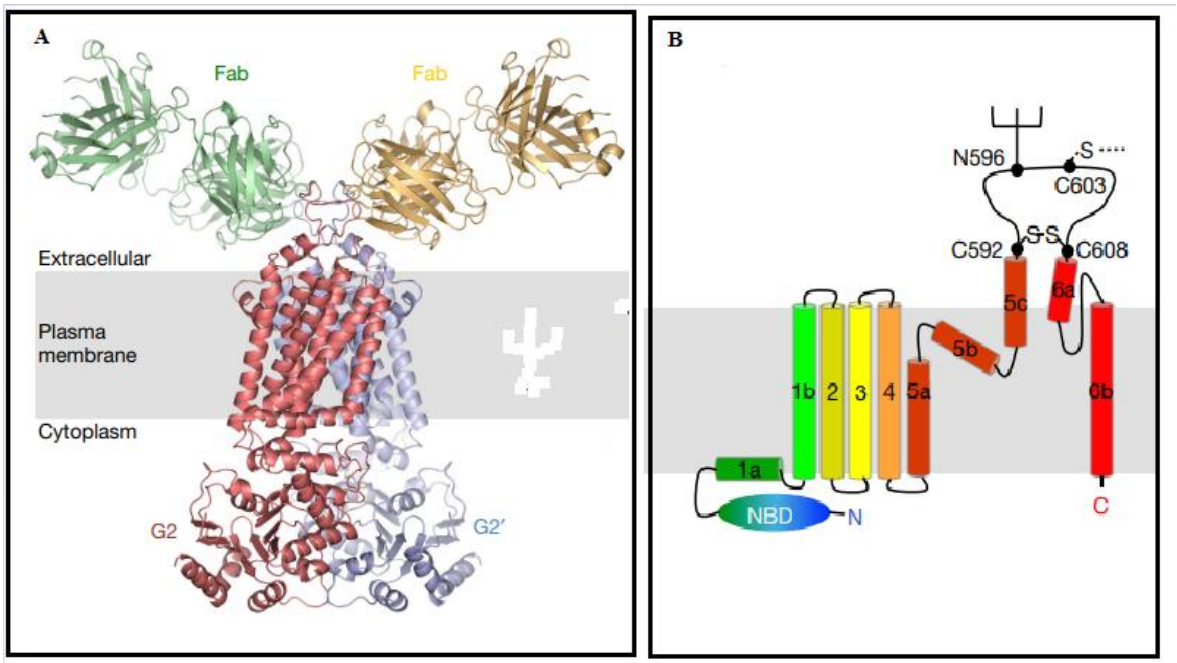


Figure 2.22: An illustration depicting the structure of ABCG2 (A) A ribbon diagram of *ABCG2* complexed with 5D3-Fab. The monomers in the ABCG2 structure are demarcated as G2 and G2'. (B) Depiction of the topology of ABCG2; the intra and intermolecular disulfide bonds are formed by cysteines (C592, C603, &C608) so as is the N-glycosylated residue (Taylor *et al.*, 2017)

2.14.3 Localization and Expression of ABCG2 gene

BCRP is widely expressed in normal human tissues such as the gastrointestinal tract, placenta, blood-brain barrier, kidney, liver, testis, lung, and lactating breast. *ABCG2* is present in the apical membrane of the colon and small intestine epithelia. The expression has also been reported in the endothelial cells of the brain (Aronica *et al.*, 2005). *BCRP* is also positioned on the luminal surface of the renal tubules. Studies have shown that drug distribution was impacted by the suppression of BCRP activity in several experimental studies. These findings point to a potential function for BCRP in regulating the uptake and distribution of its substrate molecules. BCRP expression is more commonly detected in adenocarcinomas of the lungs, endometrium, and digestive tract (Mo & Zhang, 2012). Elevated levels of *BCRP* mRNA expression are detected in the colon, prostate, spinal cord, adrenal gland, and uterus. The expression of ABCG2 is found in the luminal membrane of epithelial cells of a healthy gallbladder, sebaceous glands, alveolar pneumocytes,

islet & acinar cells of the pancreas, and squamous epithelium of the cervix (Maliepaard *et al.*, 2001).

According to reports, ABCG2 is widely expressed in lymphoid cells and malignant hematopoietic cells; its expression in various types of leukemia has been studied. Additionally, various studies have shown that ABCG2 is associated with increased expression in lung cancer. A study in the past showed that cisplatin-resistant non-small-cell lung cancer cells that overexpressed ABCG2 were more likely to divide symmetrically, whereas cells with silenced ABCG2 experienced an upsurge in the asymmetric division. So, this study concluded that ABCG2 could regulate the cell division pattern in the cisplatin-resistant NSCLC cell lines. According to numerous studies, platinum-based chemotherapy for NSCLC patients with elevated ABCG2 expression results in decreased response rates, lower overall survival, and poorer progression-free survival (Tang *et al.*, 2014).

2.14.4 Gene regulation of ABCG2 gene

The *ABCG2* gene has a TATA-less promoter along with various sites for Sp1, AP-1, and AP-2. Multiple Sp1 sites are a characteristic of TATA-less promoters. Preliminary characterization discovered the presence of CpG island in 500 nucleotides upstream of the transcription start codon. Reports have revealed that the region among nucleotides -1285 and -628 comprises positive regulatory elements, while the region between nucleotides -628 and -312 consists of harmful regulatory elements (Austin Doyle & Ross, 2003). So, concerning this, the author reported that the basal promoter activity is conferred by the 312-bp stretch immediately upstream of the *BCRP* transcription start site. The length of 3'UTR of ABCG2 mRNA is approximately 2kb and longer than the average 770bp detected for human mRNAs. This implies that it might play one or more significant roles in controlling the expression of genes. Other than Sp1, many other transcription factors are reported to play a crucial role in regulating the expression of ABCG2 (Hamada *et al.*, 2012). Such transcription factors comprise but are not confined to peroxisome proliferator-activated receptor gamma (PPARγ), hypoxia-inducible factor 1 (HIF-1), estrogen receptor alpha (ERα), aryl hydrocarbon receptor (AHR), and progesterone receptor (PGR) (Mo & Zhang, 2012). Additionally, estrogen response element between locations -188 to -172 of ABCG2 promotor has been identified through sequence analysis of the 5' flanking region of the *ABCG2* gene. Site-

directed mutagenesis and deletions analysis have also assured the presence of estrogen response elements in this particular region. Further, it was shown that 17β-estradiol could also enhance the mRNA expression of ABCG2 via activation of the estrogen receptor α, which binds to the estrogen response element in the ABCG2 promoter (Moitra *et al*., 2011).

MicroRNAs also have a role in the post-transcriptional regulation of ABCG2. miR-328 targets the *ABCG2* gene and reduces *ABCG2 mRNA* and protein levels through mRNA cleavage. In several cancer cell lines, the ABCG2 proximal miRNA response element is found in the 3'-UTR of the ABCG2 mRNA. It was noteworthy that drug-resistant cancer cells lacked this putative ABCG2 miRNA response element, which enabled them to evade miRNA-mediated inhibition of ABCG2 protein synthesis and mRNA degradation, resulting in high ABCG2 expression (To *et al.*, 2009).

2.14.5 Substrate specificity of ABCG2 gene

Transporter ABCG2 has a broad capacity to translocate a wide range of substrates. The substrate spectrum of ABCG2 considerably overlaps with that of P-gp or ABCC1. Though more than 200 ABCG2 substrates have been identified, their chemical compositions and biological activities are pretty diverse (Mao & Unadkat, 2015). BCRP substrates are recognized by vesicular or cellular transport assays or cytotoxicity assays, encompassing various anti-cancerous drugs, toxins, xenobiotics, and antibiotics. Various chemotherapeutic agents are translocated by ABCG2 transporters, such as taxanes (docetaxel and paclitaxel), anthracyclines (daunorubicin, epirubicin, pirarubicin, and doxorubicin), vinca alkaloids (vincristine, vinorelbine, vinblastine, and vindesine), camptothecin derivatives (SN-38, topotecan, CPT-11, and methotrexate), anthracenes (bisantrene and mitoxantrone), epipodophyllotoxin derivatives (teniposide and etoposide), tyrosine kinase inhibitor (gefitinib, erlotinib, imatinib) (Orlando & Liao, 2020).

Other substrates of ABCG2 are cimetidine, prazosin, rosuvastatin, glyburide, vitamin K3, vitamin B2, and various chemical carcinogens (Mo & Zhang, 2012). An effective inhibitor of BCRP, elacridar is also an inhibitor of P-gp (Karbownik *et al.*, 2020). ABCG2 is also involved in the translocation of natural substrates such as pheophorbide α, 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine (PhIP), phosphatidylserine, and protoporphyrin IX (PPIX). Additionally, inhibitors of P-gp, such as valspodar, tariquidar, cyclosporine A and reserpine, also inhibit the

activity of BCRP. TKI (Tyrosine kinase inhibitors), such as imatinib, erlotinib, nilotinib, gefitinib, and lapatinib, inhibit BCRP-mediated drug resistance. These TKIs are the substrates of BCRP, demonstrating that all these compounds might work as competitive inhibitors of BCRP (S. Wu & Fu, 2018).

2.15 Influence of ABC transporters on the intracellular distribution of drug

ABC transporters mediate the absorption and metabolism of drugs and are involved in drug transportation across the membrane. These transporters are the decision-makers of the drug accumulation in the cells, which is directly associated with drug toxicity, drug-drug interactions, and therapeutic efficacy. Besides their participation in drug disposal and its clinical toxicity, these transporters play a crucial role in maintaining cell homeostasis by regulating the transport of different endogenous compounds.

2.15.1 Drug absorption

The small intestine is the main organ of absorption, which decides the availability of nutrients from the diet. Drug absorption from the gastrointestinal (GI) tract is vital in deciding oral bioavailability (Pang *et al.*, 2010). Intestinal absorptive cells and enterocytes have a range of influx transporters localized at the luminal/apical membrane to absorb drugs and food components. The ABC transporters that influence drug trafficking are ABCB1/MDR1/P-gp, ABCG2/MXR/BCRP, ABCC2/MRP2, and ABCC4/MRP4, respectively. The expression level of these transporters differs between the various segments of the intestine (Crawford, & Schuetz, 2018). ABCB1, ABCC2, and ABCG2 have high expression in the intestine and act as a rate-limiting blockade to oral drug absorption. ABCB1 is the most characterized transporter, and studies have reported the role of P-gp in the gut, thus limiting the bioavailability of paclitaxel that is administered orally. It is also claimed that P-gp could expect the same action if any other toxic substance is ingested. P-gp in the intestine eliminates the parenteral drug administered by directly secreting the drug into the intestine lumen (Gavhane & Yadav, 2012). Even though ABCC2 and ABCG2 are higher in the intestine than ABCB1, significantly less data is on hand describing their role in drug absorption.

2.15.2 Drug metabolism

After the absorption of a drug in an organism, drug metabolism is mediated by several sequential reactions. Drug metabolism occurs through phases, i.e., Phase I and II. Phase I reactions include reduction, oxidations, and hydrolysis and are illustrated as functionalization reactions. In this reaction, a new functional group is added to the parent drug (oxidation), followed by modification of the functional group in the parent drug to be more polar (reduction) and unmask the existing functional group (hydrolysis) (Taxak & Bharatam, 2014). Phase II involves the conjugation of partially detoxified metabolites in order to make them less harmful and water-soluble. Phase III eliminates the detoxified molecules carried by the multiple transporters. The intestine is the leading site for significant non- renal removal of numerous drugs. The part of excreted molecules is reabsorbed in enterocytes because of this repeated reabsorption and extrusion; there is an extension in the residence time within the cell, enhancing the possibility of intestinal metabolism (Coleman, 2020). P-gp is a phase 1 drug-metabolizing enzyme present in high levels in enterocytes of the small intestine (Xie, Ding, & Zhang, 2016). The inflow and efflux of xenobiotics and endogenous substances into and out of the cell are crucially regulated by hepatic transporter proteins. P-gp in the small intestine is the primary route for eliminating intravenous or oral administration of the drugs. ABCB1 is a well-studied canalicular transporter with wide substrate specificity and is correlated with the outflow of drugs. Other transporters of the ABC family, such as *ABCB4*, *ABCB11*, and members of the *ABCC* subfamily, are also expressed in the liver. Efflux transporters positioned at the basolateral surface of the hepatic cells, *i.e.*, MRP3 and MRP4, play a crucial role in the excretion of drug metabolites in the sinusoid for urinary excretion by the kidneys (Corsini & Bortolini, 2013).

2.15.3 Drug excretion

The liver and kidney mainly control drug excretion, and biliary & renal excretion displays some similarities. To enter both the tissues, drugs must enter through the basolateral membrane of hepatic cells or tubular epithelial cells. Biotransformation often occurs before the drug or metabolite reaches the apical membrane, where it is actively efflux by one of the apically localized transporters or diffuses passively across the membrane. ABC transporters are widely expressed in the liver and kidney and play a crucial role in drug elimination (Masereeuw & Russel, 2012). In the kidney, P-gp expression is restricted to the proximal tubule apical membrane, consistent with

a urine efflux role. Besides this, among MRPs, ABCC2 and ABCC4 are the most crucial drugs for eliminating drugs (Glavinas *et al.*, 2004). It is unknown whether ABCC1 is expressed in the kidney's proximal tubule; however, it is claimed that it is present on the basolateral membranes of Henle cells and the distal and collecting duct tubule cells (van de Water *et al.*, 2005). It is likely essential in protecting against the toxic substances that accumulate and may reach substantial concentrations due to hormone-controlled water absorption. Additionally, ABCG2 is the only member of the ABCG subfamily involved in the drug efflux other than cholesterol transport and is located at the apical surface of proximal tubule cells (Masereeuw & Russel, 2012).

2.16 Role of ABC transporters in cancer

ATP binding cassette transporter has been studied intensely over the past years for their role in causing multidrug resistance. Chemotherapy resistance is the main problem in effectively treating numerous tumour types. Resistance may occur before the drug treatment as innate/primary resistance or can develop some time following the exposure as acquired resistance. Prolonged exposure to a particular chemotherapeutic agent might develop resistance to various other structurally distinct compounds (Housman *et al.*, 2014). Drug resistance mechanisms include drug delivery by excessive metabolism, poor absorption, poor penetration to specific sites, alteration in the apoptotic pathway, mutation of the target, environmental changes, or increased expression of drug efflux pumps, which have been reported (Ray *et al.*, 2017). One of the most effective MDR mechanisms is an elevated efflux rate of the anti-cancerous drug from the cancerous cells by the ABC transporters. Three ABC proteins are overexpressed in the tumour cells, MDR1, MRP1, and BCRP, and seven other transporters are known to cause resistance to at least a single agent (Robey *et al.*, 2018). However, in recent years, some studies have demonstrated that ABC transporters play additional, essential roles in tumour biology. There are substantial reports that these proteins are implicated in the transport of molecules that promote tumorigenesis and in a protein-protein interplay that affects the aggressiveness, development, and prognosis of cancer patients, irrespective of resistance caused by the efflux of the chemotherapeutic drugs (Muriithi *et al.*, 2020).

A growing number of studies are contributed to this scientific area, and even though the majority of the research findings are correlational, they have demonstrated a relationship between intrinsic dysregulated ABC protein expression as well as more aggressive tumour phenotype that is assessed by tumour stage, size, the likelihood of metastasis, and patient survival rate. A list of the primary ABC transporters expressed in various cancers is depicted in **Table 2.5.** The possibility that these proteins have a pro-tumour functionality cannot be ruled out, notwithstanding the likelihood that the abnormal expression results from genetic changes associated with more aggressive cancers (Muriithi *et al.*, 2020). In one of the investigations on the relationship between ABC transporters and carcinogenesis, Mochida *et al.* found that the absence of P-glycoprotein (ABCB1) inhibits the development of intestinal polyps and slows tumour growth in mice. Although the P-gp knockout mice in this work displayed substantial levels of DNA damage, likely due to increased xenobiotic accumulation, tumour development was four times less than in wild-type mice, indicating a role for P-gp in tumour formation (Mochida *et al.*, 2003).

Transporters ABCB1, ABCC1 and ABCG2 have been studied in numerous articles as affecting treatment and patient prognosis in gliomas (Balça-Silva *et al.*, 2017; Matias *et al.*, 2018). A study by de Faria *et al.* reported the expressional differences in the pattern of P-gp (ABCB1) and MRP1 (ABCC1) in low- and high-grade gliomas. The expression of ABCC1 was lower in grade I and II glioma; on the contrary, its expression was higher in grade III and IV glioma. Along with this, this study showed that the expression of ABCB1 was higher in grade I and II glioma. This ABC protein is associated with higher malignancy and tumour grade due to the elevated expression of ABCC1 in glioma grade III or glioblastoma. It may serve as a marker for the progressively undifferentiated phenotype of glial cells prevalent in high-grade gliomas (de Faria *et al.*, 2008).

Researchers in the past have evaluated the expression of ABCB1 in prostate cancer. Bandariaviciute *et al.* reported that the *MDR1* transporter gene is frequently downregulated in patients having prostate cancer and showed a substantial correlation with the clinical variables; also, they concluded that the deregulation of the *ABCB1* gene should be studied for its prognostic potential in prostate cancer (Bandariavičiūtė *et al.*, 2015). Numerous other studies have also reported the downregulation of the *ABCB1* gene in prostate cancer (Mishra *et al.*, 2010; Bhangal *et al.*, 2000). Additionally, various studies in the past have reported the elevated expression of

ABCB1 doxorubicin and paclitaxel-resistant carcinoma cell lines as well as tumour tissues. In the case of ovarian cancer, high expression of MDR1 at both mRNA and protein levels was associated with poor clinical outcomes, resistance to the therapy based on paclitaxel, lower median survival time, and short treatment response (Seborova *et al.*, 2019).

The lower MDR1 mRNA expression is associated with more prolonged survival in the kidney or ovarian cancer and thymoma. Also, in lung, kidney, and breast cancer, the expression of ABC transporters is correlated with various tumour stages and ethnic populations (Kadioglu *et al.*, 2020). Sun *et al.* reported that high *ABCB1* expression is associated with poor prognosis and chemoresistance in epithelial ovarian cancer. The study showed no evidence regarding the association between *MDR1* expression and prognosis in the epithelial ovarian cancer patients administered with a taxane-containing regimen. Although, they reported a severe association between the *MDR1* expression and survival in the patients who were not given a taxane-based regimen, mainly platinum in conjunction with cyclophosphamide and doxorubicin (S. Sun *et al.*, 2016). Other than this, studies in the past have reported the correlation between overexpression of *ABCB1* in lung cancer and associating it with lung carcinoma (Zawadzka *et al.*, 2020)

As mentioned earlier, ABCC/ MRP subfamily is the largest group of drug-efflux pumps. ABCC1 has garnered maximum interest because of its dysregulation in numerous carcinomas such as NSCLC, glioma, acute lymphocytic leukemia, and acute myeloid leukemia. Also, high levels of ABCC1 have been well reported in neuroblastoma. *ABCC1* has been associated with a predictor of poor response to chemotherapy (Cole, 2014). Additionally, in astrocytic tumours the overexpression of *ABCC1* has been reported, while the author has also reported the undetectable or low expression in the normal astrocytes (Haga *et al.*, 2001).

On the contrary, the reverse of this has been reported where the down-regulation of transporter expression in more-aggressive tumour phenotypes has been claimed (Bandariavičiūtė *et al.*, 2015). *ABCC1* expression was also correlated with invasive level V melanoma and substantially with lymph node metastasis (Walsh *et al.*, 2010). The overexpression of *ABCC1* in the tumour tissue is generally claimed to be a characteristic feature of NSCLC (Hanssen *et al.*, 2021).

Carcinoid tumours and SCLC also express MRP1, but the levels are lower than the NSCLC. Regarding lung cancer, conflicting reports claim overexpression (Poku & Iram, 2022)

and low expression of MRP1 in NSCLC. Remarkably, the expression of ABCC1 in the malignant tissues is highest in early-stage cancer while it declines with the disease progression. The overexpression of ABCC1 is likely an inherited characteristic of the naïve epithelial cells which is further activated at the time of malignant transformation while getting downregulated when the disease progresses (Berger *et al.*, 2005). Also, the ABCC1 expression has been associated with improved prognosis, particularly in patients not pre-treated with chemotherapy. A poor prognosis in the NSCLC patients in patients expressing high levels of ABCC1 and in patients treated with chemotherapy has also been discussed before (Berger *et al.*, 2005).

Also, up-regulation of MRP1 has been reported in glioma, neuroblastoma, pancreatic, prostate, ovarian, and colorectal cancer. Results published in the past from various studies imply that MRP1 expression is correlated with metastatic breast carcinoma and poor outcomes (Rudas *et al.*, 2003; Shen *et al.*, 2011). Remarkably a recent study showed that *ABCC1*, *ABCB1,* and *ABCG2* are frequently and intensely expressed in triple-negative breast cancer before the administration of chemotherapy (Nedeljković *et al.*, 2021).

ABCC2 polymorphism has been widely studied in liver cancer or pancreatic cancer. Very few reports are there on the other carcinomas. Though, few studies in lung carcinoma have shown that *ABCC2* is overexpressed in lung cancer, contributing to the reduced survival rates in the white population (Kadioglu *et al.*, 2020). Studies have also shown that after chronic usage of platinum drugs in patients with colon cancer, lung cancer, and ovarian cancer, the *MRP2* gene is commonly upregulated in certain parts of such patients, and this overexpression often leads to poor survival rates (Lee *et al.*, 2007; Zhang *et al.*, 2006). The overexpression of *MRP2* has been reported in hepatocellular carcinoma (Nies *et al.*, 2001)and renal cell carcinoma (Schaub *et al.*, 1999). Also, aberrant expression has been reported in a few cancers such as lung, stomach, breast, bowel, and ovary (Sandusky *et al.,* 2002). A recent study assessed the role of MRP2 in the cisplatin resistance of the NSCLC cells. The outcomes correlated that the high *ABCC2* expression is associated with the resistance of cisplatin in the NSCLC patients and also suggested that the reduced expression of *ABCC2* should be an opted strategy to enhance the sensitivity of the NSCLC cells to drug cisplatin (Chen *et al.*, 2021).

The other extensively studied transporter in ABCG2 was initially identified in breast cancer. It has been demonstrated that this transporter is overexpressed in stem cells, cancer stem

cells, and tumour cells localized in hypoxic areas and is predominant in side populations. The expression of ABCG2 is reported to be elevated in hepatocellular carcinoma and was also said to be further increased after chemotherapy in hepatoblastoma patients (Sukowati *et al.*, 2012). In patients with triple-negative breast carcinoma cells, elevated levels of ABCG2 appeared to be less aggressive as the elevated expression of ABCG2 was correlated with the improved outcomes in the univariate analysis (Nedeljković *et al.*, 2021). The same pattern was seen in intrahepatic cholangiocarcinoma, according to Larbcharoensub and colleagues (Larbcharoensub *et al.*, 2011). They discovered that lymph node invasion and a worse prognosis were related to the loss of *ABCG2* expression.

Similarly, lower *ABCG2* expression was found to be substantially associated with increased tumour size and worse OS in a study by Namisaki and colleagues (Namisaki *et al.*, 2014). It is puzzling how elevated *ABCG2* expression might result in a favorable outcome. Additionally, a study revealed the high expression of ABCG2 in pancreatic adenocarcinoma and the low expression of ABCG2 in colorectal cancer (Mohelnikova-Duchonova *et al.*, 2013). *BCRP* expression was an independent prognostic factor in one investigation using real-time RT-PCR in adult acute myeloid leukemia (Benderra *et al.*, 2004).

Table 2.5: A list of the primary ABC transporters expressed in various cancers

TRANSPORTER	Up-regulation in cancer type	Down-regulation in cancer type	References
ABCB1	Colorectal cancer Liver cancer Lung cancer Renal carcinoma Ovarian cancer Breast cancer Adrenocortical cancer	Breast cancer Colorectal cancer Prostate cancer Kidney cancer Thymic carcinoma	(Kadioglu *et al.*, 2020) (Mochida *et al.*, 2003) (Bandariavičiūtė *et al.*, 2015) (Sun *et al.*, 2016)
ABCC1	Lung (NSCLC) Glioma Neuroblastoma Ovarian cancer Breast cancer Colorectal cancer Prostate cancer Pancreatic cancer Chronic lymphoblastic leukaemia acute lymphocytic leukaemia	Lung (NSCLC)	(Nooter *et al.*, 1996) (Wright *et al.*, 1998) (Sugawara *et al.*, 1996) (de Faria *et al.*, 2008) (Haga *et al.*, 2001) (Nedeljković *et al.*, 2021)
ABCC2	Lung cancer (NSCLC) Ovarian Cancer Colon cancer Hepatocellular cancer Renal cell carcinoma		(Kadioglu *et al.*, 2020) (Nies *et al.*, 2001) (Schaub *et al.*, 1999) (Sandusky *et al.*, 2002)
ABCG2	NSCLC Cervical cancer Liver cancer cholangiocarcinoma Melanoma Pancreatic cancer Testicular cancer Glioma Ovarian Cancer Glioma	Prostate Colorectal cancer	(Sukowati *et al.*, 2012) (Nedeljković *et al.*, 2021) (Larbcharoensub *et al.*, 2011) (Namisaki *et al.*, 2014) (Mohelnikova-Duchonova *et al.*, 2013) (Benderra *et al.*, 2004)

2.17 Impact of single nucleotide polymorphisms in the ABC genes on lung cancer

Among the numerous polymorphisms identified in the *ABCB1* gene; $C^{1236}T$ (exon 12), $G^{2677}T/A$ (exon 21), and $C^{3435}T$ (exon 26) are the most common and relevant studied SNPs in the exon region of the MDR1 and thereby affect the functionality, substrate distribution and expression levels (Ganoci *et al.*, 2020). The synonymous $C^{1236}T$ ($Gly^{412}Gly$) SNP is found in exon 12 that encodes for the TM6 region, this area is crucial for substrate binding, and any modulation in this region can modulate the transportation of drugs and other compounds. The $C^{1236}T$ polymorphism correlates with impaired MDR1 protein function (Fung & Gottesman, 2009). The stability of the mRNA may be indirectly impacted by the $C^{1236}T$ polymorphism (Frittitta *et al.*, 2001). Allele-specific variations in RNA folding affect the control and regulation of downstream mRNA splicing, processing, or translation. The reduced translational activity might also occur (Shen *et al.*, 1999).

Another synonymous mutation, $C^{3435}T$, in which the C-to-T variation at position ^{3435} ($Ile^{1145}Ile$) does not change the amino acid sequence of ABCB1, is said to be connected with ABCB1 expression and function. Despite being a synonymous variant, the *T* allele of the synonymous $C^{3435}T$ polymorphism results in decreased expression level and reduced activity, according to numerous studies (Hitzl *et al.*, 2001; Hoffmeyer *et al.*, 2000). The polymorphism $C^{3435}T$ leads to the ribosome stalling and affects the folding of the protein, eventually modifying the topology of the substrate interaction sites. Therefore, poor interaction between the variant protein and anti-cancerous drug might lead to a reduced amount of being eliminated from the tumour cells, thereby decreasing the detrimental effect of ABCB1 transporter in the clinical outcomes of the chemotherapy treatment (Lara Jr *et al.*, 2009).

The triallelic variant $G^{2677}T/A$ is present in the native-type sequence with *G* nucleotide at ^{2677} and the mutant sequence with *A* or *T*. As three alternative nucleotides (*G, T, or A*) can be found at this position, the non-synonymous $G^{2677}T/A$ polymorphism represents a region well before TM9 and TM104 and causes an amino acid change from alanine to serine or threonine ($Ala^{893}Ser/Thr$). The $G^{2677}T/A$ polymorphism correlates with impaired protein function (Fung & Gottesman, 2009). In terms of functional impact, these SNP has produced contentious results.

The two common non-synonymous *ABCC1* polymorphisms in the Asian population are $G^{2168}A$ and $G^{3173}A$. The $G^{2168}A$ mutation is an arginine to glutamine substitution that occurs at

amino acid position 723, and $G^{3173}A$ is also an arginine to glutamine substitution at amino acid position 1058. $G^{2168}A$ polymorphism is localized in the first intracellular loop and first NBD, while G^{3173}A polymorphism is positioned in the seventh intracellular loop and the MSD 2. $G^{2168}A$ polymorphism affects the protein function by disturbing the ATPase activity of ABCC1 (Yin *et al.*, 2009).

The *ABCC2* mutation $G^{4544}A$ is a cysteine into tyrosine substitution at amino acid site 1515 in ABCC2's last cytoplasmic loop. This mutation decreases ATPase activity, significantly impacting cellular drug disposal (Elens *et al.*, 2011). Numerous studies have suggested that this mutation can cause variations in pharmacological effects, intracellular substrate levels, and toxicity (Wojnowski *et al.*, 2005; Ni *et al.*, 2010; Elens *et al.*, 2009).

The most common SNP of the *ABCG2* gene is *s* and is positioned in the transporter's Walker A motif, which affects the ATP binding domain. Numerous in vitro and in vivo investigations show that this mutation resulted in a general decrease in ABCG2 protein expression, and some of these studies also revealed a decrease in ATPase activity, which leads to reduced transportation activity (Imai *et al.*, 2002; Kobayashi *et al.*, 2005;Tamura *et al.*, 2007; Sarankó *et al.*, 2013). The neutral Q141 in the transmembrane domain is changed to a positively charged lysine, which results in folding errors in the ABCG2 protein and enhanced protein degradation, which could account for these effects on expression and function (Bruckmueller & Cascorbi, 2021). The *A* allele of $C^{421}A$ incidence is very high amongst Asian people, including approximately 25–35% of healthy populations, compared to about 10% prevalence in healthy Caucasians (Kim *et al.*, 2008). Critical studies on various *ABCB1*, *ABCC1/C2*, and *ABCG2* polymorphisms in lung cancer patients are highlighted in **Table 2.7.**

2.17.1 Association of ABC gene (ABCB1, ABCC1/C2, and ABCG2) polymorphism with prognosis in lung cancer patients

The association between the *ABCB1* polymorphism and overall survival has been evaluated in a few lung cancers studies. However, the data retrieved is not conclusive as the results are conflicting. Such reports are discussed in this section. A study in the past assessing the role of $C^{3435}T$ polymorphism in NSCLC patients did not observe any substantial differences while studying overall survival (Gandara *et al.*, 2009). Szczyrek *et al.* studied the effect of $C^{3435}T$

polymorphism in advanced NSCLC and observed no effect of age, stage, or gender on the prognosis of NSCLC patients treated with docetaxel (Szczyrek *et al.*, 2017). In similar lines, the lack of association of *C*^{3435}*T* polymorphism was also reported by Wei *et al.* when the ^{3435}*CC* genotypes were compared with the ^{3435} *CT* and *TT* genotypes (HR: 1.11; 95% CI: 0.78-1.56) (Wei *et al.*, 2011). A recent meta-analysis by Chen *et al.* assessed the prognostic value of *ABCB1 C*^{1236}*T* and *C*^{3435}*T* polymorphism in patients treated with platinum-based taxane chemotherapy. Fifteen studies having 3320 patients from different cancer were evaluated for the impact on overall survival. The study concluded that the carrier allele *T* of *C*^{1236}*T* polymorphism depicted better survival after platinum-based taxane chemotherapy (Chen *et al.*, 2021). A study by Vinolas *et al.* showed better survival in the mutant genotype of *C*^{3435}*T* as compared to the wild-type genotype (12.86 vs 10.23, p=0.75); however, substantial differences were not achieved, and no association was observed between *C*^{3435}*T* polymorphism in predicting prognosis in NSCLC patients undergoing platinum-based doublet chemotherapy.

There are reports on the impact of *ABCB1* polymorphism in SCLC patients treated with cisplatin-based regimens. Knez *et al.* studied the effect of *ABCB1 C*^{3435}*T* and *G*^{2677}*T/A* polymorphism in predicting the overall survival in SCLC patients. The study reported a median OS of 10.8 months and a lack of association between *C*^{3435}*T* and *G*^{2677}*A/T* polymorphism. However, the study also assessed the role of these polymorphisms by applying different genetic models. In the dominant model, for *C*^{3435}*T* polymorphism, the heterozygous and mutant genotypes showed better overall survival than the wild-type genotype. This study concluded the prognostic value of *C*^{3435}*T* polymorphism in Caucasian SCLC patients undergoing cisplatin-based chemotherapy (Knez *et al.*, 2012).

On the other hand, Weissfeld *et al.* reported a specific association of *ABCB1* polymorphism with the prognosis in lung carcinoma patients who underwent chemotherapy. Also, they concluded that the survival of advanced-stage lung cancer patients might be affected by the inherited variation in the *ABCB1* gene (Weissfeld *et al.*, 2014). Vaclavikova *et al.*, found that the expression level of the *ABCB1* gene in 79.5% of carcinoma tissues was down-regulated and related to polymorphic mutation of *C*^{3435}T and *C*^{1236}*T*; that is, the *ABCB1* SNP could change the function of P-gp by influencing its expression levels (Vaclavikova *et al.*, 2008; B. Yin *et al.*, 2017). A study by Bosch *et al.* showed that variant alleles of *C*^{1236}*T* polymorphism were correlated with the reduced

clearance of docetaxel, signifying the drug accumulation in the cell (Bosch *et al.*, 2006). A meta-analysis study by Chen *et al.* investigated six studies with 1217 patients for their association with OS and *ABCB1* $C^{1236}T$ polymorphism. The outcomes showed that the *CC* genotype of $C^{1236}T$ polymorphism is associated with poor survival compared to the *TT* genotype. Also, $C^{1236}T$ may substantially act as a prognostic factor in NSCLC patients treated with platinum-based chemotherapy (Chen *et al.*, 2020). Comparable observations were seen in an Asian cohort where ^{3435}TT carriers were detected with higher overall survival than carriers of the *C* allele (Kim *et al.*, 2015).

Patients with high ABCC1 expression have poorer prognoses and treatment responses (Kadioglu *et al.*, 2020). It has been reported that the *ABCC1* mutation may impact chemotherapeutic efficacy in lung carcinoma patients. However, due to the fewer reports on *ABCC1* $G^{3173}A$ and $G^{2168}A$ polymorphism and its associated role in predicting survival in carcinoma patients, we could not find literature regarding the impact of these polymorphisms in predicting overall survival. It has been widely reported that $G^{2168}A$ mutation substantially reduces MRP1 activity in resistance to etoposide, vinca, alkaloids, and anthracyclines (Yin *et al.*, 2009).

Another transporter upregulated in NSCLC patients is *ABCC2*, which is involved in platinum resistance. Only a few reports have shown an association between *ABCC2* $G^{4544}A$ and prognosis. A recent study by Cuffe *et al.* has published data discussing the role of $G^{4544}A$ polymorphism in the overall survival in stage IV NSCLC patients. The study showed that the A allele of $G^{4544}A$ is associated with substantially poor survival (HR=2.22, p=0.009) compared to the G/G or G/A allele (Cuffe *et al.*, 2016). Also, the studies have shown that the *ABCC2* gene is highly expressed and poorly differentiated in lung carcinoma compared to normal lung tissues. Filipits *et al.* enrolled early-stage NSCLC patients in the International Adjuvant lung study, and their results revealed *ABCC2* expression as an independent prognostic factor for the worse survival in patients with lung cancer (Filipits *et al.*, 2007). Additionally, no correlation was reported between ABCC2 $G^{4544}A$ polymorphism and overall survival in a cohort of platinum-treated NSCLC patients, though, as reported by the authors, the research had a limited substantial power when 125 patients were recruited (Sprowl *et al.*, 2012).

A meta-analysis was conducted by Fu *et al.* to study the correlation between *ABCG2* $C^{421}A$ polymorphism and lung cancer outcomes. The features of correlational reports are depicted in

Table 2.6. Most of these investigations were carried out in China, Germany, Japan, Korea, and Italy. The majority of participants were in stages III-IV. The meta-analysis outcomes were observed, and the authors concluded no significant correlation between *ABCG2* $C^{421}A$ polymorphism in predicting prognosis or any other clinical outcomes in lung cancer patients undergoing chemotherapy (Fu *et al.*, 2020). Another study by Muller *et al.* investigated the impact of *ABCG2* $C^{421}A$ polymorphism on the prognosis of lung carcinoma patients. The study showed worse survival in the patients carrying the *A* allele of $C^{421}A$ polymorphism and who underwent platinum-based chemotherapy (Müller *et al.*, 2009). On the contrary, Chen *et al.* did not show any substantial correlation between $C^{421}A$ variation and prognosis in Chinese advanced NSCLC patients (X. Chen *et al.*, 2015)

Table 2.6: Features of the study included in the meta-analysis (Fu *et al.*, 2020)

Author	Year	Region (Ethnicity)	No. of Patients	Stage	Evaluation Criteria
Akasaka	2010	Japan (Asian)	75	I-IV	WHO
Cusatis	2006	Italy (Caucasian)	173	I-IV	WHO
Fukudo	2013	Japan (Asians)	88	IIIB-IV	ECOG
Chen	2015	China (Asian)	70	I-II	ECOG
Han	2007	Korea (Asian)	107	IIIB-IV	ECOG
Lemos	2011	Italy (Caucasian)	94	IIIB-IV	ECOG
Kobayashi	2014	Japan (Asian)	31	IIIB-IV	CTCAE
Muller	2009	Germany (Caucasian)	187	II-IV	RECIST
Ma	2017	China (Asian)	59	IIIB-IV	ECOG
Tamura	2012	Japan (Asian)	83	I-IV	CTCAE

2.17.2 Association of ABC gene (ABCB1, ABCC1/C2, and ABCG2) polymorphism with clinical outcomes and response towards chemotherapy in lung cancer patients

Few reports have claimed that in $C^{3435}T$ and $C^{1236}T$, the *T* -allele (Caronia *et al.*, 2011) and homozygous *C* allele (Schaich *et al.*, 2009) is significantly correlated with improved outcomes after chemotherapy. *ABCB1* $C^{1236}T$ polymorphism was correlated with the chemotherapy response in the carcinoma patients, and the patients with heterozygous and mutant genotypes showed a better response to the chemotherapy compared to individuals with *CC* genotype (Zhou *et al.*, 2015). A study in the past assessed the predictors of benefit from the second-line treatment benefit in the NSCLC phase III report, where the efficacy of docetaxel and pemetrexed was compared. Results showed better survival in the women in stage IIIB having good performance status ≤ 1 and

having an objective response to the first-line treatment (Hanna *et al.*, 2004). A meta-analysis study in 419 patients having $C^{3435}T$ polymorphism and undergoing platinum-based chemotherapy conducted by Wei *et al.* showed a substantial correlation between *CC* genotype of $C^{3435}T$ polymorphism and objective response to platinum in the overall pool analysis in the Asians (OR: 2.63; 95% CI: 1.56-4.45), however, no significant correlation was reported in terms of response in the Caucasians (OR: 1.61, 95% CI: 0.79-3.28). (Wei *et al.*, 2011). Another meta-analysis study was conducted by Yin *et al.* to assess the response to chemotherapeutic agents in advanced NSCLC. The study evaluated 379 patients with $C^{3435}T$ polymorphism and patients having $G^{2677}T/A$ variation. Overall results showed that $C^{3435}T$ carriers showed a better response to chemotherapy in the case of Asians (OR: 1.97, 95% CI:1.11-3.5), and also the wild type genotype of $G^{2677}T/A$ showed higher drug response in the Asians (OR: 2.6, 95% CI: 1.44-4.74) (Yin *et al.*, 2012). Additionally, Pan *et al.* evaluated the $C^{3435}T$ and $G^{2677}T/A$ polymorphism to predict the response to cisplatin-vinorelbine-based chemotherapy in NSCLC patients. The results depicted a substantially higher response rate in the homozygous wild allele *(C/C)* of $C^{3435}T$ polymorphism compared to the heterozygous and mutant genotype (OR= 1.76, p=0.03). In the case of $G^{2677}T/A$ polymorphism, a better response rate of approximately 61% was noted in the homozygous wild alleles *(G/G)* as compared to the other genotype (2.0, p=**0.012**) (Pan *et al.*, 2008). Furthermore, correlation of *MDR1* polymorphism with response to the cisplatin-based chemotherapy in the NSCLC patients was evaluated by Chen and colleagues. The study initially stratified the patients on the basis of baseline characteristics to assess the response rate, however, the chemotherapeutic regimen and clinical parameters showed lack of association in the response rate as per any factor. The response rate in the individuals carrying ^{2677}T allele ($^{2677}T/G$, $^{2677}T/A$, $^{2677}T/T$) was lower as compared to the other allele bearers (OR=1.84, p=0.04).

A report by Sohn *et al.* studied the effect of *ABCB1* $C^{3435}T$ and $G^{2677}T/A$ polymorphism in predicting the response to cisplatin-based chemotherapy in SCLC patients. The study recruited 54 Korean patients, and the authors reported a poor response rate to cisplatin-based chemotherapy in the heterozygous genotype of the $C^{3435}T$ and $G^{2677}A/T$ allele (Sohn *et al.*, 2006). Another study on SCLC patients undergoing cisplatin-based irinotecan chemotherapy reported no correlation between response rate or overall survival and the *ABCB1* polymorphisms (Lara Jr *et al.*, 2009). On the contrary, *et al.* proposed an association between ABCB1 variations and clinical treatment outcomes in the SCLC patients with $C^{3435}T$ and $G^{2677}A/T$ polymorphism (Knez *et al.*, 2012).

In the case of *ABCC1* $G^{2168}A$ polymorphism, the data have shown that this mutation changes the functionality of the ABCC1 transporter (Słomka *et al.*, 2015). Also, the studies have reported an association between $G^{2168}A$ mutation and chemotherapy response in ovarian cancer patients (Obata *et al.*, 2006). However, Yin *et al.* reported no association between patients with this SNP and chemotherapeutic response in lung cancer patients (Yin *et al.*, 2011). Studies have shown that the allelic frequency of $G^{2168}A$ is higher in the mainland Chinese population than in other SNPs, diminishing ABCC1 activity in MDR (Yin *et al.*, 2009). No significant association was observed in ovarian cancer between protein expression and the response to chemotherapy or disease-free survival time for $G^{2168}A$ polymorphism (Obata *et al.*, 2006). It was also reported that $G^{3173}A$ polymorphism is deleterious using Sorting Intolerant From Tolerant (SIFT) analysis but does not affect the ABCC1 function (Jiye & Jianting, 2011). In a few studies, $G^{3173}A$ polymorphism has been reported to increase response to drug etoposide (Conrad *et al.*, 2002; Yin *et al.*, 2009).

For *ABCG2* $C^{421}A$ polymorphism, Qiao *et al.* found that *ABCG2* ^{421} *A/A* was associated with a higher response rate toward paclitaxel platinum-based chemotherapy (Qiao *et al.*, 2016).

2.17.3 Association of ABC gene polymorphism and toxicity

A study by Han *et al.* investigated the impact of *ABCB1, ABCC2,* and *ABCG2* polymorphism in advanced NSCLC patients undergoing a combination of platinum and irinotecan regimens. The outcomes suggested that polymorphism $G^{2677}T/A$ was correlated with severe grade neutropenia. Also, during the first cycle, the ^{2677}GG carriers depicted substantially low neutrophil count (p=0.012) and the same was found during the entire chemotherapy (p=0.042). Additionally, ^{3435}TT was strongly correlated with grade 3 diarrhea (p=0.047) (Han *et al.*, 2007). Another study by Isla *et al.* reported the significant association of the ^{3435}TT genotype with grade ≥2 diarrhea (p=0.03). This study also evaluated the other haematological, renal, hepatic, and gastrointestinal toxicity, but no correlation was found in $C^{3435}T$ polymorphism (Isla *et al.*, 2004). Han *et al.* evaluated the impact of $C^{3435}T$ and $G^{2677}T/A$ polymorphism on toxicity caused by cisplatin-vinorelbine in NSCLC patients. This study did not find any substantial relationship between $C^{3435}T$ and $G^{2677}T/A$ polymorphism and adverse events caused by the drugs (Pan *et al.*, 2008). A study by Chen *et al.* evaluates the role of $C^{3435}T$ and $G^{2677}T/A$ polymorphism with toxicity to platinum-based chemotherapy in NSCLC patients. The study showed no association between the *ABCB1*

polymorphism and the risk of gastrointestinal, haematological, nephrotoxicity, or hepatotoxicity. Though, a gastrointestinal toxicity grade≥1 was observed in the individuals harboring heterozygous genotype with a substantially elevated risk (p=0.03) (Chen *et al.*, 2010). A recent study by Rierra *et al.* showed a significant association between $C^{1236}T$ polymorphism with grade 3-4 gastrointestinal toxicity in patients undergoing irinotecan-based chemotherapy. The patients with $C^{1236}T$ polymorphism and wild-type genotype had a high incidence of grade 3-4 mucositis (p=0.002) and diarrhea (p=0.014) compared to the other genotypes. Other studies also reported the association of *ABCB1* polymorphism with toxicity in patients undergoing irinotecan therapy. The study showed a substantial correlation of *ABCB1* variants with the overall toxicity (p<0.01) and also with higher haematological toxicity (Salvador-Martín *et al.*, 2018). Cortejoso *et al.*, concluded that patients with *CC* genotype in the $C^{3435}T$ variant had a higher probability of diarrhoea than patients with *TT* and *CT* genotypes (Cortejoso *et al.* 2013). In addition, patients with a homozygous $C^{3435}T$ polymorphism in the *ABCB1* gene are four-fold more likely to develop grade 3 diarrhea's when treated with a combination of cisplatin and irinotecan (Sekine *et al.*, 2008). A meta-analysis by Zair *et al.* concluded that $G^{2677}A$ is associated with decreased risk of irinotecan-induced neutropenia (Zair & Singer, 2016a). On the other hand, it is seen that ^{2677}TT is associated with higher efflux activity as well as with grade 4 neutropenia, while ^{3435}TT variants are associated with higher efflux activity only when compared with the $^{2677}GG/^{3435}CC$ genotype of *ABCB1* gene (Han *et al.*, 2007). For G^{2677} *T/A*, it is associated with risk of toxicity and reduced survival when in haplotype combination with ^{1236} and ^{3435}. At the same time, $C^{3435}T$ is associated with increased toxicity, risk of toxicity and reduced survival when in haplotypic combination with ^{1236} and ^{2677} (Marsh & Hoskins, 2010). Analysis with *ABCB1* SNPs revealed a lack of significant associations with docetaxel related adverse events including hematological, neurological and hypersensitivity (Rizzo *et al.*, 2010). Additionally, Choi *et al.*, studied the role of ABC transporter polymorphisms in predicting response and adverse events in patients undergoing docetaxel-based therapy. The results depicted that the variant genotype of $C^{3435}T$ was substantially correlated with increased risk of anemia and neutropenia (p=0.044 and 0.029, respectively). Also, they reported strong correlation between $^{2677}G/T$ polymorphism and docetaxel-induced leukopenia in the patients (p=0.025) (Choi *et al.*, 2015).

Studies have shown that the *ABCC2* $G^{4544}A$ mutation has impaired ATPase activity, resulting in a higher accumulation of drugs in the cell (Wen *et al.*, 2017). However, the reports

for *ABCC2* gene polymorphism concerning lung cancer are very trivial, as the *ABCC2* gene has been extensively studied in the context of Dubin Johnson Syndrome. However, few of the literature available has been cited below. Sprowl *et al.* investigated the adverse events of cisplatin drug and *ABCC2* $G^{4544}A$ polymorphism in solid tumour patients and concluded no substantial correlation between $G^{4544}A$ polymorphism and platinum in causing nephrotoxicity in 112 patients who underwent cisplatin therapy (Sprowl *et al.*, 2012). A study by Laure *et al.* showed that mutation $G^{4544}A$ in the *ABCC2* gene leads to the impairment of the ATPase activity and is correlated with the increased cellular accumulation of the substrates of ABCC2, which might lead to cellular toxicity (Elens *et al.*, 2011).

Many studies have been reported regarding the association of *ABCG2* $C^{421}A$ polymorphism and toxicity due to anti-cancerous drugs. In one such study, Akasaka *et al.* assessed 75 patients having NSCLC treated with gefitinib for severe toxicity effects due to the treatment; however, no substantial correlation was found between $C^{421}A$ polymorphism and adverse effects (Akasaka *et al.*, 2010). Another study by Cusatis *et al.* pointed out that $C^{421}A$ variation is responsible for the reduced activity of ABCG2 and depicted the role of this variation in the elevated risk of developing diarrhea in NSCLC patients (Cusatis *et al.*, 2006). Another meta-analysis study recently published depicted whether $C^{421}A$ variation is associated with the risk of chemotherapy (gefitinib)-induced toxicity in NSCLC patients. The outcomes did not correlate with $C^{421}A$ polymorphism and gefitinib-induced toxicity in lung cancer patients (Tang *et al.*, 2018). A study on the Asian population (Indian, Malaysia and Chinese) concluded a non-significant influence of $C^{421}A$ polymorphism on SN-38 and the severity of neutropenia (Jada *et al.*, 2007). Additionally, Cha *et al.* reported that genetic variation of *ABCG2* may play a role in the alteration of transporter activity for the chemotherapeutic drug and enhance the systemic circulation of the drugs, which leads to myelosuppression (Cha *et al.*, 2009).

Table 2.7: Highlighting critical studies on various *ABCB1, ABCC1/C2,* and *ABCG2* polymorphisms in lung cancer patients

Gene	Polymorphism	Country or Ethnicity	Drugs	Parameters	References
ABCB1	*$^{1236}C > T$, $^{2677}G > T/A$, $^{3435}C > T$*	Korea	Irinotecan	Toxicity, tumour response, survival	(J. Han *et al.*, 2007)
	$C^{3435}T$	Spain	Docetaxel	Toxicity	(Isla *et al.*, 2004)
	$C^{1236}T$, $G^{2677}G/T$, and $C^{3435}T$	Korea	Docetaxel	Toxicity, the response rate	(Choi *et al.*, 2015)
	$C^{1236}T$, $G^{2677}G/T$, and $C^{3435}T$	Korea	Irinotecan	Toxicity	(J.-Y. Han, Lim, Park, Lee, & Lee, 2009)
	$C^{1236}T$, $G^{2677}G/T$, and $C^{3435}T$	Japanese	Gefitinib	Toxicity	(H. Kobayashi *et al.*, 2015)
	$C^{1236}T$ (Meta-analysis)	-	-	Response rate	(Z. Zhou *et al.*, 2015)
	$C^{3435}T$, $C^{1236}T$, $G^{2677}A/T$	84 White, 1 African–American, 2 American Indian/Alaska Native, 3 Unknown	Cisplatin-based regimens	Toxicity	(Lamba *et al.*, 2014)
	$C^{3435}T$, $G^{2677}A/T$	Han Chinese	Cisplatin +Vinorelbine	Response rate, Leukopenia, Nausea and vomiting	(Pan *et al.*, 2008)
	$C^{3435}T$, $G^{2677}A/T$	China	Cisplatin based regimens (docetaxel/paclitaxel/gemcitab	Response rate, Toxicity	(S. Chen *et al.*, 2010)

			ine/vinorelbine)		
	$C^{1236}T$, $C^{3435}T$ *(Meta-analysis)*	-	Cisplatin-based taxane therapy	Overall Survival, Response rate	(Q. Chen *et al.*, 2020)
	$C^{3435}T$	Spain	Cisplatin-based regimen	OS, response, rate, toxicity	(Vinolas *et al.*, 2011)
	$C^{3435}T$	China	Cisplatin-based regimen	OS, response, rate	(Yan, Huang, Yan, Xu, & Jiang, 2011)
	$G^{2677}T/A$, $C^{3435}T$ (SCLC patients)	Caucasian	cisplatin–etoposide or cyclophosphamide–epirubicin–vincristine chemotherapy.	OS, response rate	(Knez *et al.*, 2012)
	$C^{3435}T$	Turkey	Cisplatin + paclitaxel/docetaxel/gemcitabine/vinorelbine	OS, response rate	(Dogu *et al.*, 2012)
	$G^{2677}T/A$, $C^{3435}T$ (SCLC patients)	North America	Cisplatin-based regimen	OS, response rate	(Lara Jr *et al.*, 2009)
	$G^{2677}T/A$, $C^{3435}T$ (SCLC patients)	Korea	Cisplatin-based regimen	Response rate	(Sohn *et al.*, 2006)
ABCC1	*$G^{2168}A$*	Chinese	Platinum-based chemotherapy	Response rate	(J. Yin *et al.*, 2011)
ABCC2	*$G^{4544}A$*	Caucasian	Cisplatin-based regimen	Toxicity, overall survival	(Sprowl *et al.*, 2012)

		Canada	Cisplatin-based regimen	Overall survival	(Cuffe *et al.*, 2016)
		Austria	Cisplatin-based regimen	Overall survival	(Filipits *et al.*, 2007)
ABCG2	$C^{421}A$	Japanese	Gefitinib	Toxicity	(Akasaka *et al.*, 2010)
		Caucasian	Gefitinib	Toxicity	(Cusatis *et al.*, 2006)
		Caucasian	Cisplatin based regimen	Overall Survival	(Müller *et al.*, 2009)
		China	Gefitinib, erlotinib and icotinib	Overall Survival	(X. Chen *et al.*, 2015)
		-	Cisplatin-based regimen	Response rate	(Qiao *et al.*, 2016)
		Korea	Irinotecan	toxicity, tumour response, survival	(J. Han *et al.*, 2007)

2.18 Solute Carrier Transporters

The transmembrane transporters, characterized as solute carrier (SLC) proteins, mediate the solute inflow of small molecules across plasma and intracellular membranes (Hediger *et al.*, 2004). Nevertheless, specific SLC transporters act as influx or a bidirectional transporter. SLC transporters are essential for several physiological functions, such as nutrition intake and disposal, neurotransmission, acid-base homeostasis regulation, and metabolic waste excretion. They also participate in the disposal of numerous therapeutically relevant drugs, including antidiuretics and chemotherapeutics. During the first mini-review on SLCs, 298 genes were categorized into 43 SLC families (Hediger *et al.*, 2004). The SLC superfamily currently consists of more than 60 families and 400 genes, while new transporter genes are continuously being found and incorporated into the SLC superfamily (Schumann *et al.*, 2020). The SLC transporter superfamily is categorized based on biological function and sequence similarity. Transporters from the SLC family are not directly reliant on the hydrolysis of ATP and are often responsible for the majority of substrate uptake. Instead, these proteins function as secondary active or passive facilitative transporters **(Figure 2.23)**. They act as exchangers, facilitate passive diffusion of particular small molecules, or use ion gradients to induce flow against gradients (Schaller & Lauschke, 2019). **Table 2.8** summarizes a list of all SLC genes that are currently known.

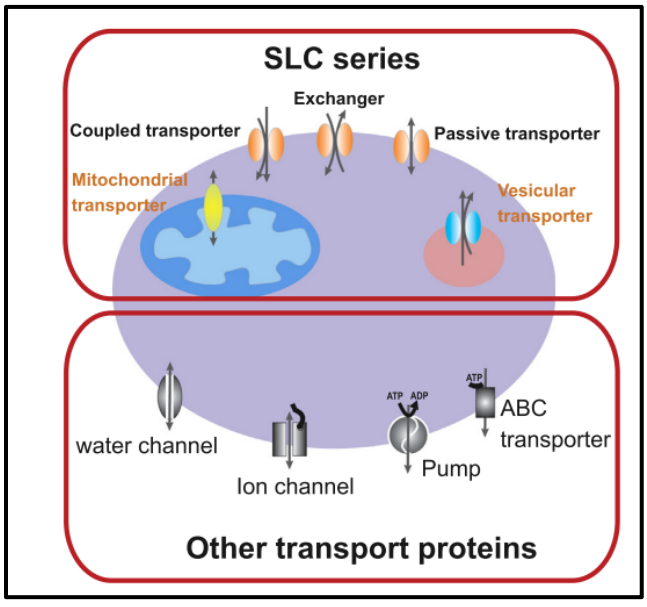


Figure 2.23: Diagrammatic Illustration showing SLC and non-SLC transporters in the plasma membrane or within intracellular compartments (Hediger *et al.*, 2013)

Table 2.8: A summary of all SLC genes that are currently known (Schumann *et al.*, 2020)

SLC name	Family	Members	SLC name	Family	Members
SLC1	High-affinity glutamate and neutral amino acid transporter family	7	**SLC33**	Acetyl-CoA transporter family	2
SLC2	Facilitative GLUT transporter family	18	**SLC34**	Type II Na^+-phosphate cotransporter family	3
SLC3	Heavy subunits of the heteromeric amino acid transporters	2	**SLC35**	Nucleoside-sugar transporter family	31
SLC4	Bicarbonate transporter family	11	**SLC36**	Proton-coupled amino acid transporter family	4
SLC5	Sodium-glucose cotransporter family	12	**SLC37**	Sugar-phosphate/phosphate exchanger family	4
SLC6	Sodium- and chloride-dependent neurotransmitter transporter family	22	**SLC38**	System A and System N sodium-coupled neutral amino acid transporter family	11
SLC7	Cationic amino acid transporter/glycoprotein-associated family	15	**SLC39**	Metal ion transporter family	14
SLC8	Na^+/Ca^{2+} exchanger family	4	**SLC40**	Basolateral iron transporter family	1
SLC9	Na^+/H^+ exchanger family	18	**SLC41**	MgtE-like magnesium transporter family	3
SLC10	Sodium bile salt cotransport family	7	**SLC42**	Rh ammonium transporter family	3
SLC11	Proton-coupled metal ion transporter family	2	**SLC43**	Na+-independent, system-L-like amino acid transporter family	3
SLC12	Electroneutral cation-coupled Cl cotransporter family	9	**SLC44**	Choline-like transporter family	5
SLC13	Human Na^+-sulfate/carboxylate cotransporter family	5	**SLC45**	H+/sugar cotransporter family	4
SLC14	Urea transporter family	8	**SLC46**	Folate transporter family	3
SLC15	Proton oligopeptide cotransporter family	4	**SLC47**	Multidrug and Toxin Extrusion (MATE) family	2
SLC16	Monocarboxylate transporter family	14	**SLC48**	Heme transporter family	1
SLC17	Vesicular glutamate transporter family	9	**SLC49**	FLVCR-related transporter family	4
SLC18	Vesicular amine transporter family	4	**SLC50**	Sugar efflux transporters	1
SLC19	Folate/thiamine transporter family	3	**SLC51**	Transporters of steroid-derived molecules	2
SLC20	Type III Na^+-phosphate cotransporter family	2	**SLC52**	Riboflavin transporter family	3
SLC21	Organic anion transporter family	11	**SLC53**	Phosphate carriers	1
SLC22	Organic cation/anion/zwitterion transporter family	29	**SLC54**	Mitochondrial pyruvate carriers	3

SLC23	Na^+-dependent ascorbic acid transporter family	4	**SLC55**	Mitochondrial cation/proton exchangers	3
SLC24	$Na^+/(Ca^{2+}\text{-}K^+)$ exchanger family	5	**SLC56**	Sideroflexins	5
SLC25	Mitochondrial carrier family	60	**SLC57**	NiPA-like magnesium transporter family	6
SLC26	Multifunctional anion exchanger family	11	**SLC58**	MagT-like magnesium transporter family	2
SLC27	Fatty acid transporter family	6	**SLC59**	Sodium-dependent lysophosphatidylcholine symporter family	2
SLC28	Na^+-coupled nucleoside transport family	3	**SLC60**	Glucose transporters	2
SLC29	Facilitative nucleoside transporter family	4	**SLC61**	Molybdate transporter family	1
SLC30	Zinc efflux family	10	**SLC62**	Pyrophosphate transporters	1
SLC31	Copper transporter family	3	**SLC63**	Sphingosine-phosphate transporters	3
SLC32	Vesicular inhibitory amino acid transporter family	1	**SLC64**	Golgi Ca^{2+}/H^+ exchangers	1
			SLC65	NPC-type cholesterol transporters	2

2.19 Location, expression, and functional role of SLC transporters

2.19.1 Solute carrier family 19, member 1

Human solute carrier family 19, member one annotated as *SLC19A1* encodes a transporter protein, reduced folate carrier 1 demarcated as RFC1. *SLC19A1* is a bi-directional, high-capacity transporter of thiamine monophosphate and 5-methyl-tetrahydrofolate. Additionally, the *SLC19A1* role extends to the influx of various chemotherapeutic agents into the cells. Also, *RFC1* is the primary carrier of systemic folate cofactors across membranes (Yee *et al.*, 2010).

2.19.2 Genomic organization

SLC19A1 is localized on chromosome 21q22.3, and **figure 2.24** depicts its location on chromosome 21. The human SLC19A1 protein, which contains 591 amino acids, has been cloned by several research groups from the placenta, small intestine, and lymphoblast cDNA libraries. The cDNAs are approximately 2.7 kb in length. *SLC19A1* gene comprises five exons, from exon 2 to exon 6, which encodes the reduced folate carrier (RFC1) protein (Hou & Matherly, 2014). The mRNA transcript for RFC1 is produced using at least four 5' alternative exons. These are spliced exclusively to exon 2, which comprises the translational point for RFC1 protein. The results of semi-quantitative PCR demonstrate that exon one is typically integrated into the transcript (Yee *et al.*, 2010). According to one of the studies, RFC1 shows alternative splicing (Whetstine *et al.*, 2002). In particular, three RFC1 splice variants were found in a human liver genome library due to the incorporation of distinct 3' sequences and three alternatives of exon 1. The *SLC19A1* gene's region upstream of the transcriptional initiation site underwent functional deletion analysis, which resulted in the discovery of two TATA-less promoters with distinctly different transcriptional effectiveness (Matherly *et al.*, 2007).

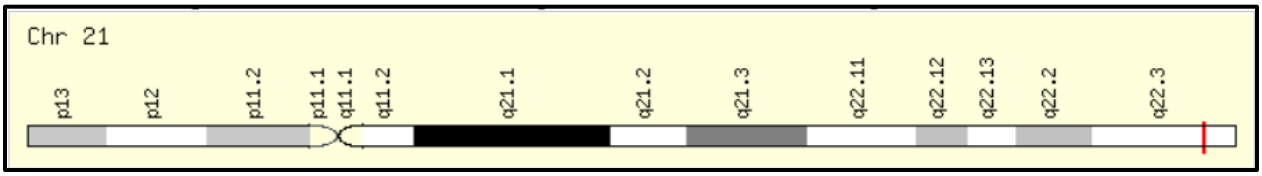


Figure 2.24: Localization of *SLC19A1* on chromosome 21(https://www.genecards.org/)

2.19.3 Structure of RFC *protein*

SLC19A1 gene encoded RFC protein, which 12 TMDs characterize with N- and C-termini oriented in the cytoplasm. Human RFC constitutes 591 amino acids, while N-glycosylated at site Asn58 in the loop linking TMD1 and TMD2 domain. RFCs from different species are conserved (between rats and humans is 64–66%), with the TMDs showing the most conservation and the lowest homologies occurring in the loop domain joining TMD6 and TMD7, as well as in the N- and C-termini (Matherly *et al.*, 2007;Matherly & Hou, 2008). An illustration of SLC19A1/Reduced folate carrier-1 is depicted in the **figure 2.25).** Researchers have looked for domains and residues that lead to the binding and translocation of anti-folate substrates in RFC structure-function investigations and TMD helix packing correlations that aid folate substrate interaction and movement. Due to the large deletions in the RFC in the TMD region abolishes surface targeting. In RFC, the linker domain is connected by TMD6 and 7, which is not well conserved except for the segment Lys204-Arg214(Liu *et al.*, 2003).

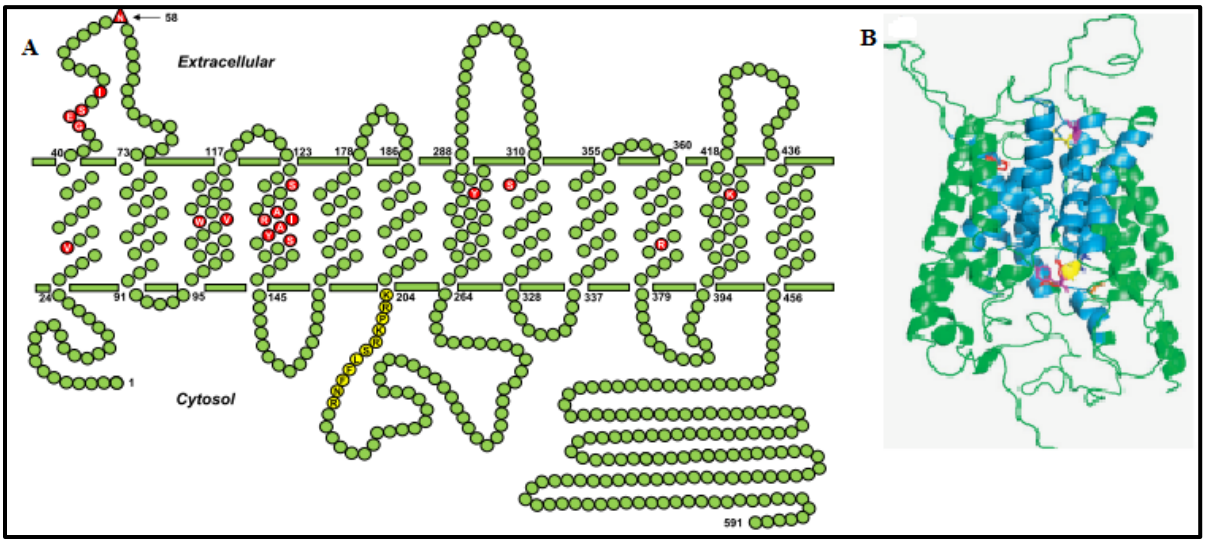


Figure 2.25: Human RFC model; (A) A topological representation of human RFC protein consisting of 12 transmembrane domains, linker domain (joining TMD 6 and 7, and internal N- & C- termini. Yellow circles represent a conserved stretch of the amino acids necessary for translocation activity. Red circles represent functionally significant amino acids. (B) A side-view of a 3-dimensional homology model of the human RFC (Hou & Matherly, 2014)

The transportation activity of human RFC was abrogated by removing certain portions of the TMD6 and TMD7 linker (amino acids from positions 215-263 and 204-263). Research suggests

that none of these the N- or C-termini or TMD6 and TMD7 linkers is necessary for the binding and transporting the folate substrate. The TMD6-TMD7 loop domain's primary function is to ensure proper gaps between the TMD1-6 & TMD7-12 segments for the optimum carrier function (Hou & Matherly, 2014).

2.19.4 Expression of SLC19A1 gene

SLC transporters are widely distributed in the human body. The expression profiles in human RFC in tissues suggest its involvement in several transport processes, notably in intestinal absorption via luminal epithelial cells. RFC1 is also associated with the transport of folates through the retinal epithelium. Studies have shown the role of human RFC in the translocation of substances across the basolateral membrane of the renal proximal tubular cells (Ganapathy *et al.*, 2004). Significant amounts of RFC were also found in the liver, kidney, leukocytes, bone marrow, lung, and the parts of the intestine (*i.e.,* maximum in the duodenum region), and also in the brain and certain parts of the central nervous system (Matherly *et al.*, 2014).

In contrast, the skeletal muscle and heart had low but detectable levels of RFC. A report in the past has shown differential expression of RFC in the fetal tissues, with the lowest levels in the heart and highest in the fetal lung (Whetstine *et al.*, 2002). *SLC19A1* has been detected in endocrine organs such as adipose tissues (Petrus *et al.*, 2018). A shred of evidence also suggests that distinct immune cell types express unique SLC transporters and that these transporters may influence physiological processes related to growth, homeostasis, & activation/differentiation (Song *et al.*, 2020). A deficiency of folate and an excess of folate seem to induce and suppress RFC expression.

2.19.5 Gene regulation of SLC19A1 gene

The TATA-less regulates the human SLC19A1 gene, two functional GC-rich promoters that govern the transcription of the two upstream non-coding exons (A and B), which are known for their numerous splice variants and alternative transcription start sites (Yee *et al.*, 2010). Previous reports have revealed essential transcription factors that play a vital role in controlling the expression of *SLC19A1* from the minimal *RFC-A* and *-B* promoters, containing leucine zipper and DNA-binding proteins (promoter A) and stimulating protein (SP) family of proteins (promoter B)

(Zhao *et al.,* 2009). *SLC19A1* transcription appears to be mediated by at least six different promoters, producing 15 different 5'-UTRs with the same human RFC open reading frame. Both tissue-specific controls these promoters (*e.g.,* CCAAT enhancer-binding proteins, Activator protein 1) nuclear factors and ubiquitous (upstream stimulatory factor, SP) resulting in varying amounts of expression in various tissues and cell types. The pattern of promoter and exon activity in various cells and tissues is likely the outcome of numerous transcription elements and epigenetic changes that can respond to particular stimuli and assure sufficient RFC levels for cell growth and tissue repair (Whetstine *et al.,* 2002). This could factor in the increase of *SLC19A1* expression and transportation in several cell lines cultured in folate-restrictive conditions, which could mimic molecular changes occurring in particular tissues. Studies in the past also claim that chromatin structure and methylation might affect the functionality of the human RFC promoters. *SLC19A1* promoter B is responsible for regulating the transcription of variant I, the most common human RFC splice variant found in the human intestine (Said & Nexo, 2012).

2.19.6 Substrate specificity of SLC19A1

The cellular inflow of folate and its byproducts is mediated by SLC19A1 (Al-Abdulla *et al*., 2019). RFC transports a variety of substrates in a uniport, symport, or antiport manner, including amino acids, carbohydrates, neurotransmitters, nucleosides, organic phosphate, and vitamins (Chang *et al.*, 2004). The transporter prefers reduced folates over non-reduced folates. Compared to folate, the derivatives of tetrahydrofolate, mainly N^5-formyltetrahydrofolate, N^5-methyltetrahydrofolate , and methotrexate, have a substantially higher potential for transport across this transporter. It is interesting to note that although SLC19A1 does not recognize thiamine as a substrate, it may translocate thiamine's phosphate esters to a specific limit (Zhao *et al*., 2001). There is strong evidence to support the idea that a transmembrane pH gradient ($pH_{out}<pH_{in}$) stimulates the SLC19A1 (Rajgopal & Goldman, 2001). This implies that the translocation mechanism entails the influx of anionic substrates coupled to either the antiport of OH- out from the cells or symport of H+ in the cells. However, research on native tissue preparations has revealed that SLC19A1's pH gradient-dependent transport function is electroneutral. This shows that this transporter prefers the monovalent anionic forms of the folate as well as its derivatives (Ganapathy *et al.*, 2004). At physiological pH, all of the SLC19A1 substrates occur mainly as anions. Antifolates such as pemetrexed, methotrexate, pralatrexate, and raltitrexed are translocated by SLC19A1. Hence, the

loss of *SLC19A1* expression in tumour cells is associated with the poor tumour response to the antifolates (Hou & Matherly, 2014).

2.20 Solute Carrier Organic Anion Transporter family members 1B1 and 1B3

The solute carrier organic anion (SLCO) family is one of the subfamilies of the solute carrier (SLC) superfamily that includes pharmacologically significant uptake transporters. Members of the SLCO subfamily are also referred to as organic anion transporting polypeptides (OATP). OATPs translocate a broad spectrum of amphipathic substrates across the cell membrane. Certain significant representatives of this transporter superfamily are expressed only in the livers of rodents and humans, where they play a role in the hepatic elimination of albumin-bound substances from peripheral blood plasma. Most OATPs are expressed in tissues such as the lung, intestine, heart, blood-brain barrier, placenta, kidney, and testis.

Only a small percentage of the OATPs discovered so far have undergone thorough structural, functional, and genomic characterization. According to the sequence similarity, 11 OATP transporters are organized in six families and ten subfamilies in humans. SLCOs are anticipated to have 12 TMDs that function in a sodium-independent way, thus regulating the cellular uptake of various exogenous and endogenous substances, notably clinically effective drugs. Several clinically effective drugs are transported via the hepatic transporters SLCO1B1/OATP1B1 and SLCO1B3/OATP1B3. The expression of *SLCO1B1* has been well-reported in the basolateral membrane of the hepatic cells, whereas expression of *SLCO1B3* is reported in the hepatocytes closer to the central vein of the liver (Svoboda *et al.*, 2011). Increasing literature points out that the translocate function of SLCO1B1 and SLCO1B3 can be mediated at many levels, such as genetic mutations, transcriptional regulation, and post-transcriptional regulation.

2.20.1 Genomic Organization

The *SLCO1B1* gene encodes organic anion transport polypeptide 1B1 (OATP1B1), a transmembrane protein that translocate various anionic drugs. The *SLCO1B1* gene is positioned on chromosome 12 (locus 12912.2) **(Figure 2.26 (A).** The entire length of the gene is 10.85kb. The *SLCO1B1* gene comprises 2,076 bases that encode 691 amino acids. This gene also contains 15 exons and introns (Zhang *et al.,* 2015). Another crucial member of the SLCO family is the *SLCO1B3* gene that encodes for organic anion transport polypeptide 1B3 (OATP1B3) (König *et al.,* 2000). Like *SLCO1B1* gene, *SLCO1B3* gene is also localized on chromosome 12 (gene locus 12p12-31.7 to 12p12-37.2). The encoding protein contains 702 amino acids. Six transcripts for the *SLCO1B3* gene, two of which, SLCO1B3-001 & SLCO1B3-201, possess a consensus coding sequence. The SLCO1B3-001 contains 16 exons, while SLCO1B3-201 consists of 15 exons.

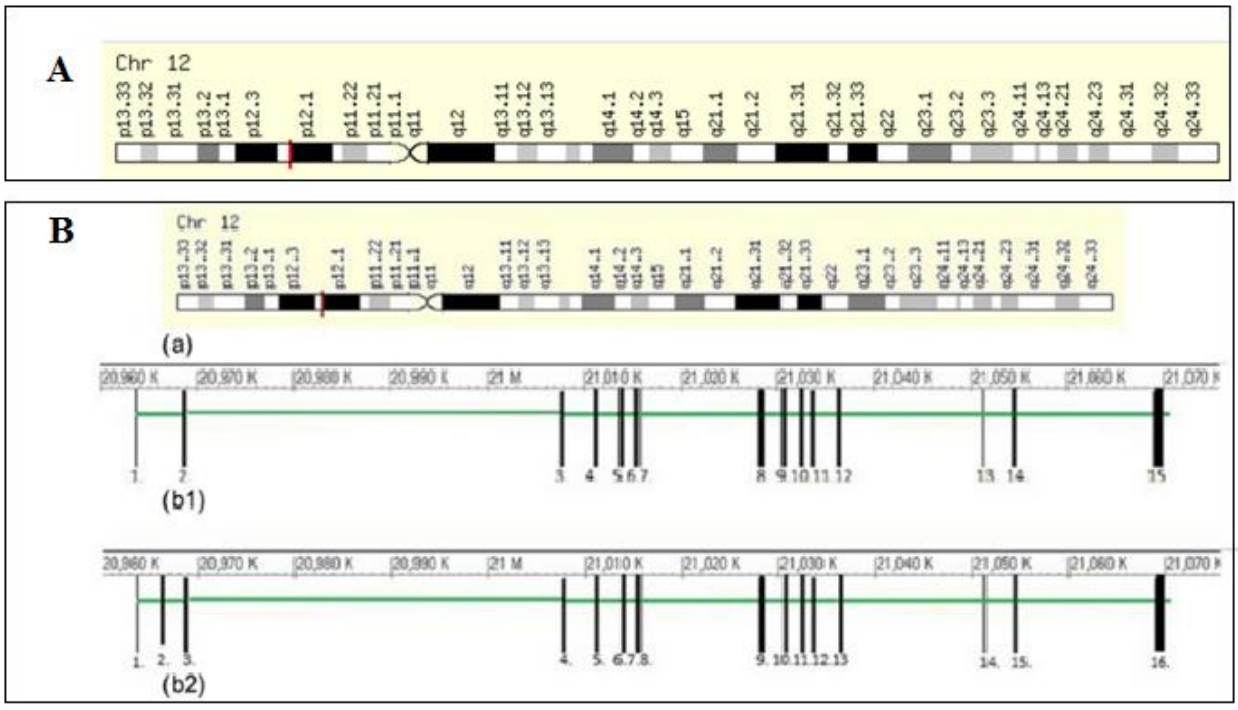


Figure 2.26: Localization of (A) SLCO1B1 on chromosome 12 (https://www.genecards.org/) (B) SLCO1B3 on chromosome 12 (Evangeli *et al.*, 2017)

The first and second exons of a 001 transcript are the primary distinction between the two transcripts **(Figure 2.26 (B)** (Evangeli *et al.,* 2017)**.** They have 12 transmembrane regions along with N- and C- termini localized on the cytoplasmic side. Reports have also shown that amino

acids in the TMD region 2,8,6, 9, 10, and the extracellular loop are crucial for the transport functioning of both the OATP substrates (Hagenbuch & Meier, 2003).

2.20.2 Structure of OATP1B1 and OATP1B3

All OATPs possess 12 transmembrane domains per hydropathy analysis (Yue *et al.*, 2019). A common structural characteristic shared by all OATPs comprises (1) the long extracellular domain spanning from transmembrane domains 9 and 10 (known as extracellular loop 5), which contains various conserved cysteine residues which mimic the zinc finger regions of the DNA binding proteins (2) N-glycosylation regions are located in the extracellular loop two and extracellular loop 5 (3) the "superfamily signature" of OATP at the junction of transmembrane domain six and extracellular loop 3 (Huber, 2005). Predominantly, conserved amino acids are located in transmembrane domains 2 – 6, intracellular loops 1, 2, & 4, and extracellular loops 1, 3, and 5 **(Figure 2.27)**. Most conserved charged amino acid residues are found along the borders of the transmembrane domains, particularly on the cytoplasmic side of the membrane. The charged amino acids conserved within the membrane are present in transmembrane domains 2, 11, & 12 (Hagenbuch & Meier, 2003).

TMDs have been postulated to play a crucial role in the effective functioning of other transporters, including organic anion transporters, and are fundamental structural components of membrane proteins (Hong *et al.*, 2010). It has been demonstrated that OATP1B1's transmembrane 8 and 9 are essential for recognizing its substrate. Additionally, several significant amino acids were found in transmembrane domain 10 of OATP1B1(Gui & Hagenbuch, 2009). According to previous studies, OATP1B1's transmembrane domain 2 contains four amino acids crucial for the uptake of prototype OATP1B1 substrate estrone-3-sulfate. Phe73 and Asp70 interact with the substrate, while Gly76 and Glu74 are crucial for ensuring the appropriate structure of the OATPs (Li *et al.*, 2013). The computer-based hydropathy assessment predicts that OATP1B1 and the other OATP members have a substantial extracellular loop 5 containing conserved cysteine amino acid residues and a homologous 12 transmembrane domain architecture (Hagenbuch & Meier, 2004). At the interface of extracellular loop 3 & transmembrane domain 6, an OATP family signature sequence is found, i.e., D-X-RW-(I, V)-GAWWX-G-(F, L)-L that is conserved in human, mouse, and rat OATPs/Oatps. However, it is still unknown what function this highly conserved domain serves in OATPs (Hong, 2017).

Additionally, OATP1B3 is a member of the OATP family with significant similarity to OATP1B1. The sequence similarity between OATP1B3 and OATP1B1 is 80% (König, 2011). Studies have put forth that the transmembrane domain 3,9,6 and 12 are buried in bilayer while transmembrane domains 1, 2, 4, 5 of N-terminal as well as helices 8, 7, 10, and 11 of C-terminal of OATPs face the pore which engages with the substrate (Meier-Abt *et al.*, 2006). The report suggests that transmembrane domain 6 of OATP1B1 is primarily embedded in the lipid bilayer, similar to that of OATP1B3. However, some of the extracellular parts appear to be involved in generating the substrate binding pore. The lysine residues at locations 361 and 399 of the OATP1B3 protein are well conserved in the human OATP family. Also, 361 residues are positioned in the projected pore-facing transmembrane helix 7, while 399 residues are localized in the fourth intracellular loop (Mandery *et al.*, 2011).

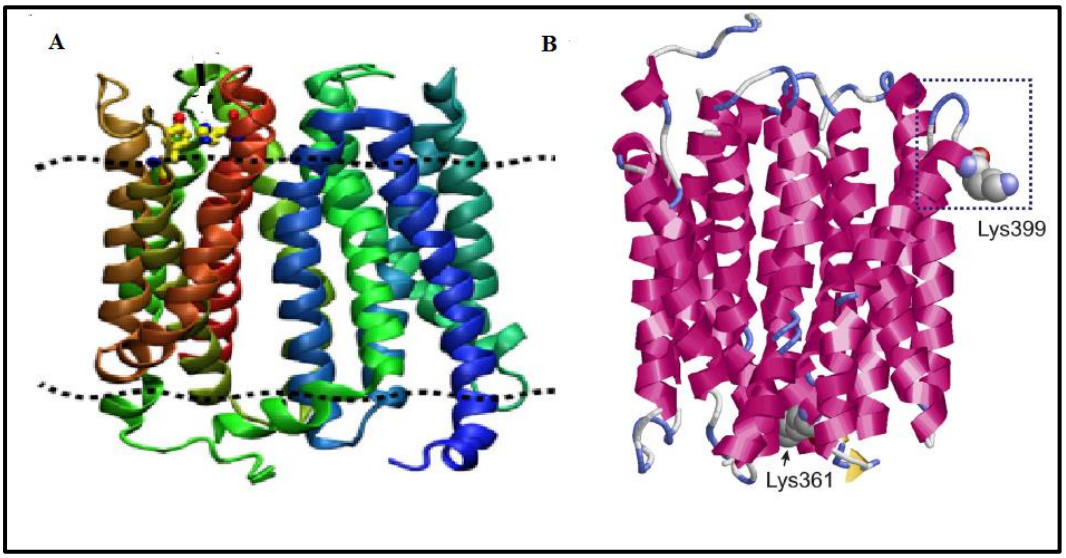


Figure 2.27: Structural modelling of (A) OATP1B1 protein and (B) OATP1B3 protein (Gruetz *et al.*, 2016;Mandery *et al.*, 2011)

2.20.3 Gene regulation of SLCO1B1 and SLCO1B3

Recently, researchers have looked at the expression and mRNA levels of OATP1B1 and OATP1B3. OATP1B1 and OATP1B3 expression levels varied with age in research by Thomson *et al.*, where OATP1B3 expression changed, ranging from being higher at birth to being lower in the infant years and then again increasing in the preadolescent years. OATP1B1 expression was generally lowered at younger ages (Thomson *et al.*, 2016). The expression of OATP1B1 and 1B3

is regulated at both translational and post-translational levels. At the transcriptional level, it has been demonstrated that several transcription factors interact with the promotor of the *SLCO1B1* and *SLCO1B3* genes. *SLCO1B1* appears explicitly expressed in the liver via the major hepatocyte nuclear factors HNF4α (Kamiyama *et al.*, 2007) and HNF1α (Jung *et al.*, 2001). Although, the interindividual expression levels of *SLCO1B1* may be regulated by the nuclear liver X receptor (LXR), nuclear farnesoid X receptor (FXR), and oxysterol (Schwabedissen *et al.*, 2010). HNF1α, FXR, and prolactin regulate the SLCO1B3 promoter- and growth factor-induced transcription factor STAT5. Also, the SLCO1B3 transcription may be inhibited by HNF-3β. *SLCO1B1* expression is decreased by FXR-dependent suppression of HNG-1 and elevated by the upregulation of FXR (Alam *et al.*, 2018). *SLCO1B1* expression can be reduced by microRNAs, notably miR-206, which function at the post-transcriptional level. Additionally, epigenetic regulation has been proposed to regulate the tissue-specific expression of *SLCO1B1* (Sandoval & Hagenbuch, 2021).

SLCO1B1 and SLCO1B3 are regulated at post-translational, transcriptional, and post-transcriptional levels. The transporter proteins in the plasma membrane can undergo various post-translational modifications and protein-protein interactions in response to different physiological stressors or stimuli, which can change the protein's functional activity. Three modifications at the post-translational level, such as phosphorylation, *N*-glycosylation, and ubiquitination, are generally associated with cellular processing and trafficking of SLCO1B1 and 1B3 (W. Lee, Ha, & Sugiyama, 2020). OATP1B1 and OATP1B3 are regulated by N-linked glycosylation at asparagine residues (Murray & Zhou, 2017). It has been predicted that OATP1B1 and OATP1B3 are phosphorylated utilizing human liver tissue phosphoproteomic studies (Bian *et al.*, 2014). According to the studies, enhanced phosphorylation of OATP1B3 was correlated with the downregulation of the OATP1B3 transporter function. Additionally, insufficient research has been done on how ubiquitination affects the OATP1B3 and 1B1 transport functions (Alam *et al.*, 2018).

2.20.4 Substrate specificity of SLCO1B1 and SLCO1B3

The transporter of the 1B family, i.e., SLCO1B1 and SLCO1B3, translocate similar substrates across the transporter, such as rifampicin, bosentan, olmesartan, valsartan, statins, and bromosulphophthalein (Alam *et al.*, 2018). Some anticancer drugs, such as docetaxel,

methotrexate, and irinotecan metabolite SN-38, are translocated by both OATP1B1 and OATP1B3 **(Figure 2.28).**

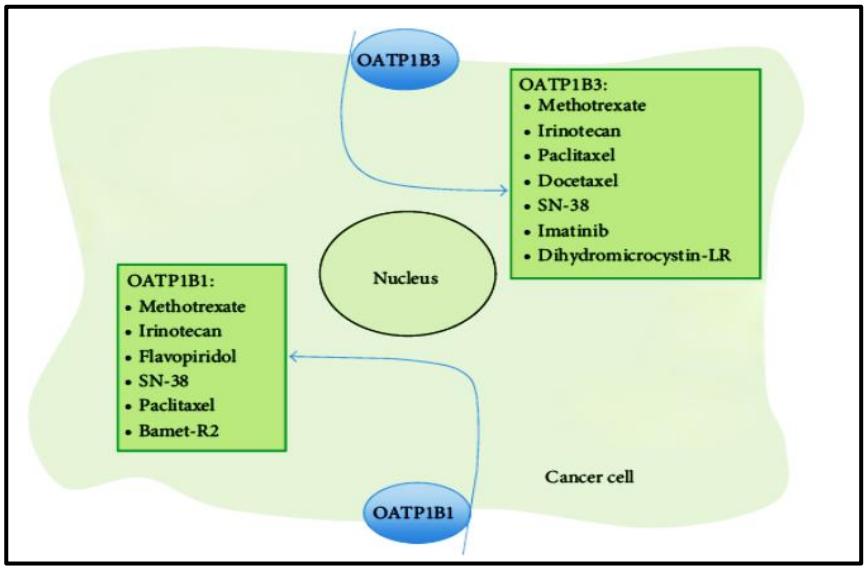


Figure 2.28: Anticancer drugs as substrates for OATP1B1 and OATP1B3 (Retrieved from Buxhofer-Ausch *et al.*, 2013 and then modified)

A study has mentioned that digoxin, paclitaxel, and docetaxel are translocated into the hepatocytes, particularly by OATP1B3 and not by OATP1B1 (Sun *et al*., 2020). A detailed list of exogenous and endogenous substrates has culminated in **table 2.9.** Various endogenous compounds such as steroid sulfates, thyroid hormones, bile acids, glucuronide conjugates, and peptides are well-known substrates of both the OATP1B1 and 1B3 (Niemi, 2007;König, 2011; Kalliokoski *et al.*, 2008). Also, in the case of endogenous substrates, OATP1B1 and OATP1B3 are commonly accountable for hepatic uptake of the unconjugated bilirubin for glucuronidation and subsequent reabsorption of conjugated bilirubin by the downstream hepatocytes after the secretion of bilirubin by MRP3 at the sinusoidal membrane (Keppler, 2014). Few substrates, mainly estrone-3-sulfate, are preferentially transported through OATP1B1, whereas compounds like peptide deltorphin, telmisartan, the cardiac glycoside ouabain, and hepatotoxic cyclic peptide amanitin are preferentially translocated by OATP1B3 (Schulte & Ho, 2019).

Table 2.9: Overview of selected substrates of SLCO1B1 and SLCO1B3 (Schulte & Ho, 2019)

Gene symbol	Exogeneous substrates	Other xenobiotics	Endogenous substrates
SLCO1B1	Daunorubicin, mitoxantrone, atrasentan, chemotherapy-bile acid conjugates (i.e., Bamet-R2, Bamet-UD2), cytarabine, etoposide, flavopiridol, gimatecan, hydroxyurea, methotrexate, microcystin-LR, carboplatin, cisplatin, oxaliplatin, SN-38 (irinotecan active metabolite), sorafenib-b-D-glucuronide, docetaxel, paclitaxel	Olmesartan, valsartan, atrasentan, temocaprilat, pitavastatin, pravastatin, cerivastatin, atorvastatin, rosuvastatin, rifampin, caspofungin, enalapril, BSP, phalloidin, troglitazone, bosentan, benzylpenicillin, nafcillin, some cephalosporins	Bilirubin, Bilirubin-glucuronides, Glycocholate, Leukotriene C4, Dehydroepiandrosterone sulfate, Thromboxane B2, Triiodothyronine, Thyroxine, Prostaglandin, Taurocholate, Estradiol-17β-glucuronide, Estrone-3-sulfate
SLCO1B3	Atrasentan, hydroxyurea, methotrexate, microcystin-LR, carboplatin, cisplatin, oxaliplatin, SN-38, docetaxel, paclitaxel, imatinib, sorafenib- β-D-glucuronide, Doxorubicin, Lapatinib, Rapamycin, Demethylphalloin, Dihydromicrocystin-LR	Fexofenadine, Deltorphin, ouabain, digoxin, Rosuvastatin, valsartan, pitavastatin, Bromosulfophthalein (BSP), rifampin, repaglinide, Telmisartan, olmesartan, enalapril, [D-penicillamine2,5] encephalin	Bilirubin Conjugates, Dehydroepiandrosterone sulfate, triiodothyronine, thyroxine), estradiol-17β-glucuronide, estrone-3-sulfate, Glycocholate, taurocholate, cholecystokinin 8

2.21 Role of SLC (*SLC19A1, SLCO1B1,* and *SLCO1B3*) transporters in cancer

SLC transporters are widely studied for their expression in tumour cells. These transporters play a crucial role in the cellular uptake of anticancer drugs, which might be a fundamental factor in evaluating their effectiveness against the disease. Consequently, compared to normal cells, tumour cells are more liable to exhibit significantly altered expression of the SLC transporter. Additionally, SLC proteins might influence the efficacy of chemotherapeutics (resistance or sensitivity) by modifying the efficiency of drug absorption into cells or altering cell survival mechanisms by controlling the transport of vital nutrition molecules and modifying the electrochemical gradient across the membrane. In some circumstances, cancer cells may have an increased expression of SLC transporters for particular nutritional needs, giving them an edge over normal cells in terms of proliferation when nutrients are scarce. Understanding the differential expression of these SLC transporters in different cancer cells may help us identify new therapeutic approaches for cancer treatment. Transporters like *SLC19A1, SLCO1B1,* and *SLCO1B3* were studied and reported as modulating the expression of SLC transporters and thereby affecting the efficacy of the chemotherapy in various tumour types. A list of these SLC transporters expressed in various cancers is depicted in **Table 2.10**.

Studies have shown that the primary folate transporter system, SLC19A1, has enhanced expression in cancer, which is congruent with the necessity to maximize the folates' cellular retention to cater to the needs of the continuously proliferating and replicating tumour cells (Abdel-Haleem *et al.*, 2011). A study by Abdel-Haleem reported an approximately 14-fold increase in the mRNA expression of SLC19A1 in urothelial cancer and a 4-fold increase in non-urothelial cancer. This study also correlated the increase in the mRNA expression with the enhancement of chemosensitivity to the anti-folates while promoting RFC expression, which could result in acquired resistance (Abdel-Haleem *et al.*, 2011). This speculation was further strengthened by the study by Gorlick *et al.*, stating that loss of the folate transporter may lead to antifolates resistance due to the increase in the folate pool size (Gorlick, 2000).

Similar to this, few studies have reported that a poor prognosis was linked to low RFC levels in primary acute lymphoblastic leukemia (Ge *et al.*, 2007; Levy *et al.*, 2003). A study by Galbiatti *et al.* reported enhanced expression of the *SLC19A1* gene in the laryngeal carcinoma cells treated with methotrexate chemotherapy. The probable reason reported by the author was that an increased chemotherapeutic dose results in more formation of SLC19A1, which is necessary for

transporting methotrexate (Galbiatti *et al.*, 2013). A study reported elevated expression of *SLC19A1* in the breast tumour cell lines treated with chemotherapy. A recent study by Li *et al.* showed elevated expression of SLC19A1 in the osteosarcoma cells. Also, this study showed poor overall survival in osteosarcoma patients with high expression of SLC19A1 as compared to the group with low SLC19A1 expression (Li *et al.*, 2020). A study was conducted to evaluate the impact of SLC19A1 expression on lung cancer patients. The authors reported the elevated expression of SLC19A1 in lung adenocarcinoma patients and showed a significant association between high expression and worse survival in adenocarcinoma patients (Ciribilli *et al.*, 2016).

A significant amount of information suggests the alteration in the expression of OATPs in the different cancer types. OATP1B1 and OATP1B3 are expressed in the liver in the normal tissues; however, these liver-specific OATPs get expressed in the pancreas, colon, prostate, lung, breast, and ovarian cancer (Buxhofer-Ausch *et al.*, 2013). Reports in the past have shown reduced expression of OATP1B1 in hepatocellular carcinoma compared to non-malignant liver. The initial report on the reduced OATP1B1 protein levels was conducted in the hepatocellular tumour cell lines (Cui *et al.*, 2003); till now, numerous ensuing types of research have also assured similar outcomes in both cell lines and tissue samples of hepatocellular tumours (Yi *et al.*, 2008). Contrary to these reports, a study has also reported no substantial decrease of SLCO1B1 in the hepatocellular carcinoma tissues (Vavricka *et al.*, 2004).

The expression of OATP1B1 has also been documented in colon polyps and colon carcinoma tissues (Ballestero *et al.*, 2006). High expression of OATP1B1 and OATP1B3 has been reported in patients with ovarian cancer (Svoboda *et al.*, 2011). Breast cancer cells have been revealed to express OATP1B1 and OATP1B3 at similar or greater levels than healthy breast tissue (Buxhofer-Ausch *et al.*, 2013). A study by Muto *et al.* demonstrated that OATP1B3 expression in breast cancer is linked to a poor prognosis (Muto *et al.*, 2007). Few studies have claimed to have enhanced protein or/and mRNA expression of both OATP1B1 (Buxhofer-Ausch *et al.*, 2013) and OATP1B3 in prostate carcinoma specimens (Lancaster *et al.*, 2013;Buxhofer-Ausch *et al.*, 2013).

Table 2.10: List of relevant SLC transporters in different tumour types

Transporter	Up-regulation in cancer type	Down-regulation in cancer type	References
SLC19A1	Urothelial Cancer Laryngeal cancer Breast Cancer Osteosarcoma Lung adenocarcinoma	Osteosarcoma Acute lymphoblastic leukemia	(Abdel-Haleem *et al.*, 2011); (Ge *et al.*, 2007)(Gorlick *et al.*, 1997)(Levy *et al.*, 2003); (Galbiatti *et al.*, 2013); (Li *et al.*, 2020);(Ciribilli *et al.*, 2016)
SLCO1B1	Colon polyps Colon Cancer Ovarian cancer Prostate cancer Breast cancer Pancreatic cancer Lung cancer Ovary Cancer	Hepatocellular cancer	(Cui *et al.*, 2003) (Ballestero *et al.*, 2006) (Svoboda, Wlcek, *et al.*, 2011) (Buxhofer-Ausch *et al.*, 2013) (Kounnis *et al.*, 2011)
SLCO1B3	Colon cancer Pancreatic cancer Lung cancer Breast Cancer Prostate cancer Ovary cancer Stomach cancer Testicular Cancer	Liver cancer	(Ballestero *et al.*, 2006) (Svoboda, Wlcek, *et al.*, 2011) (Lancaster *et al.*, 2013) (Pressler *et al.*, 2011) (Kounnis *et al.*, 2011) (Lockhart *et al.*, 2008) (Muto *et al.*, 2007)

Study in the past has reported enhanced expression of OATP1B3 with the increased tumour grade in prostate cancer (Pressler *et al.*, 2011). The expression of OATP1B1 and OATP1B3 is higher in the malignant tumours from prostate cancer who have not responded to the therapy than in those prostate cancer patients who did not receive any treatment (Wright *et al.*, 2011). Studies have shown an elevated level of OATP1B3 in the colon in association with the primary tumour stage.

OATP1B3 expression is linked to increased 5-year survival in lower tumour grades; however, in individuals with poorly differentiated malignancies, cancer recurrence is unrelated to OATP1B3 expression (Lockhart *et al.*, 2008).

2.22 Impact of single nucleotide polymorphisms in the *SLC19A1, SLCO1B1, and SLCO1B3* genes on lung cancer

In the past, many theories have been put forth to explain the observed discrepancies regarding several therapeutic drugs that may increase or inhibit the function or expression of solute carrier protein, thus altering the phenotypic activity of the SLC transporters. Specified the effect of such variations on SLC transporter activity, it is essential to look into their role in lung carcinoma, as their expression levels could control the extent and duration of chemotherapeutic drug inflow, affecting patients' responses. This section discusses the impact of *SLC19A1, SLCO1B1,* and *SLCO1B3* polymorphism in predicting clinical outcomes in lung cancer patients undergoing platinum-based doublet chemotherapy.

SLC19A1 is highly polymorphic in humans, and five variants of the *SLC19A1* gene are usually studied in different clinical conditions. Among them, $G^{80}A$ (rs1051266), which is a non-synonymous polymorphism located in exon 2, is widely studied. The polymorphism substitutes arginine with amino acid histidine at residue 27 in protein sequence ($Arg^{27}His$), which leads to alteration in the SLC19A1 transporter structure, affecting its function, thus modifying the drug bioavailability and influencing the therapeutic outcome. (Whetstine *et al.*, 2001). SLC19A1 is liable for transporting the pemetrexed drug inside the cell and may affect the transport of the drug. Besides this, it plays a significant role in folate metabolism. The assessed genotypic frequencies in Caucasians are *AA*=0.23, *GA*=0.47, and *GG*=0.29 (Rady *et al.*, 2001). Allele *A* was more common amongst Caucasians (0.473), African Americans (0.564), and Hispanics (0.472).

Various genetic polymorphisms are described in the *SLCO1B1* gene; among them, the most relevant SNPs related to drug disposition are $A^{388}G$ (rs2306283) and $T^{521}C$ (rs4149056). A necessary missense mutation of *SLCO1B1* gene $A^{388}G$ is positioned on exon 4. The *A* to *G* transition leads to the substitution of asparagine with aspartic acid at residue 130 in protein sequence ($Asn^{130}Asp$) (Michalska *et al.*, 2021). *SLCO1B1* $A^{388}G$ polymorphism is correlated with the altered transporter function that leads to modifications in the structure of transmembrane spanning regions. The *G* allele of $A^{388}G$ might increase the function of OAT1B1, thereby leading

to increased transporter activity; however, reports have suggested some controversial results with the $A^{388}G$ variant as some studies have shown unaltered transporter function by this substitution (Sissung *et al.*, 2012).

In vivo absolute protein quantification depicted that the OATP1B1 protein levels were substantially high in mutant genotype *(GG)* as compared to wild-type genotype *(AA)* genotype, assuring the enhanced transport function of $A^{388}G$-OATP1B1 (Lee & Ho, 2017). Another crucial non-synonymous variant of the *SLCO1B1* gene is $T^{521}C$ which is localized on exon 5 (Niemi *et al.*, 2011). The *T* to *C* transition leads to the substitution of valine with alanine at residue 174 in the protein sequence ($Val^{174}Ala$). This alanine substitution is correlated to reduced transport function and high statin concentration in the plasma (Hou *et al.*, 2015). Studies have shown that mutant genotype *(CC)* of $T^{5s21}C$ is associated with low function, and heterozygous genotype *(TC)* is correlated with intermediate function as compared to the wild type *(TT)* genotype (Ramsey *et al.*, 2014).

SLCO1B3 is a highly polymorphic gene that displays notable allele frequency variations among ethnic populations. One non-synonymous variant of *SLCO1B3* $A^{1683\text{-}5676}G$ (rs11045585) has been reported at intron 12 (Song *et al.*, 2021). Studies have reported that $A^{1683\text{-}5676}G$ variation is associated with the reduced transporter activity of the OATP1B3 (Shitara *et al.*, 2013). Polymorphism in the *SLCO1B3* gene has been correlated with the altered OATP activity that often leads to drug-associated adverse events (Yamada *et al.*, 2011). The frequency of the *SLCO1B3 rs11045585* variation was substantially higher in the Hungarian samples.

2.22.1 Association of SLC genes (SLC19A1, SLCO1B1, and *SLCO1B3) polymorphism with prognosis and clinical outcomes in lung cancer patients*

The SLC19A1 protein translocate folate drugs like pemetrexed in the cell. Pemetrexed is often administered with platinum compounds. Variations in the *SLC19A1* gene might vary the cellular inflow of chemotherapeutic drugs or may affect the folate levels, consequently affecting the cancer patients' clinical outcomes (Corrigan *et al.*, 2014). Most of the past studies have evaluated the impact of *SLC19A1* $G^{80}A$ polymorphism in predicting clinical outcomes in acute lymphoblastic leukemia, and many few studies have been carried out in lung cancer regarding this polymorphism. The literature from the past has been depicted further. Studies have reported the association of

RFC $G^{80}A$ polymorphism with decreased plasma folate and increased homocysteine levels in an average person.

Tiseo *et al.* assessed the impact of *SLC19A1* $G^{80}A$ polymorphism in predicting prognosis in NSCLC patients undergoing carboplatin and pemetrexed chemotherapy. The results of the study showed better survival in the patients harbouring one mutant allele *(GA)* for G80A polymorphism as compared to the wild-type genotype *(GG)* (OS=11.5 months vs 9.4 months). However, despite the survival trends between both the groups, the substantial differences between *SLC19A1* $G^{80}A$ polymorphism were not established (p=0.634). In a similar study, the authors evaluated the association of $G^{80}A$ polymorphism with the response rate towards pemetrexed-based chemotherapy, though no significant association was noted between *RFC1* $G^{80}A$ variation on modulation of clinical response rate (Tiseo *et al.*, 2012). A study in the past evaluated the impact of *RFC* $G^{80}A$ polymorphisms on the overall survival of NSCLC patients undergoing platinum-based chemotherapy. The study claimed that patients harbouring mutant genotype *(AA)* for *SLC19A1* $G^{80}A$ polymorphism had a high risk of mortality as compared to the patients having wild-type genotype *(GG)* (HR=1.86, p=**0.04**, 95% CI=1.02-3.41). The median OS for the mutant carriers was lower as compared to the patients with wild-type carriers (MST=24.5 vs 32.2 months). Also, the study correlated the mutant genotype of *SLC19A1* $G^{80}A$ with the high risk of progression (Pérez-Ramírez *et al.*, 2019).

Most studies in SLCO1B1 polymorphism have assessed the role of $A^{388}G$ and $T^{521}C$ variants in the toxicity and tumour response in lung cancer. Literature in context to impact of these polymorphisms in predicting prognosis in lung cancer patients is very slender. Han *et al.* assessed the impact of *SLCO1B1* $A^{388}G$ and $T^{521}C$ polymorphic genotypes on tumour response and found no substantial significance (Han *et al.*, 2008). A study by Huang *et al.* reported that the wild-type *(AA)* and heterozygous *(GA)* genotypes of SLCO1B1 $A^{388}G$ polymorphism correlate with the rapid response rate to chemotherapy in colorectal cancer. This study also reported a lack of association of $A^{388}G$ polymorphism in predicting overall survival in metastatic colorectal cancer (Huang *et al.*, 2013).

SLCO1B1 $T^{521}C$ polymorphism has been evaluated in breast cancer patients, where outcomes have shown that the wild-type genotype *(TT)* of $T^{521}C$ exhibit better overall survival as compared to the mutant genotype *(CC)* (p=**0.034**). On the contrary, no association between *SLCO1B1* $A^{388}G$ polymorphism and prognosis was reported in breast cancer patients (Zhang *et al.*,

2015). Similarly, when $A^{388}G$ polymorphism was evaluated in multiple myeloma, a report showed a lack of association between genotypes of this polymorphism and overall survival; however, lower survival was reported in the wild-type genotype *(AA)* as compared to the mutant genotype *(GG)*. The researchers claim that the *G* allele of the $A^{388}G$ variant is responsible for the high expression of the OATP1B1 protein, which is accountable for the translocation of chemotherapeutic agents, assuring efficacious transport and greater concentration of the drug into the cell, thereby making therapy more effective (Michalska *et al.*, 2021).

Choi *et al.* assessed the correlation between the role of *SLCO1B3* $A^{1683-5676}G$ polymorphism and tumour response in lung cancer patients. The authors reported a marginal association between patients having mutant genotype *(GG) of SLCO1B3* $A^{1683-5676}G$ and tumour response, though the data lacked substantial significance (p=0.07) (Choi *et al.*, 2015).

2.22.2 Association of SLC19A1, SLCO1B1, and SLCO1B3 gene polymorphism and toxicity in lung cancer

In the case of *SLC19A1* $G^{80}A$ polymorphism, the variant genotype of this polymorphism was correlated with reduced haematological toxicity in patients with rectal cancer (Ulrich *et al.*, 2014). A study by Tiseo *et al.* reported adverse effects as rare events in NSCLC patients undergoing pemetrexed-based chemotherapy. The study reported that only 6% of patients had severe toxicity while 10% had severe leukopenia. Though, no substantial correlation was reported between the different genotypes of *SLC19A1* $G^{80}A$ polymorphism and pemetrexed-based chemotherapy (Tiseo *et al.*, 2012). A study by Ramirez *et al.* assessed the impact of *SLC19A1* $G^{80}A$ polymorphism in modulating toxicity in NSCLC patients undergoing platinum-based chemotherapy. The report evaluated adverse events like nephrotoxicity, haematological, and gastrointestinal toxicity. Increased risk of gastrointestinal toxicity was observed in variant genotype *(AA)* of SLC*19A1* $G^{80}A$ polymorphism compared to the other genotypes (p=**0.01**). Besides this, $G^{80}A$ polymorphism did not reveal any impact on nephrotoxicity and haematological toxicity (Pérez-Ramírez *et al.*, 2016). No association was reported between the *SLC19A1* $G^{80}A$ SNP and chemotherapy-related toxicity for patients on pemetrexed-carboplatin versus pemetrexed (Smit *et al.*, 2009).

Han *et al.* assessed the impact of *SLCO1B1* $A^{388}G$ and $T^{521}C$ polymorphism with irinotecan-induced toxicity. The authors reported that patients having mutant genotype *(CC)* and heterozygous genotype *(TC)* showed a higher prevalence of grade 4 (severe) neutropenia as

compared to the patients harbouring wild type *(TT)* genotype. (p=**0.008**). Also, the study mentioned that the lung cancer patients harbouring mutant type genotype (GG) developed grade 3 diarrhea as compared to the wild type genotype *(GG)* (p=**0.046**) (Han *et al.*, 2008). Another study evaluated the association between mutant genotype *(GG)* of *SLCO1B1* $A^{388}G$ polymorphism and severe neutropenia in NSCLC patients undergoing irinotecan-based chemotherapy. The same study mentioned that in multivariate analysis of *SLCO1B1* $T^{521}C$ polymorphism, *TC* and *CC* genotypes were independent predictive factors for severe neutropenia (Han *et al.*, 2009).

A meta-analysis study was carried out by Zair *et al.*, where the authors culminated 16 publications from the past on the variants of influx transporters and their impact on chemotherapy-induced toxicity. A total of 1510 lung cancer patients were selected for the review and analysis. The study depicted promising findings in which $T^{521}C$ was correlated with an elevated risk of irinotecan-induced neutropenia and lower excretion of irinotecan. Also, the meta-analysis report mentioned a lack of literature correlating the risk of adverse drug reactions with lung cancer patients expressing *SLCO1B1* $A^{388}G$. The authors reported a small-scale study regarding $A^{388}G$ polymorphism but found a significant association with reduced risk of irinotecan-induced neutropenia and diarrhea. This speculation was attributed to the fact that $A^{388}G$ polymorphism reduces OATP1B1 activity, leading to reduced drug uptake and less drug efficacy, thereby reducing toxicity (Zair & Singer, 2016b). The salient features of the critical studies from different publications chosen for meta-analysis are depicted in **table 2.11.** Another meta-analysis study was conducted recently to assess the role of *SLCO1B1* $T^{521}C$ in causing methotrexate-induced toxicity in different malignancies. The analysis included five studies comprising 465 patients for the review. The outcomes revealed that *C* allele bearers of *SLCO1B1* $T^{521}C$ variation are associated high risk of hepatotoxicity as compared to the homozygous T alleles (Han *et al.*, 2022).

Table 2.11: Salient features of the critical study from different publications chosen for meta-analysis (Zair & Singer, 2016b)

Year	Patient number	Ethnicity /Country	Chemotherapy regimen	Influx transporter	Reference
2006	71 cancer patients-5 NSCLC	Chinese, Malayas, Indian, Non-Asian NSCLC patients-not mentioned	Cisplatin and Irinotecan	*SLCO1B1*	(Xiang *et al.*, 2006)
2008	32 Lung cancer patients	Japan	Docetaxel – regimen not given	*SLCO1B1*	(Kiyotani *et al.*, 2008)
2008	17 (Stage III) 64 (Stage IV)	Korea	Cisplatin and Irinotecan	*SLCO1B1*	(Han *et al.*, 2008)
2009	12 (stage I) 2 (stage II)	Japan	Irinotecan	*SLCO1B3*	(Cha *et al.*, 2009)
2009	107 NSCLC	Korea	Irinotecan	*SLCO1B1*	(Han *et al.*, 2009)
2009	86 cancer patients-26 with lung cancer	11 African–American 67 White 4 Hispanic 1 Pacific Islander 2 Asian	Irinotecan	*SLCO1B1* *SLCO1B3*	(Innocenti *et al.*, 2009)
2010	48 NSCLC (Stage III) 45 (Stage IV)	USA	Pemetrexed Followed by bevacizumab	*SLC19A1*	(Adjei *et al.*, 2010)
2010	54 chemo-naïve 20(stage IIIB) 132 (Stage IV)	USA	Pemetrexed and gemcitabine	*SLC19A1*	(Adjei *et al.*, 2010)
2015	92 patients-55 with lung cancer	Korea	Docetaxel	*SLCO1B3*	(Choi *et al.*, 2015)

In the case of *SLCO1B3* polymorphism, Choi *et al.* assessed the role of $A^{1683\text{-}5676}G$ polymorphism in causing adverse events in NSCLC patients undergoing docetaxel chemotherapy. The study found no association between *SLCO1B3* $A^{1683\text{-}5676}G$ polymorphism and frequencies of neutropenia. Additionally, in this study, a multivariate analysis was carried out in which the impact of tumour stage, gender, chemotherapy regimen, and *SLCO1B3* polymorphism was evaluated to look for docetaxel-induced toxicity. The outcomes suggested substantial differences between variant

genotype *(GG)* of *SLCO1B3* $A^{1683\text{-}5676}G$ polymorphism and docetaxel-induced toxicity (OR=9.44, 95% CI=0.98-21.12, p=**0.02**). Alongside, a substantial correlation was also reported between chemotherapy regimen and toxicity. The study concluded that *SLCO1B3* $A^{1683\text{-}5676}G$ is a genetic predictor of docetaxel-induced toxicity in lung cancer patients undergoing chemotherapy (Choi *et al.*, 2015). Kiyotani *et al.* reported a substantial correlation between *SLCO1B3* $A^{1683\text{-}5676}G$ with severe grade docetaxel-induced leukopenia or neutropenia (OR=5.44, p=0.0001). Authors also claimed that this SNP alters the *SLCO1B3* activity and might increase the docetaxel AUC due to reduced hepatic elimination of docetaxel (Kiyotani *et al.*, 2008).Yamada *et al.* reported that the increased rate of the $A^{1683\text{-}5676}G$ variant might contribute to the reduced *SLCO1B3* function that may alter therapeutic efficacy. This also helps to correlate that elevated risk of neutropenia is commonly caused by the increase in the systemic exposure to the drug docetaxel due to the reduced OATP1B3 function (Yamada *et al.*, 2011). Shitara *et al.* also reported that neutropenia and leucopenia induced by docetaxel are correlated with $A^{1683\text{-}5676}G$ polymorphism as docetaxel clearance is reduced in patients with this polymorphism (Shitara *et al.*, 2013). A recent study by Sone *et al.* assessed *SLCO1B3* $A^{1683\text{-}5676}G$ polymorphism in 69 NSCLC patients undergoing docetaxel therapy samples. The outcomes did not correlate this polymorphism with the prevalence of neutropenia in lung cancer patients (Sone *et al.*, 2019).

2.23 Thymidylate synthase gene

The *thymidylate synthase (TYMS or TS)* gene encodes a tightly regulated enzyme that mediates the catalyzes of deoxyuridylate (dUMP) and converts it into thymidylate (dTMP). A carbon atom from 5,10-methylenetetrahydrofolate is transferred during this reaction to the fifth location of the pyrimidine ring (Zhao *et al.*, 2021). This transformation is a part of the thymidylate cycle and is the only *de novo* source of thymidylate. Other enzymes involved in the thymidylate synthase pathway are dihydrofolate reductase (DHFR) and serine hydroxymethyl transferase (SHMT). SHMT is involved in converting tetrahydrofolate to 5,10-methylenetetrahydrofolate, along with the formation of glycine from serine. So serine transfers a single carbon atom to tetrahydrofolate with the help of the enzyme SHMT and gets reduced to 5,10-methylenetetrahydrofolate (Liu *et al*., 2002). Now, this 5,10-methylenetetrahydrofolate donates its carbon (methylene) group to dUMP to form dTMP with the help of the enzyme *thymidylate synthase* that is utilized to synthesize thymidine. At the same time, 5,10-methylenetetrahydrofolate is transformed into dihydrofolate (Figure 2.29). So, the resultant dihydrofolate can again be reduced to tetrahydrofolate and 5,10-methylenetetrahydrofolate to continue the cycle. Further, dTMP is metabolized intracellularly into dTTP, an essential precursor for the biosynthesis of DNA (Wilson *et al*., 2014).

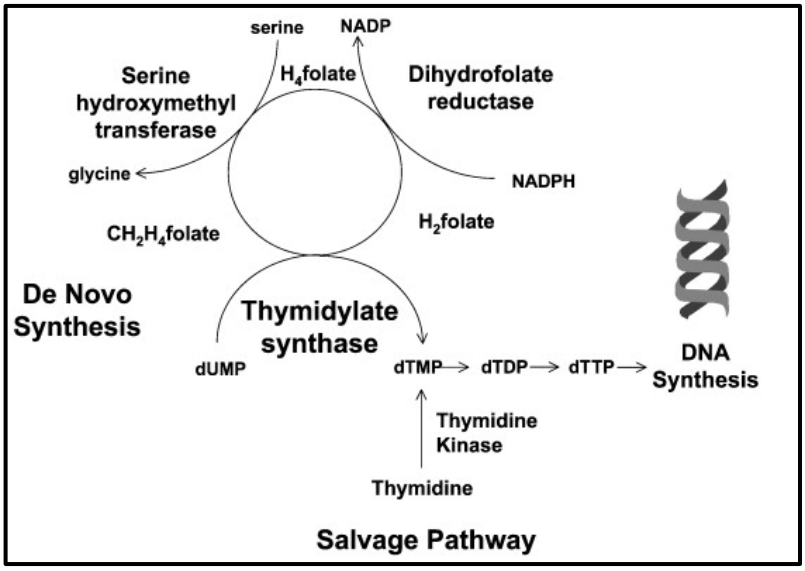


Figure 2.29: An illustration of the Thymidylate synthase pathway(Liu *et al*., 2002)

2.23.1 Genomic organization

The Thymidylate synthase gene is localized in chromosome 18p11.32 **(Figure 2.30)**. A biologically active unit of thymidylate synthase is about a 16 kilobase pair that encodes a protein of 313 amino acids. The gene consists of 7 exons (approximately 30Kb) and six introns. TS gene contains a complete tandem repeat sequence at 5'-UTR. It has been proposed that this variable region physiologically controls the translation of TS. The 5'-UTR region of TS mRNA has unique structural characteristics, such as the G+C content is very high in this region (80%), and it also comprises triple tandem repeat elements closely upstream of the ATG start codon (Ghosh *et al.*, 2011).

The first and second element contains similar 28-nucleotide sequence, whereas the third element comprises 6-bp insertion and a single-base substitution (Hammad *et al.*, 2012). It was reported for the first time that the nucleotide sequence in the 5'UTR region of TS cDNA forms three interconvertible 2^{o} structures, each consisting of a stem-loop structure generated by the combination of two CGCCGCG sequences. It is also hypothesized that this 6-bp insert regulates the TS expression. The second study reported that the transcriptional start site is localized 160-180 bp upstream from the ATG start codon, whereas the promoter region is approximately 400bp from the ATG codon. The 5' flanking region lacks "CAAT," "TATA," or consensus sequence for the Sp1 binding. Intron 1 comprises three GC boxes (Kaneda *et al.*, 1990).

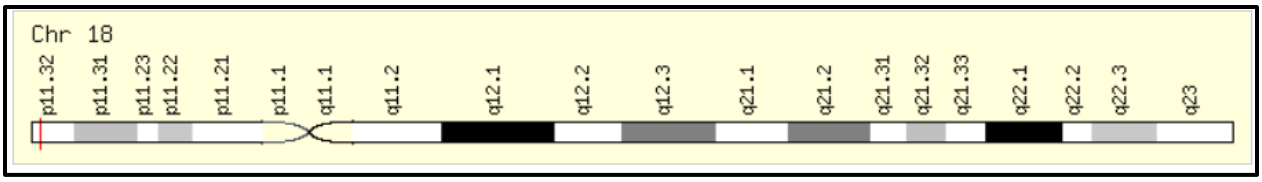


Figure 2.30: Localization of Thymidylate synthase gene on chromosome 18 (https://www.genecards.org/)

2.23.2 Structure of TS

The reported crystal structures of TS are in various molecular states, such as apoprotein, complexes with substrates, inhibitors, dUMP, and dTMP. A recent study has reported the X-ray structure of native human TS without inhibitors and substrates (**Figure 2.31)**. The average pairwise RMSD was 0.25 A˚ (Deschamps *et al.*, 2017). TS exists in a homodimeric form and has two active sites,

each produced by the residues of both monomers. The protein codes for 313 amino acids, and sequences are highly conserved. There are two domains in each monomer: a larger domain is conserved and spans from residues 1 to 98, and 130 to 313, whereas a smaller domain is a variable that extends from residues 99 to 129 in humans. Each monomer exhibits alpha and beta (α+β) folds along with eight β strands, five α helices, and two 3_{10} helices in the large domain (Costi *et al.*, 2005). The dimer interface is formed with five stranded β sheets in each large domain. Human TS binds dUMP and folate substrate 5,10-methylenetetrahydrofolate in a pocket close to the catalytic residue, Cys195.

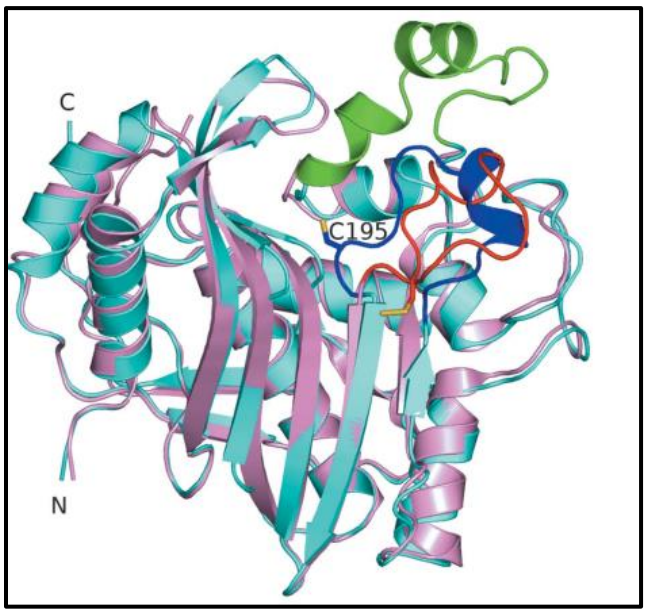


Figure 2.31: The structure of human Thymidylate synthase in active form (Active state of human TS is shown in cyan, with loops 181–198 (blue) and loops 107–128 (green). The inactive form of human TS is depicted in pink color with loops 181–197 (red*)* (Deschamps *et al.*, 2017)

The catalytic loop is made up of the amino acids 181 to 197, which also can adopt two different conformations linked by a 180^{o} rotation (Deschamps *et al.*, 2017). Cys195 is proximal to the active site when it is in the active conformation, but it is too far away from the active site in the inactive conformation as it is rotated (Pozzi *et al.*, 2019). Another vital region detected in the TS structure is the loop around R50 spanning from residue 47-54, the loop at the interface from residue 144-158, and the C-terminal region from 308-313 (Costi *et al.*, 2005).

2.24 Role of Thymidylate synthase in cancer

TS is the main target of chemotherapeutic agents, like fluoropyrimidines and numerous antifolate analogues (Chu & Sartorelli, 2018). Numerous vital pieces of evidence suggest that TS is a significant chemotherapeutic target. The first is based on *in vivo*, *in vitro*, and clinical investigations that show a direct relationship between fluoropyrimidine responsiveness and TS enzyme activity and protein expression (Wilson *et al.*, 2014). It is widely known that fluoropyrimidines and antifolate analogues, which target TS, have cytotoxic and anticancer effects on cancer cell lines or malignant tumours. However, these effects are substantially less pronounced in cancer cell lines and tumours expressing a higher amount of TS. Second, there is a significant association between the degree of TS enzyme inhibition in patient tumour specimens after 5-FU therapy and the subsequent clinical response (Vodenkova *et al.*, 2020).

Additionally, pemetrexed has been extensively studied regarding its role in inhibiting TS. *In vitro* experiments show that high baseline expression levels imparted pemetrexed resistance. Similarly, specific clinical investigations have correlated elevated TS expression levels with the poor chemotherapeutic response to the pemetrexed treatment, including colorectal cancer (Rose *et al.*, 2002), breast cancer (Gomez *et al.*, 2006), and malignant pleural mesothelioma (Righi *et al.*, 2010), and head and neck cancer. The critical studies and findings regarding TS expression in different cancers are depicted in **table 2.12.**

A meta-analysis study in the past assessed the TS expression and overall survival in colorectal cancer patients. The analysis analyzed thirteen studies with 887 cases of advanced colorectal cancer. The patients expressing a high level of TS were associated with poorer prognoses than those with tumours with low levels of TS (Popat *et al.*, 2004). Another study by Chamizo *et al.* reported that overexpression of the *TS* gene is associated with reduced response to pemetrexed therapy in advanced NSCLC patients (Chamizo *et al.*, 2015). A recent study by Song *et al.* reported elevated *TS* expression in breast cancer compared to the normal tissues and correlated this high TS expression with poor prognosis in patients (Song *et al.*, 2021). A recent study by Donner *et al.* assessed the TS expression in correlation to prognosis in hepatocellular carcinoma patients who underwent resection. The elevated TS expression was correlated with poor overall survival (Donner *et al.*, 2019). A meta-analysis study by Gao *et al.* reported high expression of TS with overall adverse survival and drug resistance in gastric cancer patients who received

fluoropyrimidine-based chemotherapy (Gao *et al.*, 2016). Another notable meta-analysis study was conducted by Wang *et al.* on NSCLC patients undergoing pemetrexed-based chemotherapy to assess the association of *TS* expression with response rate. The study analyzed 526 patients and claimed that -/low expression of *TS* correlates with higher objective response in lung cancer patients who received pemetrexed-based chemotherapy compared to patients with +/high expression of *TS* (Wang *et al.*, 2014). A study in the past reported higher expression of the *TS* gene in lung cancer patients compared to the normal lung epithelial cells. Normal cells such as endothelial cells and bronchial smooth muscle displayed *TS* expression; however, their expression was not noted in normal lung epithelial cells. The probable reason for this could be that TS is a crucial enzyme involved in the thymidine production for DNA synthesis, so its expression is upregulated in the rapidly proliferating tumour cells. A report has shown high TS expression levels in SCLC, while in case of NSCLC, adenocarcinoma patients were reported to have low TS expression, whereas SQCC patients had elevated *TS* expression levels as compared to ADCC, also in case of LCC, TS expression was lower as compared to SCLC but higher with respect to adenocarcinoma and squamous cell carcinoma (SCLC>LCC>SQCC>ADCC) (Tanaka *et al.*, 2011).

Table 2.12: Highlighting critical studies on TS expression in different cancer types

Gene	Cancer	Expression	Key Findings	References
Thymidylate synthase	Colorectal cancer	↑ TS expression	Poor overall survival	(Popat *et al.*, 2004)
	NSCLC	↑ TS expression	Reduced response rate	(Chamizo *et al.*, 2015)
		↓ TS expression	High objective response	(L. Wang *et al.*, 2014)
	Breast cancer	↑ TS expression	Worse overall survival	(S. Song *et al.*, 2021)
	Gastric cancer	↑ TS expression	Poor overall survival	(L. Wang *et al.*, 2014)
		↑ TS expression	Adverse prognosis	(Gao *et al.*, 2016)
	Pancreatic cancer	↑ TS expression	Worse overall survival	(Gao *et al.*, 2016)
	Hepatocellular carcinoma	↑ TS expression	Poor overall survival	(Donner *et al.*, 2019)

2.25 Impact of single nucleotide polymorphisms in the *TS* gene on lung cancer

Reports have suggested two well-studied *TS* gene polymorphisms in the TS enhancer region *(TSER) 2R/3R (rs34743033)* and TS *1494del6 (rs151264360)* in the 5'- and 3'-untranslated regions (UTRs) (Sharma *et al.*, 2022). Polymorphisms in the 5'-UTR and 3'-UTRs are thought to have a close relationship with *TS* gene expression regulation. In the 5'UTR region, a variable tandem repeat of the 28bp sequence is present either in duplicate *(2R)* or triplicate *(3R)* in the *TSER* region. When compared to double 28-bp tandem repeats (*2R*) in the 5'-UTR, triple (*3R*) not only confers enhanced mRNA stability but also increases transcriptional and translational efficiency and elevates TS mRNA levels that are associated with poor clinical outcomes in cancer patients (Ishida *et al.*, 2002).

TS 1494del6 polymorphism is an insertion/deletion polymorphism of 6 bp (CTTTAA) localized at the nucleotide 1494. The *1494del6* deletion or insertion polymorphism might alter enzyme activity and function, thus varying susceptibility to cancer and altering the sensitivity of carcinoma patients to chemotherapeutic drugs (Sharma *et al.*, 2022). Studies in the past have correlated this polymorphism with TS mRNA stability and reduced thymidylate synthase protein expression (Ulrich *et al.*, 2000). The presence of homozygous 6bp deletion in 3'UTR of TS reduces the mRNA stability and is correlated with low TS expression, which is associated with enhanced treatment efficacy. According to the literature, the *TS 1494del6* polymorphism is in linkage disequilibrium with the *TSER 2R/3R* polymorphisms. Thus, it has been proposed that *TS* polymorphisms may influence the effectiveness of chemotherapy treatment and can be vital in determining the overall survival of NSCLC patients (Lima *et al.*, 2014). Table 2.13 summarizes the impact of TS SNPs in lung cancer patients.

A study in the past enrolled 106 ADCC patients who underwent pemetrexed treatment and assessed the role of *TS 1494del6* polymorphism in assessing the prognosis and response. The outcomes revealed a short-term response to the pemetrexed treatment in heterozygous and mutant genotype patients, ensuring a better response rate to chemotherapy in the patients with mutant genotype *(-6/-6bp).* Better overall survival was reported in the mutant genotype *(-6/-6bp)* for *1494del6* polymorphism, and it was evaluated as the prognostic marker for personalized chemotherapy in ADCC (Wang *et al.*, 2013). A recent study showed no association between the TS polymorphisms and overall survival in NSCLC patients who underwent cisplatin and

pemetrexed chemotherapy (Abbasian *et al.*, 2022). Elevated expression of TS protein and VNTR variations of TS gene displayed substantial correlation with TNM stage, differentiation, and metastasis. Also, NSCLC patients with mutant genotype *(3R/3R)* for *TSER* polymorphism showed a high rate of thymidylate synthase protein overexpression. The elevated expression in mutant genotype (*3R/3R*) was correlated with shorter overall survival in patients. The article reported a high prevalence of mutant genotype(*-6/-6bp*) o*f 1494del6* polymorphism in NSCLC patients compared to others (Chen *et al*., 2021).

Table 2.13: Summarizing critical studies on TS polymorphism in lung cancer patients

Gene polymorphism	Cancer type		Chemotherapy regimen	Key Findings	References
Thymidylate synthase	NSCLC	106	Pemetrexed	Overall survival, Response rate	(Wang *et al.*, 2013)
		40	Pemetrexed + Cisplatin	Overall survival	(Abbasian *et al.*, 2022)
		160	-	Overall survival, Clinicopathological characteristics	(Chen *et al*., 2021)
		25	Pemetrexed-based regimen	Overall survival, Response rate, Toxicity	(Arévalo *et al.*, 2014)
		90	Pemetrexed-based regimen	Overall survival, Response rate	(Hu *et al.*, 2012)
		130	Platinum-based chemotherapy	Overall survival	(Lima *et al.*, 2014)

The impact of *TS gene* polymorphism in predicting clinical outcomes was also evaluated in the European population with advanced NSCLC patients receiving pemetrexed. The results of *TSER* polymorphism showed an association of mutant genotype *(3R/3R)* with a higher overall response rate over the rest of *the TSER* genotypes. Also, the *3R/3R* genotype patients showed superior overall survival (p=**0.019**) compared to the other genotypes. This study showed a lack of association of *TSER* and *1464del* polymorphism with adverse events (Arévalo *et al.*, 2014). Hu *et al.* reported a lack of correlation in OS, overall response rate, and different genotypes of TS polymorphism in advanced NSCLC patients (Hu *et al.*, 2012).

CHAPTER 3

GAPS IN THE STUDY & OBJECTIVES

3.1 Gaps in the study

To the best of our knowledge, no studies in the North Indian population have examined the impact of transporter genes, *i.e.*, ABC and SLC and folate metabolism gene polymorphisms. The influence of *ABC, SLC,* and *TS* gene polymorphisms on lung cancer prognosis and their association with adverse events and clinical outcomes in north Indian lung cancer patients receiving platinum-based doublet chemotherapy has also not been reported. In the past, studies have claimed that if chemotherapy cannot control tumor growth, the detrimental effects of chemotherapy may reduce patients' quality of life. Many transporter genes and *thymidylate synthase* gene alteration carry polymorphisms with the potential to modulate gene function, hence modulating the effect of drugs and toxicity. Also, it is undebatable that genetic polymorphisms in the transporters gene and drug targets can influence the chemotherapeutic response in patients with lung cancer. Therefore, identifying such polymorphisms in transporters and folate metabolism genes may help establish the correlation in predicting clinical outcomes in patients with lung cancer. This would yield insights on how the transporter gene polymorphism adversely or favorably influences the chemotherapy outcomes in the north Indian population. Also, this will help us understand that choosing a chemotherapeutic regimen might increase cancer patients' response and, as a result, their overall survival. These SNPs could also be exploited as biomarkers, assisting in the formulation of individualized chemotherapy regimens depending on genotype and determining the relationship between these polymorphisms and clinical outcomes for the development of personalized therapeutics. Therefore, a better understanding of prognostic factors would help minimize the resistance and toxicity caused by chemotherapy and will help individualize treatment for lung cancer patients.

3.2 Objectives

1. To study the SNP variants in *ABC, SLC,* and *TS* genes
2. To evaluate the overall survival and clinicopathological features
3. To evaluate the clinical outcomes and toxicity profiles of lung cancer patients undergoing platinum-based chemotherapy

CHAPTER 4

MATERIAL AND METHODS

4.1 Study Design

A retrospective case study was designed to assess the North Indian population correlation with genetic polymorphisms of Thymidylate synthase, ATP-binding cassette, and Solute carrier transporter genes. Blood samples of lung cancer patients were collected in this research study from the Department of Pulmonary Medicine, Post Graduate Institute of medical education and research (PGIMER) Chandigarh, India. The Institute Ethics Committee of PGIMER reviewed and authorized this study (Approval No. IEC-04/2018-884), and it was conducted in agreement with the ethical standards outlined in the 1964 Declaration of Helsinki and its subsequent amendments. An ethical clearance letter has been attached in Appendix-I. All patients or their representatives provided written informed consent, which is attached in Appendix-II.

Patients newly diagnosed with primary lung cancer were included in the current study. Based on the patient's histological diagnosis, the patients were classified into ADCC, SQCC, and SCLC. There was no age, smoking, gender, histology, or TNM stage limitations. The following factors were considered in the inclusion criteria:

a) Histology or cytology is used to diagnose lung cancer (NSCLC or SCLC) accurately.
b) Age, gender, smoking status, or staging restrictions were no constraints.
c) Patients in stage III or stage IV
d) Informed written consent was obtained.
e) Untreated to undergo definitive chemotherapeutic treatment (administered with platinum-based agents, *i.e.*, cisplatin/carboplatin
f) A performance status (PS) of 0–2 as per Eastern Cooperative Oncology Group (ECOG).
g) According to the Response Evaluation Criteria in Solid Tumor Group (RECIST) criteria, there must be at least one bi-dimensionally quantifiable lesion.
h) Appropriate organ function, such as platelet count >100,000/L, absolute neutrophil count >1500/L, creatinine levels, and liver enzymes such as alanine aminotransferase (ALT) levels less than two times the upper limits of normal (ULN).

The following were among the exclusion criteria:

a) Patients with immunosuppression or active infection
b) Patients had a history of any other type of cancer

c) Patients undertaking any systemic steroids
d) Patients experiencing persistent diarrhea due to a variety of factors
e) Patients who are not being treated with chemotherapy

Though, plentiful physiological tests were done before the patients' recruitment, such as a complete blood count (CBC), which assesses neutrophil count, and biochemical testing such as renal and liver function tests. Additionally, the patients had a chest X-ray and a contrast-enhanced computed tomography (CT) scan of the thorax and the upper abdomen.

Also, a trained interviewer filled out a detailed questionnaire for each case. The questionnaire inquired about the patient's age, region, gender, smoking habits, and intake of alcohol, among other things. Smokers confirmed they used cigarettes and beedis (a native cheaper than a cigarette-like stick of unprocessed tobacco wrapped in a dry tembuhurni leaf). To compute pack years as a measure of cumulative smoking exposure, the following formula was used:

Pack years= {(Number of beedis or cigarette per day/20) * Number of years smoked}

The critical patient information for the cases was procured from the hospital's medical records, including TNM classification, histology, clinical staging, ECOG-PS, KPS, objective response, and chemotherapeutic regimen, using RECIST criteria.

4.1.1 Chemotherapy regimen

In the current study, lung cancer patients were administered doublet platinum-based chemotherapy treatment. The regimen included a combination of a platinum-based compound such as cisplatin/carboplatin and different drugs such as docetaxel or pemetrexed, or paclitaxel, or irinotecan. The drugs, as mentioned above, were given intravenously at a fixed dosage based on the body's surface area. The combined chemotherapeutic dose contained the administration of 500 mg/m^2 pemetrexed, 100 mg/m^2 irinotecan, or 75 mg/m^2 docetaxel as a one-hour infusion, followed by the *iv*. Infusion of cisplatin (70 mg/m^2) for over three hours. Supplements of standard folate and Vitamin B_{12} formulations were provided to each patient. Drugs, for example, ranitidine, dexamethasone, and granisetron were also given as part of the premedication protocol. Four cycles of chemotherapy were given before the tumor response assessment, as is standard practice at PGIMER. The tumor response was monitored, and, if necessary, chemotherapy was halted if there was intolerable toxicity or clinic-radiological signs of cancer progression before the end of four

cycles. Patients who showed an objective response to chemotherapy treatment were administered two more cycles of treatment (*i.e.,* a maximum of six cycles).

4.1.2 Follow-up and tumor response assessment

All participants were followed up through telephone conversations every two months till the completion of the study period. The survival time was calculated from the date of lung carcinoma diagnosis till the last date of follow-up or death due to uncertain reasons. The primary endpoint of the research was overall survival, and the secondary endpoint was to evaluate the association of genetic polymorphism with treatment response and adverse events (AEs). RECIST criteria were utilized to assess the tumor response. Based on RECIST criteria, responses are classified as "responders" or "non-responders. "Responders" have a partial response (PR) or complete response (CR), whereas "non-responders" have stable disease (SD) or progressive disease (PD).

- CR: All target lesions have wholly disappeared.
- PR: At least a 30% reduction in the sum of target lesions' diameters, using the baseline sum diameter as a reference.
- PD: At least a 20% increase in the sum of diameters of target lesions, taking the smallest sum in the study (including the baseline sum if that is the smallest). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).
- SD: Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking the minor sum diameters while studying (Eisenhauer *et al.,* 2009).

4.2 DNA Extraction

4.2.1 Genomic DNA extraction

Genomic DNA was extracted from peripheral blood samples of lung cancer cases using the protocol of Bartlett and White (2003) with few modifications.

a) Firstly, to separate red blood cells (RBCs) from blood lymphocytes, 3ml of whole blood was washed with an equal volume of washing solution containing 320mM sucrose, 20mM Tris-HCl (pH 8.0), 5mM $MgCl_2$, and 1.5% Triton X-100. This process was performed three times. The samples were centrifuged at 3,500 rpm for 10 minutes at 4°C.

b) Furthermore, the RBC-free pellet was subjected to the lysis at 45°C in the lysis solution containing 150mM NaCl, 400mM Tris-HCl (pH 8.0), 1% SDS, 60mM EDTA, and 100 µg/ml proteinase-K.
c) An equal volume of phenol/chloroform/isoamyl alcohol (P:C: I=25:24:1) solution was used to remove proteins. After gentle mixing, the solution was centrifuged at 8000 rpm for 7 minutes at 4°C, resulting in two distinct layers. The upper aqueous layer transferred into a new vial, and this process was repeated.
d) An equal volume of chloroform/isoamyl alcohol (C:I=24:1) solution was added to the aqueous layer. To separate the aqueous layer into another vial, the mixture was gently mixed and centrifuged at 6500 rpm for 5 minutes at 4°C.
e) The DNA precipitation was done using 2.5 times the volume of ethanol or an equal volume of chilled isopropanol. The solution was kept at -20°C for 1-2 hours, followed by the centrifugation of the solution at 10,000rpm for 5 min.
f) After the centrifugation, the retrieved pellet was washed with ice-cold 70% ethanol by centrifugation at 10,000 rpm for the 5min. The obtained pellet was air-dried.
g) After that, the DNA pellet was dissolved in 200µl of sterile Tris-EDTA buffer.

4.2.2 Qualitative and quantitative estimation of genomic DNA

The quality of isolated DNA was estimated by agarose gel electrophoresis. The visualization of extracted DNA samples was examined using a 0.8% agarose gel with 0.5 µg/ml ethidium bromide (EtBr) immersed in 0.5X TBE. The samples were mixed with 6X loading dye and diluted with sterile water prior to loading in the wells. The presence or absence of DNA on the gel was determined using a UV-transilluminator (Johansson, 1972).

The quantitative estimation of extracted DNA was done using spectrophotometric analysis by Nanodrop (Nanodrop ND-100 spectrophotometer). A single unit of absorbance (1OD) of double-stranded DNA at 260 nm is equivalent to 50 ng/µl DNA. The absorbance ratio at 260 nm to absorbance at 280 nm was used to calculate the sample purity. A ratio of 1.8 must be obtained in the case of a pure DNA sample. Protein contaminants in the DNA sample are indicated by a ratio less than 1.8, whereas RNA or phenol impurities are indicated by a ratio greater than 1.8 (Desjardins

and Conklin, 2010). DNA was diluted to 100 ng/µl and stored at -20ºC for use as a template after a thorough assessment of concentration and purity.

4.3 Genotyping of polymorphic sites *of TS, ABC, and SLC* genes

4.3.1 Genotyping of TS polymorphism by PCR-RFLP

PCR-PFLP method was utilized to determine the genotype of variants as previously described (Yim *et al.*, 2010; Shi *et al.*, 2005), tabulated below in **table 4.1**. The polymorphic site was amplified using a conventional PCR method. The PCR mixture of 25 µl comprised of 1X PCR buffer, 1.5mM $MgCl_2$, 0.5µM of forward and reverse primer, 200µM dNTPs, 100 µg/ml bovine serum albumin (BSA), 1U Taq polymerase (DNAzyme, Thermo Scientific), and 200 ng DNA. PCR conditions utilized were: 95˚C for 5 min and 94˚C for the 30s (denaturation), annealing stage at an annealing temperature of the specific primer for 45s (annealing) followed by 72˚C for 30s (extension) for the 29 cycles as well as the final extension for 5 min at 72˚C.

After the PCR amplification, the PCR amplicon was processed using appropriate restriction enzymes purchased from New England Biolabs (NEB), Ipswich, MA, USA. PCR product of 10 µl was digested with 5U of enzyme in corresponding NEB buffer, thereby making a volume of 20µl for the reaction. The reaction mixture was then subjected to incubation at the desired temperature, which is particularly 37ºC for many enzymes, followed by incubation for 12-16 hours. The digested products were separated using polyacrylamide gel electrophoresis based on size. Bio-rad does the gel documentation Gel Doc™ XR+ Gel Documentation System. For scoring the SNPs, the restriction pattern for each SNP was determined by the generation or deletion of the restriction site in the lack of the variant allele. Two different people investigated the results to remove the undue biasness, and also 20% of the samples were selected randomly, and the reproducibility of the outcome was 100%.

4.3.2 Polyacrylamide gel electrophoresis (PAGE)

Polyacrylamide gel is being used to resolve proteins and nucleic acids in a solid support unit under the electric field in a buffer. A cross-linked polymer comprising acrylamide and bisacrylamide is used to make a PAGE gel. The total concentration and ratio of monomeric units (acrylamide and

bisacrylamide) determine the size of the pore. Polymerization is begun with the addition of ammonium persulphate (APS) and is catalyzed by N,N,N',N'-Tetramethyl- ethylenediamine (TEMED). PAGE has a limited separation range but an excellent resolving power. Polyacrylamide is used to separate fragments of DNA that are less than 500 bp in length. It can also be used to resolve DNA fragments that differ in length by a single base pair. The gels were visualized using ethidium bromide. Sambrook *et al.* (2001) provided the protocol, which was slightly modified.

4.3.3 Ethidium bromide (EtBr) staining

The Native-PAGE gel was subjected to staining in EtBr solution, which was made by adding ten µl of EtBr (0.5 µg/ml) to 50 ml of sterile water. The native-PAGE gel was kept in the EtBr staining solution for at least 20-25 min in the dark. After staining, the gel was examined under a UV-transilluminator to determine the bands after digestion. The genotyping was also scored in the gel documentation system Gel Doc™ XR by Bio-rad.

Table 4.1: List of primers and restriction enzymes used for *TS* genetic variants

Gene, SNP ID	Primer sequence (Forward and reverse)	Annealing temperature	Product Size (bp)	Restriction enzyme	Restriction pattern
***TSER* rs34743033 (2R/3R)**	FP=5′-CGTGGCTCCTGCGTTTCC-3′ RP=3′-GAGCCGGCCACAGGCAT-5′.	62ºC	2R=215, 3R=243	-	-
***1494del6* rs151264360 6bp ins/del**	FP=5′CAGATAAGTGGCAGTACAGA3′ RP=3′CAAATCTGAGGGAGCTGAGT5′.	58ºC	158	*DraI*	+6/+6=88, 70 bp -6/-6=152,6bp +6/6=152,88,70,6bp

4.3.4 Genotyping of ABCB1, ABCC1, ABCC2, and ABCG2 polymorphism by PCR-RFLP

The PCR-RFLP approach was used for genotyping each *ABCB1, ABCC1, ABCC2,* and *ABCG2* SNPs. The forward and reverse primers, respective restriction enzyme used, and restriction pattern are tabulated in **table 4.2**. Four SNPs of *ABCB1* were studied. Polymorphism of *ABCB1*, $C^{1236}T$ (rs1128503), $C^{3435}T$ (rs1045642), $G^{2677}A$ (rs2032582), and $G^{2677}T$ (rs2032582) were performed as previously reported by, Rustemoglu *et al.*, 2014 and Panczyk *et al.*, 2009. For *ABCC1* polymorphism, PCR-RFLP of the two polymorphisms $G^{2168}A$ (rs4148356) and $G^{3173}A$ (rs41410450) were

carried out as previously described (Yin *et al.*, 2009). For *ABCC2* $G^{4544}A$, (rs8187710) polymorphism, the genotyping was carried out as previously reported by Daly *et al.*, 2007. *ABCG2* variant, $C^{421}A$ *(*rs2231142), was genotyped by allele-specific PCR (AS-PCR) assay. The genotyping method and primers used were referred to in the previous study by Zheng *et al.*, 2016.

The PCR (25 µL) reaction contained 1X PCR buffer, 0.5 µM forward primer, 0.5 µM reverse primers, 200 µM dNTPs, 100 µg/mL bovine serum albumin (BSA), 1U Taq polymerase (DyNAzyme II, Thermo Scientific), and 200 ng DNA to amplify the required fragment. As indicated previously, the amplified product was digested with their appropriate restriction enzymes shown in **table 4.2**. The digested product was separated on a polyacrylamide gel or agarose gel and mixed with 2µl of 6X loading buffer containing sucrose, bromophenol blue, and xylene cyanol. The separated bands were stained with ethidium bromide under a UV transilluminator to determine the restriction patterns. Furthermore, the outcomes were documented by Gel Doc™ XR+ Gel Documentation System by Bio-rad. For scoring the SNPs, the restriction pattern for each SNP was determined on the generation or deletion of the restriction site in lack of the variant allele. Two individuals verified the data to ensure no errors, and 20% of the samples were randomly chosen to ensure that the results were 100% accurate.

Table 4.2: List of primers and restriction enzymes used for *ABCB1, ABCC1, ABCC2, and ABCG2* genetic variants

Gene, SNP ID	Primer sequence (Forward and reverse)	Annealing temperature	Product Size (bp)	Restriction enzyme	Restriction pattern
ABCB1, rs1128503, $C^{1236}T$	FP=TATCCTGTGTCTGTGAATTGCC RP= CCTGACTCACCACACCAATG	55ºC	366	*HaeIII*	CC: 269,97 TT:269,62,35 CT- 269, 97, 62,35
ABCB1, rs1045642 $C^{3435}T$	FP=TTGATGGCAAAGAAATAAAGC RP= CTTACATTAGGCAGTGACTCG	51ºC	206	*MboI*	CC: 130,76 TT: 206 CT=206,130,76
ABCB1, rs2032582 $G^{2677}A$	FP= TGCAGGCTATAGGTTCCAGG RP= GTTTGACTCACCTTCCCAG	55ºC	220	*BsrI*	GG:220 GA:20,206,14 AA: 206,14
ABCB1, rs2032582 $G^{2677}T$	FP= TGCAGGCTATAGGTTCCAGG RP=TTTAGTTTGACTCACCTTCCCG	55ºC	224	*BanI*	GG:198,26 GT: 224,198,26 TT: 224
ABCC1, rs4148356 $G^{2168}A$	FP=GCCTGGATTCAGAATGATTCTCTTC RP=TACTGACCTTCTCGCCAATCTCTGT	52ºC	157	*TaqI*	GG=132,25 AA=157 GA157,132,25
ABCC1, rs41410450 $\boldsymbol{G^{3173}A}$	FP= TCTGCATTGTGGAGTTTT RP= GACGAAGAAGTAGATGAGGC	52ºC	344	*PstI*	GG=300,44 AA=344 GA=344,300,44
ABCC2 rs8187710 $G^{4544}A$	FP=TCCTGGTTATTCTTATAAATGCCTA RP= ACAATCGAGGGGTTTCTCAA	44ºC	440	*RsaI*	GG=307,71,22 AA=196,111,71,22
Allele-Specific PCR (AS-PCR)					
ABCG2 rs2231142 $C^{421}A$	FP=TTGGCAAATCCTTGTATGAAGCAGT RP1=AGCCGAAGAGCTGCTGCTGAGAATTT RP2= AGCCGAAGAGCTGCTGAGAATTG	55ºC	320	-	CC(RP1) =320 TT(RP2) =320

4.3.5 Genotyping of SLC19A1, SLCO1B1, and SLCO1B3 polymorphism by PCR-RFLP

The genotype of *SLC19A1, SLCO1B1,* and *SLCO1B3* variations was determined using the PCR-PFLP method (Lima *et al.,* 2006; Liu *et al.,* 2013; Nagy *et al.,* 2015). The primers description, restriction enzyme used, and digestion pattern has been tabulated in **table 4.3**. The PCR reaction of 20µl comprised of 1X PCR buffer, 0.5µM of forward and reverse primer, 1.5mM $MgCl_2$, 100µg/ml bovine serum albumin (BSA), 200µM dNTPs, 1U Taq polymerase (DNAzyme, Thermo Scientific), and 200ng of DNA. PCR conditions (as shown in table 4.3) used for the mixture were: 95˚C for 5 min and 94˚C for the 30s (denaturation), 59˚C ($G^{80}A$/rs1051266), 55˚C

r($A^{388}G$/rs2306283), 60˚C ($T^{521}C$/rs4149056) and 57˚C($A^{1683\text{-}5676}G$/rs11045585) for 45s (annealing) followed by 72˚C for 30s (extension) for the 29 cycles as well as the final extension for 5 min at 72˚C. Digested fragments were separated on 8% Native-polyacrylamide gel electrophoresis (PAGE) and were stained with the ethidium bromide and visualized under UV trans-illuminator. Gel Doc™ XR+ Gel Documentation system did the gel documentation by Bio-rad. For scoring the SNPs, the restriction pattern for each SNP was determined by the generation or deletion of the restriction site in the lack of the variant allele. Two separate people looked into the data to eliminate any potential biases, and 20% of the samples were chosen at random, ensuring that the outcomes were 100% reproducible.

Table 4.3: List of primers and restriction enzymes used for *SLC19A1, SLCO1B1, and SLCO1B3* genetic variants

Gene, SNP ID	Primer sequence (Forward and reverse)	Annealing temperature	Product Size (bp)	Restriction enzyme	Restriction pattern
SLC19A1 rs1051266 $G^{80}A$	FP= AGTGTCACCTTCGTCCCCTC RP= CTCCCGCGTGAAGTTCTT	59˚C	230	*HhaI*	GG=125,68 AA=162,68 GA=162,125,68
SLCO1B1 rs2306283 $A^{388}G$	FP=ATAATGGTGCAAATAAAGGGG RP= ACTATCTCAGGTGATGCTCTA	55˚C	214	*TaqI*	AA=151,63 GG=128,63,23 AG=151,128,63.23
SLCO1B1 rs4149056 $T^{521}C$	FP= TTGTCAAAGTTTGCAAAGTG RP= GAAGCATATTACCCATGAGC	60˚C	209	*HhaI*	TT=209 CC=189, 20 TC=209,189,20
SLCO1B3 rs11045585 $A^{1683\text{-}5676}G$	FP= GTGGGTAAAAGGCAGGTAAATG RP=GAATTCAAACATCTCATGTGCTC	57˚C	286	*RsaI*	AA=262,24 GG=155,107,24 AG=262,155,107,24

4.4 Statistical analysis

4.4.1 Demographic analysis

Demographic variables, for example, age, smoking status, gender, and pack-years, were divided into categorical and continuous variables. Descriptive data are presented as median, mean ± standard deviation (SD), or number/percentages.

4.4.2 Genotypic and allelic Frequency analysis

Among cases, the Hardy–Weinberg equilibrium (HWE) theory ($p^2+2pq+q^2=1$; p is the frequency of the wild-type allele and q is the frequency of the mutant allele) was used to compute the genotypic frequencies of all polymorphisms by using χ^2 test. The minor allele frequency (MAF) was also computed for each polymorphic site. The Chi-square goodness-of-fit test was applied to see if the cases followed the Hardy-Weinberg equilibrium (Thakkinstian *et al.*, 2005).

4.4.3 Overall survival (OS) and Hazard ratio analysis

The association between overall survival and genetic polymorphism was studied using the Kaplan Meier and Log-rank tests. The univariate analysis called a log-rank test was utilized as a statistical method to compare the median survival time (MST) of patients based on the genotype of that particular variant. The Cox proportional hazard model was used to compute the hazard ratio (HR) and investigate the impact of genetic polymorphism on overall survival after adjusting for other factors such as age, smoking, gender, tumor stage, histology, regimen, and performance status. The hazard ratios and 95% confidence intervals (CI) were calculated using cox proportional hazards models. Cox's proportional hazards model is similar to a multiple regression model that enables the comparison between the survival time of different groups of patients to be tested while controlling for other factors (Mahimkar *et al.*, 2012). The hazards ratio was utilized to compute the death rate. The hazard is the possibility that a patient would witness relapse or die at a specific moment in time, provided that the subject has survived to that point without experiencing the event. Kaplan-Meier and cox regression approach was also applied in several subgroups stratified based on smoking habits, sex, histology, regimen, and performance status. Also, the different genotypic combinations, including the double and triple inter SNP combinations, were evaluated using the same approach. In all analyses, a p-value of <0.05 was considered significant. Medcalc version 16.8.1 (Medcalc Software, Ostend, Belgium) was used for all statistical analysis.

4.4.4 Stratified analysis

Stratified analysis was carried out to estimate risk for subgroups based on different parameters such as tumor stage, lymph node invasion, primary tumor extension, metastasis, and response rate

by odds ratios (ORs) and 95% confidence intervals (CIs) were determined. Unconditional multivariate logistic regression was used to determine the adjusted odds ratio (AOR) and 95% Confidence Intervals associated with each genotype and various genotypic combinations. The odds ratio was adjusted for age, gender, and smoking as these might act as confounding factors. All the variables in the study were dichotomous, except for the continuous variable, such as age. All p-values were two-sided, and a p-value of <0.05 was considered statistically significant (Begg and Zhang, 1994). Medcalc version 16.8.1 (Medcalc Software, Ostend, Belgium) was used for all statistical analyses.

4.4.5 Adverse events (AEs) analysis

Toxicity in lung cancer patients was assessed according to standard National Cancer Institution Criteria 3.0 (http://ctep.cancer.gov). Maximum attention was paid to hematological toxicity, such as neutropenia, anemia, leukopenia and thrombocytopenia, gastrointestinal toxicity, and nephrotoxicity (Table 4.4). Severe toxicity or severe event consisted of grade 3 or 4 toxicity. With severe hematologic toxicity, the subsequent treatment was postponed until recovery to grade 1 or grade 0. With grade 3 and 4 gastrointestinal toxicity, the doses of each drug were reduced by 25%. If creatinine clearance decreases to 59−41 ml min^{-1}, platinum was reduced by 25%. If creatinine clearance decreases below 40 ml min^{-1}, platinum was stopped. Clinical data have been systematically recorded during treatment. Analysis was done by calculating the odds ratio and 95% confidence intervals. Using multivariate logistic regression, the adjusted odds ratio (AOR) and 95% Confidence Intervals were calculated in association with each genotype and various genotypic combinations. The odds ratio was adjusted for age, gender, smoking, and regimen as these might act as confounding factors. In all analyses, a p-value of <0.05 was considered significant. Medcalc version 16.8.1 (Medcalc Software, Ostend, Belgium) was used for all statistical analysis.

Table.4.4: Summarizing adverse events grading for hematological, gastrointestinal, and nephrotoxicity (CTCAE v3.0; (Thomas *et al.*, 2009)

	Gradation of A/E	Grade I	Grade 2	Grade 3	Grade 4
Hematological toxicity	**Anemia**	Hb <12.0-10.0 gm/dL	Hb <10.0 -8.0 gm/dL	Hb <8.0 -6.5 gm/dL	Hb<6.5gm/dL
	Leukopenia	TLC <4500 -3000/µL	TLC <3000-2000/ µL	TLC <2000-1000/ µL	TLC <1000/ µL
	Neutropenia	*****ANC <2000 - 1500/ µL	ANC <1500 - 1000/ µL	ANC <1000 - 500/ µL	ANC <500/ µL
	Thrombocytopenia	Plt <1,50,000 - 75,000/ µL	Plt <75,000 - 50,000/ µL	Plt <50,000 - 25,000/uL	Plt <25,000/uL
Gastrointestinal toxicity	**Diarrhea**	≤4 stools/day	4-6 stools/day; IV fluids required <24hrs	≥7 stools/day; IV fluids required ≥24hrs	Hemodynamic collapse
	Vomiting	1 episode in 24 hrs	2-5 episodes in 24 hrs; IV fluids required <24 hrs	≥ 6 episodes in 24 hrs; IV fluids required >24 hrs	Hemodynamic collapse
	Constipation	Intermittent symptoms; occasional use of laxatives/enemas	Persistent symptoms; regular use of laxatives/enemas	Obstipation; manual evacuation required	Intestinal obstruction
	Anorexia	No alteration in oral intake	Alteration in oral intake but no significant weight loss or malnutrition	Significant weight loss or malnutrition; IV fluids or tube feedings indicated	Life-threatening consequences
Nephrotoxicity	**Chronic kidney disease**	******GFR≥90mL/min/1.73^2	GFR=89-60 mL/min/1.73^2	GFR=59-30 mL/min/1.73^2	GFR=29-15/ mL/min/1.73^2

Note: Hb- Hemoglobin; ANC- Absolute neutrophil count; Plt- Platelets; Glomerular filtration rate (GFR)

$$* \; Absolute\ neutrophil\ count\ (ANC) = \frac{(Total\ leucocyte\ count\ (TLC) * Neutrophils}{100}$$

$$** \; Glomerular\ filtration\ rate\ (GFR) = \frac{(140 - Age) * Weight(Kg)}{72 * serum\ creatinine} * 0.85(if\ female)$$

4.4.6 Survival analysis trees analysis

Furthermore, the recursive partitioning strategy accounted for high-order gene interactions. This method generates the initial split by considering the critical factor determining the patients' overall survival. The survival analysis trees (STREE) application was used to generate the decision tree (http://c2s2.yale.edu/software/stree). The log-rank approach was used as the splitting method. The generated tree was binary, with each terminal node representing a subset of individuals with a specific genotypic combination, thus displaying a range of survival times and prognosis. Multi-variate cox proportional hazard analysis was used to determine the HRs and 95% CIs for every terminal node, adjusted for gender, age, histology, ECOG, KPS, stage, and regimen.

CHAPTER 5

RESULTS

5.1 Genomic DNA Isolation

The genomic DNA was extracted and analyzed qualitatively and quantitatively. The quality of the isolated genomic DNA was done by running the DNA samples on 0.8% agarose gel electrophoresis. Figure 5.1 illustrates intact genomic DNA isolated from blood lymphocytes collected from lung cancer patients. The concentration of the isolated DNA samples was determined by measuring the absorbance at 260nm, while the purity of the nucleic acid was evaluated by taking the ratio of absorbance between 260nm and 280nm.

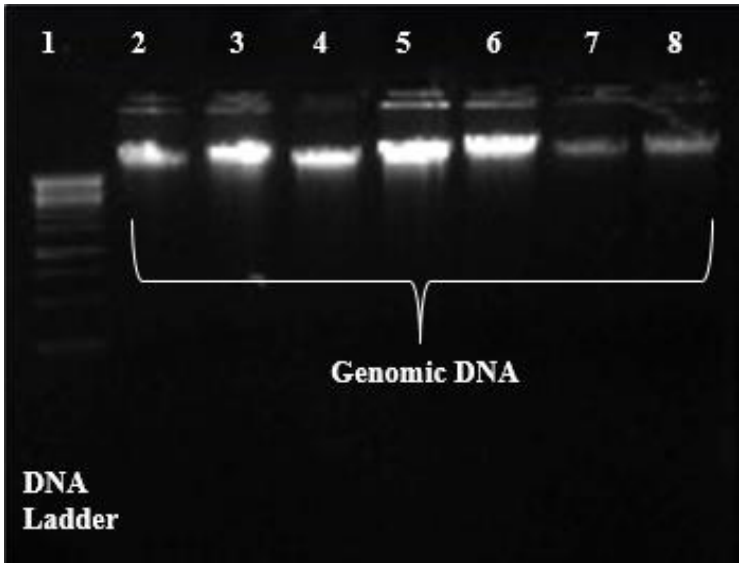


Figure 5.1: 0.8% agarose gel illustrating the isolated genomic DNA from the blood samples of patients with lung cancer (Lane 1-DNA ladder and Lane 2-8- genomic DNA)

5.2 PCR Amplification

PCR carried out the amplification of a gene. For each polymorphism, the amplified products of the PCR were confirmed by running 2.0 % agarose gel electrophoresis. A 100bp molecular weight marker was used to verify successful amplification. Figure 5.2 depicts amplification of the genomic DNA as an illustration for *TS 1494del6* polymorphism. An amplified PCR product of 158 bp was obtained and run along the 100 bp ladder.

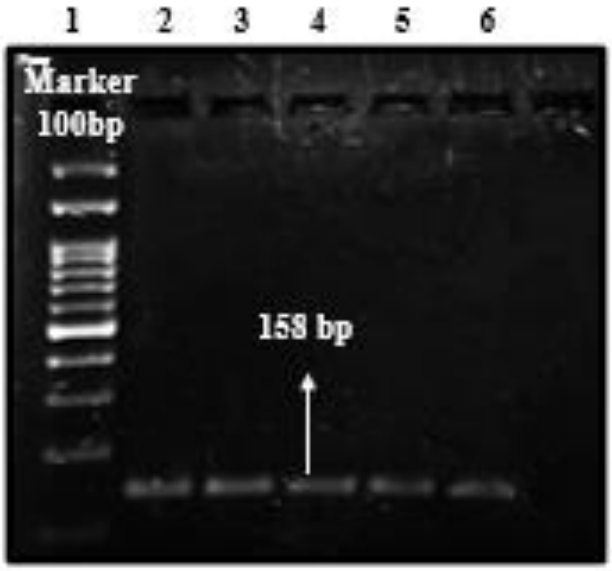


Figure 5.2: Illustration demonstrating agarose gel electrophoresis of the PCR amplification product (Lane 1-DNA ladder and Lane 2-6-amplified PCR product)

5.3 Genotyping of *Thymidylate synthase* (*TS)* transporter gene

5.3.1 *Genotyping of TS variants*

For the *TS* gene, genotyping of two polymorphisms in the 5'- and 3'-untranslated regions (UTRs) was performed. *TSER* polymorphism (*2R/3R*) present in the 5'-UTR region was studied. The amplified product of 215 bp was observed for wild-type alleles and recognized as double repeats (*2R)*, whereas a band of 243bp in the case of variant alleles was identified as triple repeats (*3R)*, and two bands of 215 bp and 243 bp were retrieved in case of heterozygous alleles (*2R/3R)*. **Figure 5.3A** depicted the amplified products *TSER* polymorphism separated on 6% polyacrylamide gel electrophoresis and stained with ethidium bromide.

TS 1494del6 (6 bp insertion/deletion) was studied in the 3'UTR, and PCR product of 158 bp was checked on 2.0% agarose gel and then digested with 5U of *Dra I* restriction enzyme (New England Biolabs) at 37˚C. Digested fragments were separated on 6% Native- polyacrylamide gel electrophoresis and were later stained with the help of ethidium bromide and visualized under a UV transilluminator (**Figure 5.3B)**. The wild type allele (*+6/+6*, homozygous 6 bp insertion) produced two bands of 88, 70 bp, variant allele (*-6/-6*, homozygous 6 bp deletion) were identified by one band of 152 bp, and heterozygous displayed all the three bands.

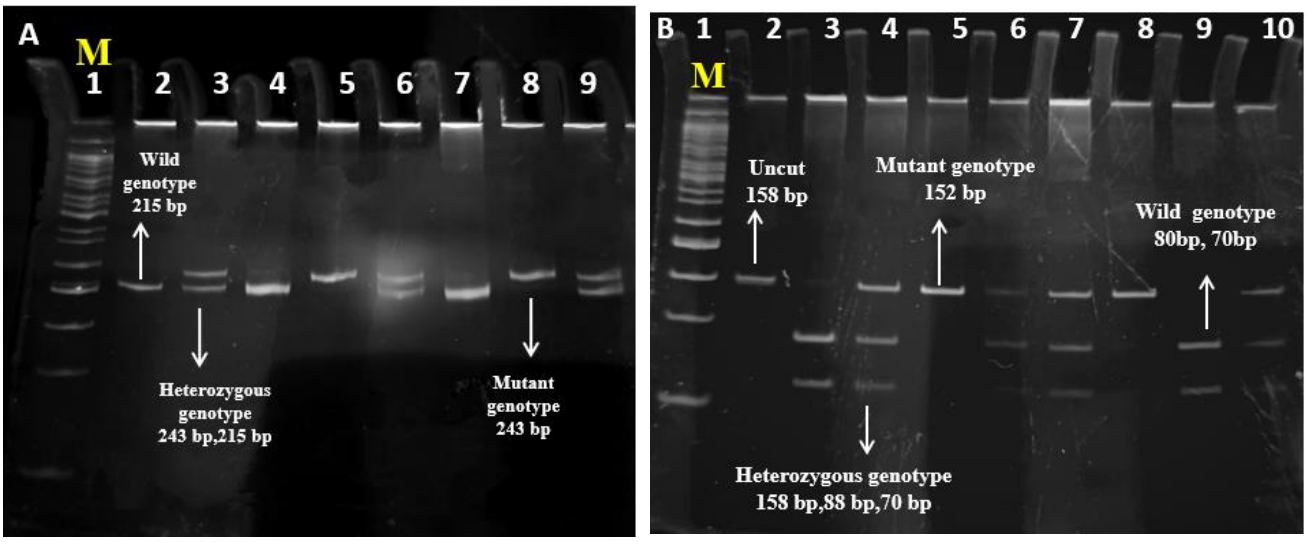


Figure 5.3: *TS* variants polymorphisms (A) 6.0% Native-PAGE of PCR products for *TSER* polymorphism. Wild genotype (*2R2R*): 215 bp; Mutant genotype (*3R3R*): 243 bp; Heterozygous genotype (*2R3R*): 215 bp, 243bp. Lane 1: Marker (M) (50 bp); Lane 2, 4, 7: Homozygous wild (*2R2R*) genotype; Lane 3,6,9: Heterozygous (*2R3R*) genotype; Lane 5,8: Homozygous variant (*3R3R*) (B) 6.0% Native-PAGE of PCR products after digestion with *DraI* for TS *1494del6* polymorphism Wild genotype *(+6/+6bp)*:80,70bp; Mutant genotype (*-6/-6bp*): 152 bp; Heterozygous genotype *(+6/-6bp)*: 158bp, 88bp, and 70bp Lane 1: Marker (M) (50 bp); Lane 2: Undigested PCR product; Lane 3, 9: Homozygous wild *(+6/+6bp)* genotype; Lane 4,6,7,10: Heterozygous *(+6/-6bp)* genotype; Lane 5,8: Homozygous variant (*-6/-6bp*) genotype

5.4 Statistical analysis

5.4.1 Demographic characteristics of the study group for TS gene

The study group recruited 700 patients, and the demographic features consisted of gender, age, smoking status, and other clinical parameters illustrated in **Table 5.1**. The mean age of the lung cancer subjects was 60±10.10 years (range 29–86) at diagnosis. There was a dominant proportion of 579 (82.71%) males compared with 121 (17.28%) females in the case group. The number of smokers was higher than non-smokers and accounted for 80.25% of the entire group. The average pack-year amongst smokers was 22.49±30.91. The patients were classified based on TNM staging: 0.71% of patients were diagnosed at stage I, 2.7% at stage II, 24% at stage III, and67.5% at stage IV lung cancer. The lung cancer subjects with T1 and T2 tumor sizes exhibited low frequencies of 5.42 % and 8.85 %, respectively, whereas patients with T3 and T4 tumor sizes constituted 19.85%

and 57%, respectively. Of all cases, 45.2% of the TNM data had no metastatic involvement (M0), while 49.28% had distant metastases (M1). For lymph node invasion, 12.4% of the lung cancer patients had no lymph node involvement, while subjects with N1, N2, N3, and N4 lymph node invasion were around 8.1, 43.4, 30.14, and 0.42%, of the total cases, respectively. Histologically, 41.4% of the lung cancer diagnosed were squamous cell carcinoma (SQCC) patients, 38.8% were detected with adenocarcinoma (ADCC), and 18.7% had small-cell lung cancer (SCLC).

Table 5.1: Demographic characteristics among case group

Variable	Total (N=700)	Cases, n (%)
Gender	700	
Male		579 (82.71)
Female		121 (17.28)
Age		
Mean ± SD	693	60±10.10
Range		29-86
Smoking status	699	
Smokers		561 (80.25)
Non-smokers		138 (19.74)
Pack years	548	
Mean ± SD		22.49±30.91
Histological types	700	
SQCC		285 (40.71)
ADCC		267 (38.14)
SCLC		129 (18.42)
Others		7 (1)
Unknown		12 (1.71)
TNM staging	700	
I		5 (0.9)
II		19 (3.78)
III		168 (24)
IV		473 (67.5)
Unclassified		35 (5)
Tumor Size	700	
Tx		23 (3.28)
T1		38 (5.42)
T2		62 (8.85)
T3		139 (19.85)
T4		399 (57)
Unknown		39 (5.57)
Lymph Node	700	
N0		87 (12.4)
N1		57 (8.1)
N2		304 (43.4)
N3		211 (30.14)
N4		3 (0.42)
Unknown		38 (5.42)
Metastasis	700	
M0		317 (45.2)
M1		345 (49.28)
Unknown		38 (5.42)

Abbreviations: SD= Standard deviation, n=total number of lung carcinoma cases, SQCC=squamous cell carcinoma, ADCC=Adenocarcinoma, and SCLC= small cell lung carcinoma

5.4.2 Clinical features of lung carcinoma patients assessed for prognostic significance of TS gene

The investigation's clinical characteristics and demographics on the overall survival of the lung carcinoma patients with variant *TSER* and *1494del6* are detailed in table 5.2. Males constituted 83.06% of the 679 individuals, while females comprised 16.93%. Since smoking is a known risk factor for lung cancer, most of the sample group were smokers (80.67%), while 19.32% were non-smokers. Clinical parameters, including histology, performance status, TNM stage, and chemotherapeutic regimen, are also included in Table 5.2. In the treated group, 571 of 700 patients were dead with median follow up time of 8.3 months. According to the histological categorization, 37.64% were adenocarcinomas, 41.8 % were squamous cell carcinomas, and 19.04% had small-cell lung carcinomas. TNM grading classified the participants in the study as follows: 3.53% stage I/stage II patients, 24.3% staged III patients, 67.01% stage IV patients, and 5.15% patients were unclassified. Additionally, the cases were classified based on Karnofsky's performance status (KPS) and the Eastern Cooperative Oncology Group (ECOG), representing the participants' performance status. In our investigation, there were 200 participants (30.48%) with KPS scores of 100-90, 340 (51.82%) with KPS scores of 80-70, and 116 subjects (19.2%) with KPS scores below 60. According to the ECOG score, 45.57% (299) of the individuals had ECOG scores ranging from 0 to 1, 38.71% (254) had ECOG 2, and 15.7% (103) had ECOG scores of 3-4. Among the case group, 485 patients were given cisplatin/carboplatin-based therapy, of which 29.27% patients received pemetrexed, 18.35% participants were administered with irinotecan, or paclitaxel, or docetaxel regimen, 6.18% patients received gemcitabine, 3.09% patients gefitinib, 3.50% patients received etoposide, and 2.68% patients were administered with other regimens.

Table 5.2: Patients characteristics and clinical features with overall survival

Variable	Total (N=679)	Cases, n (%)
Gender	679	
Male		564 (83.06)
Female		115 (16.93)
Age	672	
Mean ± SD		60.35±9.94
Range		29-86
Smoking status	678	
Smokers		547 (80.67)
Non-smokers		131 (19.32)
Histological types	672	
SQCC		281 (41.81)
ADCC		253 (37.64)
SCLC		128 (19.04)
Others		6 (0.89)
Unknown		4 (0.59)
Overall Survival	679	
Dead		571 (84.09)
Alive		108 (15.90)
TNM staging	679	
I/II		24 (3.53)
III		165 (24.3)
IV		455 (67.01)
Unclassified		35 (5.15)
ECOG performance status	656	
0-1		299 (45.57)
2		254 (38.71)
3-4		103 (15.7)
KPS performance status	656	
90-100		200 (30.48)
70-80		340 (51.82)
60 & below 60		116 (19.2)
Median Follow up (months)		**8.30**
Events, deaths		N=571 (84.09%)
Chemotherapy regimen	485	
Pemetrexed + cis/carboplatin		142 (29.27)
Irinotecan + cis/carboplatin		89 (18.35)
Docetaxel + cis/carboplatin		89 (18.35)
Paclitaxel + cis/carboplatin		89 (18.35)
Gemcitabine + cis/carboplatin		30 (6.18)
Gefitinib		15 (3.09)
Etoposide		17 (3.50)
Ceretinib		1 (0.20)
Others		13 (2.68)

Abbreviations: SD= Standard deviation, n=total number of lung carcinoma cases

5.5 Epidemiological statistics of Northern Indian lung carcinoma patients

5.5.1 Regional distribution of lung carcinoma patients

Lung cancer patients who visited the Postgraduate Institute of Medical Education and Research (PGIMER), Department of Pulmonary Medicine, were recruited for the current study, and blood samples were collected from the patients. PGIMER, Chandigarh is a multi-specialist public hospital that receives referrals from North India. The study included 700 lung cancer patients reported from Himachal Pradesh, Punjab, Haryana, Uttarakhand, Chandigarh, Jammu, and Kashmir. The PGIMER institutional ethics board has reviewed and approved this study (IEC-04/2018–884). As shown in figure 5.4, the highest proportion of patients was found in Himachal Pradesh (215, 30.71%), Haryana (139, 19.86%), and Punjab (167, 23.86%). The other lung carcinoma patients in the research came from Chandigarh (53, 7.57%), Uttar Pradesh (77, 11.00%), Jammu, and Kashmir (21, 3%), and Uttarakhand (22, 3.14%). The lowest proportion of lung cancer patients were from Bihar (1, 0.14%), Nepal (1, 0.14%), and Delhi (1, 0.14%).

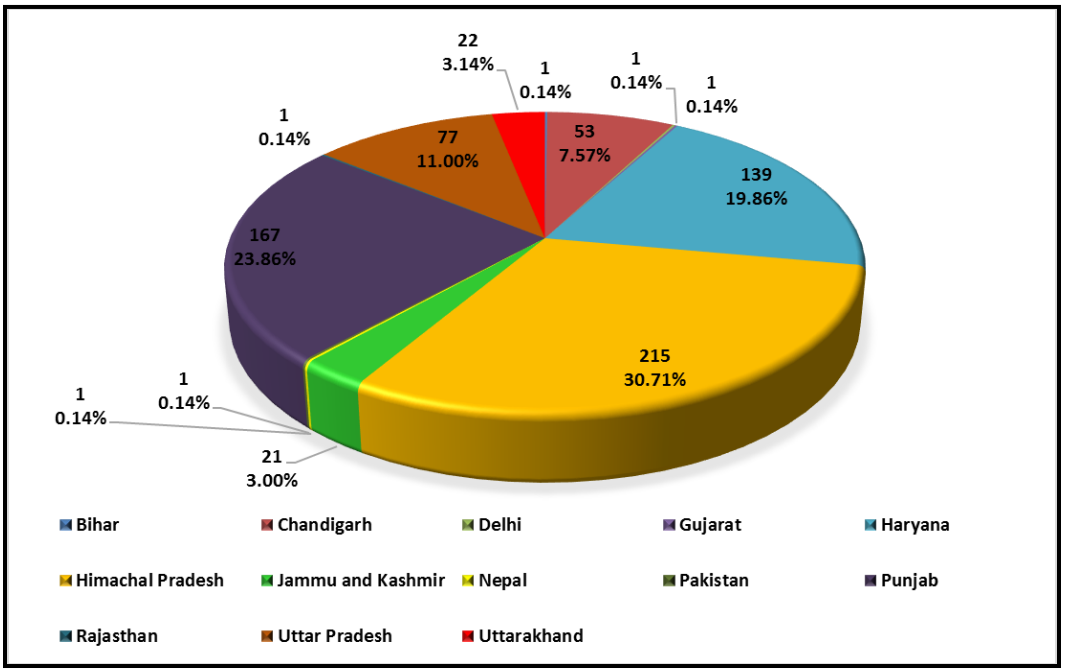


Figure 5.4: The regional distribution of Northern Indian lung carcinoma patients

The patients were further assessed for their gender-based regional distribution. In cases, the sample population consisted of 579 men (82.71%) and 121 females (17.28%). Himachal Pradesh (181, 31.26%), Haryana (116, 20.03%), and Punjab (141, 24.35%) regions had the highest proportion of men (Figure 5.5 (a)) while in the case of females' (Figure 5.5 (b)) highest proportion was from the region of Uttar Pradesh (19), Himachal Pradesh (34), Haryana (23), and Punjab (26). Figure 5.5 (a,b) depicts a diagrammatic illustration of the distribution of females and males in the study population.

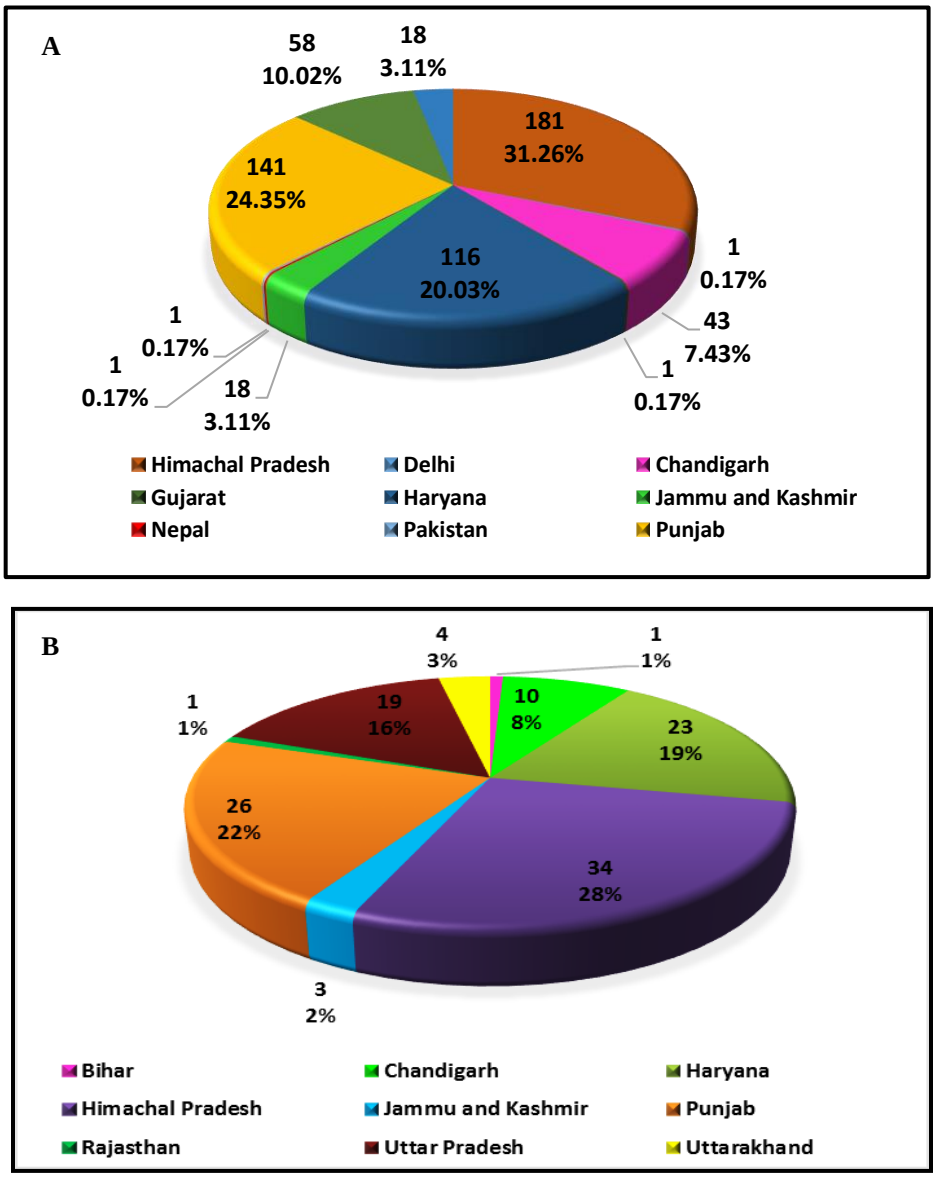


Figure 5.5: Regional distribution of Northern Indian Lung carcinoma patients based on gender (a) Males (b) Females

5.5.2 Distribution of lung carcinoma patients based on smoking status

One of the essential epidemiological data acquired was the smoking status of all patients. Based on their smoking behaviors, the study subjects were divided into smokers and non-smokers as smoking is a recognized risk factor for lung cancer, with more smokers among cases than non-smokers. Among 700 patients, the smoking status of 699 patients was known, while the smoking habit of one patient was not known. Our data showed that smokers contributed to 80.25% of all cases, whereas non-smokers accounted for 19.74%. Figure 5.6 illustrates a quantitative estimate of smoking by computing the pack years for the study subject. The mean pack-years for smokers were 22.49±30.91 years in the case group.

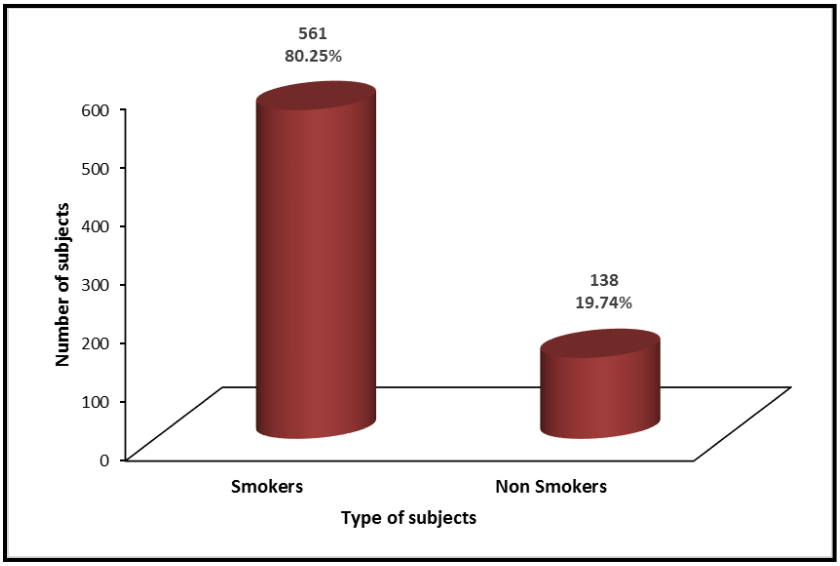


Figure 5.6: Distribution of Northern Indian Lung carcinoma patients based on smoking status

Furthermore, detailed information about the type of smoking was also logged. This data demonstrates that cigarette, beedi, and a combination of beedi and cigarette were among the several types of smoking used by study participants. However, in the case group, it was claimed that the study participants had also been exposed to beedi. The smoking habits of the study participants were also stratified based on their gender. In the studied patients, male smokers outnumbered female smokers by 522 (90.31%) to 39 (32.2%). As indicated in figure 5.7, males (9.68%) had a lower percentage of non-smokers than females (67.7%).

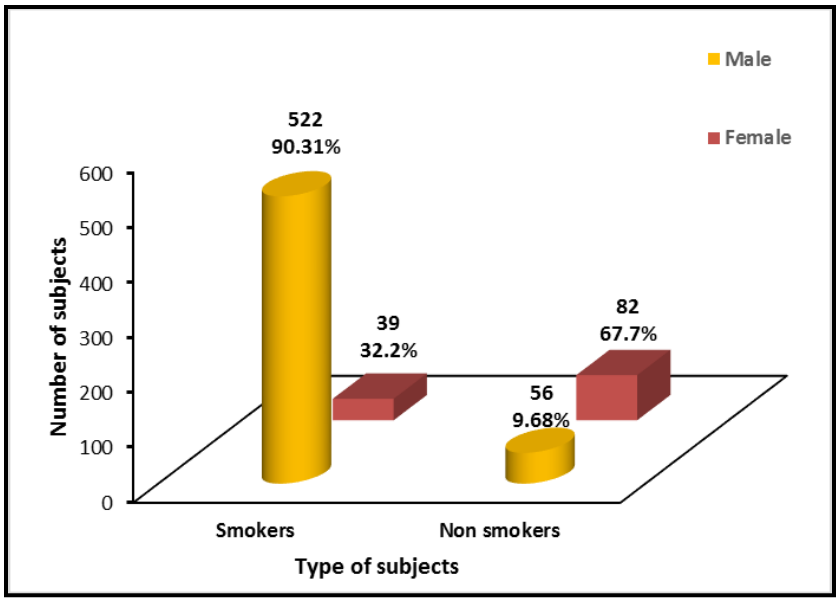


Figure 5.7: Graph illustrating the distribution of male and female study subjects based on smoking status

5.5.3 Distribution of lung carcinoma patients based on age

Age is an essential demographic characteristic associated with the onset of numerous diseases. The age of the study subjects was recorded in the current study; The mean age of lung cancer patients was calculated to be 60±10.10 (range 29-86). The median age in the case group was calculated to be 60 years. Males over the age of 60 were over-represented compared to those ≤ over the age of 60 (53.4 % versus 46.5%) among lung carcinoma patients. Similarly, 60% of females were reported to have an age ≤ 60, while 40% of females were found to have an age of more than 60 years. Figure 5.8 depicts a schematic representation of the gender distribution of study participants.

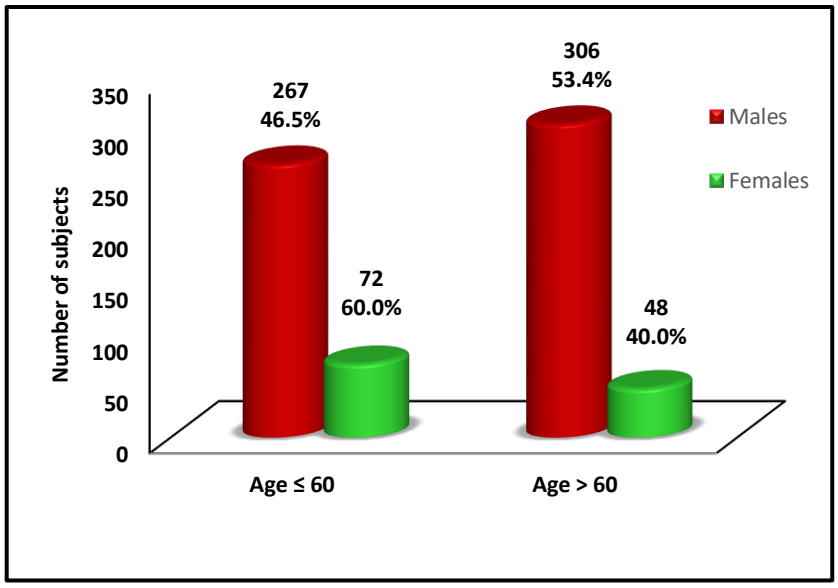


Figure 5.8: Graph illustrating the distribution of lung cancer patients based on age

5.5.4 Distribution of lung carcinoma patients based on TNM staging

All the clinical-pathological features of lung carcinoma patients were recorded from the hospital medical files. Depending on the clinical stages of the tumor, the lung cancer patients were categorized in the following: stage I, stage II, stage III, and stage IV. Data for 665 lung cancer patients were available, of which the lowest proportion of patients was in stage I and stage II, *i.e.*, 0.75% and 2.85%, respectively. Most lung cancer patients were noted to be stage III (168, 25.26%) and stage IV (473,71.12%). Figure 5.9 shows the proportion of lung cancer patients in different tumor stages. Additionally, the lung cancer subjects were also categorized based on various parameters of TNM, for example, direct tumor extension (T), lymph node invasion, and metastasis (M). Low frequencies of T1 and T2 were noted, *i.e.*, 5.42% and 8.85%, respectively, while T3 and T4 tumor sizes had high frequencies of 19.85% percent and 57%, respectively. Some patients were noted in N0 with 12.4% for lymph node involvement, while N1, N2, N3, and N4 had a rate of 8.1%, 43.4%, 30.14%, and 0.42%, respectively. Three hundred forty-five patients had already presented metastasis at the time of diagnosis, while 317 subjects had no metastatic involvement.

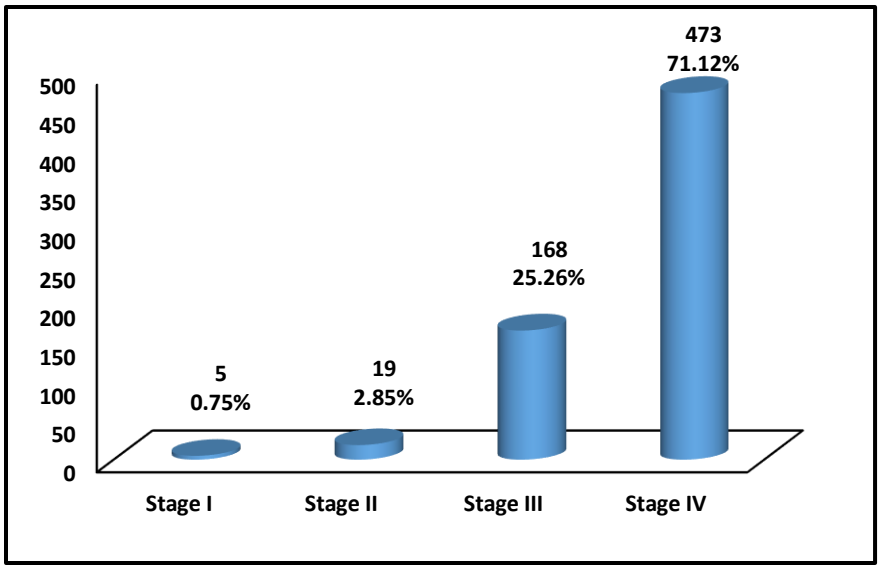


Figure 5.9: Graph illustrating the distribution of lung cancer patients based on tumor stage in North Indians

5.5.5 Distribution of lung carcinoma patients based on the tumor histology

Pathologists classify lung cancer into ADCC, SQCC, and SCLC histological subtypes. In this study, all patients were classified in the same manner. The occurrence of SQCC patients (41.36%) was highest among all the other histological subtypes in this analysis, preceded by ADCC (38.75%) and SCLC (18.72%), as depicted in figure 5.10. However, seven other lung cancer patients were classified with other histological subtypes such as giant cell carcinoma, LCC, NSCLC undifferentiated, and malignant mesothelioma, while the histology of 0.20% of patients was ambiguous. Our results show that SQCC is the common prevailing lung carcinoma in Northern Indians. We have evaluated the incidence of smokers in each histological subtype, and our results showed that 57.30% of people having ADCC were smokers; similarly, the highest proportion of smokers was noted in patients having SQCC (95.7%) while 92.9% of people with SCLC were recorded for the same. So, it is apparent that smoking is a prevalent cause of lung carcinoma.

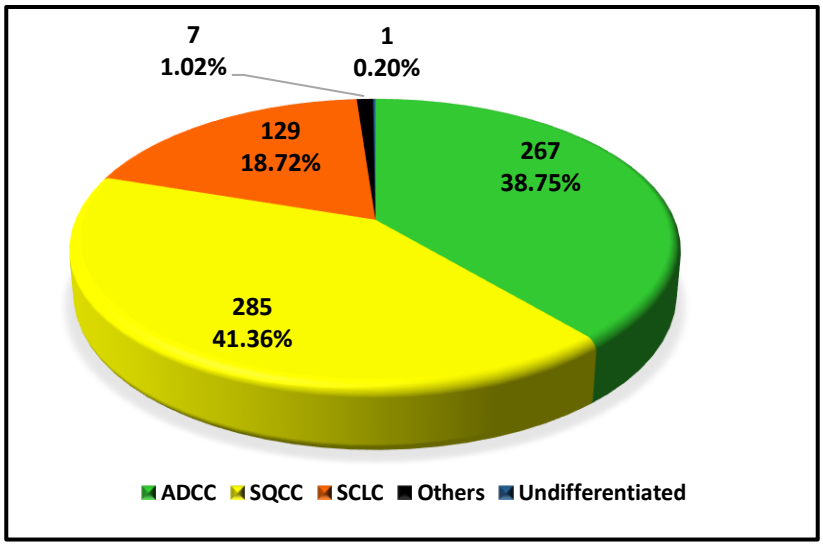


Figure 5.10: Distribution of Northern Indian Lung carcinoma patients based on histology

5.5.6 Distribution of lung carcinoma patients based on performance status

Lung carcinoma patients were also evaluated based on their performance status during the therapy. The Eastern Cooperative Oncology Group (ECOG)- performance status and Karnofsky performance status (KPS) are the two methods employed to determine the functional status of lung cancer patients. Based on ECOG performance status, 8.14% of lung cancer patients had ECOG status (0), 36.14% of subjects had ECOG status (1), whereas the majority of patients had ECOG status 2 (37%) while 15 % of subjects had ECOG status 3-4 as shown in figure 5.11.

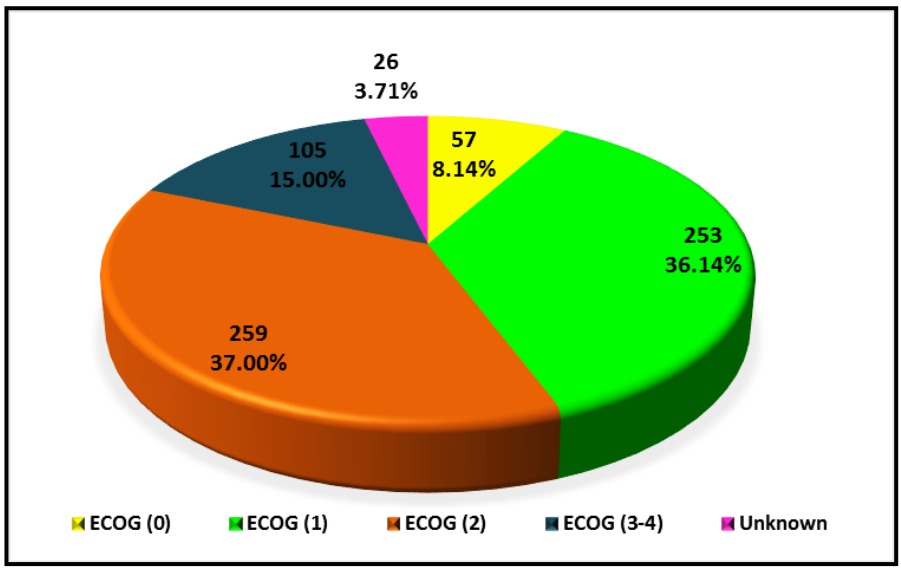


Figure 5.11: Distribution of Northern Indian Lung carcinoma patients based on ECOG performance status

When the lung cancer patients were analyzed based on KPS scores, our results demonstrated that 16.86% of patients had a KPS score of 90-100, specifying good performance status, 49.57% of patients had a KPS score of 70-80, while 29.71% lung cancer patients had a KPS score of 30-60, signifying poor performance as depicted in figure 5.12.

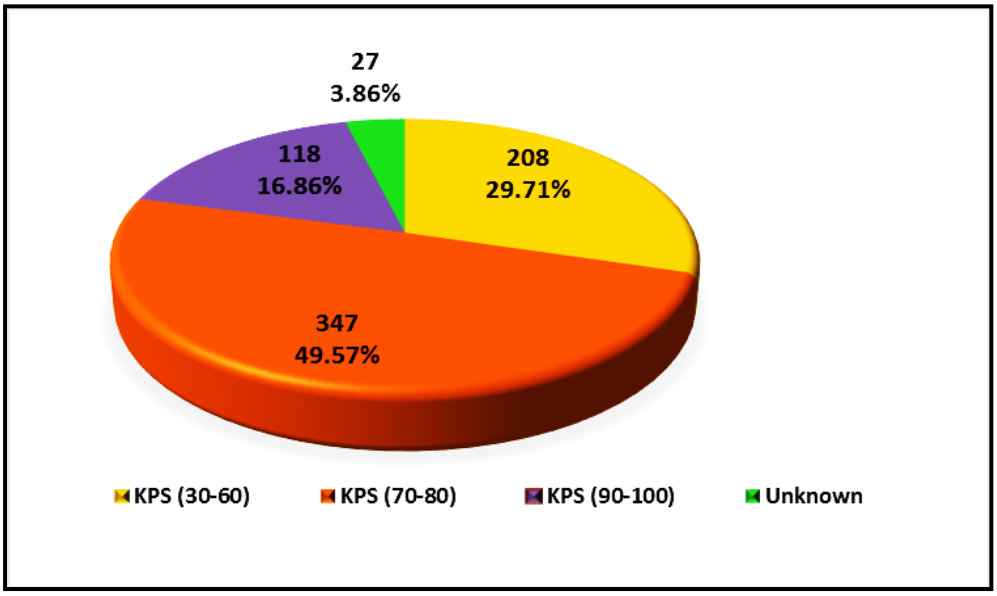


Figure 5.12: Distribution of Northern Indian lung carcinoma patients based on KPS score

5.5.7 Distribution of lung carcinoma patients based on platinum-based doublet chemotherapeutic regimen

Patients with lung cancer were treated with platinum-doublet chemotherapy in the present study (Figure 5.13). A second-line chemotherapeutic drug was administered based on the patient's histological classification of lung cancer and other fundamental clinical factors. Chemotherapeutic drugs like paclitaxel, docetaxel, pemetrexed, irinotecan, and gemcitabine were combined with platinum-based drugs like cisplatin or carboplatin. The data for the chemotherapy regimen was available for 510 lung cancer patients. Most patients were administered pemetrexed in combination with cisplatin/carboplatin (21.54%), followed by 12.84% of patients with paclitaxel and carboplatin/cisplatin.

Further, 12.98% of patients were treated with irinotecan and cisplatin/carboplatin, and 12.98% of patients were administered with docetaxel and cisplatin/carboplatin. Also, 4.42% received gemcitabine and cisplatin/carboplatin, 2.14% of patients were administered with gefitinib

and cisplatin/carboplatin, and 2.43% were subjected to etoposide and cisplatin/carboplatin. The patients treated with ceritinib and cisplatin/carboplatin were less represented (0.14%), and 3.28% of lung cancer patients were administered with other chemotherapy regimens such as crizotinib and erlotinib. The medical records for 27.25% of patients were not available, so such patients were placed in the unknown category.

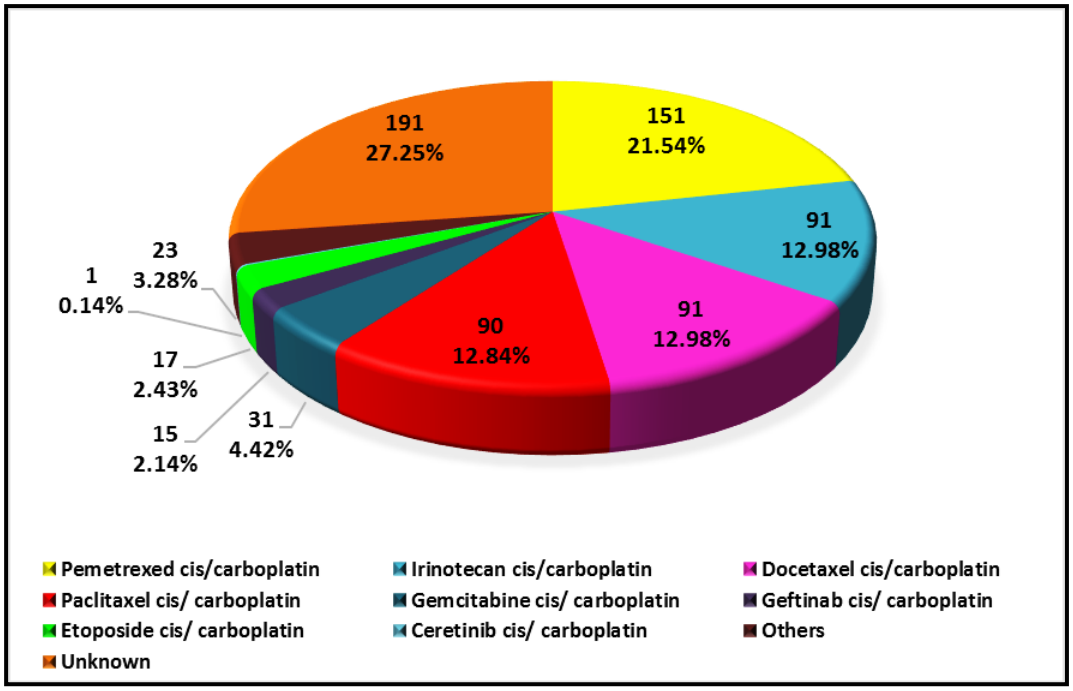


Figure 5.13: Distribution of Northern Indian lung carcinoma patients based on the chemotherapeutic regimen

5.6 Statistical analysis of *TS* gene

5.6.1 Genotype Frequencies of TS Variants

The distribution of genotypes for 5'- and 3'-untranslated regions (UTRs) in lung cancer patients has been illustrated in **Table 5.3**. Among the patients, *TS* distribution of 5' UTR (*TSER/ TS VNTR/* rs34743033) polymorphism was 18.57% in wild homozygous (*2R2R*), 45.28% in heterozygous (*2R3R*), and 36.14% in variant homozygous (*3R3R*) genotypes, which were consistent with Hardy-Weinberg equilibrium with a p-value 0.08 and χ^2=2.99. Minor allele frequency (MAF) for *TSER* polymorphism was calculated to be 0.58. Furthermore, for 3'UTR (*1494 ins/del6/ rs151264360*) polymorphism, a high prevalence of patients (24%) harbored homozygous wild genotype (*+6/+6bp)*, 57.71% had heterozygous genotype (*+6/-6bp),* and the lowest proportion of patients

(18.28%) had the variant homozygous genotype (-6/-6bp). The MAF for the *1494 ins/del* variant was 0.47.

Table 5.3: Genotypic and allelic frequencies of TS polymorphism in lung carcinoma patients

Genotype rs34743033 *TS* (VNTR)	**No. patients (%) n=700**	**χ2**	**P-value**
2R2R	130 (18.57)		
2R3R	317 (45.28)		
3R3R	253 (36.14)		
2R3R+3R3R	570 (81.4)	2.99	0.08
2R (allele)	577		
3R (allele)	823		
MAF	0.58		
Genotype rs151264360 (1494 ins/del6)	**No. patients (%) n=700**	**χ2**	**P-value**
+6/+6	168 (24)		
+6/-6	404 (57.71)		
-6/-6	128 (18.28)		
+6/-6 + -6/-6	532 (76)	17.48	<0.05
+6 (allele)	740		
-6 (allele)	660		
MAF	0.47		

5.7 Elucidation of overall survival (OS) of lung carcinoma patients with *TS* polymorphism

5.7.1 Overall survival analysis for TSER and 1494 ins/del6 polymorphism in lung cancer patients

Survival analysis for 679 lung cancer samples and its association with the *TS* polymorphisms has been studied and depicted in **table 5.4**. After three years of follow-up, 84.09% of patients were dead and 15.9% alive. After adjusting for gender, smoking status, age, tumor stage, histology, regimen, ECOG, and KPS, the association of the *TS* SNPs with the lung carcinoma survival was evaluated using both univariate and multivariate analysis. In our study, we have applied four different models (*i.e.,* co-dominant, dominant, recessive, and additive) to find the association of overall survival in lung carcinoma patients regarding *TS* polymorphism. The results have been presented as the best-fitted model, as shown in **Table 5.4.**

For *TSER* polymorphism, in the co-dominant model, we observed a higher median survival time (MST) in patients harboring both wild-type (*2R2R*) alleles than subjects who were carrying both the mutant (*3R3R*) alleles (MST=9.77 *vs.*7.57 months; log-rank p=0.11; HR=0.82). After

applying the Cox regression model, results showed poor survival outcomes in subjects carrying mutant (*3R3R*) alleles when compared with patients carrying wild (*2R2R*) allele (HR=1.29, 95%CI=1.01-1.66; p=**0.04**) as shown in the **figure 5.14.**

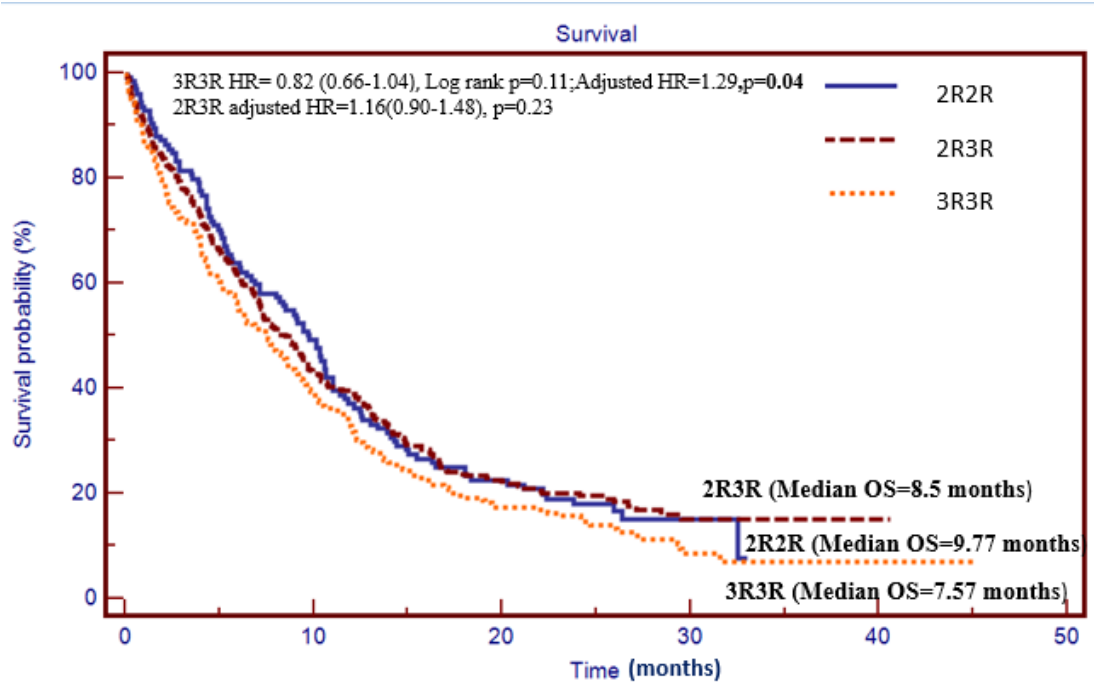


Figure 5.14: Kaplan Meier curves illustrating the association between overall survival in different genotypes of *TSER* polymorphism in lung cancer patients. (Note: *2R2R* represents wild-type genotype, *2R3R* represents heterozygous genotype, and *3R3R* represents homozygous for mutant genotype)

Further, our data showed no significance between the *TSER* (5' UTR) genotypes and the survival of lung cancer patients in both the dominant and additive models. However, in the recessive model, 63.7% of patients had homozygous wild and heterozygous genotype (*2R2R+2R3R)* and were compared with 36.2% of individuals having homozygous variant genotype (*3R3R)*. Our results showed a shorter survival time for lung cancer patients harboring mutant genotype (*3R3R)* than subjects carrying the wild type (*2R2R*) genotype (MST=8.97 *vs.* 7.57 months; log-rank p=**0.03**).

Table 5.4: Univariate and Multivariate analysis for *TS* variants

Genotype rs34743033 *TS*(VNTR)	**CASES n(%), N=679**	**DEAD n(%), N=571**	**ALIVE n(%), N=108**	**Univariate analysis**			**Multivariate analysis**	
				MST(months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b](95% CI)**	***p***
Codominant model								
2R2R	124(18.2)	104(18.2)	20(18.5)	9.77	-	1.0(reference)		
2R3R	309(45.5)	251(43.9)	58(53.7)	8.5	0.92	0.98(0.78-1.24)	1.16(0.90-1.48)	0.23
3R3R	246(36.2)	216(37.8)	30(27.7)	7.57	0.11	0.82(0.66-1.04)	1.29(1.01-1.66)	**0.04**
Dominant model								
2R2R	124(18.2)	104(18.2)	20(18.5)	9.77	-	1.0(reference)		
2R3R+3R3R	555(81.7)	467(82.7)	88(81.4)	7.9	0.41	0.91(0.74-1.12)	1.22(0.97-1.53)	0.08
Recessive model								
2R3R+2R2R	433(63.7)	355(62.1)	78(72.2)	8.97	-	1.0(reference)		
3R3R	246(36.2)	216(37.8)	30(27.7)	7.57	**0.03**	0.83(0.69-0.98)	1.21(1.01-1.44)	**0.03**
Additive model								
2R2R+3R3R	370(54.4)	320(56)	50(46.2)	8.3	-	1.0(reference)		
2R3R	309(45.5)	251(43.9)	58(53.7)	8.5	0.16	1.12(0.95-1.32)	0.94(0.79-1.12)	0.53
Genotype rs151264360 (1494 ins/del6)	**CASES n(%), N=679**	**DEAD n(%), N= 571**	**ALIVE n(%), N= 108**	**Univariate analysis**			**Multivariate analysis**	
				MST(months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b](95% CI)**	***P***
Codominant model								
+6/+6	159(23.4)	137(23.9)	22(20.3)	7.23	-	1.0(reference)		
+6/-6	398(58.6)	329(57.61)	69(63.8)	8.6	0.36	1.09(0.89-1.34)	0.90(0.73-1.10)	0.32
-6/-6	122(17.9)	105(18.3)	17(15.7)	9	0.56	1.07(0.83-1.38)	0.94(0.72-1.22)	0.62
Dominant model								
+6/+6	159(23.4)	137(23.9)	22(20.3)	7.23	-	1.0(reference)		
+6/-6 + -6/-6	520(76.5)	434(76)	86(79.6)	8.67	0.36	1.09(0.89-1.33)	0.90(0.74-1.09)	0.31
Recessive model								
+6/-6 + +6/+6	557(82.0)	466(81.6)	91(84.2)	8.2	-	1.0(reference)		
-6/-6	122(17.9)	105(18.3)	17(15.7)	9.0	0.95	1.00(0.81-1.24)	0.98(0.79-1.22)	0.98
Additive model								
+6/+6 + -6/-6	281(41.3)	242(42.3)	39(36.1)	7.7	-	1.0(reference)		
+6/-6	398(58.6)	329(57.6)	69(63.8)	8.6	0.47	1.06(0.89-1.25)	0.93(0.78-1.11)	0.44

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, histology, KPS, ECOG

Multivariate Cox proportional hazards analyses further revealed that, besides gene polymorphisms, the OS was also associated with the ECOG and smoking of patients with lung cancer. It should be noted that the overall hazard ratio of patients carrying the *3R/3R* genotype at *TS VNTR* were 1.21 times higher than in patients carrying the *2R/2R+2R/3R* genotype (**Table 5.5**). Hence, ECOG, stage, smoking, and *3R/3R* genotype in the VNTR site of the *TS* gene could be key factors contributing to the development of lung cancer. Moreover, we found that gender, age, KPS score, and stage did not correlate with OS (gender, P=0.688; age, P=0.305; KPS score, P=0.655).

Table 5.5: Cox proportional hazards regression model analysis of overall survival in lung cancer subjects

N=679	b	SE	P-value	Adjusted HR (Exp b)	95% CI of Exp (b)
Age	0.0048	0.0047	0.3056	1.0048	0.995 to 1.014
Sex	-0.055	0.139	0.6886	0.9456	0.720 to 1.241
Smoking	0.299	0.137	0.02913	1.349	1.032 to 1.763
Stage	0.159	0.081	0.0509	1.1723	1.00 to 1.374
Regimen	0.0090	0.0525	0.8639	1.009	0.9107 to 1.11
ECOG	0.3770	0.075	0.00000058	1.4579	1.258 to 1.689
KPS	0.0348	0.078	0.6554	1.0355	0.889 to 1.205
TS_*TSER*	0.194	0.090	0.0322	1.2142	1.017 to 1.448

For *1494 ins/del6* polymorphism, Kaplan Meier curve analysis showed higher survival in patients harboring mutant (*-6/-6bp)* genotype when compared to the subjects having wild (*+6/+6bp)* type genotype (MST=9 *vs.* 7.23 months, log-rank p=0.62; HR=0.94). Better survival was observed in patients having heterozygous genotype (*+6/-6bp)* when compared to wild-type genotype (*+6/+6bp)* (MST=8.6 *vs.* 7.23 months; log-rank p-value=0.32; HR=0.90). However, the results were not significant, as shown in **table 5.4**. None of the other models showed prognostic significance for *1494del6* polymorphism for overall survival in lung cancer subjects. Therefore, we summarize that our data revealed no significant contribution of *1494del6* polymorphism in predicting OS and individual genotype in the lung cancer subjects.

5.7.2 Association of TS polymorphism with histology and OS

We further evaluated the role of TS polymorphisms in predicting the overall survival of lung cancer patients based on histological subtypes. The data for the same has been presented in **Table 5.6**. In the present study, patients having SQCC and *TSER* polymorphism showed a majority of patients having variant genotype (*3R3R)* as that of wild type genotype (*2R2R)* (37.3% *vs.* 19.2%). Our results showed the reduced survival in the homozygous variant genotype (*3R3R)* in SQCC patients compared to the homozygous wild genotype (*2R2R)*. However, results were not substantially significant even after the multivariate cox-regression model (MST=7.93 *vs.* 8.37, p=0.13). On the contrary, SQCC patients having *1494ins/del6* variations showed better survival in patients having both copies of the mutant allele (*-6/-6bp)* as compared to the SQCC patients having homozygous wild allele (*+6/+6bp)* (MST=8.03 *vs.* 5.97); however, our results did not show any significant association between the genotypes as shown in **table 5.6**.

Additionally, the ADCC patients harboring the mutant genotype (*3R3R*) showed poor survival outcomes compared to those patients with wild-type genotype (*2R2R*) for *TSER* (MST= 6.1 months *vs.* 10.3 months; log-rank p=0.39). After multivariate cox analysis, the HR was 1.25 (95% CI=0.83-1.89), though the results were not statistically significant in either of the models. For *1494 ins/del6* polymorphism, our data showed a higher survival time in patients carrying the mutant (*-6/-6bp)* genotype as compared to subjects with wild genotype *(+6/+6bp)* (MST= 11.87 *vs.* 7.7 months; p=0.42; HR=0.83, 95% CI=0.52-1.30).

Furthermore, upon analyzing the survival trend in SCLC patients having *TSER* polymorphism, our results showed that 39% of people harbored variant genotype (*3R3R*) and had poor survival as compared to wild-type genotype (*2R2R*) (MST=8.67 *vs.* 10.6, p=0.27). Contrastingly, SCLC patients with *1494del6* polymorphism showed a marginal increase in survival in patients having mutant genotype (*-6/-6bp)* as compared to the wild-type genotype (*+6/+6bp)* (MST=9.93 *vs.* 9.3, p=0.51). However, this polymorphism did not have any substantial role in predicting the survival in SCLC patients. Therefore, these results show that none of the histological parameters such as SQCC, ADCC, and SCLC were associated with predicting any prognostic significance for the *TSER* and *1494 ins/del6* polymorphisms, as illustrated in table 5.6.

Table 5.6: Association of *TS* SNPs on overall survival according to histology

SQCC								
Genotype rs34743033 *TS* (VNTR)	**CASES n (%), N=281**	**DEAD n (%), N=236**	**ALIVE n (%), N=45**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
2R2R	54 (19.2)	46 (19.4)	8 (17.7)	8.37	-	1.0 (reference)		
2R3R	122 (43.4)	97 (41.1)	25 (55.5)	7.33	0.75	1.05 (0.74-1.50)	1.41 (0.94-2.1)	0.09
3R3R	105 (37.3)	93 (39.4)	12 (26.6)	7.93	0.61	0.91 (0.64-1.29)	1.34 (0.91-1.96)	0.13
Dominant model								
2R2R	54 (19.2)	46 (19.4)	8 (17.7)	8.37	-	1.0 (reference)		
2R3R+3R3R	227 (80.7)	190 (80.5)	37 (82.2)	7.5	0.94	0.98 (0.71-13.63)	1.34 (0.94-1.89)	0.09
Recessive model								
2R3R+2R2R	176 (62.6)	143 (60.5)	33 (73.3)	7.33	-	1.0 (reference)		
3R3R	105 (37.3)	93 (39.4)	12 (26.6)	7.93	0.30	0.87 (0.66-1.13)	1.22 (0.93-1.59)	0.14
Genotype rs151264360 (1494 ins/del6)	**CASES n (%), N=281**	**DEAD n (%), N=236**	**ALIVE n (%), N=45**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
+6/+6	73 (25.9)	64 (27.1)	9 (20)	5.97	-	1.0 (reference)		
+6/-6	156 (55.5)	127 (53.8)	29 (64.4)	8.63	0.17	1.22 (0.90-1.69)	0.88 (0.64-1.19)	0.42
-6/-6	52 (18.5)	45 (19.0)	7 (15.5)	8.03	0.46	1.15 (0.78-1.68)	1.05 (0.70-1.57)	0.78
Dominant model								
+6/+6	73 (25.9)	64 (27.1)	9 (20)	5.97	-	1.0 (reference)		
+6/-6 + -6/-6	208 (74.0)	172 (72.8)	36 (80)	8.57	0.19	1.21 (0.90-1.65)	0.92 (0.68-1.23)	0.58
Recessive model								
+6/-6 + +6/+6	229 (81.4)	191 (80.9)	38 (84.4)	7.3	-	1.0 (reference)		
-6/-6	52 (18.5)	45 (19.0)	7 (15.5)	8.03	0.92	0.98 (0.71-1.36)	1.11 (0.79-1.54)	0.53
ADCC								
Genotype rs34743033 *TS* (VNTR)	**CASES n (%), N=253**	**DEAD n (%), N=204**	**ALIVE n (%), N=49**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
2R2R	51 (20.1)	42 (20.5)	9 (18.3)	10.3	-	1.0 (reference)		
2R3R	117 (46.2)	92 (45.0)	25 (51.0)	9.23	0.89	1.0 (0.71-1.48)	0.96 (0.65-1.42)	0.87
3R3R	85 (33.5)	70 (34.3)	15 (30.6)	6.1	0.39	0.84 (0.58-1.23)	1.25 (0.83-1.89)	0.28
Dominant model								
2R2R	51 (20.1)	42 (20.5)	9 (18.3)	10.3	-	1.0 (reference)		
2R3R+3R3R	202 (79.8)	162 (79.4)	40 (81.6)	7.9	0.75	0.94 (0.67-1.32)	1.08 (0.76-1.54)	0.65

Recessive model								
2R3R+2R2R	168 (66.4)	134 (65.6)	34 (69.3)	9.43	-	1.0 (reference)		
3R3R	85 (33.5)	70 (34.3)	15 (30.6)	6.1	0.16	0.81 (0.59-1.09)	1.30 (0.96-1.77)	0.08
Genotype rs151264360 (1494 ins/del6)	**CASES n (%), N=253**	**DEAD n (%), N=204**	**ALIVE n (%), N=49**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
+6/+6	61 (24.1)	49 (24.0)	12 (24.4)	7.7	-	1.0 (reference)		
+6/-6	148 (58.4)	120 (58.8)	28 (57.1)	8.23	0.89	0.97 (0.70-1.36)	0.86 (0.61-1.22)	0.41
-6/-6	44 (17.3)	35 (17.1)	9 (18.3)	11.87	0.74	1.07 (0.69-1.65)	0.83 (0.52-1.30)	0.42
Dominant model								
+6/+6	61 (24.1)	49 (24.0)	12 (24.4)	7.7	-	1.0 (reference)		
+6/-6 + -6/-6	192 (75.8)	155 (75.9)	37 (75.5)	8.73	0.99	1.0 (0.72-1.38)	0.84 (0.60-1.18)	0.33
Recessive model								
+6/-6 + +6/+6	209 (82.6)	169 (82.8)	40 (81.6)	8.23	-	1.0 (reference)		
-6/-6	44 (17.3)	35 (17.1)	9 (18.3)	11.87	0.61	1.09 (0.76-1.56)	0.93 (0.63-1.35)	0.71
SCLC								
Genotype rs34743033 *TS* (VNTR)	**CASES n (%), N=128**	**DEAD n (%), N=119**	**ALIVE n (%), N=9**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
2R2R	17 (13.2)	16 (13.4)	1 (11.1)	10.6	-	1.0 (reference)		
2R3R	61 (47.6)	55 (46.2)	6 (66.6)	7.33	0.68	0.89 (0.51-1.53)	1.32 (0.71-2.46)	0.36
3R3R	50 (39.0)	48 (40.3)	2 (22.2)	8.67	0.30	0.74 (0.44-1.29)	1.41 (0.76-2.61)	0.27
Dominant model								
2R2R	17 (13.2)	16 (13.4)	1 (11.1)	10.6	-	1.0 (reference)		
2R3R+3R3R	111 (86.7)	103 (86.5)	8 (88.8)	8.26	0.49	0.83 (0.51-1.38)	1.36 (0.77-2.41)	0.28
Recessive model								
2R3R+2R2R	78 (60.9)	71 (59.6)	7 (77.7)	9.33	-	1.0 (reference)		
3R3R	50 (39.0)	48 (40.3)	2 (22.2)	8.67	0.22	0.79 (0.53-1.15)	1.19 (0.80-1.76)	0.39
Genotype rs151264360 (1494 ins/del6)	**CASES n (%), N=128**	**DEAD n (%), N=119**	**ALIVE n (%), N=9**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
+6/+6	21 (16.4)	20 (16.8)	1 (11.1)	9.3	-	1.0 (reference)		
+6/-6	81 (63.2)	74 (62.1)	7 (77.7)	8.4	0.92	1.02 (0.62-1.68)	0.94 (0.55-1.61)	0.83
-6/-6	26 (20.3)	25 (21.0)	1 (11.1)	9.93	0.64	0.87 (0.47-1.58)	0.79 (0.39-1.59)	0.51

Dominant model								
+6/+6	21 (16.4)	20 (16.8)	1 (11.1)	9.3	-	1.0 (reference)		
+6/-6 + -6/-6	107 (83.5)	99 (83.1)	8 (88.8)	8.67	0.99	0.99 (0.61-1.61)	0.92 (0.54-1.56)	0.77
Recessive model								
+6/-6 + +6/+6	102 (79.6)	94 (78.9)	8 (88.8)	8.67	-	1.0 (reference)		
-6/-6	26 (20.3)	25 (21.0)	1 (11.1)	9.93	0.61	0.89 (0.56-1.40)	0.85 (0.52-1.39)	0.52

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex smoking, stage, regimen, KPS, and ECOG

5.7.3 Association of TS polymorphism with chemotherapeutic regimen and OS

The lung cancer patients in this study were treated with platinum-based chemotherapy along with docetaxel, paclitaxel, pemetrexed, and irinotecan. So, we looked into the modulating effects of the *TS* polymorphism and its correlation to chemotherapy treatment and overall patient survival. **Table 5.7** shows the effect of the *TS* polymorphisms on overall survival when stratified based on chemotherapeutic regimens. Chemotherapy consisted of four different regimens that were given to different patient groups. All lung cancer patients were treated with a platinum-based therapy (cisplatin/carboplatin) and a second-line chemotherapeutic treatment such as pemetrexed, docetaxel paclitaxel, or irinotecan.

Patients who were administered with cisplatin/carboplatin along with docetaxel and carrying the *TSER* wild type alleles (*2R2R*) showed marginal survival time differences when compared with subjects harboring the mutant type alleles (*3R3R*) (MST= 8.37 *vs.* 8.63; p=0.68). In the case of the *1494 ins/del6* polymorphism, the subjects carrying both the copies of the wild type allele (*+6/+6bp*) showed better survival when compared with mutant type alleles (*-6/-6bp)* (MST=12.56 *vs.* 9.13 months; p=0.06), but both the *TSER* polymorphism and *1494del6* variations were not found to be substantially associated in predicting overall survival in lung cancer patients undergoing docetaxel along with cisplatin/carboplatin combined therapy.

Additionally, for the patients administered with carboplatin/cisplatin and pemetrexed, a survival trend was observed in *TSER* polymorphism, but there was no relationship in OS between different genotypes for *TSER 1494 ins/del6* polymorphism. Even in the case of lung carcinoma patients receiving cisplatin/carboplatin along with paclitaxel or irinotecan, no significant relationship was seen between OS and *TSER* polymorphism (**Table 5.7**). So, on analyzing the survival outcomes in both the polymorphism, we conclude that none of the *TS* polymorphisms studied were associated with prognosis in lung cancer patients undergoing platinum-based doublet chemotherapy (docetaxel, paclitaxel, pemetrexed, and irinotecan).

Table 5.7: Association of *TS* SNPs on overall survival based on the regimen

Genotype rs34743033 *TS* (VNTR)	CASES n (%), N=89	DEAD n (%), N=75	ALIVE n (%), N=14	Univariate analysis MST (months)	Log rank *p*	Unadjusted HR[a]	Multivariate analysis Adjusted HR[b] (95% CI)	*P*
REGIMEN- Docetaxel cisplatin/carboplatin								
Codominant model								
2R2R	26 (29.2)	21 (28)	5 (35.7)	8.37	-	1.0 (reference)	-	-
2R3R	30 (33.7)	23 (30.6)	7 (50)	10.4	0.24	1.41 (0.78-2.64)	0.75 (0.36-1.54)	0.44
3R3R	33 (37.0)	31 (41.3)	2 (14.2)	8.63	0.88	0.96 (0.55-1.67	1.13 (0.60-2.13)	0.68
Dominant model								
2R2R	26 (29.2)	21 (28)	5 (35.7)	8.37	-	1.0 (reference)		
2R3R+3R3R	63 (70.7)	54 (72)	9 (64.2)	9.77	0.55	1.16 (0.69-1.97)	0.94 (0.53-1.66)	0.84
Recessive model								
2R3R+2R2R	56 (62.9)	44 (58.6)	12 (85.7)	9.73	-	1.0 (reference)	-	-
3R3R	33 (37.0)	31 (41.3)	2 (14.2)	8.63	0.30	0.78 (0.48-1.25)	1.37 (0.83-2.26	0.20
Genotype rs151264360 (1494 ins/del6)	**CASES n (%), N=89**	**DEAD n (%), N=75**	**ALIVE n (%), N=14**	**Univariate analysis MST (months)**	**Log-rank *p***	**Unadjusted HR[a]**	**Multivariate analysis Adjusted HR[b] (95% CI)**	***P***
Codominant model								
+6/+6	11 (12.3)	7 (9.3)	4 (28.5)	12.56	-	1.0 (reference)		
+6/-6	56 (62.9)	47 (62.6)	9 (64.2)	8.63	0.17	0.58 (0.32-1.23)	1.92 (0.70-5.24)	0.20
-6/-6	22 (24.7)	21 (28)	1 (7.14)	9.13	0.06	0.46 (0.22-1.05)	2.95 (0.95-9.11)	0.06
Dominant model								
+6/+6	11 (12.3)	7 (9.3)	4 (28.5)	12.56	-	1.0 (reference)		
+6/-6 + -6/-6	78 (87.6)	68 (90.6)	10 (71.4)	8.8	0.11	0.54 (0.3-1.1)	2.21 (0.89-5.51)	0.08
Recessive model								
+6/-6 + +6/+6	67 (75.2)	54 (72)	13 (92.8)	9.73		1.0 (reference)		
-6/-6	22 (24.7)	21 (28)	1 (7.14)	9.13	0.26	0.75 (0.42-1.26)	1.63 (0.93-2.86)	0.08
REGIMEN- Paclitaxel cisplatin/carboplatin								
Genotype rs34743033 *TS* (VNTR)	**CASES n (%), N=89**	**DEAD n (%), N=75**	**ALIVE n (%), N=14**	**Univariate analysis MST (months)**	**Log rank *p***	**Unadjusted HR[a]**	**Multivariate analysis Adjusted HR[b] (95% CI)**	***P***
Codominant model								
2R2R	15 (16.8)	13 (17.3)	2 (14.2)	5.67	-	1.0 (reference)		
2R3R	40 (44.9)	32 (42.6)	8 (57.1)	7.27	0.56	1.20 (0.61-2.40)	0.77 (0.38-1.57)	0.48
3R3R	34 (38.2)	30 (40)	4 (28.5)	6.9	0.62	1.17 (0.59-2.33)	0.65 (0.31-1.37)	0.26
Dominant model								
2R2R	15 (16.8)	13 (17.3)	2 (14.2)	5.67	-	1.0 (reference)		
2R3R+3R3R	74 (83.1)	62 (82.6)	12 (85.7)	7.33	0.54	1.19 (0.64-2.30)	0.69 (0.36-1.31)	0.26
Recessive model								
2R3R+2R2R	55 (61.7)	33 (44)	10 (71.4)	6.1	-	1.0 (reference)		
3R3R	34938.2)	30 (40)	4 (28.5)	6.9	0.98	0.99 (0.62-1.58)	0.70 (0.42-1.17)	0.18
Genotype rs151264360 (1494 ins/del6)	**CASES n (%), N=89**	**DEAD n (%), N=75**	**ALIVE n (%), N=14**	**Univariate analysis MST (months)**	**Log-rank *p***	**Unadjusted HR[a]**	**Multivariate analysis Adjusted HR[b] (95% CI)**	***P***
Codominant model								
+6/+6	27 (30.3)	24 (32)	3 (21.4)	5.67	-	1.0 (reference)		
+6/-6	51 (57.3)	42 (56)	9 (64.2)	7.5	0.45	1.20 (0.72-2.04)	0.79 (0.47-1.34)	0.39
-6/-6	11 (12.3)	9 (12)	2 (14.2)	7.27	0.27	1.50 (0.72-3.14)	0.75 (0.29-1.91)	0.55
Dominant model								
+6/+6	27 (30.3)	24 (32)	3 (21.4)	5.67	-	1.0 (reference)		
+6/-6 + -6/-6	62 (69.6)	51 (68)	11 (78.5)	7.5	0.34	1.26 (0.76-2.13)	0.79 (0.48-1.31)	0.37
Recessive model								
+6/-6 + +6/+6	78 (87.6)	66 (88)	12 (85.7)	6.3	-	1.0 (reference)		
-6/-6	11 (12.3)	9 (12)	2 (14.2)	7.27	0.58	1.21 (0.62-2.29)	1.01 (0.48-2.11)	0.96
REGIMEN- Pemetrexed cisplatin/carboplatin								
Genotype	**CASES**	**DEAD**	**ALIVE**	**Univariate analysis**			**Multivariate analysis**	

rs34743033 *TS* (VNTR)	**n (%), N=142**	**n (%), N=119**	**n (%), N=23**	**MST (months)**	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
2R2R	29 (20.4)	25 (21.0)	4 (17.3)	10.6	-	1.0 (reference)		
2R3R	70 (49.2)	56 (47.0)	14 (60.8)	8.8	0.97	0.99 (0.61-1.59)	1.05 (0.64-1.73)	0.83
3R3R	43 (30.2)	38 (31.9)	5 (21.7)	6.1	0.43	0.82 (0.49-1.35)	1.31 (0.77-2.22)	0.31
Dominant model								
2R2R	29 (20.4)	25 (21.0)	4 (17.3)	10.6	-	1.0 (reference)		
2R3R+3R3R	113 (79.5)	94 (78.9)	19 (82.6)	7.57	0.71	0.92 (0.59-1.42)0	1.12 (0.71-1.77)	0.61
Recessive model								
2R3R+2R2R	99 (69.7)	81 (68.0)	18 (78.2)	9.43	-	1.0 (reference)		
3R3R	43 (30.2)	38 (31.9)	5 (21.7)	6.1	0.25	0.80 (0.52-1.18)	1.37 (0.92-2.05)	0.11
Genotype	**CASES**	**DEAD**	**ALIVE**	**Univariate analysis**			**Multivariate analysis**	
rs151264360 (1494 ins/del6)	**n (%), N=142**	**n (%), N=119**	**n (%), N=23**	**MST (months)**	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
+6/+6	41 (28.8)	35 (29.4)	6 (26.0)	7.57	-	1.0 (reference)	-	-
+6/-6	77 (54.2)	63 (52.9)	14 (60.8)	9.23	0.65	1.09 (0.72-1.67)	0.80 (0.51-1.25)	0.33
-6/-6	24 (16.9)	21 (17.6)	3 (13.0)	9.33	0.71	0.90 (0.52-1.56)	0.68 (0.36-1.29)	0.24
Dominant model								
+6/+6	41 (28.8)	35 (29.4)	6 (26.0)	7.57	-	1.0 (reference)		
+6/-6 + -6/-6	101 (71.1)	84 (70.5)	17 (73.9)	9.23	0.80	1.05 (0.70-1.56)	0.82 (0.54-1.25)	0.37
Recessive model								
+6/-6 + +6/+6	118 (83.0)	98 (82.3)	20 (86.9)	8.33	-	1.0 (reference)		
-6/-6	24 (16.9)	21 (17.6)	3 (13.0)	9.33	0.52	0.86 (0.51-1.40)	1.02 (0.63-1.66)	0.91
REGIMEN- Irinotecan cisplatin/carboplatin								
Genotype	**CASES**	**DEAD**	**ALIVE**	**Univariate analysis**			**Multivariate analysis**	
rs34743033 *TS* (VNTR)	**n (%), N=89**	**n (%), N=78**	**n (%), N=11**	**MST (months)**	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
2R2R	10 (11.2)	9 (11.5)	1 (9.0)	11	-	1.0 (reference)		
2R3R	46 (51.6)	39 (50)	7 (63.6)	10.4	0.66	0.85 (0.42-1.71)	1.52 (0.63-3.65)	0.34
3R3R	33 (37.0)	30 (38.4)	3 (27.2)	8.67	0.36	0.71 (0.36-1.44)	2.47 (0.93-6.54)	0.07
Dominant model								
2R2R	10 (11.2)	9 (11.5)	1 (9.0)	11	-	1.0 (reference)		
2R3R+3R3R	79 (88.7)	69 (88.4)	10 (90.9)	8.67	0.51	0.79 (0.42-1.53)	1.77 (0.78-3.97)	0.16
Recessive model								
2R3R+2R2R	56 (62.9)	48 (61.5)	8 (72.7)	10.57	-	1.0 (reference)		
3R3R	33 (37.0)	30 (38.4)	327.2)	8.67	0.34	0.80 (0.49-1.27)	1.55 (0.92-2.60)	0.09
Genotype	**CASES**	**DEAD**	**ALIVE**	**Univariate analysis**			**Multivariate analysis**	
rs151264360 (1494 ins/del6)	**n (%), N=89**	**n (%), N=78**	**n (%), N=11**	**MST (months)**	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
+6/+6	11 (12.3)	10 (12.8)	1 (9.0)	9.86	-	1.0 (reference)		
+6/-6	64 (71.9)	55 (70.5)	9 (81.8)	9.35	0.66	0.86 (0.44-1.66)	1.03 (0.49-2.17)	0.92
-6/-6	14 (15.7)	13 (16.6)	1 (9.0)	10.4	0.45	0.74 (0.30-1.70)	0.83 (0.27-2.54)	0.75
Dominant model								
+6/+6	11 (12.3)	10 (12.8)	1 (9.0)	9.86	-	1.0 (reference)		
+6/-6 + -6/-6	78 (87.6)	68 (87.1)	10 (90.9)	10.1	0.60	0.84 (0.4-1.60)	1.10 (0.52-2.31)	0.78
Recessive model								
+6/-6 + +6/+6	75 (84.2)	65 (83.3)	10 (90.9)	9.86	-	1.0 (reference)		
-6/-6	14 (15.7)	13 (16.6)	1 (9.0)	10.4	0.58	0.84 (0.44-1.58)	0.86 (0.45-1.65)	0.66

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, gender, smoking, stage, histology, KPS, and ECOG

5.7.4 Association of TS polymorphism with gender and OS

In lung cancer patients, 84.21% and 83.47% of males and females were dead, respectively, at the time of statistical analysis. After using univariate analysis, the patients having wild genotype (*2R2R*) of *TSER* polymorphism in the case of both the males (MST= 9.1 *vs.* 7.67 months, log-rank p= 0.30) and females (MST=13.4 *vs.* 6 months, log-rank p=0.16) were associated with better survival when compared to mutant genotype (*3R3R*), though was not statistically significant (**Table 5.8**). However, in the recessive model, males showed better survival in the group where wild type and heterozygous genotypes (*2R2R+2R3R*) were combined and compared to the patients with the mutant genotype (*3R3R*) (8.83 *vs.* 7.67 months; log-rank p= **0.04**) as shown in **figure 5.15.**

Additionally, those subjects possessing mutant genotype (*-6/-6bp)* for *1494del6* polymorphism showed higher survival with respect to subjects with the homozygous wild alleles *(+6/+6bp*) in both males (MST= 8.73 *vs.* 6.96, log-rank p=0.58) and females (MST=14 *vs.* 7.37, log-rank p= 0.63) (**Table 5.8**). Therefore, *1494del6* polymorphism did not show any association in overall survival among different genotypes when lung cancer patients were stratified based on gender.

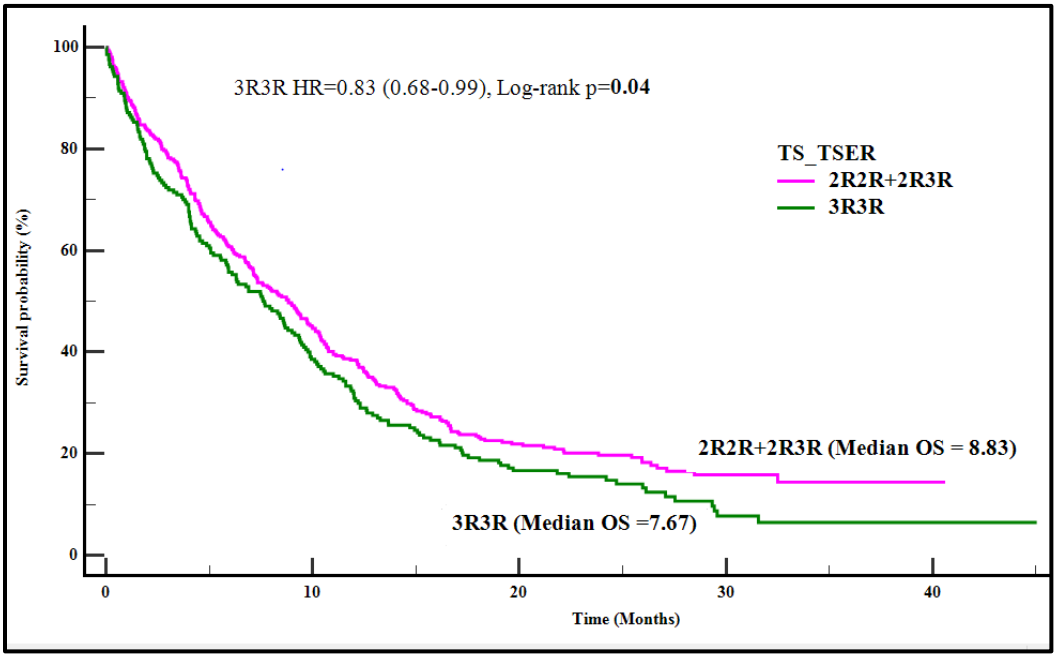


Figure 5.15: Kaplan Meier curves illustrating the association between overall survival in different genotypes of *TSER* polymorphism in males with lung cancer. (Note: *2R2R* represents wild-type genotype, *2R3R* represents heterozygous genotype, and *3R3R* represents homozygous for mutant genotype)

Table 5.8: Association of *TS* SNPs on overall survival based on gender

MALE								
Genotype rs34743033 *TS* (VNTR)	**CASES n(%), N=564**	**DEAD n(%), N=475**	**ALIVE n(%), N=89**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
2R2R	105(18.6)	89(18.7)	16(17.9)	9.1	-	1.0(reference)		
2R3R	249(44.1)	200(42.1)	49(55.0)	8.71	0.55	1.07(0.83-1.39)	1.15(0.87-1.50)	0.30
3R3R	210(37.2)	186(39.1)	24(27.9)	7.67	0.30	0.87(0.68-1.12)	1.2(0.92-1.59)	0.15
Dominant model								
2R2R	105(18.6)	89(18.7)	16(17.9)	9.1	-	1.0(reference)		
2R3R+3R3R	459(81.3)	386(81.2)	73(82.0)	8.03	0.87	0.98(0.78-1.23)	1.18(0.92-1.51)	0.17
Recessive model								
2R3R+2R2R	354(62.7)	289(60.8)	65(73.0)	8.83	-	1.0(reference)		
3R3R	210(37.2)	186(39.1)	24(26.9)	7.67	**0.04**	0.83(0.68-0.99)	1.17(0.96-1.42)	0.10
Genotype rs151264360 (1494 ins/del6)	**CASES n(%), N=564**	**DEAD n(%), N=475**	**ALIVE n(%), N=89**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
+6/+6	128(22.6)	112(23.5)	16(17.9)	6.96	-	1.0(reference)		
+6/-6	331(58.6)	271(57.0)	60(67.4)	8.63	0.16	1.16(0.93-1.47)	0.89(0.71-1.11)	0.32
-6/-6	105(18.6)	92(19.3)	13(14.6)	8.73	0.58	1.07(0.81-1.42)	0.95(0.71-1.27)	0.75
Dominant model								
+6/+6	128(22.6)	112(23.5)	16(17.9)	6.96	-	1.0(reference)		
+6/-6 + -6/-6	436(77.3)	363(76.4)	73(82.0)	8.63	0.20	1.14(0.92-1.43)	0.89(0.72-1.11)	0.34
Recessive model								
+6/-6 + +6/+6	459(81.3)	383(80.6)	76(85.3)	8.26	-	1.0(reference)		
-6/-6	105(18.6)	92(19.3)	13(14.6)	8.73	0.70	0.95(0.75-1.20)	1.02(0.80-1.29)	0.86
FEMALE								
Genotype rs34743033 *TS* (VNTR)	**CASES n(%), N=115**	**DEAD n(%), N=96**	**ALIVE n(%), N=19**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b](95% CI)**	***P***
Codominant model								
2R2R	19(16.5)	15(15.6)	4(21.0)	13.4	-	1.0(reference)		
2R3R	60(5.8)	51(53.1)	9(47.3)	7.6	0.12	0.64(0.39-1.11)	1.33(0.70-2.52)	0.37
3R3R	36(31.3)	30(31.2)	6(31.5)	6	0.16	0.65(0.36-1.19)	1.74(0.87-3.5)	0.11
Dominant model								
2R2R	19(16.5)	15(15.6)	4(21.0)	13.4	-	1.0(reference)		
2R3R+3R3R	96(83.4)	81(84.3)	1578.9)	7.37	0.12	0.64(0.42-1.10)	1.49(0.81-2.7)	0.19
Recessive model								
2R3R+2R2R	79(68.6)	66(68.7)	13(68.4)	9.43	-	1.0(reference)		
3R3R	36(31.3)	30(31.2)	6(31.5)	6	0.44	0.84(0.53-1.31)	1.50(0.94-2.37)	0.08
Genotype rs151264360 (1494 ins/del6)	**CASES n(%), N=115**	**DEAD n(%), N=96**	**ALIVE n(%), N=19**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b](95% CI)**	***P***
Codominant model								
+6/+6	31(26.9)	25(26.04)	6(31.5)	7.37	-	1.0(reference)		
+6/-6	67(58.2)	58(60.4)	9(47.3)	8.23	0.45	0.83(0.53-1.32)	0.96(0.56-1.65)	0.89
-6/-6	17(14.7)	13(13.5)	4(21.05)	14	0.63	1.17(0.60-2.26)	0.68(0.28-1.61)	0.38
Dominant model								
+6/+6	31(26.9)	25(26.04)	6(31.5)	7.37	-	1.0(reference)		
+6/-6 + -6/-6	84(73.0)	71(73.9)	13(68.4)	8.8	0.63	0.89(0.57-1.40)	0.93(0.55-1.57)	0.80
Recessive model								
+6/-6 + +6/+6	98(85.2)	83(86.4)	15(78.9)	7.97	-	1.0(reference)		
-6/-6	17(14.7)	13(13.5)	4(21.05)	14	0.32	1.33(7.66-2.22)	0.82(0.44-1.50)	0.52

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, smoking, stage histology, regimen, KPS, ECOG

5.7.5 Association of TS polymorphism with OS based on smoking habits

Smoking is the primary cause of lung cancer, and many lung carcinoma-related deaths are attributed to the smoking habits of an individual. So, we have assessed the role of *TS* polymorphism in modulating prognosis when stratified based on smokers and non-smokers, as depicted in **table 5.9**. A high prevalence of smokers was present among the case group compared to the non-smokers. According to our analysis, 85.5% of smokers were dead, while only 14.4% were alive. For *TSER* polymorphism, a reduced survival trend was observed in both heterozygous (*2R3R)* (MST=7.93 *vs.* 8.43, Log-rank-p=0.65) and mutant (*3R3R)* (MST=7.6 *vs.* 8.43, Log-rank p=0.43) genotype as compared to the wild type genotype (*2R2R)*, however, the data lacked statistical significance. In the case of *1464del6* polymorphism, the smokers having at least one copy of the mutant allele (*-6/-6bp)* had better survival as compared to the subjects having wild-genotype *(+6/+6bp)*, though no association was noted between *TS 1494del6* polymorphism and prognosis among smokers (MST=8.03 *vs.* 6.93, Log-rank p=0.17).

In the case of non-smoker lung cancer patients, 32.06% of individuals harbored homozygous mutant genotype (*3R3R)*, while 21.37% of patients had wild-type genotype (*2R2R)*. Patients with mutant genotype had poor survival compared to the wild type genotype, but lack of association between *TSER* and overall survival was noted (MST=6.10 *vs.* 12.46, Log-rank p=0.15). On the contrary, in case *1494del6* polymorphism, when 17.56% variant genotype (*-6/-6bp)* was compared with 27.48% wild genotype *(+6/+6bp)*, better survival and lower death ratio was noted in the former group as compared to the latter (MST=9.43 *vs.* 7.6, HR=0.81, 95% CI=0.44-1.49, Log-rank p=0.50). We conclude that despite observing survival trends in both polymorphisms, we did not find any correlation between *TS* polymorphism in predicting prognosis in lung carcinoma patients based on smoking status.

Table 5.9: Association of *TS* SNPs on overall survival based on smoking status

Smokers								
Genotype rs34743033 *TS* (VNTR)	**CASES n(%), N=547**	**DEAD n(%), N=468**	**ALIVE n(%), N=79**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
2R2R	96(17.55)	83(17.73)	13(16.45)	8.43	-	1.0(reference)	-	-
2R3R	248(45.33)	205(43.80)	43(54.43)	7.93	0.65	1.06(0.81-1.37)	1.08(0.76-1.53)	0.65
3R3R	203(37.11)	180(38.46)	23(29.11)	7.6	0.43	1.1(0.85-1.43)	1.14(0.80-1.62)	0.44
Dominant model								
2R2R	96(17.55)	83(17.73)	13(16.45)	8.43	-	1.0(reference)	-	-
2R3R+3R3R	451(82.44)	385(82.26)	56(70.88)	7.7	0.90	1.01(0.80-1.28)	1.07(0.78-1.47)	0.65
Recessive model								
2R3R+2R2R	344(62.88)	288(61.53)	26(70.88)	8.03	-	1.0(reference)	-	-
3R3R	203(37.11)	180(38.46)	23(29.11)	7.6	0.12	1.16(0.95-1.40)	1.12(0.87-1.45)	0.35
Genotype rs151264360 (1494 ins/del6)	**CASES n(%), N=547**	**DEAD n(%), N=468**	**ALIVE n(%), N=79**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
+6/+6	123(22.49)	110(23.50)	13(16.46)	6.93	-	1.0(reference)	-	-
+6/-6	325(59.41)	272(58.12)	53(67.09)	8.03	0.17	0.85(0.67-1.07)	0.91(0.66-1.24)	0.55
-6/-6	99(18.10)	86(18.38)	13(16.46)	8.73	0.33	0.87(0.65-1.15)	1.09(0.72-1.63)	0.67
Dominant model								
+6/+6	123(22.49)	110(23.50)	13(16.46)	6.93	-			
+6/-6 + -6/-6	424(77.51)	358(77.48)	66(83.54)	8.93	0.16	0.85(0.6-1.06)	0.93(0.69-1.26)	0.67
Recessive model								
+6/-6 + +6/+6	448(81.9)	382(81.6)	66(83.54)	7.57	-	1.0(reference)		
-6/-6	99(18.10)	86(18.38)	13(16.46)	8.73	0.84	0.97(0.77-1.23)	1.13(0.82-1.54)	0.44
Non-smokers								
Genotype rs34743033 *TS* (VNTR)	**CASES n(%), N=131**	**DEAD n(%), N=102**	**ALIVE n(%), N=29**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**UnadjustedHR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
2R2R	28(21.37)	21(20.58)	7(24.13)	12.46	-	1.0(reference)	-	-
2R3R	61(46.56)	46(45.09)	15(51.72)	9.43	0.43	1.22(0.74-2.01)	1.03(0.49-2.15)	0.92
3R3R	42(32.06)	35(34.31)	7(24.13)	6.10	0.15	1.47(0.87-2.49)	1.83(0.83-4.05)	0.13
Dominant model								
2R2R	28(21.37)	21(20.58)	7(24.13)	12.46	-	1.0(reference)	-	-
2R3R+3R3R	103(78.62)	81(79.41)	22(75.86)	8.2	0.25	1.29(0.83-2.03)	1.27(0.66-2.43)	0.46
Recessive model								
2R3R+2R2R	89(67.93)	67(65.68)	22(75.86)	11.03	-	1.0(reference)	-	-
3R3R	42(32.06)	35(34.31)	7(24.13)	6.1	0.14	1.38(0.89-2.13)	1.73(0.99-3.02)	0.05
Genotype rs151264360 (1494 ins/del6)	**CASES n(%), N=131**	**DEAD n(%), N=102**	**ALIVE n(%), N=29**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
+6/+6	36(27.48)	27(26.47)	9(31.03)	7.6	-	1.0(reference)	-	-
+6/-6	72(54.96)	56(54.90)	16(55.17)	9.43	0.78	1.06(0.671.67)	1.41(0.68-2.91)	0.34
-6/-6	23(17.56)	19(18.63)	4(13.79)	9.43	0.50	0.81(0.44-1.49)	0.91(0.32-2.56)	0.85
Dominant model								
+6/+6	36(27.48)	27(26.47)	9(31.03)	7.6	-	1.0(reference)	-	-
+6/-6 + -6/-6	95(72.5)	75(73.52)	20(68.9)	9.46	0.71	1.08(0.70-1.67)	1.44(0.73-2.83)	0.28

Recessive model								
+6/-6 + +6/+6	108(82.4)	83(81.37)	25(86.20)	9.40	-	1.0(reference)	-	-
-6/-6	23(17.56)	19(18.63)	4(13.79)	11.87	0.78	1.07(0.64-1.78)	1.19(0.61-2.32)	0.60

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, stage histology, regimen, KPS, and ECOG

5.7.6 Association of TS polymorphism with OS based on tumor stage

To understand the prognostic impact of the cancer stage in lung carcinoma patients, we have stratified the patients based on their respective tumor stages III and IV, as shown in **table 5.10**. In the case of *TSER* polymorphism, patients in stage IV showed a strong association in predicting prognosis in lung cancer patients. In the recessive model, the stage IV patients having mutant genotype (*3R3R*) showed reduced survival as compared to the combined wild and heterozygous genotype (*2R2R+3R3R*) (MST=6.1 *vs.* 8.83, HR=1.37, 95% CI=1.10-1.69, p=**0.003**). After adjusting with multiple covariates such as age, gender, smoking, histology, regimen, and performance status, the death ratio increased to 1.34 times in stage IV patients harboring both copies of mutant alleles (*3R3R*) (HR=1.34, 95% CI=1.03-1.75. p=**0.02**) **(Figure 5.16)**. However, we did not find any association of *1494del6* polymorphism in predicting overall survival in stage IV lung carcinoma patients.

Lung cancer patients in stage IV outnumbered the stage III patients (455 *vs.* 165). In stage III, prevalence of at least one mutant allele was high in the case of both *TSER* (*2R3R*) and *1494del6 (+6/-6)* polymorphism, *i.e.,* 48.4 and 60%, respectively. In the case of *TSER* polymorphism, patients in stage III harboring heterozygous genotype (*2R3R*) had reduced survival compared to the individual with a wild-type genotype, though the substantial significance between the genotypes was not reached (MST=7.57 *vs.* 10.33, HR=1.50, 95% CI=0.71-3.20, p=0.28). On the contrary, in *1494del6* polymorphism, stage III patients having at least one copy of mutant allele *(+6/-6)* showed better survival as compared to the wild-type genotype *(+6/+6)* (MST=9.43 *vs.* 7.3, p=0.30) but lack of association was found in this scenario.

Table 5.10: Association of *TS* SNPs on overall survival based on tumor stage

Tumor stage III								
Genotype rs34743033 *TS*(VNTR)	**CASES n(%), N=165**	**DEAD n(%), N=132**	**ALIVE n(%), N=33**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b](95% CI)**	***P***
Codominant model								
2R2R	28(16.96)	21(15.90)	7(21.21)	10.33	-	1.0(reference)	-	-
2R3R	80(48.48)	63(47.72)	17(51.51)	7.57	0.40	1.22(0.76-1.95)	1.50(0.71-3.20)	0.28
3R3R	57(34.54)	48(36.36)	9(27.27)	9.87	0.51	1.18(0.71-1.94)	1.08(0.50-2.34)	0.82
Dominant model								
2R2R	28(16.96)	21(15.90)	7(21.21)	10.33	-	1.0(reference)	-	-
2R3R+3R3R	137(83.03)	111(84.09)	26(78.78)	8.8	0.41	1.19(0.77-1.86)	1.23(0.63-2.41)	0.52
Recessive model								
2R3R+2R2R	108(65.45)	84(63.63)	24(72.72)	8.5	-	1.0(reference)	-	-
3R3R	57(34.54)	48((36.36)	9(27.27)	9.87	0.90	1.02(0.71-1.45)	0.82(0.48-1.39)	0.47
Genotype rs151264360 (1494 ins/del6)	**CASES n(%), N=165**	**DEAD n(%), N=132**	**ALIVE n(%), N=33**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b](95% CI)**	***P***
Codominant model								
+6/+6	40(24.24)	34(25.75)	6(18.18)	7.3	-	1.0(reference)	-	-
+6/-6	99(60)	76(57.57)	23(69.69)	9.43	0.30	0.80(0.52-1.22)	0.72(0.38-1.37)	0.32
-6/-6	26(15.75)	22(16.66)	4(12.12)	10.53	0.52	0.84(0.49-1.42)	0.77(0.27-2.17)	0.63
Dominant model								
+6/+6	40(24.24)	34(25.75)	6(18.18)	7.3	-	1.0(reference)	-	-
+6/-6 + -6/-6	125(75.75)	98(74.24)	27(81.81)	9.7	0.29	0.80(0.53-1.21)	0.71(0.38-1.32)	0.28
Recessive model								
+6/-6 + +6/+6	139(84.24)	110(83.33)	29(87.87)	8.8	-	1.0(reference)	-	-
-6/-6	26(15.75)	22(16.66)	4(12.12)	10.53	0.9	1.01(0.63-1.60)	0.89(0.43-1.86)	0.76
Tumor stage IV								
Genotype rs34743033 *TS*(VNTR)	**CASES n(%), N=455**	**DEAD n(%), N=395**	**ALIVE n(%), N=60**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b](95% CI)**	***P***
Codominant model								
2R2R	83(18.24)	73(18.48)	10(16.66)	9.7	-	1.0(reference)	-	-
2R3R	204(44.83)	169(42.78)	35(58.33)	8.23	0.73	0.95(0.72-1.25)	1.02(0.71-1.46)	0.88
3R3R	168(36.92)	153(38.73)	15(25)	6.1	0.06	1.28(0.98-1.68)	1.25(0.87-1.80)	0.21
Dominant model								
2R2R	83(18.24)	73(18.48)	10(16.66)	9.7	-	1.0(reference)	-	-
2R3R+3R3R	372(81.75)	322(81.51)	50(83.33)	7.27	0.49	1.09(0.85-1.39)	1.05(0.78-1.49)	0.61
Recessive model								
2R3R+2R2R	287(63.07)	242(61.26)	45(75)	8.83	-	1.0(reference)	-	-
3R3R	168(36.92)	153(38.73)	15(25)	6.1	**0.003**	1.37(1.10-1.69)	1.34(1.03-1.75)	**0.02**
Genotype rs151264360 (1494 ins/del6)	**CASES n(%), N=455**	**DEAD n(%), N=395**	**ALIVE n(%), N=60**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b](95% CI)**	***P***
Codominant model								
+6/+6	107(23.51)	94(23.79)	13(21.66)	6.93	-	1.0(reference)	-	-
+6/-6	260(57.14)	225(56.96)	35(58.33)	7.97	0.71	0.95(0.74-1.21)	0.96(0.69-1.34)	0.84
-6/-6	88(19.34)	76(19.24)	12(20)	9.06	0.44	0.88(0.65-1.20)	1.11(0.73-1.68)	0.62
Dominant model								
+6/+6	107(23.51)	94(23.79)	13(21.66)	6.93	-	1.0(reference)	-	-
+6/-6 + -6/-6	348(76.48)	301(76.20)	47(78.33)	8.03	0.60	0.93(0.74-1.18)	1.0(0.73-1.38)	0.95
Recessive model								
+6/-6 + +6/+6	367(80.65)	319(80.75)	48(80)	7.47	-	1.0(reference)	-	-
-6/-6	88(19.34)	76(19.24)	12(20)	9.13	0.48	0.91(0.71-1.16)	1.10(0.80-1.51)	0.53

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, histology, regimen, KPS, ECOG

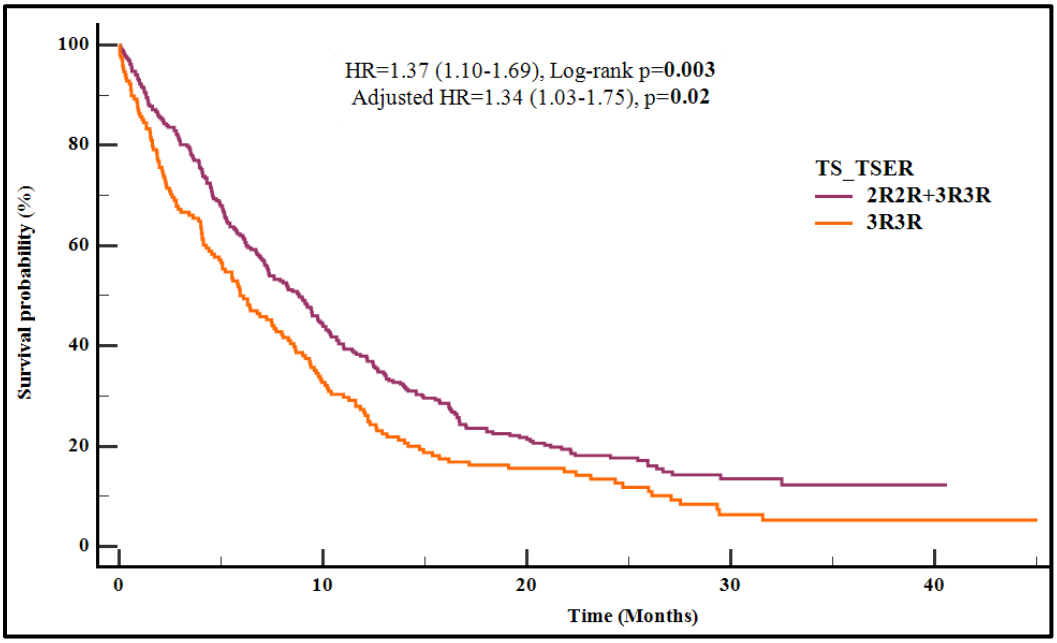


Figure 5.16: Kaplan Meier curves illustrating the association between overall survival in different genotypes of *TSER* polymorphism in Stage IV lung cancer patients. (Note: 2R2R represents wild-type genotype, 2R3R represents heterozygous genotype, and 3R3R represents homozygous for mutant genotype)

5.7.7 Association of TS polymorphism with OS based on performance status

ECOG performance status (ECOG-PS) reflects a patient's ability to care for themselves, their everyday activities, and physical ability (walking, working). To assess the impact of ECOG-PS in association with *TS* polymorphism to predict survival in lung cancer patients, the individuals were categorized based on ECOG-PS score 0,1 (good/restricted performance status) and ECOG-PS score 2,3,4 (limited/bad performance status), as shown in **table 5.11**. For ECOG-PS 0,1, in the case of *TSER* polymorphism, 15.71% of patients had wild-type genotype (*2R2R)*, 47.82% patients had heterozygous genotype (*2R3R)*, and 36.45% patients had mutant genotype (*3R3R)*. When the recessive model was applied, mutant genotype (*3R3R)* showed reduced survival as compared to combined heterozygous and wild type genotype (*2R2R+2R3R)* (MST=9.36 *vs.* 12.63, HR=1.45, 95% CI=1.10-1.92, p=**0.007**). After adjusting with multiple covariates, the death ratio increased to 1.82 times in the mutant genotype with ECOG score 0,1 (MST=9.36 *vs.* 12.63, HR=1.82, 95% CI=1.27-2.59, p=**0.0009**) **(Figure 5.17)**. In similar lines, patients with ECOG-PS 2,3,4 having *TSER* polymorphism showed reduced survival in combined mutant and heterozygous genotype

(*3R3R+2R3R)* as compared to the wild type genotype (*2R2R)* (MST=5.93 *vs.* 8.3, HR=1.30, 95% CI=1.01-1.68, p=**0.04**). Therefore, we summarize that *TSER* polymorphism and ECOG-PS are associated in predicting survival in lung cancer patients, whereas *1494del6* polymorphism did not show any association in predicting prognosis when stratified based on ECOG-PS.

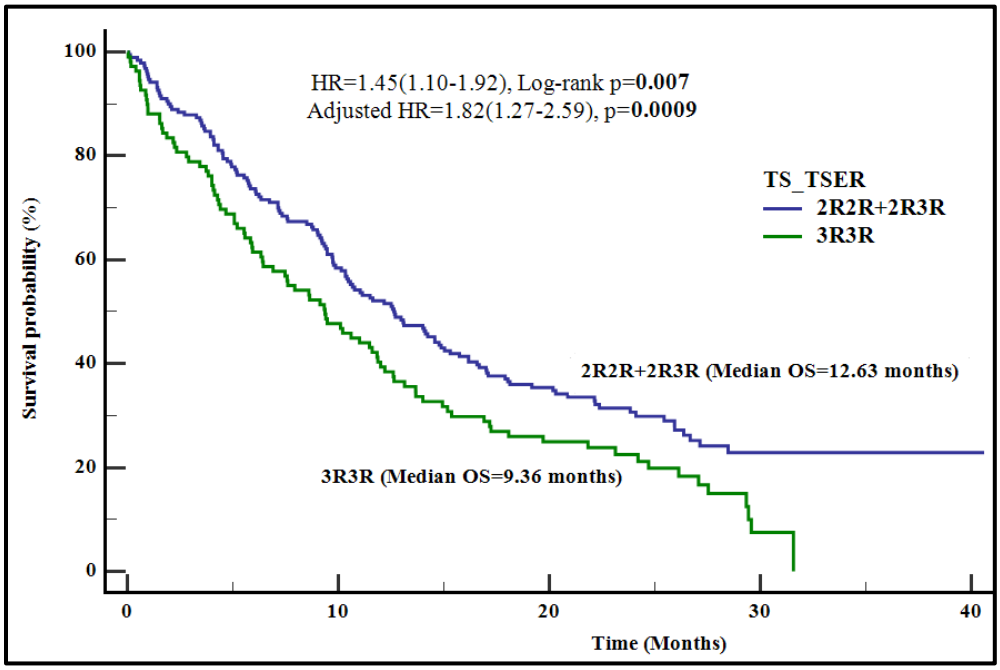


Figure 5.17: Kaplan Meier curves illustrating the association between overall survival in different genotypes of *TSER* polymorphism in ECOG-PS lung cancer patients. (Note: 2R2R represents wild-type genotype, 2R3R represents heterozygous genotype, and 3R3R represents homozygous for mutant genotype)

Table 5.11: Association of *TS* SNPs on overall survival based on performance status

ECOG 0,1								
Genotype rs34743033 *TS* (VNTR)	**CASES n(%), N=299**	**DEAD n(%), N=229**	**ALIVE n(%), N=70**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
2R2R	47(15.71)	37(16.15)	10(14.28)	10.57	-			
2R3R	143(47.82)	100(43.66)	43(61.42)	14	0.35	0.82(0.55-1.23)	0.75(0.45-1.23)	0.25
3R3R	109(36.45)	92(40.17)	17(24.28)	9.36	0.25	1.23(0.851.78)	1.49(0.90-2.47)	0.11
Dominant model								
2R2R	47(15.71)	37(16.15)	10(14.28)	10.57	-	1.0(reference)	-	-
2R3R+3R3R	252(84.28)	192(83.84)	60(85.71)	11.5	0.9	0.98(0.69-1.40)	0.99(0.62-1.56)	0.97
Recessive model								
2R3R+2R2R	190(63.54)	137(59.82)	53(75.71)	12.63	-	1.0(reference)	-	-
3R3R	109(36.45)	92(40.17)	17(24.28)	9.36	**0.007**	1.45(1.10-1.92)	1.82(1.27-2.59)	**0.0009**
Genotype rs151264360 (1494 ins/del6)	**CASES n(%), N=299**	**DEAD n(%), N=229**	**ALIVE n(%), N=70**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
+6/+6	68(22.74)	51(22.27)	17(24.28)	12.06	-	1.0(reference)	-	-
+6/-6	175(58.52)	135(58.95)	40(57.14)	9.73	0.46	1.12(0.82-1.54)	0.94(0.60-1.48)	0.81
-6/-6	56(18.72)	43(18.77)	13(18.57)	12.7	0.64	0.90(0.60-1.36)	0.87(0.48-1.57)	0.65
Dominant model								
+6/+6	68(22.74)	51(22.27)	17(24.28)	12.06	-	1.0(reference)	-	-
+6/-6 + -6/-6	231(77.25)	178(77.72)	53(75.71)	11	0.66	1.07(0.78-1.45)	0.94(0.611.44)	0.78
Recessive model								
+6/-6 + +6/+6	243(81.27)	186(81.22)	57(81.42)	10.33	-	1.0(reference)	1.0(reference)	-
-6/-6	56(18.72)	43(18.77)	13(18.57)	12.7	0.27	0.83(0.61-1.14)	0.82(0.53-1.28)	
ECOG 2,3,4								
Genotype rs34743033 *TS* (VNTR)	**CASES n(%), N=357**	**DEAD n(%), N=323**	**ALIVE n(%), N=34**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
2R2R	74(20.72)	64(19.81)	10(29.41)	8.3	-	1.0(reference)	-	-
2R3R	155(43.41)	142(43.96)	13(38.23)	5.93	0.05	1.32(0.99-1.75)	1.32(0.89-1.95)	0.15
3R3R	128(35.85)	117(36.22)	11(32.35)	5.8	0.06	1.31(0.98-1.77)	1.0(0.66-1.51)	0.9
Dominant model								
2R2R	74(20.72)	64(19.81)	10(29.41)	8.3	-	1.0(reference)	-	-
2R3R+3R3R	283(79.27)	259(80.18)	24(70.58)	5.93	**0.04**	1.30(1.01-1.68)	1.16(0.81-1.65)	0.40
Recessive model								
2R3R+2R2R	229(64.14)	206(63.77)	23(67.64)	6.8	-	1.0(reference)	-	-
3R3R	128(35.85)	117(36.22)	11(32.35)	5.93	0.43	1.09(0.87-1.38)	0.86(0.63-1.16)	0.33
Genotype rs151264360 (1494 ins/del6)	**CASES n(%), N=357**	**DEAD n(%), N=323**	**ALIVE n(%), N=34**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
+6/+6	87(24.36)	83(25.69)	4(11.76)	5.27	-	1.0(reference)	-	-
+6/-6	210(58.82)	183(56.65)	27(79.41)	6.76	**0.03**	0.74(0.56-0.98)	0.81(0.56-1.16)	0.25
-6/-6	60(16.80)	57(17.64)	3(8.82)	5.8	0.93	0.98(0.70-1.38)	1.22(0.75-1.98)	0.40
Dominant model								
+6/+6	87(24.36)	83(25.69)	4(11.76)	5.27	-	1.0(reference)	-	-
+6/-6 + -6/-6	270(75.63)	240(74.30)	30(88.23)	6.76	0.07	0.78(0.60-1.02)	0.88(0.62-1.24)	0.47
Recessive model								
+6/-6 + +6/+6	297(83.19)	266(82.35)	31(91.17)	6.3	-	1.0(reference)	-	-
-6/-6	60(16.80)	57(17.64)	3(8.82)	5.8	0.21	1.21(0.89-1.64)	1.34(0.92-1.95)	0.11

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, histology, KPS, and regimen

5.7.8 Association of combined genotypes in TS polymorphism with OS

Combined genotypes of *TS* polymorphisms in lung cancer patients were compared to assess the interrelationship between 5' and 3' UTR polymorphism and survival, as shown in table 5.12. Our data observed that 15.5% of the patients were carrying wild genotype for both polymorphisms *[(2R2R)* + *(+6/+6)*], 21.1% had mutant genotype (*3R3R* + *-6/-6*), and 63.3% of the subjects had heterozygous genotype (*2R3R* + *+6/-6)* for both polymorphisms. In the co-dominant model, a high median survival time was noted in patients with the genotypic combination of *2R2R* + *+6/+6* compared to subjects carrying the homozygous variant genotype (*3R3R* + *-6/-6*) (MST=10.33 *vs.* 7.81; log-rank p=0.13; HR=0.74). On the contrary, on applying the recessive model, we observed a better prognosis in subjects carrying both the wild and heterozygous alleles [*(2R3R)* + *(+6/-6)* and *(2R2R)* + *(+6/+6)]* as compared to patients having the homozygous variant alleles for both the genotypes (*3R3R*+ *-6/-6 bp*) (MST=9.4 *vs.* 7.81; log-rank **p=0.03**; HR=0.73) (Figure 5.18). Patients with the latter genotype had a higher death ratio (HR=1.37, 95%CI=1.01-1.86, **p=0.04**) upon adjusting with various covariates in the Cox regression model. **(Table 5.12).**

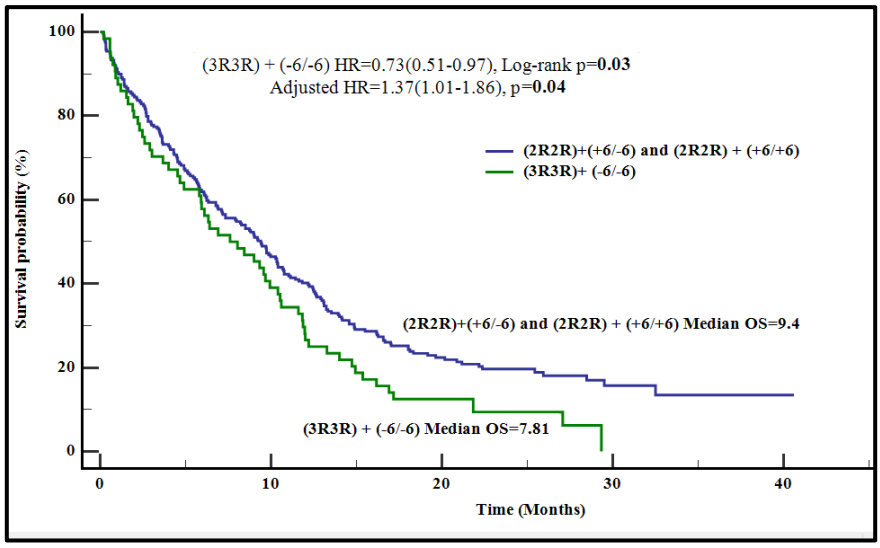


Figure 5.18: Kaplan Meier curves illustrating the combinatorial analysis of the *TS* SNPs on overall survival (recessive model) in lung cancer patients. (Note: *2R2R* represents wild-type genotype of *TSER*, *2R3R* represents heterozygous genotype of *TSER*, and *3R3R* represents homozygous for the mutant genotype of *TSER*); *(+6/+6)* represents the wild-type genotype of *1494 ins/del6*, *(+6/-6)* represents the heterozygous genotype of *1494 ins/del6*, and *(-6/-6)* represents homozygous for the mutant genotype of *1494 ins/del6*

Being well aware that both 5'-UTR and 3'-UTRs have a close relationship with TS gene expression regulation, we have made a decent attempt to evaluate the impact of both these polymorphisms on patients' survival. It is well known that increased TS expression has been reported in the mutant allele of *TSER* (*3R3R*) and wild type alleles of *1494del6* (+6/+6), and reduced TS expression has been stated in wild type alleles of *TSER* (*2R2R*) and mutant type alleles of *1494del6* (*-6/-6bp)* polymorphism. Keeping this fact in view, we have compared the survival differences of patients having the genotypic combination of the homozygous variant of rs34743033 and wild type alleles of rs151264360 polymorphism, *i.e.* (*3R3R+6/+6*), and compared it with those subjects who were carrying the wild type alleles for rs34743033 and mutant alleles of the rs151264360 *TSER* polymorphism, *i.e.* (*2R2R+-6/-6bp*) (**Table 5.12**). We observed a substantial improvement in patients' survival time and prognosis with the genotypic combination of *2R2R &-6/-6* (low TS expression) compared to patients harboring the *3R3R+ +6/+6bp* genotype (MST=12.17 *vs.* 5.57 months; log-rank p=0.10). After applying the cox regression model, we observed that such a group of patients (*2R2R + -6/-6*) had a low death ratio concerning subjects with *3R3R+ +6/+6* genotype (HR=0.47, 95%CI=0.22-1.00, **p=0.04**) **(Figure 5.19).** Our results conclude that reduced TS expression in lung cancer patients may lead to better survival outcomes and prognosis than in patients with increased TS expression.

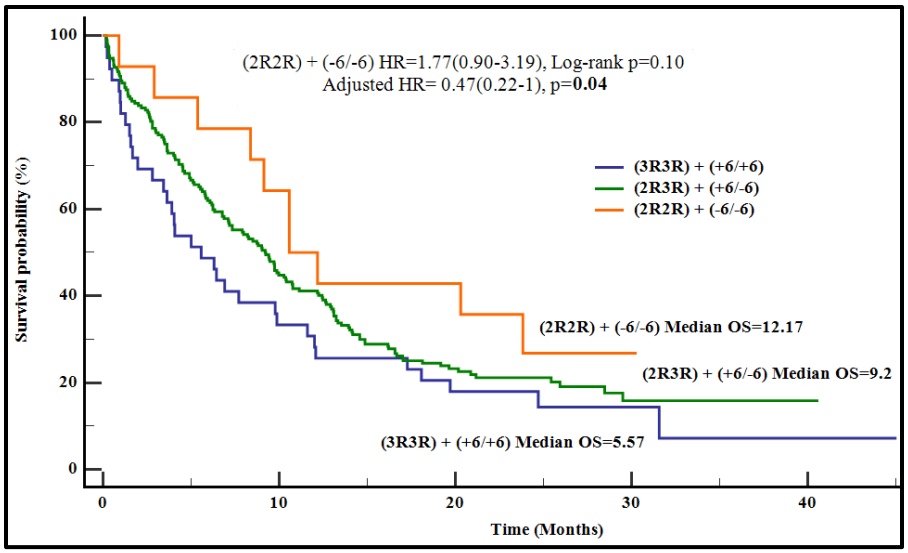


Figure 5.19: Kaplan Meier curves illustrating the combinatorial analysis of the *TS* SNPs on overall survival in lung cancer patients. (Note: *2R2R* represents wild-type genotype of *TSER*, *2R3R* represents heterozygous genotype of *TSER*, and *3R3R* represents homozygous for the mutant genotype of *TSER*); (+6/+6) represents the wild-type genotype of 1494 ins/del6, (+6/-6) represents the heterozygous genotype of 1494 ins/del6, and (-6/-6) represents homozygous for the mutant genotype of 1494 ins/del6

Table 5.12: Combinatorial analysis of the *TS* SNPs on overall survival

Genotype *TSER*+1496del6	CASES n (%), N=303	DEAD n (%), N=253	ALIVE n (%), N=50	Univariate analysis			Multivariate analysis	
				MST (months)	Log-rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
Codominant model								
(*2R2R*) + (+6/+6)	47 (15.5)	41 (16.2)	6 (12)	10.33	-	1.0 (reference)	1.0 (reference)	-
(*2R3R*) + (+6/-6)	192 (63.3)	153 (5.92)	39 (78)	9.2	0.69	1.07 (0.75-1.52)	1.02 (0.71-1.46)	0.90
(*3R3R*) + (-6/-6)	64 (21.1)	59 (23.3)	5 (10)	7.81	0.13	0.74 (0.49-1.09)	1.44 (0.92-2.25)	0.10
Dominant model								
(*2R2R*) + (+6/+6)	47 (15.5)	41 (16.2)	6 (12)	10.33	-	1.0 (reference)	1.0 (reference)	-
2R3R) + (+6/-6) and (*3R3R*) + (-6/-6)	256 (84.4)	212 (83.7)	44 (88)	9	0.93	0.98 (0.70-1.37)	1.12 (0.79-1.59)	0.50
Recessive model								
(*2R3R*) + (+6/-6) and (*2R2R*) + (+6/+6)	239 (78.8)	194 (76.6)	45 (9)	9.4	-	1.0 (reference)	1.0 (reference)	-
(*3R3R*) + (-6/-6)	64 (21.1)	59 (23.3)	5 (10)	7.81	**0.03**	0.73 (0.51-0.97)	1.37 (1.01-1.86)	**0.04**
Additive model								
(*2R2R*) + (+6/+6) and (*3R3R*) + (-6/-6)	111 (36.6)	100 (39.5)	11 (22)	8.97	-	1.0 (reference)	1.0 (reference)	-
(*2R3R*) + (+6/-6)	19263.3)	153 (60.4)	39 (78)	9.2	0.10	1.23 (0.95-1.61)	0.85 (0.66-1.11)	0.26
***TSER*+1496del6**	**CASES n (%), N=248**	**DEAD n (%), N=197**	**ALIVE n (%), N=48**	**MST (months)**	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
(*3R3R*) + (+6/+6)	39 (15.7)	34 (17.2)	5 (10.4)	5.57	-	1.0 (reference)	-	-
(*2R3R*) + (+6/-6)	192 (77.4)	153 (77.6)	39 (81.2)	9.2	0.17	1.29 (0.88-1.98)	0.76 (0.51-1.12)	0.17
(*2R2R*) + (-6/-6)	14 (5.64)	10 (5.07)	4 (8.33)	12.17	0.10	1.77 (0.90-3.19)	0.47 (0.22-1.00)	**0.04**

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, histology, stage, KPS, ECOG

5.8 Impact of *TS* polymorphisms on clinical outcomes in lung cancer patients

5.8.1 Association of TS polymorphism and chemotherapy response

Univariate logistic regression was used to examine the relationship between *TS* polymorphisms and the clinical outcome of response rate. The correlation of *TS* polymorphism with the chemotherapeutic response has been illustrated in **Table 5.13**. The subjects demonstrating stable (SD) and progressive disease (PD) were categorized as poor responders, and those exhibiting complete (CR) and partial responses (PR) were classified as good responders. According to the platinum-based chemotherapy treatment response for *TSER* polymorphism, our results showed an odds of 1.40 times in the mutant genotype (*3R3R)* of poor responders as compared to the mutant genotype (*3R3R)* of good responders (AOR=1.40, 95% CI=0.77-2.56,p=0.26); however, no statistical significance was shown between the *TSER* polymorphism and platinum-based chemotherapeutic treatment response. Similarly, for the *1494del6* variation, 20.4% of poor responders with homozygous mutant alleles (*-6/-6)* had an adjusted odd of 1.21 times that of 16.1% of good responders (AOR=1.21, 95% CI=0.62-2.35, p=0.56). However, no substantial difference was found. We summarize that none of the *TS* polymorphisms were involved in the significant prediction of chemotherapeutic response or clinical benefit.

Table 5.13: Association of *TS* SNPs and chemotherapeutic response

	Response of chemotherapy		**AOR (95% CI)** [a]	***p*** [b]
Genotype rs34743033 *TS* (VNTR)	**CR+PR (161), n (%)**	**SD+PD (205), n (%)**		
2R2R	34 (21.1)	33 (16.0)	1.0 (Reference)	
2R3R	70 (43.4)	99 (48.2)	1.49 (0.83-2.65)	0.17
3R3R	57 (35.4)	73 (35.6)	1.40 (0.77-2.56)	0.26
2R3R+3R3R	127 (78.8)	172 (83.9)	1.44 (0.84-2.46)	0.17
Genotype rs151264360 (1494 ins/del6)	**CR+PR (161), n (%)**	**SD+PD (205), n (%)**	**AOR (95% CI)** [a]	***p*** [b]
+6/+6	36 (22.3)	47 (22.9)	1.0 (Reference)	
+6/-6	99 (61.4)	116 (56.5)	0.92 (0.55-1.55)	0.78
-6/-6	26 (16.1)	42 (20.4)	1.21 (0.62-2.35)	0.56
+6/-6 + -6/-6	125 (77.6)	158 (77.0)	0.98 (0.60-1.62)	0.96

a)-Adjusted Odds ratios, 95% confidence intervals, and corresponding p-values were calculated by logistic regression analysis after adjusting for age, gender, regimen, and smoking. (b)-Two-sided $\chi 2$ test for either genotype distribution or allelic frequencies between the responders and non-responders. The number in bold indicates the significant values in the table

5.8.2 Association of clinicopathological features TS polymorphic variants

The effect of the *TS* SNPs on various clinicopathological features such as tumor stage, primary tumor extension, lymph node invasion, and distant metastasis was also calculated and is illustrated in **table 5.14**. The subjects were segregated according to the clinical stage (III *vs.* IV), tumor extension (T3+T4 *vs.* T0+T1+T2), lymph node invasion (N2+N3+N4 *vs.* Nx+N0+N1), and metastatic stats (positive *vs.* negative). The lung cancer patients were divided into stages III and IV based on the clinical stage. The genotypic frequency of all the polymorphisms has been evaluated. The incidence of patients in stage IV is 73.79%, whereas 26.2% of patients were in stage III of lung cancer. For *TSER* polymorphism, the prevalence of mutant type genotype in stage IV (36.9%) was more than that of stage III (33.9%); also, in the case of *1494del6* polymorphism, the frequency of mutant type genotype *(-6/-6)* was higher in stage IV patients (19.6%) as compared to stage III patients (16%). No risk was observed for both *TSER* mutant (AOR=1.01, 95% CI=0.59-1.74, p=0.9) and *1494del6* mutant genotypes (AOR=1.35, 95% CI= 0.75-2.41, p=0.30). Therefore, no significant association was found between *TS* polymorphisms (*TSER and 1494del6*) and tumor stage in all the models assessed, as shown in **table 5.14**.

Further, the patients were stratified based on primary tumor extension (T3 *vs.* T4). The frequency of each genotype is mentioned in **table 5.14**. For *TSER* polymorphism, it was observed that the frequency of homozygous variant allele with T4 extension was slightly higher than patients with T3 extension. Also, no significant association of this polymorphism was found with direct tumor extension (AOR=0.86, p=0.62). For *1494del6* polymorphism, 56.3% of patients with at least one mutant allele (+6/-6) and T4 extension had no risk compared to the 60.4% of patients with T3 tumor extension, though the results did not show any substantial differences (AOR=0.75, 95% CI=0.46-1.22, p=0.25).

Furthermore, no significant association was found between *TS* polymorphisms (*TSER* and *1494del6*) and lymph node invasion in all the models assessed, as shown in **table 5.14**. In the case of *1494 ins/del6* polymorphism and its association with metastasis, we observed that individuals harboring heterozygous *(+6/-6)* genotype was less likely to manifest metastasis as compared to subjects carrying wild type *(+6/+6)* genotype (AOR=0.58, 95%CI=0.39-0.86; p=**0.006**). There was no significant association between tumor stage, primary tumor extension, and lymph node invasion with any *TS* polymorphic variants **(Table 5.14).**

Table 5.14: Relationship of different *TS* polymorphic genotypes with clinic-pathological parameters

CLINICAL STAGE					LYMPH NODE INVASION				
Genotype rs34743033 *TS* (VNTR)	**Stage III (168), n (%)**	**Stage IV (473), n (%)**	**AOR (95% CI)** [a]	***p*** [b]	**Genotype** rs34743033 *TS* (VNTR)	**Nx+N0+N1 (144), n (%)**	**N2+N3+N4 (518), n (%)**	**AOR (95% CI)** [a]	***p*** [b]
2R2R	28(16.6)	89(18.8)	1.0(Reference)		*2R2R*	29(20.1)	94(18.1)	1.0(Reference)	
2R3R	83(49.4)	209(44.1)	0.82(0.49-1.38)	0.46	*2R3R*	61(42.3)	240(46.3)	1.18(0.71-1.95)	0.52
3R3R	57(33.9)	175(36.9)	1.01(0.59-1.74)	0.95	*3R3R*	54(37.5)	184(35.5)	1.07(0.63-1.80)	0.79
2R3R+3R3R	140(83.3)	384(81.1)	0.91(0.56-1.47)	0.70	*2R3R+3R3R*	115(79.8)	424(81.8)	1.11(0.70-1.78)	0.63
Genotype rs151264360 (1494 ins/del6)	**Stage III (168), n (%)**	**Stage IV (473), n (%)**	**AOR (95% CI)** [a]	***p*** [b]	**Genotype** rs151264360 (1494 ins/del6)	**Nx+N0+N1 (144), n (%)**	**N2+N3+N4 (518), n (%)**	**AOR (95% CI)** [a]	***p*** [b]
+6/+6	41(24.4)	115(24.3)	1.0(Reference)		+6/+6	37(25.6)	125(24.1)	1.0(Reference)	
+6/-6	100(59.5)	265(56.0)	0.99(0.64-1.54)	0.98	+6/-6	79(54.8)	300(57.9)	1.10(0.70-1.71)	0.66
-6/-6	27(16.0)	93(19.6)	1.35(0.75-2.41)	0.30	-6/-6	28(19.4)	93(17.9)	0.96(0.54-1.68)	0.88
+6/-6 + -6/-6	127(75.5)	358(75.8)	1.06(0.69-1.61)	0.78	+6/-6 + -6/-6	107(74.3)	393(75.8)	1.06(0.69-1.63)	0.76
TUMOR SIZE					**METASTASIS**				
Genotype rs34743033 *TS* (VNTR)	**T3 (139), n (%)**	**T4 (399), n (%)**	**AOR (95% CI)** [a]	***p*** [b]	**Genotype** rs34743033 *TS* (VNTR)	**M0 (317), n (%)**	**M1 (345), n (%)**	**AOR (95% CI)** [a]	***p*** [b]
2R2R	26(18.7)	78(19.5)	1.0(Reference)		*2R2R*	58(18.2)	65(18.8)	1.0(Reference)	
2R3R	63(45.3)	181(45.3)	0.92(0.54-1.57)	0.78	*2R3R*	149(47.0)	152(44.0)	0.93(0.59-1.46)	0.76
3R3R	50(35.9)	140(35.0)	0.86(0.49-1.52)	0.62	*3R3R*	110(34.7)	128(37.1)	1.16(0.73-1.85)	0.52
2R3R+3R3R	113(81.2)	321(80.4)	0.90(0.55-1.48)	0.69	*2R3R+3R3R*	259(81.7)	280(81.1)	1.02(0.67-1.54)	0.92
Genotype rs151264360 (1494 ins/del6)	**T3 (139), n (%)**	**T4 (399), n (%)**	**AOR (95% CI)** [a]	***p*** [b]	**Genotype** rs151264360 (1494ins/del6)	**M0 (317), n (%)**	**M1 (345), n (%)**	**AOR (95% CI)** [a]	***p*** [b]
+6/+6	30(15.5)	103(25.8)	1.0(Reference)		+6/+6	63(19.8)	99(28.6)	1.0(Reference)	
+6/-6	84(60.4)	225(56.3)	0.75(0.46-1.22)	0.25	+6/-6	198(62.4)	181(52.4)	0.58(0.39-0.86)	**0.006**
-6/-6	25(17.9)	71(17.7)	0.83(0.45-1.55)	0.57	-6/-6	56(17.6)	65(18.8)	0.77(0.46-1.27)	0.31
+6/-6 + -6/-6	109(78.4)	296(74.1)	0.77(0.48-1.23)	0.28	+6/-6 + -6/-6	254(80.1)	246(71.3)	0.62(0.42-0.90)	**0.014**

a)-Adjusted Odds ratios, 95% confidence intervals, and corresponding p-values were calculated by logistic regression analysis after adjusting for age, gender, regimen, and smoking. (b)-Two-sided $\chi 2$ test for either genotype distribution or allelic frequencies between different clinical-pathological parameters. The number in bold indicates the significant values in the table

5.9 Association between TS genotypes and toxicity

One of the current objectives of interest in this investigation was to assess the development of toxicity in lung cancer patients administered with platinum-based doublet chemotherapy along with pemetrexed, docetaxel, paclitaxel, and irinotecan. Univariate logistic regression analysis evaluated the odds ratio (OR), and 95% CI was used to assess the risk of acquiring different toxicity grades after therapy in subjects with each genotype. The multivariate regression model was also employed to evaluate the adjusted ORs, using the toxicity grades as the dependent variable. Gender, age, performance status, and regimen were added as covariates in the multivariate model and were assessed for potential interaction or confounding effects. We used three distinct analysis methods to see how polymorphisms affected different toxicity levels. In the first assessment, we compared subjects with any toxicity grade (grades 1-4) versus no toxicity (grade 0), in the second analysis, we compared patients with intermediate/ severe toxicity (grades 2-4) versus no/low-grade (grade 0-1) toxicity, and in the third analysis, we compared severe toxicity (grade 3-4) with no/low/intermediate-grade (grade 0-2) to broadly assess whether the *TS* polymorphisms were correlated with severe toxicities. In this manner, cases were grouped into different toxicity grades.

5.9.1 Association between TS genotypes and hematological toxicity

All chemotherapy-related adverse events (AEs) were documented for each treatment cycle. We have assessed leukopenia, anemia, thrombocytopenia, and absolute neutropenia (ANC) in hematological toxicities, as illustrated in **table 5.15**. In leukopenia, a total of 410 patients' toxicity data was available. The table shows that 13.6% of subjects were classified as grade 1-4 leukopenia, and 86.3% of patients were categorized as grade 0 leukopenia. A comparison was made between patients with any toxicity grade (grade 1-4) and with no toxicity grade (grade 0). For *TSER* polymorphism, 57.1% of patients harbored single copy mutant allele (*2R3R*). **Table 5.15** shows that, after adjusting with multiple covariates, the patients with heterozygous genotype (*2R3R*) showed 2.30-fold increased risk of developing leukopenia (AOR=2.30, 95% CI=0.96-5.52; p=0.05) when compared to the subjects with wild type genotype (*2R2R*). For *1494 ins/del6* polymorphism, 26.7% of patients categorized in grade 1-4 leukopenia harbored mutant *(-6/-6)* genotype and showed a 2.40-fold elevated risk of developing leukopenia (OR=2.40, 95% CI=1.03-5.57; p=**0.04**) as compared to the wild type genotype *(+6/+6)*. After adjusting for confounding, factors like gender, age, regimen, and performance status, a 2.57-fold risk of leukopenia was

observed compared to the wild-type genotype (*+6/+6bp)* (AOR=2.57, 95% CI=1.07-6.15; p=**0.03**). On comparing patients with intermediate/ severe toxicity (grades 2-4) versus no/low-grade (grade 0-1) toxicity, we observed 6.44-fold high risk in mutant genotype (*-6/-6bp)* as compared to wild type genotype (+6/+6bp) (AOR=6.44, 95% CI=1.16-35.6; p=**0.03**).

In anemia, we compared intermediate/severe toxicity (grades 2-4) versus no/low-grade (grade 0-1) toxicity as shown in **table 5.15**. Lung cancer patients with mutant genotype (*-6/-6bp)* (AOR=1.90, 95% CI=1.02-3.56, p=**0.04**) for *1494 ins/del6* polymorphism had a roughly 2-fold risk of developing anemia as compared to patients with wild genotype (*+6/+6bp)*. On the contrary, in *TSER* polymorphism, when we assessed severe toxicity (grade 3-4) grades with no/low/intermediate grades (grade 0-2), we observed that 36.6% with wild type genotype (*2R2R*), 33.3%, with heterozygous genotype (*2R3R*) and 30% patients with mutant genotype (*3R3R*) had grade 3-4 anemia. The patients harboring heterozygous genotype (*2R3R*) had a reduced risk of anemia as compared to the wild type (*2R2R*) (OR=0.38, 95%CI=0.15-0.95; p=**0.03).** Also, we observed a decreased risk of anemia in patients with mutant type genotype (*3R3R*) as compared to wild type genotype (*2R2R*) (OR=0.40, 95% CI=0.15-1.01, p=0.05). Further, the combined genotype (*2R3R+3R3R*) for *TSER* polymorphism also showed a reduced risk of anemia on adjusting to the confounding factors such as age, gender, regimen, and performance status as compared to wild type genotype (*2R2R*) (AOR=0.42, 95% CI=0.18-0.94; p=**0.03).** Additionally, we did not find any statistically significant relationship between other hematological toxicities such as neutropenia, thrombocytopenia, and *TS* polymorphism (**Table 5.15**).

In absolute neutrophil count, we compared low/intermediate/severe toxicity (grade 1-4) with no toxicity (grade 0) as depicted in **table 5.15**. Lung cancer patients with mutant (*-6/-6)* genotype showed a 2.36-fold elevated risk of developing neutropenia (OR=2.36, 95% CI=0.89-6.25; p=0.08) as compared to the wild type genotype *(+6/+6)*. After adjusting for confounding, factors like gender, age, regimen, and performance status, a 2.60-fold risk of neutropenia was observed as compared to the wild-type genotype (*+6/+6bp)* (AOR=2.60, 95% CI=0.94-7.18; p=0.06).

Table 5.15: Association of hematological toxicity (grade 0 *vs.* 1-4, grade 0-1 *vs.* 2-4, and grade 0-2 *vs.* 3-4) with *TSER* and *1494del6* polymorphism

Hematological toxicity													
Leukopenia													
Genotype rs34743033 *TS* (VNTR)	**Grade of toxicity 0, n(%) N=354**	**Grade of toxicity 1-4, n(%) N=56**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype** rs34743033 *TS* (VNTR)	**Grade of toxicity 0-1, n(%) N=393**	**Grade of toxicity 2-4, n(%) N=17**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
2R2R	73(20.6)	8(14.2)	1.00 (Reference)		1.00 (Reference)		*2R2R*	77(19.5)	4(23.5)	1.00 (Reference)		1.00 (Reference)	
2R3R	145(40.9)	32(57.1)	2.01(0.88-4.59)	0.09	2.30(0.96-5.52)	0.05	*2R3R*	169(43.0)	8(47.0)	0.91(0.26-3.11)	0.88	1.18(0.29-4.75)	0.80
3R3R	136(38.4)	16(28.5)	1.07((0.43-2.62)	0.87	1.14(0.44-2.94)	0.78	*3R3R*	147(37.4)	5(29.4)	0.65(0.17-2.50)	0.53	0.95(0.21-4.22)	0.94
2R3R+3R3R	281(79.3)	48(85.7)	1.55(0.70-3.43)	0.27	1.74(0.75-4.04)	0.19	*2R3R+3R3R*	316(80.4)	13(76.4)	0.79(0.25-2.49)	0.69	1.05(0.28-3.86)	0.93
Genotype rs151264360 (1494 ins/del6)	**Grade of toxicity 0, n(%) N=354**	**Grade of toxicity 1-4, n(%) N=56**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype** rs151264360 (1494 ins/del6)	**Grade of toxicity 0-1, n(%) N=393**	**Grade of toxicity 2-4, n(%) N=17**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
+6/+6	104(29.3)	11(19.6)	1.00 (Reference)		1.00 (Reference)		+6/+6	113(28.7)	2(11.7)	1.00 (Reference)		1.00 (Reference)	
+6/-6	191(53.9)	30(53.5)	1.4(0.71-3.08)	0.28	1.40(0.66-2.93)	0.37	+6/-6	212(53.9)	9(52.9)	2.39(0.50-11.29)	0.26	2.26(0.45-11.15)	0.31
-6/-6	59(16.6)	15(26.7)	2.40(1.03-5.57)	**0.04**	2.57(1.07-6.15)	**0.03**	-6/-6	68(17.3)	6(35.2)	4.98(0.97-25.4)	0.05	6.44(1.16-35.6)	**0.03**
+6/-6 + -6/-6	250(70.6)	45(80.3)	1.70(0.84-3.41)	0.13	1.63(0.81-3.3)	0.16	+6/-6 + -6/-6	280(71.2)	15(88.2)	3.02(0.68-13.45)	0.14	2.97(0.65-13.52)	0.15
Anaemia													
Genotype rs34743033 *TS* (VNTR)	**Grade of toxicity 0 n(%) N=102**	**Grade of toxicity 1-4, n(%) N=310**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype** rs151264360 (1494 ins/del6)	**Grade of toxicity 0-1 n(%) N=250**	**Grade of toxicity 2-4 n(%) N=162**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
2R2R	22(21.5)	60(19.3)	1.00 (Reference)		1.00 (Reference)		+6/+6	76(30.4)	40(24.6)	1.00 (Reference)		1.00 (Reference)	
2R3R	41(40.1)	136(43.8)	1.21(0.66-2.21)	0.52	1.31(0.70-2.46)	0.38	+6/-6	135(54)	86(53.0)	1.21(0.75-1.93)	0.42	1.22(0.76-1.98)	0.39
3R3R	39(38.2)	114(36.7)	1.07(0.58-1.97)	0.82	1.20(0.64-2.27)	0.55	-6/-6	39(15.6)	36(22.2)	1.75(0.96-3.17)	0.06	1.90(1.02-3.56)	**0.04**
2R3R+3R3R	80(78.4)	250(80.6)	1.14(0.66-1.98)	0.62	1.23(0.70-2.17)	0.46	+6/-6 + -6/-6	174(69.6)	122(75.3)	1.33(0.85-2.08)	0.18	1.35(0.85-2.14)	0.18
Genotype rs151264360 (1494 ins/del6)	**Grade of toxicity 0 n(%)102**	**Grade of toxicity 1-4, n(%)302**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype** rs34743033 *TS* (VNTR)	**Grade of toxicity 0-2 n(%)382**	**Grade of toxicity 3-4 n(%)30**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
+6/+6	36(35.29)	80(25.8)	1.00 (Reference)		1.00 (Reference)		*2R2R*	71(18.5)	11(36.6)	1.00 (Reference)		1.00 (Reference)	
+6/-6	51(50)	170(54.8)	1.5(0.90-2.47)	0.11	1.42(0.84-2.39)	0.18	*2R3R*	167(43.7)	10(33.3)	0.38(0.15-0.95)	**0.03**	0.41(0.16-1.03)	0.06
-6/-6	15(14.7)	60(19.3)	1.80(0.90-3.58)	0.09	1.58(0.77-3.23)	0.21	*3R3R*	144((37.6)	9(30)	0.40(0.15-1.01)	0.05	0.42(0.16-1.10)	0.07
+6/-6 + -6/-6	66(64.7)	230(74.1)	1.56(0.97-2.53)	0.06	1.46(0.89-2.41)	0.13	*2R3R+3R3R*	311(81.4)	19(63.33)	0.39(0.17-0.86)	**0.02**	0.42(0.18-0.94)	**0.03**
Genotype rs34743033 *TS* (VNTR)	**Grade of toxicity 0-1 n(%)250**	**Grade of toxicity 2-4 n(%)162**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype** rs151264360 (1494 ins/del6)	**Grade of toxicity 0-2 n(%) N=382**	**Grade of toxicity 3-4 n(%) N=30**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
2R2R	43(17.2)	39(24.0)	1.00 (Reference)		1.00 (Reference)		+6/+6	108(28.2)	8(26.6)	1.00 (Reference)		1.00 (Reference)	
2R3R	111(44.4)	66(40.7)	0.65(0.38-1.11)	0.11	0.69(0.40-1.19)	0.18	+6/-6	203(53.1)	18(60)	1.19(0.50-2.84)	0.68	1.19(0.49-2.87)	0.68
3R3R	96(38.4)	57(35.18)	0.65(0.38-1.12)	0.12	0.68(0.39-1.19)	0.18	-6/-6	71(18.5)	4(13.33)	0.76(0.22-2.62)	0.66	0.95(0.26-3.47)	0.94
2R3R+3R3R	207(82.8)	123(75.9)	0.65(0.40-1.06)	0.08	0.68(0.41-1.12)	0.13	+6/-6 + -6/-6	274(71.7)	22(73.3)	1.08(0.46-2.50)	0.85	1.12(0.48-2.64)	0.78

Thrombocytopenia													
Genotype rs34743033 *TS* (VNTR)	**Grade of toxicity 0 n(%) N=348**	**Grade of toxicity 1-3, n(%) N=56**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype** rs151264360 (1494 ins/del6)	**Grade of toxicity 0 n(%)**	**Grade of toxicity 1-3, n(%)**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
2R2R	70(20.1)	8(14.2)	1.00 (Reference)	-	1.00 (Reference)	-	+6/+6	99(28.4)	13(23.2)	1.00 (Reference)	-	1.00 (Reference)	-
2R3R	150(43.1)	25(44.6)	1.45(0.62-3.39)	0.38	2.01(0.81-5.01)	0.13	+6/-6	189(59.4)	30(53.5)	1.20(0.60-2.42)	0.59	1.25(0.61-2.58)	0.52
3R3R	128(36.7)	23(41.07)	1.57(0.66-3.69)	0.29	1.92(0.76-4.84)	0.16	-6/-6	60(17.2)	13(23.2)	1.65(0.71-3.79)	0.23	1.82(0.77-4.31)	0.17
2R3R+3R3R	278(79.8)	48(85.7)	1.51(0.68-3.33)	0.30	1.81(0.78-4.24)	0.16	+6/-6 + -6/-6	249(71.5)	43(76.7)	1.31(0.67-2.55)	0.41	1.39(0.70-2.76)	0.34
ANC													
Genotype rs34743033 *TS* (VNTR)	**Grade of toxicity 0 n(%) N=338**	**Grade of toxicity 1-4, n(%) N=35**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype** rs34743033 *TS* (VNTR)	**Grade of toxicity 0-1 n(%) 355**	**Grade of toxicity 2-4 n(%)18**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
2R2R	68(20.1)	7(20)	1.00 (Reference)		1.00 (Reference)		*2R2R*	71(20)	4(22.2)	1.00 (Reference)		1.00 (Reference)	
2R3R	146(43.1)	17(48.5)	1.13(0.44-2.85)	0.79	1.30(0.48-3.48)	0.59	*2R3R*	154(43.3)	9(50)	1.03(0.30-3.48)	0.95	1.08(0.31-3.70)	0.89
3R3R	124(36.6)	11(31.4)	0.86(0.31-2.32)	0.76	0.93(0.32-2.71)	0.90	*3R3R*	130(36.6)	5(27.7)	0.68(0.17-2.62)	0.57	0.82(0.20-3.35)	0.79
2R3R+3R3R	270(79.8)	28(80)	1.25(0.53-2.95)	0.59	1.12(0.44-2.83)	0.81	*2R3R+3R3R*	284(80)	14(77.7)	0.87(0.27-2.73)	0.81	0.93(0.29-2.97)	0.91
Genotype rs151264360 (1494 ins/del6)	**Grade of toxicity 0 n(%) N=338**	**Grade of toxicity 1-4, n(%) N=35**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype** rs151264360 (1494 ins/del6)	**Grade of toxicity 0-1 n(%) N=355**	**Grade of toxicity 2-4 n(%) N=18**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
+6/+6	93(27.5)	8(22.8)	1.00 (Reference)		1.00 (Reference)		+6/+6	99(27.8)	2(11.1)	1.00 (Reference)		1.00 (Reference)	
+6/-6	191(56.5)	16(45.7)	0.97(0.40-2.35)	0.95	0.91(0.37-2.24)	0.84	+6/-6	196(55.2)	11(61.1)	2.77(0.60-12.7)	0.18	2.96(0.63-13.8)	0.16
-6/-6	54(15.9)	11(31.4)	2.36(0.89-6.25)	0.08	2.60(0.94-7.18)	0.06	-6/-6	60(16.9)	5(27.7)	4.12(0.77-21.9)	0.09	4.31(0.78-23.67)	0.09
+6/-6 + -6/-6	245(72.4)	27(77.1)	1.28(0.56-2.92)	0.55	1.22(0.53-2.82)	0.62	+6/-6 + -6/-6	256(72.1)	16(88.8)	3.09(0.69-13.7)	0.13	3.21(0.72-14.29)	0.12

OR[1] Crude odds ratio, 95% CI: 95% confidence interval, **AOR[2]**Adjusted Odds ratio data were calculated by unconditional logistic regression and adjusted for age, gender, regimen, and performance status. A p-value < 0.05 was considered statistically significant

5.9.2 Association between TS genotypes and nephrological toxicity

Chemotherapeutic drugs are eliminated through the kidneys and are nephrotoxic; thus, renal functions are monitored during chemotherapy. Data of 402 patients were available to evaluate the nephrotoxicity. Among 402 patients, 14.9% were categorized as no/low toxicity, whereas 85% of patients were classified in intermediate/severe toxicity grade. We have evaluated chemotherapy-induced nephrotoxicity by comparing intermediate/severe toxicity (grades 3-5) versus no/low grade (grades 1-2) toxicity. We also compared low/intermediate/severe (grades 2-5) toxicity to no grade (grades 1) toxicity in chemotherapy-induced nephrotoxicity, as shown in **table 5.16**.

When grades 2-5 were compared to grade 1, our results showed that lung cancer patients with heterozygous (*2R3R)* genotype for *TSER* polymorphism showed a higher incidence of developing nephrotoxicity when compared to individuals with wild-type genotype (*2R2R*), but the significant association was not found (OR=1.4, 95% CI=0.70-3.01; p=0.31). For *1494del6* polymorphism, 16.6% of patients with variant genotype *(-6/-6)* were categorized as low/ intermediate and severe toxicity, whereas 25% had variant genotype *(-6/-6)* showed no renal toxicity. In the co-dominant model, the mutant genotype (*-6/-6)* had an adjusted-odds of 0.52 as compared to the wild-type genotype (*+6/+6)* (AOR=0.52, 95% CI=0.21-1.30, p=0.16). We did not find any correlation between the *TSER* and *1494del6* polymorphisms and nephrotoxicity-related adverse events due to chemotherapy **(Table 5.16).**

Table 5.16: Association of TS polymorphism with nephrotoxicity toxicity

Nephrotoxicity						
GFR						
Genotype rs34743033 *TS* (VNTR)	**Grade of toxicity 1, n (%) N=60**	**Grade of toxicity 2-5, n (%) N=342**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
2R2R	14 (23.3)	67 (19.5)	1.00 (Reference)		1.00 (Reference)	
2R3R	22 (36.6)	153 (44.7)	1.4 (0.70-3.01)	0.31	1.38 (0.60-3.18)	0.44
3R3R	24 (40)	122 (35.6)	1.06 (0.51-2.18)	0.87	0.99 (0.45-2.19)	0.99
2R3R+3R3R	46 (76.6)	275 (80.4)	1.24 (0.64-2.40)	0.50	1.15 (0.55-2.39)	0.69
Genotype rs151264360 (1494 ins/del6)	**Grade of toxicity 1, n (%) N=60**	**Grade of toxicity 2-5, n (%) N=342**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
+6/+6	16 (26.6)	98 (28.6)	1.00 (Reference)		1.00 (Reference)	
+6/-6	29 (48.3)	187 (54.6)	1.05 (0.54-2.03)	0.87	1.03 (0.51-2.08)	0.92
-6/-6	15 (25)	57 (16.6)	0.62 (0.28-1.34)	0.22	0.52 (0.21-1.30)	0.16
+6/-6 + -6/-6	44 (73.3)	244 (71.3)	0.90 (0.48-1.68)	0.75	0.89 (0.46-1.7)	0.75
Genotype rs34743033 *TS* (VNTR)	**Grade of toxicity 1-2, n (%) N=209**	**Grade of toxicity 3-5, n (%) N=193**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
2R2R	45 (21.5)	36 (18.6)	1.00 (Reference)		1.00 (Reference)	
2R3R	89 (42.5)	86 (44.5)	1.20 (0.71-2.05)	0.48	1.32 (0.72-2.42)	0.36
3R3R	75 (35.8)	71 (36.7)	1.18 (0.68-2.04)	0.54	1.38 (0.74-2.57)	0.30
2R3R+3R3R	164 (78.4)	157 (81.3)	1.19 (0.73-1.95)	0.47	1.31 (0.75-2.29)	0.33
Genotype rs151264360 (1494 ins/del6)	**Grade of toxicity 1-2, n (%) N=209**	**Grade of toxicity 3-5, n (%) N=193**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
+6/+6	59 (28.2)	55 (28.4)	1.00 (Reference)		1.00 (Reference)	
+6/-6	112 (53.5)	104 (53.8)	0.99 (0.63-1.56)	0.98	1.0 (0.60-1.67)	0.99
-6/-6	38 (18.1)	34 (17.6)	0.95 (0.53-1.73)	0.89	0.92 (0.46-1.84)	0.82
+6/-6 + -6/-6	150 (71.7)	138 (71.5)	0.98 (0.63-1.52)	0.95	0.98 (0.60-1.59)	0.94

OR[1] Crude odds ratio, 95% CI: 95% confidence interval, **AOR[2]**Adjusted Odds ratio data were calculated by unconditional logistic regression and adjusted for age, gender, regimen, and performance status. A p-value < 0.05 was considered statistically significant

5.9.3 Association between TS genotypes and gastrointestinal toxicity

In the gastrointestinal (GI) toxicity, we assessed diarrhea, constipation, anorexia, and nausea/ vomiting. In the case of diarrhea, among 139 patients, 44.6% of individuals had grade 1 toxicity, and 55.3% of patients had grade 2-4 toxicity. When a comparison between both grades was made, our results showed an odds of 1.23 and 1.42 for having diarrhea for the mutant genotypes of both *TSER* (AOR=1.23, 95% CI=0.44-3.46, p=0.6) and *1494del6* (AOR=1.42, 95% CI=0.52-3.89, p=0.49) polymorphism, respectively, however, no significant association was found.

We also looked at gastrointestinal (constipation) toxicity by comparing individuals with low toxicity (grade 1) to those with intermediate-high grades (grade 2). **Table 5.17** shows that lung carcinoma patients with the heterozygous genotype *(2R3R)* (OR=5.14, 95% CI=1.5-16.9; **p=0.007**) for *TSER* polymorphism had a 5-fold risk of constipation compared to the subjects having wild type genotype (*2R2R*). After adjusting with different covariates such as age, gender, regimen, and performance status, the odds of 4.76 was observed in the heterozygous group (AOR=4.76, 95% CI=1.33-17.04, p=**0.0016**). The patients harboring mutant genotype also showed a 3-fold risk of constipation as compared to the wild type genotype (OR=3.21, 95% CI= 0.98-10.47, p=0.05). Further, lung carcinoma patients with the combined mutant type (*3R3R*) and heterozygous genotype (*2R3R*) revealed a 4.09-fold elevated risk of having constipation (OR=4.09, 95% CI=1.38-12.05; **p=0.01)** as compared to the wild type genotype (*2R2R*). Even after adjusting with multiple covariates, the risk remained 3.65 times in the combined mutant type (*3R3R*) and heterozygous genotype (*2R3R*) (AOR=3.65, 95% CI=1.18-11.27, p=**0.02**).

In nausea/vomiting, we compared intermediate/ severe toxicity (grades 2-4) versus low-grade (grade 1) toxicity. 54.6% of patients experienced grade 2-4 nausea/vomiting. For *1494 ins/del6* polymorphism, an increased risk of nausea/vomiting was observed in patients with mutant genotype (*-6/-6bp)* with an odds of 2.77 as compared to patients with wild-type genotype *(+6/+6bp)* (AOR= 2.77; 95%CI=1.10-6.96, p=**0.03**) as shown in **table 5.17**. Additionally, lung cancer patients with combined heterozygous genotype (*+6/-6)* and mutant genotype (*-6/-6)* showed an increased risk of nausea/vomiting with an odd of 2.85 as compared to wild type genotype (*+6/+6)* (AOR= 2.85; 95%CI=1.18-6.86, p=**0.01).** We did not find any association between the TS polymorphism, other gastrointestinal toxicities such as diarrhea and anorexia, and other AEs due to chemotherapy (**Table 5.17**).

Table 5.17: Association of gastrointestinal toxicity (grade 0 *vs.* 1-4, grade 0-1 *vs.* 2-4, and grade 0-2 *vs.* 3-4) with *TSER* and *1494del6* polymorphism

Gastrointestinal toxicity													
Diarrhoea													
Genotype rs34743033 *TS* (VNTR)	**Grade of toxicity 1 n(%) N=62**	**Grade of toxicity 2-4, n(%) N=77**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype** rs34743033 *TS* (VNTR)	**Grade of toxicity 1-2 n(%) N=108**	**Grade of toxicity 3-4 n(%) N=31**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
2R2R	11(17.7)	13(16.8)	1.00 (Reference)		1.00 (Reference)		*2R2R*	19(17.5)	5(16.1)	1.00 (Reference)		1.00 (Reference)	
2R3R	27(43.54)	30(38.9)	0.94(0.36-2.44)	0.89	0.82(0.30-2.22)	0.70	*2R3R*	46(42.5)	11(35.4)	0.90(0.27-2.97)	0.87	0.86(0.25-2.91)	0.81
3R3R	24(38.7)	34(44.1)	1.19(0.45-3.12)	0.71	1.23(0.44-3.46)	0.68	*3R3R*	43(39.8)	15(48.3)	1.32(0.42-4.17)	0.63	1.31(0.39-4.35)	0.65
2R3R+3R3R	51(82.2)	64(83.1)	1.06(0.43-2.56)	0.89	0.98(0.39-2.45)	0.97	*2R3R+3R3R*	89(82.4)	26(83.8)	1.11(0.37-3.26)	0.84	1.06(0.35-3.21)	0.91
Genotype rs151264360 (1494 ins/del6)	**Grade of toxicity 1 n(%) N=62**	**Grade of toxicity 2-4, n(%) N=77**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype** rs151264360 (1494 ins/del6)	**Grade of toxicity 1-2 n(%) N=108**	**Grade of toxicity 3-4 n(%) N=31**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
+6/+6	17(27.4)	18(23.3)	1.00 (Reference)		1.00 (Reference)		+6/+6	29(26.8)	6(19.3)	1.00 (Reference)		1.00 (Reference)	
+6/-6	33(53.2)	42(54.5)	1.20(0.53-2.68)	0.65	1.31(0.56-3.03)	0.52	+6/-6	58(53.7)	17(54.8)	1.41(0.50-3.97)	0.50	1.61(0.55-4.67)	0.38
-6/-6	12(19.35)	17(22.0)	1.33(0.49-3.61)	0.56	1.42(0.52-3.89)	0.49	-6/-6	21(19.4)	8(25.8)	1.84(0.55-6.10)	0.31	1.73(0.49-6.07)	0.38
+6/-6 + -6/-6	45(72.5)	59(76.6)	1.23(0.57-2.66)	0.58	1.36(0.61-3.00)	0.44	+6/-6 + -6/-6	79(73.1)	25(80.6)	1.52(0.56-4.10)	0.39	1.56(0.57-4.29)	0.38
Anorexia													
Genotype rs34743033 *TS* (VNTR)	**Grade of toxicity 1 n(%) N=8**	**Grade of toxicity 2-3, n(%) N=119**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype** rs151264360 (1494 ins/del6)	**Grade of toxicity 1 n(%) N=8**	**Grade of toxicity 2-3, n(%) N=119**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
2R2R	1(12.5)	20(16.8)	1.00 (Reference)		1.00 (Reference)		+6/+6	3(37.5)	29(24.3)	1.00 (Reference)		1.00 (Reference)	
2R3R	5(62.5)	45(37.8)	0.45(0.04-4.10)	0.47	0.45(0.04-4.30)	0.49	+6/-6	5(62.5)	67(56.3)	1.38(0.31-6.18)	0.66	1.17(0.24-5.62)	0.83
3R3R	2(25)	54(45.3)	1.35(0.11-15.71)	0.81	1.39(0.08-21.96)	0.81	-6/-6	-	23(19.3)	-	-	-	-
2R3R+3R3R	7(87.5)	99(83.1)	0.70(0.08-6.06)	0.75	0.76(0.08-6.81)	0.80	+6/-6 + -6/-6	8(100)	90(75.6)	1.86(0.41-8.27)	0.41	1.79(0.38-8.35)	0.45
Constipation													
Genotype rs34743033 *TS* (VNTR)	**Grade of toxicity 1 n(%) N=32**	**Grade of toxicity 2, n(%) N=70**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype** rs151264360 (1494 ins/del6)	**Grade of toxicity 1 n(%) N=32**	**Grade of toxicity 2, n(%) N=70**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
2R2R	10(31.2)	7(10)	1.00 (Reference)		1.00 (Reference)		+6/+6	10(31.2)	19(27.1)	1.00 (Reference)		1.00 (Reference)	
2R3R	10(31.2)	36(51.4)	5.14(1.5-16.9)	**0.007**	4.76(1.33-17.04)	**0.0016**	+6/-6	17(53.1)	39(55.7)	1.20(0.46-3.13)	0.69	1.55(0.54-4.43)	0.40
3R3R	12(37.5)	27(38.5)	3.21(0.98-10.47)	0.05	2.71(0.78-9.36)	**0.11**	-6/-6	5(15.6)	12(17.1)	1.26(0.34-4.60)	0.72	1.72(0.41-7.12)	0.45
2R3R+3R3R	22(68.7)	63(90)	4.09(1.38-12.05)	**0.01**	3.65(1.18-11.27)	**0.02**	+6/-6 + -6/-6	22(68.7)	51(72.8)	1.22(0.48-3.04)	0.66	1.47(0.56-3.86)	0.43

Nausea-Vomiting													
Genotype rs34743033 *TS* (VNTR)	**Grade of toxicity 1 n(%) N=54**	**Grade of toxicity 2-4, n(%) N=65**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype** rs151264360 (1494 ins/del6)	**Grade of toxicity 1 n(%) N=54**	**Grade of toxicity 2-4, n(%) N=65**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
2R2R	10(18.5)	13(20)	1.00 (Reference)		1.00 (Reference)		+6/+6	20(37.03)	12(18.4)	1.00 (Reference)		1.00 (Reference)	
2R3R	22(40.7)	23(35.3)	0.80(0.29-2.20)	0.67	0.90(0.29-2.71)	0.85	+6/-6	26(48.1)	40(61.5)	2.56(1.07-6.11)	**0.03**	2.77(1.10-6.96)	**0.03**
3R3R	22(40.7)	29(44.6)	1.11(0.40-3.03)	0.83	1.19(0.39-3.55)	0.75	-6/-6	8(14.8)	13(20)	2.70(0.87-8.42)	0.08	3.23(0.94-11.11)	0.06
2R3R+3R3R	44(81.4)	52(80)	0.90(0.36-2.27)	0.83	1.02(0.38-2.73)	0.95	+6/-6 + -6/-6	34(62.9)	53(81.5)	2.59(1.12-5.98)	**0.02**	2.85(1.18-6.86)	**0.01**

OR[1] Crude odds ratio, 95% CI: 95% confidence interval, **AOR[2]**Adjusted Odds ratio data were calculated by unconditional logistic regression and adjusted for age, gender, regimen, and performance status. A p-value < 0.05 was considered statistically significant

5.10 ATP-binding cassette (*ABC*) transporter genes

5.10.1 Illustration of PCR Amplification

A PCR carried out the amplification of a gene. For each polymorphism, the amplified products of the PCR were confirmed by running 1.7 % agarose gel electrophoresis. A 100bp molecular weight marker was used to verify successful amplification. Figure 5.20 depicts amplification of the genomic DNA as an illustration for *ABCB1* $C^{1236}T$ polymorphism. An amplified PCR product of 366 bp was obtained and run along the 100 bp ladder.

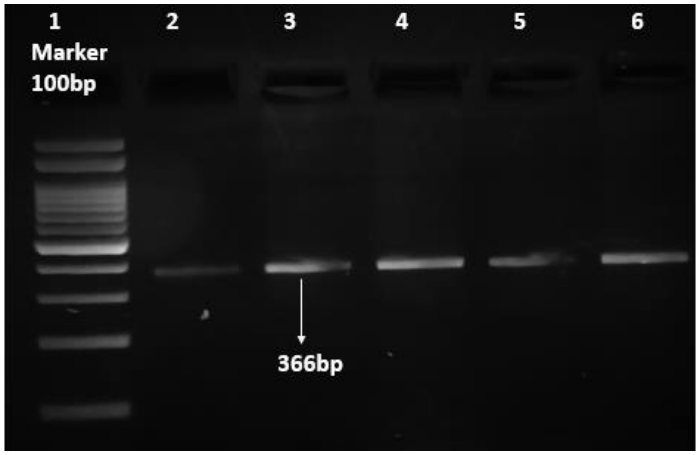


Figure 5.20: Illustration demonstrating agarose gel electrophoresis of the PCR amplification product (Lane 1-DNA ladder and Lane 2-6-amplified PCR product)

5.10.2 Genotyping of ABCB1 variants

For the *ABCB1* gene, genotyping of four polymorphisms ($C^{1236}T$, $C^{3435}T$, $G^{2677}T$, and $G^{2677}A$) was done. In the case of *ABCB1* $C^{1236}T$ amplified product of 366 bp was obtained and was separated on 1.5% agarose gel electrophoresis. The amplified product was digested with the *Hae III* enzyme, separated on 6% polyacrylamide gel electrophoresis, and stained with ethidium bromide. The wild-type allele (*C)* produced two fragments of 269 bp and 97 bp, while the mutant allele (*T)* generated three bands of 262 bp, 62 bp, and 35bp (Figure 5.21A). For *ABCB1* $C^{3435}T$ polymorphism, the amplified product was 206 bp. The wild *C* allele had a *MboI* restriction site that produced the bands of 130 bp and 76 bp, whereas the variant *T* allele did not have the restriction site for *MboI,* which generated a band of 206 bp. Figure 5.21B illustrates 6% polyacrylamide gel demonstrating the restriction pattern for *ABCB1* $C^{3435}T$ polymorphism. For *ABCB1* $G^{2677}T$, an amplified product of

224 bp was produced, and the *BanI* enzyme carried out the digestion. Upon digestion, the wild-type alleles produced a band of 198 bp and 26bp, whereas the mutant alleles had a band size of 224 bp, and the patients carrying heterozygous genotype constituted all three bands of 224 bp, 198 bp, and 26 bp (Figure 5.21C). In the case of *ABCB1* $G^{2677}A$ polymorphism, the PCR product of 220 bp was obtained and separated on 2% agarose gel electrophoresis. When PCR products were digested with *BsrI*, the variant allele produced two bands of 206 bp and 14 bp, and the wild type allele generated a single band of 220 bp (Figure 5.21D).

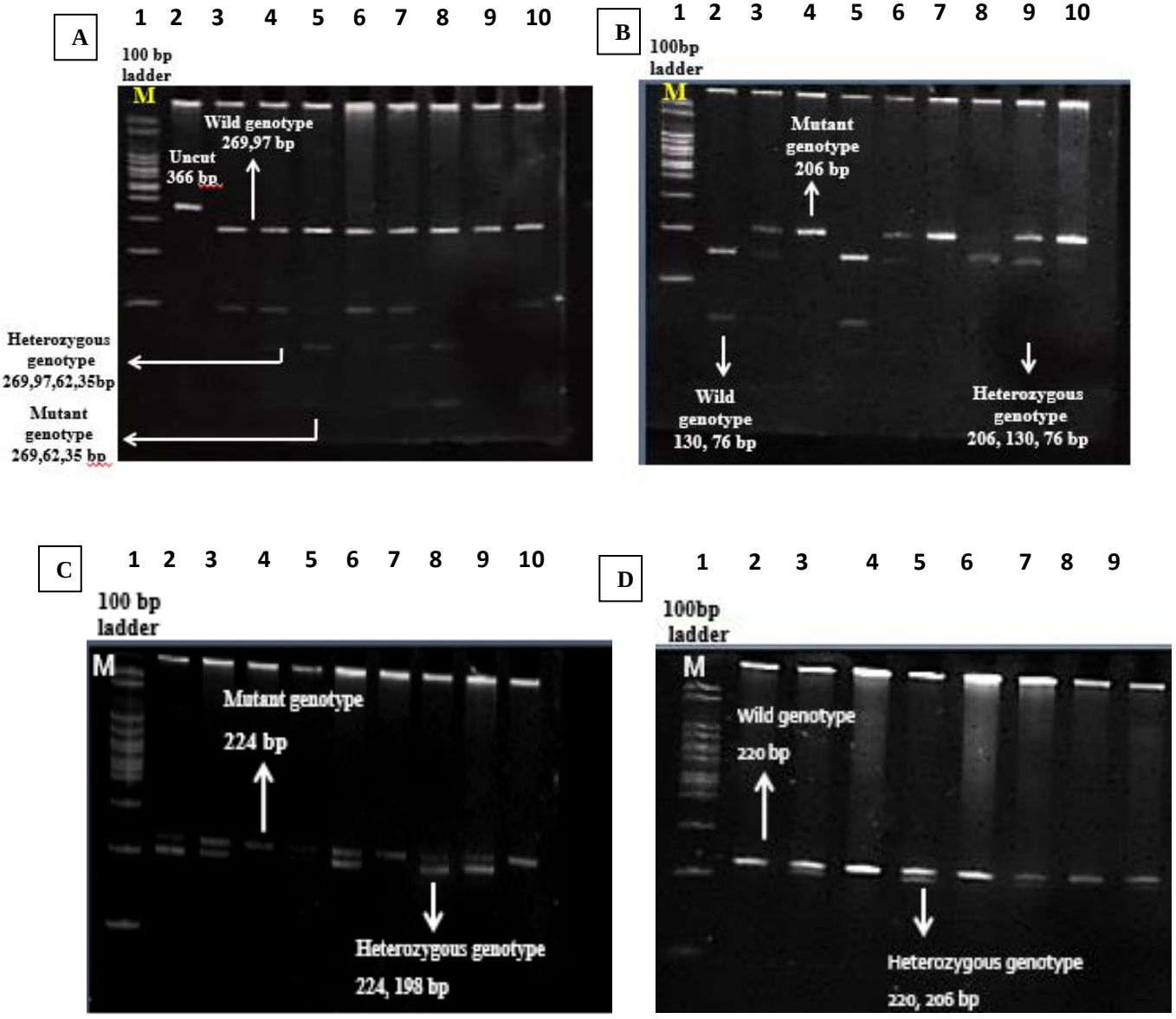


Figure 5.21: Restriction digestion pattern of *ABCB1* variants (A) $C^{1236}T$ polymorphism, Wild type (*CC*): 269,97bp; Mutant genotype (*TT*): 269,32, 35bp (the 35bp was too small to resolve accurately); Heterozygous genotype (*CT*): 269,97,62,35 bp. Lane 1: marker, Lane 2: Amplified PCR product, Lane 3,6,9:*CC* genotype; Lane 4,7,10: *CT* genotype; Lane 5,8: *TT* genotype. (B) $C^{3435}T$ polymorphism, Wild type (*CC*): 130,76bp; Mutant genotype (*TT*): 206 bp; Heterozygous genotype (*CT*): 206,10,76 bp. Lane 1: marker, Lane 2,5,8:*CC* genotype; Lane 3,6,9: *CT* genotype; Lane 4,7,10: *TT* genotype. (C) $G^{2677}T$ polymorphism, Mutant genotype (*TT*): 224 bp; Heterozygous genotype (*GT*): 224,198 bp. Lane 1: marker, Lane 2,3,6,8,9:*GT* genotype; Lane 4,5,7,10: *TT* genotype. (D) $G^{2677}A$ polymorphism, Wild genotype (*GG*): 220 bp; Heterozygous genotype (*GA*): 220,206 bp. Lane 1: marker, Lane 2,4,6,8,9:*GG* genotype; Lane 3,5,7,9: *GA* genotype

5.10.3 Genotyping of ABCC1 variants

For the *ABCC1* gene, genotypic analysis of the two polymorphisms ($G^{3173}A$ and $G^{2168}A$) was evaluated. The polymorphism *ABCC1* $G^{3173}A$ is located in the exon 23 of the gene. After amplification, a PCR product of 344 bp was retrieved. The amplified products were digested with *Pst I* enzyme, and the restriction pattern was noted. The obtained fragments were separated on 6% polyacrylamide gel and stained with ethidium bromide. The wild *G* allele had a restriction site for *Pst I* that produced bands of 300 bp and 44 bp, whereas the *A* allele lacked this restriction size, and a single band of 344 bp was obtained (Figure 5.22A). The other polymorphism of the *ABCC1* gene studied was $G^{2168}A$ localized on exon 17. The PCR product of 157 bp was obtained upon amplification and was resolved on 2% agarose gel electrophoresis. The *G* allele had a restriction site for *Taq I*, the bands of 132 bp and 25 bp were obtained, while the variant *A* allele did not have a restriction site for *Taq I*, and a single band of 157 bp was obtained (Figure 5.22B).

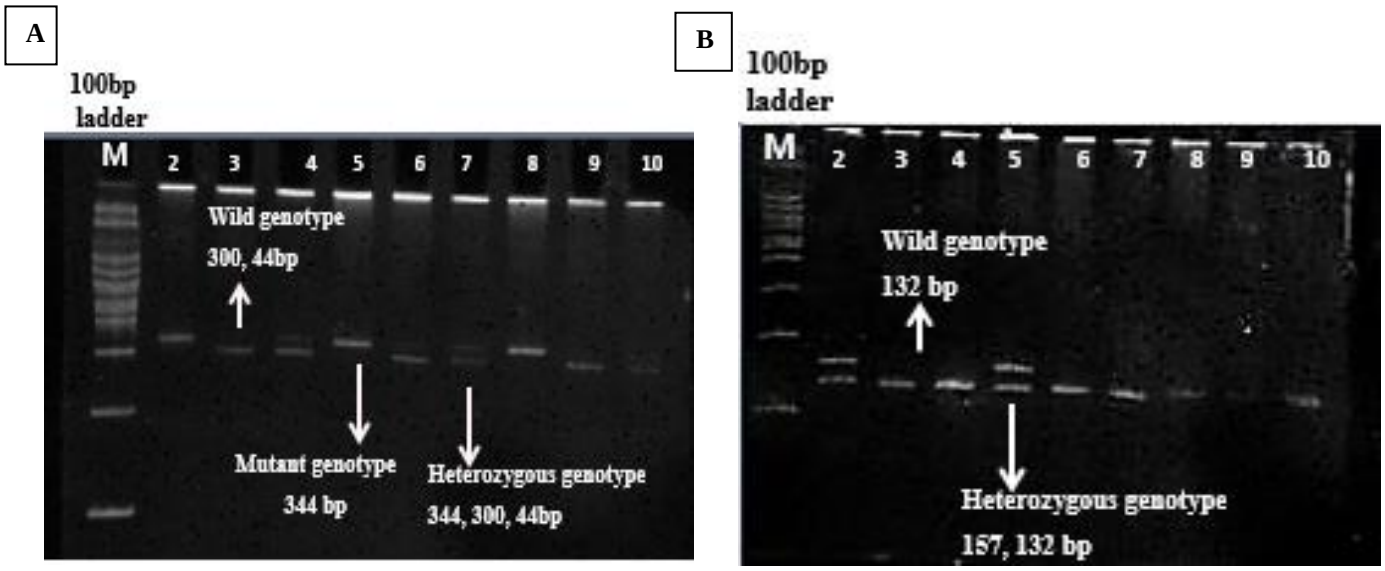


Figure 5.22: Restriction digestion pattern of *ABCC1* variants (A) $G^{3173}A$ polymorphism Wild type (*GG*): 300,44bp (the 44 bp was too small to resolve accurately); Mutant genotype (*AA)*: 344bp; Heterozygous genotype (*GA)*: 344, 300, 44 bp. Lane 1: marker, Lane 2: Amplified PCR product, Lane 3,8:*GG* genotype; Lane 4,6,7,10: *GA* genotype; Lane 5,8: *AA* genotype. B) $G^{2168}A$ polymorphism Wild type (*GG*): 132, 25bp (the 25 bp was too small to resolve accurately); Mutant genotype (*AA)*: 157 bp; Heterozygous genotype (*GA)*: 157, 132, 25 bp. Lane 1: marker, Lane 3,4,6,7,8,9,10:*GG* genotype; Lane 2,5: *GA* genotype

5.10.4 Genotyping of ABCC2 variants

The polymorphic variant of *ABCC2* $G^{4544}A$ and is localized at exon 32 of the gene. The PCR product of 440 bp was obtained after amplification. The PCR product was digested with the 5U of *Rsa I* restriction enzyme (Thermo fisher scientific, Inc.) at 37° C. The digested samples were run on 6% native polyacrylamide gel, then stained with ethidium bromide. The homozygous wild genotype (*GG)* had two sites for *Rsa I* and gave three fragments of 307,71,22 bp. On the contrary, upon digestion, the homozygous variant genotype (*AA)* generated four fragments of 196,111, 71, and 22bp, and the heterozygous genotype gave fragments of 307,196,111,71, and 22bp, respectively, as shown in figure 5.23.

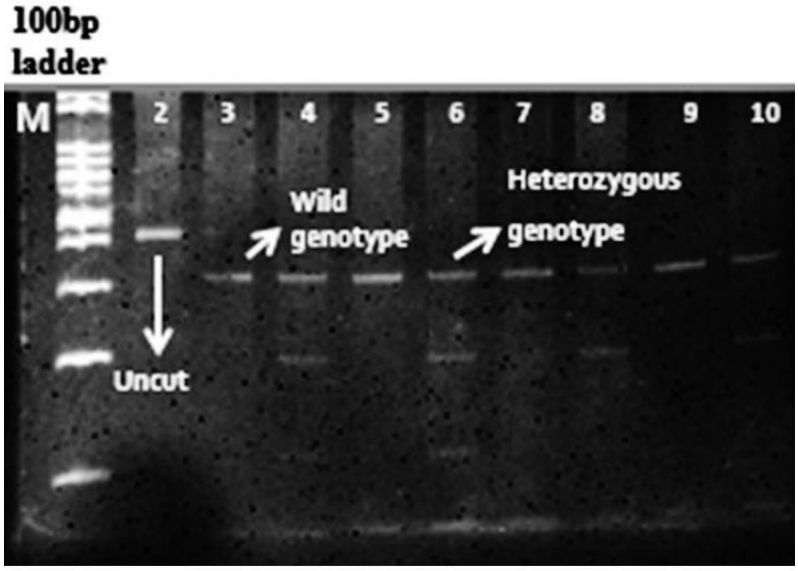


Figure 5.23: Restriction digestion pattern of *ABCC2* $G^{4544}A$ polymorphism Wild type (*GG*): 307,71, 22 bp (the 22bp was too small to resolve accurately); Mutant genotype (*AA)*: 196, 111, 71, 22bp; Heterozygous genotype (*GA)*: 307, 196, 111, 71, 22 bp. Lane 1: marker, Lane 2: Amplified PCR product of 400 bp, Lane 3,5,7,9:*GG* genotype; Lane 4,6,8,10: *GA* genotype

5.10.5 Genotyping of ABCG2 variants

The genotyping of $C^{421}A$ variant of *ABCG2* gene was carried out. The polymorphism $C^{421}A$ is located in exon 5. Allele-specific PCR was carried out for the amplification. Two specific reverse primers were constructed with complementary 3'-terminal nucleotides to the respective polymorphism. The reverse primers produced a single fragment with a standard forward primer in two distinct tubes. In homozygotes, the distinct band of 320 bp is generated in one tube, whereas,

in the case of heterozygotes, a band of 320 bp in both tubes. Figure 5.24 illustrates the genotypic pattern for the *ABCG2* $C^{421}A$ polymorphism



Figure 5.24: Restriction digestion pattern of *ABCG2* $C^{421}A$ polymorphism Wild type (*CC*): 320bp (generated from reverse primer 1 in one tube); Mutant genotype (*AA)*:320bp (generated from reverse primer 2 in one tube); Heterozygous genotype (*CA)*: 320 bp (generated from both reverse primer 1 and 2 in two distinct tubes). Lane 1: marker, Lane 2-3, 6-7, and 8-9 *CC* genotype; Lane 4-5: *CA* genotype

5.11 Statistical analysis of *ABC* genes

5.11.1 Demographic characteristics of the study group for ABCB1, ABCC1, ABCC2, and ABCG2 gene

Demographic features of the case group such as age, smoking status, gender, pack years, tumor size, lymph node invasion (N), metastases (M), tumor staging, and other clinical parameters are summarized in table 5.18. A total of 502 cases lung cancer patients were recruited to study the polymorphism of *ABCB1* ($C^{1236}T$, $C^{3435}T$, $G^{2677}A$, $G^{2677}T$), *ABCC1* ($G^{2168}A$, $G^{3173}A$), *ABCC2* ($G^{4544}A$), and *ABCG2* ($C^{421}A$) gene. The mean age of the patients was 60±9.67 (range 30-86). In the studied patients, males outnumbered females by 401 (79.8%) to 101 (20.1%). Smokers accounted for 78.8% of the group, more significant than non-smokers (21.1%). Smokers had an average pack-year of 22.81±23.52. When stratified based on TNM staging, 32.2% and 58.1% of patients were diagnosed with stage III and IV lung cancer with T1 and T2 tumor sizes. They had low frequencies of 6.17% and 8.7%, respectively, whereas those with T3 and T4 tumor sizes had high frequencies of 18.32% percent and 60.1%, respectively.

Table 5.18: Demographic characteristics among case group recruited to study ABC polymorphisms

Variable	Total (N=502)	Cases, n (%)
Gender	502	
Male		401 (79.8)
Female		101 (20.1)
Age		
Mean ± SD	502	60±9.67
Range		30-86
Smoking status	502	
Smokers		396 (78.8)
Non-smokers		106 (21.1)
Pack years	502	
Mean ± SD		22.81±23.52
Histological types	502	
SQCC		207 (41.2)
ADCC		205 (40.8)
SCLC		76 (15.1)
Others		3 (0.59)
Unknown		11 (2.19)
TNM staging	502	
I		5 (0.9)
II		19 (3.78)
III		162 (32.2)
IV		292 (58.1)
Unclassified		24 (4.7)
Tumor Size	502	
Tx		19 (3.78)
T1		31 (6.17)
T2		44 (8.7)
T3		92 (18.32)
T4		302 (60.1)
Unknown		14 (2.78)
Lymph Node	502	
N0		69 (13.7)
N1		39 (7.7)
N2		220 (43.8)
N3		160 (31.8)
N4		1 (0.19)
Unknown		13 (2.58)
Metastasis	502	
M0		215 (42.8)
M1		274 (54.5)
Unknown		13 (2.58)

Abbreviations: SD= Standard deviation, n=total number of lung carcinoma cases, SQCC=squamous cell carcinoma, ADCC=Adenocarcinoma, and SCLC= small cell lung carcinoma

N0 had 13.7% for lymph node invasion, while N1, N2, N3, and N4 had an incidence of 7.7%, 43.8%, 31.8%, and 0.19%, respectively. At the time of diagnosis, 54.5% of patients had already presented metastasis, while 42.8% had no metastatic involvement. Histologically, 41.4% of patients were diagnosed with adenocarcinoma (ADCC), 41.8% of patients had squamous cell carcinoma (SQCC), and 15.3% of patients were detected with small-cell-lung cancer (SCLC).

5.11.2 Clinical features of lung carcinoma patients assessed for prognostic significance of different ABC genes

The investigation's clinical characteristics and demographics on the overall survival of the lung carcinoma patients with variant *ABCB1, ABCC1, ABCC2,* and *ABCG2* are detailed in table 5.19. Males constituted 80.3% of the 483 individuals, while females comprised 19.6%. Since smoking is a known risk factor for lung cancer, most sample groups were smokers (78.8%), while 20.7% were non-smokers. Clinical parameters including histology, performance status, TNM stage, and chemotherapeutic regimen are also included in Table 5.19. According to the histological categorization, there were 39.5% adenocarcinomas, 42.2 % squamous cell carcinomas, 15.5% small-cell lung carcinomas, and 0.62% patients with unclassified histology. TNM grading classified the participants in the study as follows: 4.96 % stage I/stage II patients, 32.9% staged III patients, 57.1% stage IV patients, and 4.96% patients were unclassified. In addition, the cases were distinguished according to Eastern Cooperative Oncology Group (ECOG) and Karnofsky's status (KPS); 86 participants had KPS scores below 60, 249 patients had KPS scores of 70-80, and 132 subjects had KPS scores of 90-100. As per the ECOG score, 45.1% (211) of the people had ECOG scores ranging from 0 to 1, 38.9% (182) had ECOG 2, and 18.8% (74) had ECOG scores of 3-4. Chemotherapeutic information was available for 348 participants. Most of the patients were administered with pemetrexed in combination with cisplatin/carboplatin (30.1%), followed by 12.3% of patients subjected with irinotecan and cisplatin/carboplatin. Also, 12.6% of patients were administered with docetaxel along with cisplatin/carboplatin, 24.4% were subjected with paclitaxel and carboplatin/cisplatin, 8.64% received gemcitabine along with cisplatin/carboplatin, 4.31% patients were administered with gefitinib, and 4.88% were subjected with etoposide and carboplatin/cisplatin.

Table 5.19: Demographic characteristics and clinical features with overall survival

Variable	Total (N=483)	Cases, n (%)
Gender	483	
Male		388 (80.3)
Female		95 (19.6)
Age	483	
Mean ± SD		60±9.67
Range		30-86
Smoking status	483	
Smokers		383 (78.8)
Non-smokers		100 (20.7)
Histological types	483	
SQCC		204 (42.2)
ADCC		191 (39.5)
SCLC		75 (15.5)
Others		3 (0.62)
Unknown		10 (2.07)
Overall Survival	483	
Dead		408 (84.4)
Alive		75 (15.5)
TNM staging	483	
I/II		24 (4.96)
III		159 (32.9)
IV		276 (57.1)
Unclassified		24 (4.96)
ECOG performance status	467	
0-1		211 (45.1)
2		182 (38.9)
3-4		74 (18.8)
KPS performance status	467	
90-100		132 (28.2)
70-80		249 (53.3)
60 & below 60		86 (18.4)
Median Follow up (months)		8.3
Events, deaths		N=408 (84.4%)
Chemotherapy regimen	348	
Pemetrexed + cis/carboplatin		105 (30.1)
Irinotecan + cis/carboplatin		43 (12.3)
Docetaxel + cis/carboplatin		44 (12.6)
Paclitaxel + cis/carboplatin		85 (24.4)
Gemcitabine + cis/carboplatin		30 (8.62)
Gefitinib		15 (4.31)
Etoposide		17 (4.88)
Ceretinib		1 (0.28)
Others		8 (2.29)

Abbreviations: SD= Standard deviation, n=total number of lung carcinoma cases

5.12 Statistical analysis of *ABCB1* gene

5.12.1 Genotypic and allelic frequencies of *ABCB1* polymorphic variants

The distribution of genotypes and alleles for *ABCB1* polymorphisms in lung cancer patients has been illustrated in Table 5.20. Among the patients for $C^{1236}T$ (rs1128503**)** polymorphism, 54.7% of patients harbored homozygous wild genotype *(CC)*, whereas 15.3% of patients had mutant genotype (*TT)*. Minor allele frequency (MAF) was calculated to be 0.30 for the $C^{1236}T$ variant. Further, the genotypic frequencies of *ABCB1* $C^{3435}T$ (rs1045642) are also depicted in table 5.20, and the results show that patients having variant genotype (*TT)* are over-represented as compared to the subjects having wild type genotype (*CC)* in the sample population (19.3% *vs.* 39.4%). Also, in evaluating the *ABCB1* $G^{2677}A$ polymorphism, the highest proportion of patients had wild-type genotype (89.4%) in the sample population, and 10.5% of subjects had at least one copy of mutant allele (*GA)*. For *ABCB1* $G^{2677}A$ (rs2032582**)** polymorphism, the genotypic and allelic frequency of case subjects were consistent with Hardy-Weinberg equilibrium with a p-value of 0.21 and $\chi 2=1.55$. Furthermore, patients with lung cancer having *ABCB1* $G^{2677}T$ (rs2032582) polymorphism has the highest frequency of heterozygous genotype (*GT)*, whereas the 47.8% of patients had both copies of mutant alleles (*TT)* in the sample population.

Table 5.20: Genotypic and allelic distribution of *ABCB1* genetic polymorphism in lung cancer patients

Genotype rs1128503 *ABCB1 (C1236T)*	**Cases (502) N (%)**	**χ2 (df)**	**p[a]-value**
CC	275 (54.7)		
CT	150 (29.8)	42.8 (1)	<0.05
TT	77 (15.3)		
CT+TT	227 (45.2)		
C (allele)	700		
T (allele)	304		
MAF	0.30		
Genotype rs1045642 *ABCB1 (C3435T)*	**Cases (502) N (%)**	**χ2 (df)**	**p[a]-value**
CC	97 (19.3)		
CT	207 (41.2)		
TT	198 (39.4)	9.91 (1)	<0.05
CT+TT	405 (80.6)		
C (allele)	401		
T (allele)	603		
MAF	0.6		
Genotype rs2032582 *ABCB1 (G2677A)*	**Cases (502) N (%)**	**χ2 (df)**	**p[a]-value**
GG	449 (89.4)		
GA	53 (10.5)	1.55 (1)	0.21
AA	0 (0)		
GA+AA	53 (10.5)		
G (allele)	951		
A (allele)	53		
MAF	0.05		
Genotype rs2032582 *ABCB1 (G2677T)*	**Cases (502) N (%)**	**χ2 (df)**	**p[a]-value**
GG	0 (0)		
GT	262 (52.1)	62.5	<0.05
TT	240 (47.8)		
G (allele)	262		
T (allele)	742		
MAF	0.73		

5.13 Elucidation of overall survival (OS) of lung carcinoma patients with *ABCB1* polymorphism

5.13.1 Association of polymorphic ABCB1 genetic variant with OS of lung cancer patients

The correlation of the *ABC* gene polymorphisms with overall survival in 483 lung carcinoma samples was investigated in the current study. At the analysis time, 84.4% of patients were dead, while 15.5% were alive after three years of follow-up. The association of the *ABCB1* polymorphism in lung cancer subjects with survival was investigated using both univariate and multivariate analysis after adjusting for various demographic parameters such as smoking status, age, histology, tumor stage, regimen, and ECOG **(**Table 5.21**)**. In the present analysis, we have employed four potential models (dominant, recessive, co-dominant, and additive) to evaluate the relationship between *ABCB1* polymorphism and overall survival in lung cancer patients. The best-fit model presented the results as illustrated in Table 5.21.

For *ABCB1* $C^{1236}T$ polymorphism, in the co-dominant model, our data revealed a lower median survival time (MST) in patients harboring heterozygous (*CT*) genotype as compared to wild (*CC*) genotype (MST= 7.7 *vs.* 8.57; log-rank p=0.27; HR=1.13). After adjusting with the different covariates such as age, gender, stage, smoking status, histology, regimen, and performance status, the results showed poor survival outcomes in heterozygous (*CT*) genotype when compared to wild (*CC*) genotype (HR=1.34, p=**0.04**) [Figure 5.25 (a)]. The lung cancer patients with $C^{1236}T$ polymorphism and harboring mutant alleles (*TT)* had lower MST than those with wild-type alleles (*CC)*. However, substantial differences were not observed (MST 7.6 *vs.* 8.57, Log-rank-p=0.51). After, applying the dominant model where heterozygous and mutant groups were combined, reduced survival differences were found in it as compared to the wild-type genotype, though significance was not reached (MST=7.67 *vs.* 8.67, p=0.21, HR=1.18, 95% CI=0.91-1.53). Also, on using recessive and additive models, results showed survival differences between different groups, but substantial differences were not achieved, as shown in table 5.21.

Table 5.21: Univariate and Multivariate analysis for *ABCB1* variants

Genotype rs1128503 *ABCB1 (C1236T)*	CASES n (%), N=483	DEAD n (%), N=408	ALIVE n (%), N=75	Univariate analysis			Multivariate analysis	
				MST (months)	Log-rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
Co-dominant model								
CC	268 (55.4)	226 (55.3)	42 (56)	8.57	-	1.0 (reference)	-	-
CT	144 (29.8)	126 (30.8)	18 (24)	7.7	0.27	1.13 (0.90-1.41)	1.34 (1.00-1.80)	**0.04**
TT	71 (14.6)	56 (13.7)	15 (20)	7.6	0.51	0.90 (0.68-1.21)	0.98 (0.66-1.45)	0.92
Dominant model								
CC	268 (55.4)	226 (55.3)	42 (56)	8.6	-	1.0 (reference)	-	-
CT+TT	215 (44.5)	182 (44.6)	33 (44)	7.67	0.61	1.05 (0.86-1.27)	1.18 (0.91-1.53)	0.21
Recessive model								
CC+CT	412 (85.3)	352 (86.2)	60 (80)	8.37	-	1.0 (reference)	-	-
TT	71 (14.6)	56 (13.7)	15 (20)	7.6	0.35	0.88 (0.67-1.15	0.88 (0.61-1.29)	0.53
Additive model								
CC+TT	339 (70.1)	282 (69.1)	57 (76)	8.4	-	1.0 (reference)	-	-
CT	144 (29.8)	126 (30.8)	18 (24)	7.7	0.19	1.15 (0.92-1.43)	1.32 (0.99-1.75)	0.05
Genotype rs1045642 *ABCB1 (C3435T)*	**CASES n (%), N=483**	**DEAD n (%), N=408**	**ALIVE n (%), N=75**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Co-dominant model								
CC	93 (19.2)	76 (18.6)	17 (22.6)	8.4	-	1.0 (reference)	-	-
CT	199 (41.2)	171 (41.9)	28 (37.3)	7.57	0.20	1.18 (0.91-1.54)	1.32 (0.92-1.92)	0.12
TT	191 (39.5)	161 (39.4)	30 (40)	8.73	0.31	0.87 (0.66-1.14)	1.14 (0.79-1.65)	0.46
Dominant model								
CC	93 (19.2)	76 (18.6)	17 (22.6)	8.4	-	1.0 (reference)	-	-
CT+TT	390 (80.7)	332 (81.3)	58 (77.3)	8.26	0.21	1.16 (0.91-1.47)	1.21 (0.87-1.69)	0.25
Recessive model								
CC+CT	292 (60.4)	247 (60.5)	45 (60)	7.8	-	1.0 (reference)	-	-
TT	191 (39.5)	161 (39.4)	30 (40)	8.73	0.9	0.99 (0.81-1.21)	0.97 (0.74-1.27)	0.85
Additive model								
CC+TT	284 (58.7)	237 (58.0)	47 (62.6)	8.6	-	1.0 (reference)	-	-
CT	199 (41.2)	171 (41.9)	28 (37.3)	7.57	0.29	1.11 (0.91-1.35)	1.17 (0.89-1.53)	0.24
Genotype rs2032582 *ABCB1 (G2677A)*	**CASES n (%), N=483**	**DEAD n (%), N=408**	**ALIVE n (%), N=75**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Co-dominant model								
GG	431 (89.2)	363 (88.9)	68 (90.6)	8.6	-	1.0 (reference)	-	-
GA	52 (10.7)	45 (11.0)	7 (9.3)	5.37	0.12	1.31 (0.93-1.84)	1.20 (0.81-1.78)	0.35
AA	0 (0)	0 (0)	0 (0)	-	-	-	-	-
Genotype rs2032582 *ABCB1 (G2677T)*	**CASES n (%), N=483**	**DEAD n (%), N=408**	**ALIVE n (%), N=75**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Co-dominant model								
GT	250 (51.7)	211 (51.7)	39 (52)	7.5	-	1.0 (reference)	-	-
TT	233 (48.2)	197 (48.2)	36 (48)	8.8	0.52	0.93 (0.77-1.14)	0.97 (0.75-1.27)	0.8

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, regimen, histology, KPS, and ECOG

Multivariate cox proportional hazards analysis revealed that, besides gene polymorphism, the overall survival was correlated with ECOG of patients with lung cancer. It was also noted that the overall risk of patients carrying heterozygous *(CT)* genotype at *ABCB1* $C^{1236}T$ was 1.34 times higher than in patients carrying wild *(CC)* genotype (table 5.22). Hence, ECOG and *2R3R* genotype in the *1236* site of *ABCB1* gene could be key factors for contributing to the development of lung cancer. Moreover, we found that age (P= 0.28), gender (P=0.18), smoking (P=0.78), stage (P=0.13), KPS (P=0.50), histology (P=0.9), and regimen (0.17) did not correlate with OS.

Table 5.22: Cox proportional hazards regression model analysis of overall survival in lung cancer subjects

N=483	b	SE	P-value	Adjusted HR (Exp b)	95% CI of Exp (b)
ABCB1_C1236T	0.296	0.1503	**0.04**	1.3450	1.0018 to 1.8056
Age	-0.007	0.0074	0.2896	0.9922	0.9778 to 1.0067
Sex	0.295	0.2228	0.1844	1.3441	0.8685 to 2.0802
Smoking	-0.06	0.2339	0.7897	0.9395	0.5941 to 1.4859
Stage	0.20	0.1345	0.1311	1.2251	0.9412 to 15945
ECOG	0.475	0.1552	**0.0022**	1.6085	1.1866 to 2.1804
KPS	0.105	0.1565	0.5015	1.1110	0.8174 to 1.5099
Histology	0.005	0.1083	0.9562	1.0060	0.8135 to 1.2439
Regimen	-0.1114	0.08161	0.1724	0.8946	0.7624 to 1.0496

For *ABCB1* $C^{3435}T$ polymorphism, a marginal increase in the survival was observed in the patients having mutant genotype (*TT)* as compared to the wild type genotype (*CC)*; however, no substantial differences in terms of prognosis were observed (MST=8.73 *vs.* 8.4, log-rank p=0.31). The lung cancer patients having heterozygous genotype for $C^{3435}T$ polymorphism showed reduced survival compared to the wild-type genotype, but no significant differences were observed (MST=7.57 *vs.* 8.73, p=0.20, HR=1.18, 95% CI=0.91-1.54). Upon adjusting with different covariates such as age, gender, performance status, histology, tumor stage, and regimen, the death ratio increased 1.32 times in the patients harboring heterozygous genotype (*CT)* (Figure 5.25 (b)). However, substantial differences were not observed (HR=1.32, 95% CI=0.92-1.92, p=0.12). In brief, we did not find any association of *ABCB1* $C^{3435}T$ polymorphism in predicting prognosis in lung cancer patients undergoing platinum-based doublet chemotherapy.

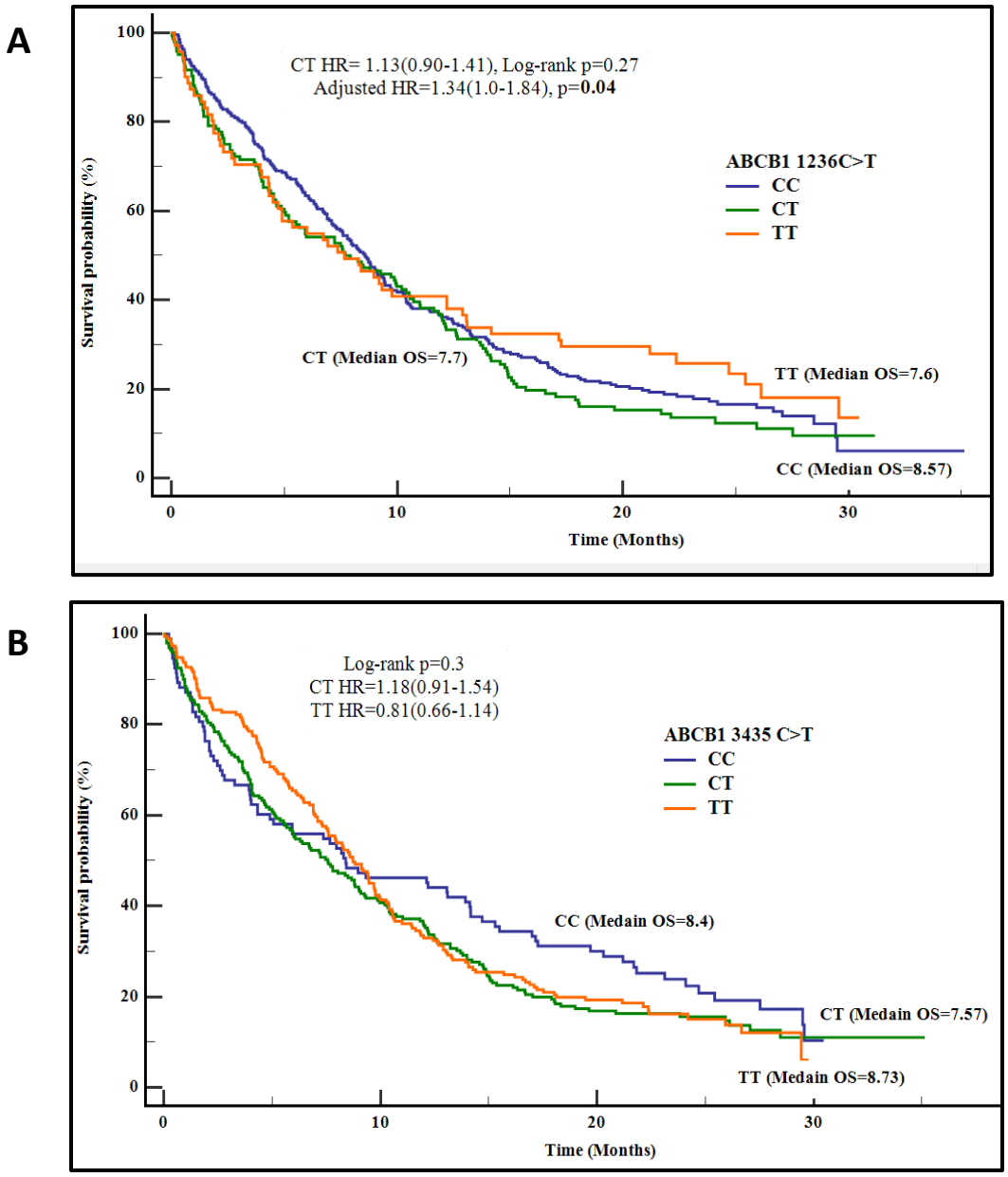


Figure 5.25: Kaplan Meier curves illustrating the association between overall survival in different genotypes of (A) *ABCB1 C1236T* polymorphism in lung cancer patients and (B) *ABCB1 C3435T* polymorphism in lung cancer patients (Note: *CC* represents wild-type genotype, *CT* represents heterozygous genotype, and *TT* represents homozygous for mutant genotype)

Patients with the heterozygous allele (*GA*) for the *ABCB1* $G^{2677}A$ variant had a poorer median survival in lung cancer patients than those with the wild type (*GG*) genotype, though significant differences were not observed (MST= 5.7 *vs.* 8.6; p=0.12) (Figure 5.26 (a)). Since, no patient had a mutant genotype (*AA*), the survival differences for this group were not assessed. For the *ABCB1*

$G^{2677}T$ polymorphism, our data demonstrated that patients having both mutant alleles (*TT*) had a greater survival rate than those having only one copy of mutant allele (*GT)* (MST=8.8 *vs.* 7.5; p=0.5) (Figure 5.26 (b)). The *ABCB1 $G^{2677}T$* mutation, on the other hand, showed no significant differences in terms of prognosis.

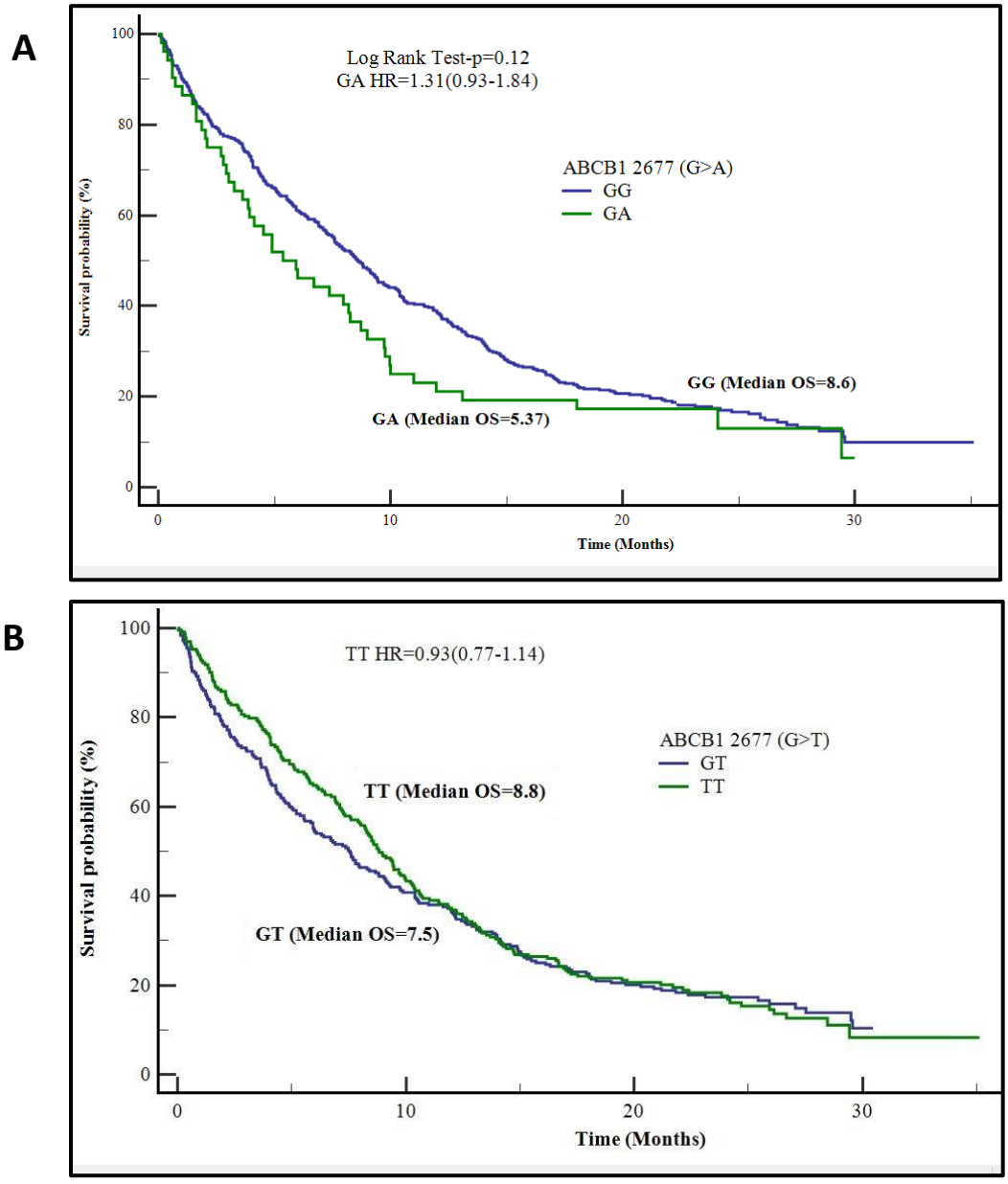


Figure 5.26: Kaplan Meier curves illustrating the association between overall survival in different genotypes of (a) *ABCB1 $G^{2677}A$* polymorphism in lung cancer patients and (b) *ABCB1 $G^{2677}T$* polymorphism in lung cancer patients (Note: *GG* represents wild-type genotype, *GA* or *GT* represents heterozygous genotype, and AA or TT represents homozygous for mutant genotype)

5.13.2 Association of ABCB1 polymorphism with OS in lung cancer histological subtypes

Furthermore, we have determined the importance of *ABCB1* polymorphism in survival in distinct subgroups stratified by histology, as illustrated in Table 5.23 (a,b,c)**.** The genotypic distribution and overall survival of ADCC subjects have been illustrated in table 5.23 (a), patients with SQCC in table 5.23 (b), and SCLC patients in table 5.23 (c). When lung cancer patients were stratified based on ADCC histology, our results showed that 79.5% of patients were dead while 20.4% were alive at the analysis time. For *ABCB1* $C^{1236}T$ polymorphism, patients with ADCC harboring heterozygous (*CT)* genotype had lower survival as compared to the patients having wild type (*CC)* genotype (MST=8.8 *vs.* 7.57 months, log-rank-p=0.32). After multivariate analysis, the death ratio was substantially increased in the patients with heterozygous genotype (*CT)* (HR=1.68, 95% CI= 1.03-2.73, p=**0.03**) [Figure 5.27 (a)]. On the contrary, patients harboring mutant genotype (*TT)* for *ABCB1* $C^{1236}T$ had longer survival as compared to the patients with wild-type genotype (*CC),* though the substantial difference among the groups was not reached (MST=9.77 *vs.* 8.8 months). So, we found a strong association between heterozygous genotype *(CT)* for *ABCB1* $C^{1236}T$ and overall survival in lung cancer patients, as is depicted in table 5.23 (a). For *ABCB1* $C^{3435}T$, in the co-dominant model, a drastic decrease in survival was observed in the ADCC patients having heterozygous genotype (*CT*) when compared with the wild type genotype (*CC*) (MST= 6.8 *vs.* 13.07; HR=1.33, 95% CI=0.87-2.03, Log-rank p=0.18). After multivariate analysis, the death ratio was substantially increased to 1.92 in the patients with heterozygous genotype (*CT)* as compared to the wild type genotype (HR=1.92, 95% CI=1.01-3.64, p=**0.04**) [Figure 5.27 (b)] as shown in table 5.23 (a).

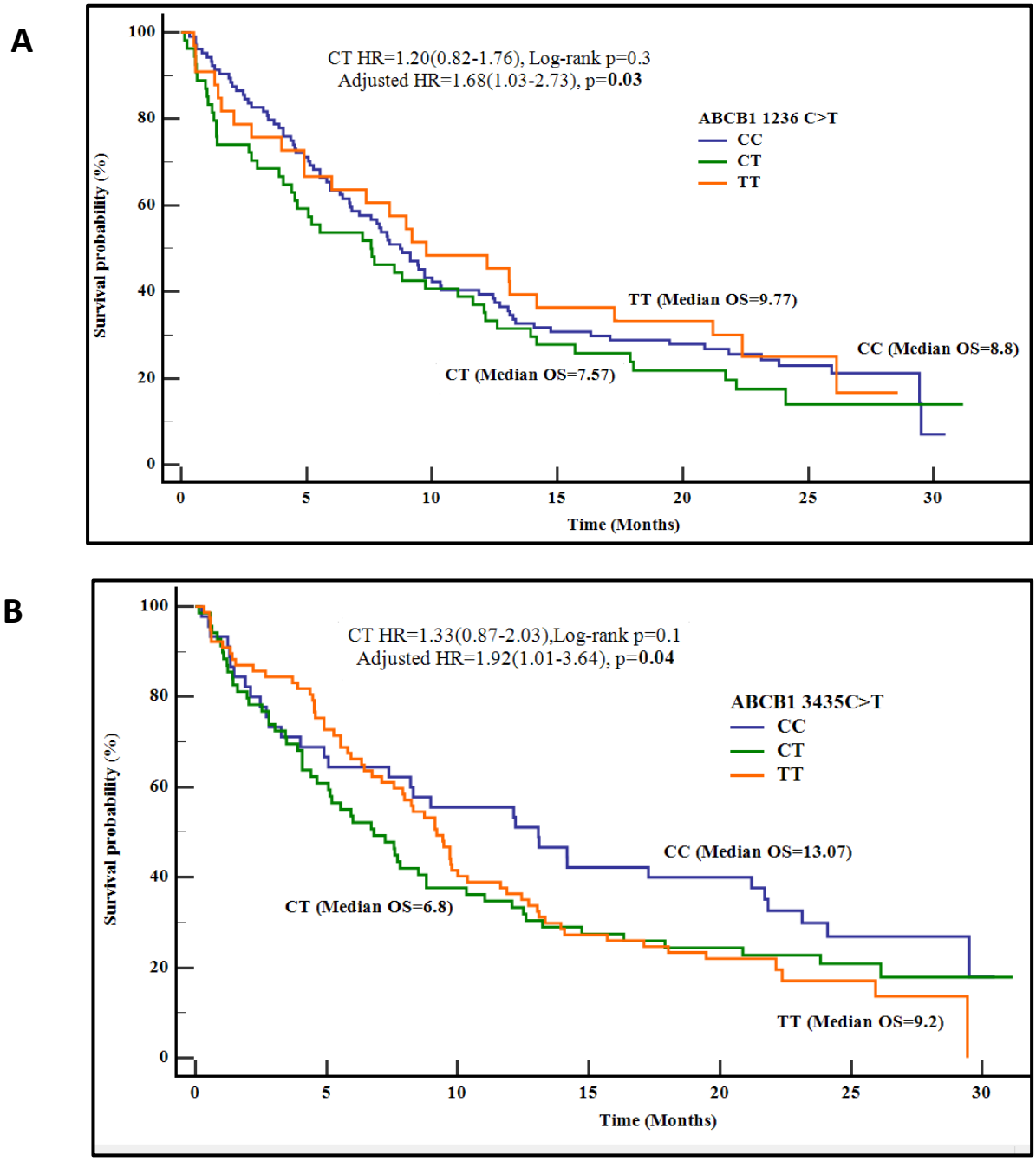


Figure 5.27: Kaplan Meier curves illustrating the association between overall survival in different genotypes of (A) *ABCB1 C[1236]T* polymorphism in ADCC patients and (B) *ABCB1 C[3435]T* polymorphism in ADCC patients.

(Note: CC represents wild-type genotype, CT represents heterozygous genotype, and TT represents homozygous for mutant genotype)

ADCC patients with $G^{2677}A$ polymorphism and harboring heterozygous genotype (*GA)* showed lower survival as compared to the patients having wild-type genotype (*GG)*; however, substantial differences among the groups were not reached (MST=6.0 *vs.* 8.97, HR=1.46, 95% CI=0.88-2.42, Log-rank p=0.13). In the case of $G^{2677}T$ polymorphism, none of the patients had wild-type genotype, and 50.2% of patients had heterozygous genotype (*GT)* while 49.7% of patients had mutant genotype (*TT)*. Lower survival was observed in the mutant genotype (*TT)* as compared to the heterozygous genotype (*GT)* (MST=8.5 *vs.* 8.96, HR=1.20, 95% CI=0.77-1.87, p=0.3). However, both $G^{2677}A/T$ polymorphisms did not show any association in predicting the survival of lung cancer patients, as tabulated in table 5.23 (a).

Table 5.23 (a): Association of *ABCB1* SNPs on overall survival according to ADCC histology

ADCC								
Genotype	**CASES**	**DEAD**	**ALIVE**	**Univariate analysis**			**Multivariate analysis**	
rs1128503	**n (%),**	**n (%),**	**n (%),**	**MST**	**Log-rank**	**Unadjusted HR[a]**	**Adjusted HR[b]**	***p***
ABCB1 (C1236T)	**N=191**	**N=152**	**N=39**	**(months)**	***p***		**(95% CI)**	
Codominant model								
CC	104 (54.4)	82 (53.9)	22 (56.4)	8.8	-	1.0 (reference)	-	-
CT	54 (28.2)	45 (29.6)	9 (23.0)	7.57	0.32	1.20 (0.82-1.76)	1.68 (1.03-2.73)	**0.03**
TT	33 (17.2)	25 (16.4)	8 (20.5)	9.77	0.79	0.94 (0.60-1.46)	1.05 (0.58-1.92)	0.85
Dominant model								
CC	104 (54.4)	82 (53.9)	22 (56.4)	8.8	-	1.0 (reference)	-	-
CT+TT	87 (45.5)	70 (46.0)	17 (43.5)	8.57	0.60	1.08 (0.79-1.50)	1.35 (0.88-2.05)	0.16
Recessive model								
CC+CT	158 (82.7)	127 (83.5)	31 (79.4)	8.23	-	1.0 (reference)	-	-
TT	33 (17.2)	25 (16.4)	8 (20.5)	9.77	0.53	0.87 (0.58-1.32)	0.86 (0.49-1.52)	0.61
Genotype	**CASES**	**DEAD**	**ALIVE**	**Univariate analysis**			**Multivariate analysis**	
rs1045642	**n (%),**	**n (%),**	**n (%),**	**MST**	**Log rank**	**Unadjusted HR[a]**	**Adjusted HR[b]**	***P***
ABCB1 (C3435T)	**N=191**	**N=152**	**N=39**	**(months)**	***p***		**(95% CI)**	
Codominant model								
CC	45 (23.5)	33 (21.7)	12 (30.7)	13.07	-	1.0 (reference)	-	-
CT	69 (36.1)	55 (36.1)	14 (35.8)	6.8	0.18	1.33 (0.87-2.03)	1.92 (1.01-3.64)	**0.04**
TT	77 (40.3)	64 (42.1)	13 (33.3)	9.2	0.10	1.40 (0.93-2.12)	1.52 (0.83-2.80)	0.17
Dominant model								
CC	45 (23.5)	33 (21.7)	12 (30.7)	13.07	-	1.0 (reference)	-	-
CT+TT	146 (76.4)	119 (78.2)	27 (69.2)	7.97	0.11	1.33 (0.93-1.91)	1.72 (0.99-3.01)	0.05
Recessive model								
CC+CT	114 (59.6)	88 (57.8)	26 (66.6)	7.8	-	1.0 (reference)	-	-
TT	77 (40.3)	64 (42.1)	13 (33.3)	9.2	0.53	0.90 (0.64-1.25)	0.94 (0.61-1.45)	0.8
Genotype	**CASES**	**DEAD**	**ALIVE**	**Univariate analysis**			**Multivariate analysis**	
rs2032582	**n (%),**	**n (%),**	**n (%),**	**MST**	**Log rank**	**Unadjusted HR[a]**	**Adjusted HR[b]**	***P***
ABCB1 (G2677A)	**N=191**	**N=152**	**N=39**	**(months)**	***p***		**(95% CI)**	
GG	165 (86.3)	129 (84.8)	36 (92.3)	8.97	-	1.0 (reference)	-	-
GA	26 (13.6)	23 (15.1)	3 (7.6)	6.0	0.13	1.46 (0.88-2.42)	1.35 (0.76-2.39)	0.29
Genotype	**CASES**	**DEAD**	**ALIVE**	**Univariate analysis**			**Multivariate analysis**	
rs2032582	**n (%),**	**n (%),**	**n (%),**	**MST**	**Log rank**	**Unadjusted HR[a]**	**Adjusted HR[b]**	***P***
ABCB1 (G2677T)	**N=191**	**N=152**	**N=39**	**(months)**	***p***		**(95% CI)**	
GT	96 (50.2)	75 (49.3)	21 (53.8)	8.96	-	1.0 (reference)	-	-
TT	95 (49.7)	77 (50.6)	18 (46.15)	8.5	0.62	1.08 (0.78-1.48)	1.20 (0.77-1.87)	0.39

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, regimen, KPS, ECOG

Lung cancer patients diagnosed with SQCC histology and carrying *ABCB1* $C^{1236}T$ polymorphism showed reduced survival in subjects having mutant genotype (*TT)* as compared to the wild-type genotype (*CC)* (MST=6.73 *vs.* 7.8, HR=0.52, 95% CI=0.26-1.07, p=0.07). In the case of *ABCB1* $C^{3435}T$ polymorphism in SQCC patients, patients harboring mutant genotype had higher survival than the wild type genotype (MST=7.5 *vs.* 5.9, HR=0.75, 95% CI=0.40-1.41, p=0.3), though no significance was found. On the other hand, SQCC patients having heterozygous genotype (*GA)* for $G^{2677}A$ showed drastically reduced survival as compared to the wild-type genotype (MST=4.13 *vs.* 7.27, HR=1.21, 95% CI=0.68-2.14, Log-rank p=0.5). In the case of $G^{2677}T$ polymorphism, SQCC patients having mutant genotype (*TT)* showed better survival as compared to the wild type genotype (*GG)* (MST=8.46 *vs.* 5.93, HR=0.88, 95% CI=0.65-1.19, Log-rank-p=0.4). Briefly, none of the SQCC patients did not reveal any prognostic significance for the *ABCB1* ($C^{1236}T$, $C^{3435}T$, $G^{2677}A$, $G^{2677}T$) polymorphisms, as illustrated in Table 5.23 (b)**.**

Table 5.23 (b): Association of *ABCB1* SNPs on overall survival according to SQCC histology

SQCC								
Genotype rs1128503 ABCB1 (C1236T)	**CASES n (%), N=204**	**DEAD n (%), N=176**	**ALIVE n (%), N=28**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
Codominant model								
CC	113 (55.3)	98 (55.6)	15 (53.5)	7.8	-	1.0 (reference)	-	-
CT	62 (30.3)	56 (31.8)	6 (21.4)	5.93	0.47	1.13 (0.80-1.58)	0.95 (0.58-1.55)	0.8
TT	29 (14.2)	22 (12.5)	7 (25)	6.73	0.30	1.25 (0.80-1.95)	0.52 (0.26-1.07)	0.07
Dominant model								
CC	113 (55.3)	98 (55.6)	15 (53.5)	7.8	-	1.0 (reference)	-	-
CT+TT	91 (44.6)	78 (44.3)	13 (46.4)	5.93	0.9	1.0 (0.74-1.35)	0.81 (0.52-1.26)	0.35
Recessive model								
CC+CT	175 (85.7)	154 (87.5)	21 (75)	7.27	-	1.0 (reference)	-	-
TT	29 (14.2)	22 (12.5)	7 (25)	6.73	0.21	1.29 (0.85-1.95)	0.62 (0.32-1.18)	0.14
Genotype rs1045642 ABCB1 (C3435T)	**CASES n (%), N=204**	**DEAD n (%), N=176**	**ALIVE n (%), N=28**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
CC	35 (17.1)	30 (17.04)	5 (17.8)	5.9	-	1.0 (reference)	-	-
CT	87 (42.6)	77 (43.7)	10 (35.7)	6.4	0.68	1.08 (0.71-1.65)	1.26 (0.65-2.43)	0.49
TT	82 (40.1)	69 (39.2)	13 (46.4)	7.5	0.8	1.05 (0.67-1.63)	0.75 (0.40-1.41)	0.38
Dominant model								
CC	35 (17.1)	30 (17.04)	5 (17.8)	5.93	-	1.0 (reference)	-	-
CT+TT	169 (82.8)	146 (82.9)	23 (82.1)	7.27	0.9	1.01 (0.68-1.50)	0.98 (0.56-1.72)	0.9
Recessive model								
CC+CT	122 (59.8)	107 (60.7)	15 (53)	5.97	-	1.0 (reference)	-	-
TT	82 (40.1)	69 (39.2)	13 (46.4)	7.5	0.4	0.88 (0.65-1.2)	0.94 (0.61-1.45)	0.79
Genotype rs2032582 ABCB1 (G2677A)	**CASES n (%), N=204**	**DEAD n (%), N=176**	**ALIVE n (%), N=28**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GG	186 (91.1)	161 (91.4)	25 (89.2)	7.27	-	1.0 (reference)	-	-
GA	18 (8.8)	15 (85.2)	3 (10.7)	4.13	0.50	1.21 (0.68-2.14)	0.89 (0.39-2.02)	0.79
Genotype rs2032582 ABCB1 (G2677T)	**CASES n (%), N=204**	**DEAD n (%), N=176**	**ALIVE n (%), N=28**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GT	108 (52.9)	94 (53.4)	14 (50)	5.93	-	1.0 (reference)	-	-
TT	96 (47.05)	82 (46.5)	14 (50)	8.46	0.42	0.88 (0.65-1.19)	0.90 (0.59-1.37)	0.64

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, regimen, KPS, ECOG

SCLC patients having *ABCB1 $C^{1236}T$* polymorphism and carrying mutant genotype (*TT)* showed drastically reduced survival with a 2.48 times high death ratio as compared to the wild type genotype (*CC)* (MST=4.5 *vs.* 9.4, HR=2.48, 95% CI=0.78-7.90, p=0.12). In the case of *ABCB1 $C^{3435}T$,* our results showed high survival in SCLC patients harboring mutant genotype (*TT)* as compared to the wild type genotype (*CC)* (MST=9.7 *vs.* 7.67, HR=1.03), though significance was not reached. In the case of SCLC, patients with *ABCB1 $G^{2677}A$* polymorphism and carrying one copy of mutant allele (*GA)* showed poor survival when compared with the patients having both copies wild type alleles (*GG)* (MST=4.53 *vs.* 10.4, HR=3.72, 95% CI=1.08-12.83, p=**0.03**). On applying cox regression model and adjusting with various covariates, the heterozygous genotype

(GA) showed the death ratio of 5.54 (HR=5.54, 95% CI=1.69-18.06, p=**0.004**) (Figure 5.28). Other than this, *ABCB1* polymorphisms (*$C^{1236}T$*, *$C^{3435}T$*, and *$G^{2677}T$)* did not show any association with the prognosis of SCLC patients (Table 5.23 (c)).

Table 5.23 (c): Association of *ABCB1* SNPs on overall survival according to SCLC histology

SCLC								
Genotype	**CASES**	**DEAD**	**ALIVE**	**Univariate analysis**			**Multivariate analysis**	
rs1128503 ABCB1 (C1236T)	**n (%), N=75**	**n (%), N=71**	**n (%), N=4**	**MST (months)**	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
Codominant model								
CC	44 (58.6)	40 (56.3)	4 (100)	9.4	-	1.0 (reference)	-	-
CT	23 (30.6)	23 (32.3)	0 (0)	11.0	0.35	1.29 (0.74-2.25)	1.51 (0.58-3.89)	0.39
TT	8 (10.6)	8 (11.2)	0 (0)	4.5	0.17	1.91 (0.75-4.85)	2.48 (0.78-7.90)	0.12
Dominant model								
CC	44 (58.6)	40 (56.3)	4 (100)	9.4	-	1.0 (reference)	-	-
CT+TT	31 (41.3)	31 (43.6)	0 (0)	9.93	0.21	1.37 (0.83-2.24)	1.59 (0.73-3.45)	0.23
Recessive model								
CC+CT	67 (89.3)	63 (88.7)	4 (100)	10.4	-	1.0 (reference)	-	-
TT	8 (10.6)	8 (11.26)	0 (0)	4.5	0.19	1.82 (0.73-4.52)	1.59 (0.62-4.05)	0.32
Genotype	**CASES**	**DEAD**	**ALIVE**	**Univariate analysis**			**Multivariate analysis**	
rs1045642 ABCB1 (C3435T)	**n (%), N=75**	**n (%), N=71**	**n (%), N=4**	**MST (months)**	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
CC	11 (14.6)	11 (15.4)	0 (0)	7.67	-	1.0 (reference)	-	-
CT	36 (48)	34 (47.8)	2 (50)	10.73	0.85	0.93 (0.46-1.89)	1.08 (0.47-2.46)	0.84
TT	28 (37.3)	26 (36.6)	2 (50)	9.7	0.9	1.03 (0.51-2.07)	0.97 (0.32-2.92)	0.9
Dominant model								
CC	11 (14.6)	11 (15.4)	0 (0)	7.67	-	1.0 (reference)	-	-
CT+TT	64 (85.3)	60 (84.5)	4 (100)	9.93	0.9	0.96 (0.49-1.86)	1.39 (0.55-3.50)	0.48
Recessive model								
CC+CT	47 (62.6)	45 (63.3)	2 (50)	9.3	-	1.0 (reference)	-	-
TT	28 (37.3)	26 (36.6)	2 (50)	9.7	0.43	1.22 (0.73-2.02)	1.31 (0.61-2.83)	0.47
Genotype	**CASES**	**DEAD**	**ALIVE**	**Univariate analysis**			**Multivariate analysis**	
rs2032582 ABCB1 (G2677A)	**n (%), N=75**	**n (%), N=71**	**n (%), N=4**	**MST (months)**	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GG	69 (92)	65 (91.5)	4 (100)	10.4	-	1.0 (reference)	-	-
GA	6 (8)	6 (8.4)	0 (0)	4.53	**0.03**	3.72 (1.08-12.83)	5.54 (1.69-18.06)	**0.0045**
Genotype	**CASES**	**DEAD**	**ALIVE**	**Univariate analysis**			**Multivariate analysis**	
rs2032582 ABCB1 (G2677T)	**n (%), N=75**	**n (%), N=71**	**n (%), N=4**	**MST (months)**	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GT	39 (52)	38 (53.5)	1 (25)	7.6	-	1.0 (reference)	-	-
TT	36 (48)	33 (46.4)	3 (75)	10.6	0.12	0.68 (0.42-1.10)	0.73 (0.37-1.43)	0.36

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, regimen, KPS, ECOG

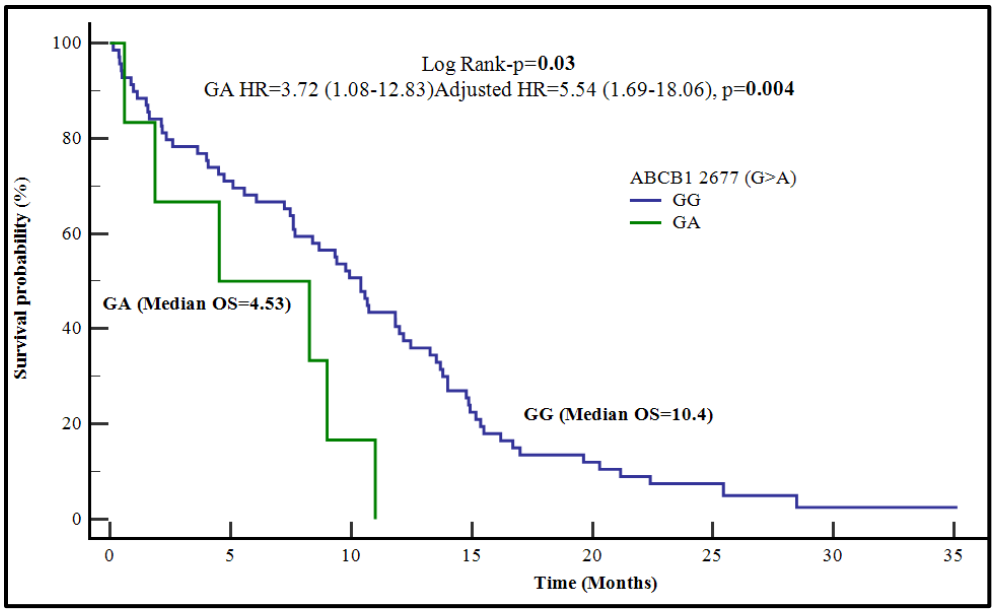


Figure 5.28: Kaplan Meier curves illustrating the association between overall survival in different genotypes of *ABCB1 G^{2677}A* polymorphism in SCLC patients

5.13.3 Prognosis of lung cancer patients treated with various chemotherapy regimens

Patients with lung carcinoma were given platinum-based chemotherapy along with pemetrexed, paclitaxel, docetaxel, and irinotecan.

5.13.3.1 Association of ABCB1 polymorphisms on OS according to cisplatin/carboplatin and docetaxel regimen

As shown in table 5.24 (a), the patients administered with cisplatin/carboplatin and docetaxel and having both copies of the mutant allele (*TT)* for *ABCB1 C^{1236}T* polymorphism showed substantially higher survival as compared to the wild type genotype (*CC)* (MST 9.7 *vs.*8.7, HR=0.54, 95% CI=0.22-1.30, p=0.17). After adjusting with different covariates such as age, gender, smoking habits, performance status, histology, tumor stage, and regimen, the death ratio of 0.30 was observed, which signified better prognosis in the patients carrying mutant genotype *(TT)* for *ABCB1 C^{1236}T* polymorphism (HR=0.30, 95% CI=0.09-0.98, p=**0.04**). Even in the recessive model, survival remained significantly high in patients who had mutant genotype (*TT)* as compared to subjects with combined wild and heterozygous genotype (*CC+CT*) (HR=0.34, 95% CI=0.12-0.97, p=**0.04)** (Figure 5.29).

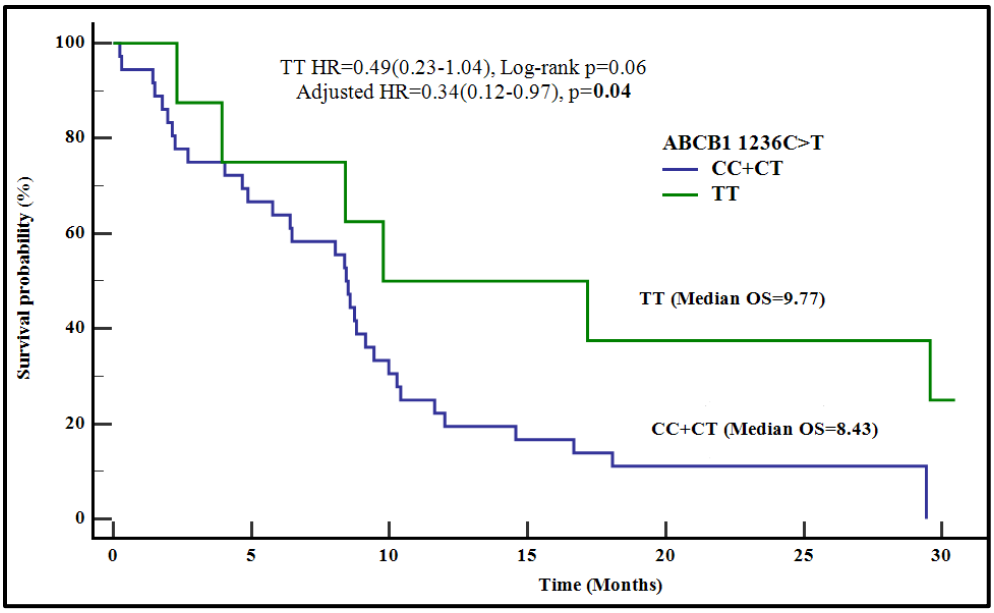


Figure 5.29: Kaplan Meier curves illustrating the association between overall survival in different genotypes of *ABCB1 C^{1236}T* polymorphism in lung cancer patients undergoing cisplatin/ carboplatin and docetaxel chemotherapy

Patients administered with cisplatin/carboplatin and docetaxel having *ABCB1 C^{3435}T* and harboring one allele of mutant genotype (*CT)* showed higher survival than the wild type genotype (MST=8.5 *vs.* 4.03, p=0.43). Patients having *G^{2677}A* polymorphism undergoing cisplatin/ carboplatin and docetaxel therapy and harboring heterozygous genotype (*GA)* showed better survival as compared to the wild type genotype (*GG)* (MST=9.7 *vs.* 8.4, HR=0.42, 95% CI= 0.13-1.38, p=0.15). Patients administered with cisplatin/carboplatin and docetaxel and having both copies of the mutant allele (*TT)* for *ABCB1 G^{2677}T* polymorphism showed borderline higher survival as compared to the wild type genotype (*CC)* (MST =8.57 *vs.*8.4, HR=1.15, 95% CI=0.61-2.19, p=0.6). However, no significant association was found for *G^{2677}T* in predicting the prognosis in the lung patients undergoing cisplatin/carboplatin and docetaxel combined therapy.

Table 5.24 (a): Association of *ABCB1* SNPs on overall survival according to Cisplatin/carboplatin + docetaxel regimen

Cisplatin/carboplatin + Docetaxel								
Genotype rs1128503 ABCB1 (C1236T)	**CASES n (%), N=44**	**DEAD n (%), N=39**	**ALIVE n (%), N=5**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
Codominant model								
CC	22 (50.0)	19 (48.7)	3 (60.0)	8.7	-	1.0 (reference)	-	-
CT	14 (31.8)	14 (35.8)	0 (0)	4.6	0.08	1.82 (0.86-3.88)	1.88 (0.76-4.67)	0.16
TT	8 (18.1)	6 (15.3)	2 (40.0)	9.7	0.17	0.54 (0.22-1.30)	0.30 (0.09-0.98)	**0.04**
Dominant model								
CC	22 (50)	19 (48.7)	3 (60.0)	8.73	-	1.0 (reference)	-	-
CT+TT	22 (50)	20 (51.2)	2 (40.0)	8.4	0.78	1.09 (0.57-2.07)	0.84 (0.40-1.78)	0.66
Recessive model								
CC+CT	36 (81.8)	33 (84.6)	3 (60.0)	8.43	-	1.0 (reference)	-	-
TT	8 (18.1)	6 (15.3)	2 (40.0)	9.77	0.06	0.49 (0.23-1.04)	0.34 (0.12-0.97)	**0.04**
Genotype rs1045642 ABCB1 (C3435T)	**CASES n (%), N=44**	**DEAD n (%), N=39**	**ALIVE n (%), N=5**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
CC	8 (18.1)	8 (20.5)	0 (0)	4.03	-	1.0 (reference)	-	-
CT	19 (43.1)	16 (41.02)	3 (60)	8.5	0.43	0.69 (0.27-1.73)	1.05 (0.34-3.23)	0.9
TT	17 (38.6)	15 (38.4)	2 (40)	9.13	0.7	0.88 (0.36-2.11)	1.19 (0.35-4.03)	0.77
Dominant model								
CC	8 (18.1)	8 (20.5)	0 (0)	4.03	-	1.0 (reference)	-	-
CT+TT	36 (81.8)	31 (79.4)	5 (100)	8.73	0.46	0.72 (0.30-1.71)	1.02 (0.40-2.58)	0.9
Recessive model								
CC+CT	27 (61.3)	24 (61.5)	3 (60)	8.40	-	1.0 (reference)	-	-
TT	17 (38.6)	15 (38.4)	2 (40)	9.13	0.9	0.9 (0.51-1.90)	1.13 (0.50-2.51)	0.76
Genotype rs2032582 ABCB1 (G2677A)	**CASES n (%), N=44**	**DEAD n (%), N=39**	**ALIVE n (%), N=5**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GG	38 (86.3)	34 (87.1)	4 (80)	8.4	-	1.0 (reference)	-	-
GA	6 (13.6)	5 (12.8)	1 (20)	9.7	0.30	0.65 (0.29-1.47)	0.42 (0.13-1.38)	0.15
Genotype rs2032582 ABCB1 (G2677T)	**CASES n (%), N=44**	**DEAD n (%), N=39**	**ALIVE n (%), N=5**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GT	19 (43.1)	16 (41.02)	3 (60)	8.4	-	1.0 (reference)	-	-
TT	25 (56.8)	23 (58.9)	2 (40)	8.57	0.64	1.15 (0.61-2.19)	0.95 (0.47-1.93)	0.9

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, histology, KPS, ECOG

5.13.3.2 Association of ABCB1 polymorphisms on OS according to cisplatin/carboplatin and paclitaxel regimen

As shown in Table 5.24 (b), the patients administered with cisplatin/carboplatin and paclitaxel and having one copy of the mutant allele (*TT)* for *ABCB1 $C^{1236}T$* polymorphism showed higher survival as compared to the wild type genotype (*CC)* (MST 7.5 *vs.*6.9, HR=0.81, 95% CI=0.48-1.37, p=0.43). Lung cancer patients administered with cisplatin/carboplatin and paclitaxel having *ABCB1 $C^{3435}T$* and harboring both the alleles of mutant genotype (*TT)* showed drastically higher survival than the wild type genotype (MST= 8.3 *vs.* 3.97, p=0.20). Also, no association was found

for either of the *ABCB1 G*^{2677}*A/T* polymorphisms in predicting the prognosis of lung cancer patients undergoing cisplatin/carboplatin and paclitaxel doublet chemotherapy.

Table 5.24 (b): Association of *ABCB1* SNPs on overall survival according to Cisplatin/carboplatin + paclitaxel regimen

Cisplatin/carboplatin+paclitaxel								
Genotype	**CASES**	**DEAD**	**ALIVE**	**Univariate analysis**			**Multivariate analysis**	
rs1128503 ABCB1 (C1236T)	**n (%), N=85**	**n (%), N=72**	**n (%), N=13**	**MST (months)**	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
Codominant model								
CC	48 (56.4)	43 (59.7)	5 (38.4)	6.9	-	1.0 (reference)	-	-
CT	24 (28.2)	19 (26.3)	5 (38.4)	7.5	0.43	0.81 (0.48-1.37)	0.75 (0.42-1.34)	0.34
TT	13 (15.2)	10 (13.8)	3 (23.07)	4.3	0.9	0.98 (0.49-1.98)	0.95 (0.43-2.09)	0.91
Dominant model								
CC	48 (56.4)	43 (59.7)	5 (38.4)	6.9	-	1.0 (reference)	-	-
CT+TT	37 (43.5)	29 (40.2)	8 (61.5)	5.2	0.58	0.87 (0.55-1.40)	0.87 (0.52-1.43)	0.58
Recessive model								
CC+CT	72 (84.7)	62 (86.1)	10 (76.9)	7.2	-	1.0 (reference)	-	-
TT	13 (15.2)	10 (13.8)	3 (23.07)	4.33	0.82	1.07 (0.54-2.14)	1.12 (0.55-2.28)	0.75
Genotype	**CASES**	**DEAD**	**ALIVE**	**Univariate analysis**			**Multivariate analysis**	
rs1045642 ABCB1 (C3435T)	**n (%), N=85**	**n (%), N=72**	**n (%), N=13**	**MST (months)**	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
CC	18 (21.1)	14 (19.4)	4 (30.7)	3.97	-	1.0 (reference)	-	-
CT	32 (37.6)	28 (38.8)	4 (30.7)	5.67	0.62	1.16 (0.62-2.19)	1.69 (0.76-3.76)	0.19
TT	35 (41.1)	30 (41.6)	5 (38.4)	8.3	0.6	0.86 (0.44-1.66)	0.59 (0.26-1.32)	0.20
Dominant model								
CC	18 (21.1)	14 (19.4)	4 (30.7)	3.97	-	1.0 (reference)	-	-
CT+TT	67 (78.8)	58 (80.5)	9 (69.2)	7.27	0.9	1.01 (0.56-1.82)	1.01 (0.51-1.99)	0.9
Recessive model								
CC+CT	50 (58.8)	42 (58.3)	8 (61.5)	4.3	-	1.0 (reference)	-	-
TT	35 (41.1)	30 (41.6)	5 (38.4)	8.3	0.42	0.82 (0.52-1.31)	0.79 (0.47-1.33)	0.38
Genotype	**CASES**	**DEAD**	**ALIVE**	**Univariate analysis**			**Multivariate analysis**	
rs2032582 ABCB1 (G2677A)	**n (%), N=85**	**n (%), N=72**	**n (%), N=13**	**MST (months)**	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GG	74 (87.05)	62 (86.1)	12 (92.3)	7.27	-	1.0 (reference)	-	-
GA	11 (12.9)	10 (13.8)	1 (7.6)	4.13	0.12	1.89 (0.83-4.31)	1.88 (0.78-4.50)	0.15
Genotype	**CASES**	**DEAD**	**ALIVE**	**Univariate analysis**			**Multivariate analysis**	
rs2032582 ABCB1 (G2677T)	**n (%), N=85**	**n (%), N=72**	**n (%), N=13**	**MST (months)**	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GT	45 (52.9)	38 (52.7)	7 (53.8)	5.2	-	1.0 (reference)	-	-
TT	40 (47.05)	34 (47.2)	6 (46.1)	8.3	0.5	0.85 (0.53-1.35)	0.78 (0.46-1.32)	0.35

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, histology, KPS, ECOG

5.13.3.3 Association of ABCB1 polymorphisms on OS according to cisplatin/carboplatin and pemetrexed regimen

As shown in table 5.24 (c), the lung cancer subjects harboring heterozygous genotype (*CT)* for *ABCB1 $C^{1236}T$* and treated with the pemetrexed and cisplatin/carboplatin had substantially poor survival and 2-times high death ratio as compared to the patients with wild type *(CC)* genotype (MST=3.9 *vs.* 7.9 months, HR=2.0, 95% CI=1.17-3.43; p=**0.01**). After adjusting with different covariates such as age, smoking, stage, performance status, and histology, the hazard ratio was 1.78 (HR=1.78, 95% CI=1.05-2.99, p=**0.02**) (Figure 5.30 (a)). In the same line, patients treated with the pemetrexed and cisplatin/carboplatin and having heterozygous genotype (MST=6.0 *vs.* 14.17, HR=1.94, 95% CI=1.11-3.38, Log-rank-p=**0.018**) for *ABCB1 $C^{3435}T$* had approximately two times poor survival when compared with the wild type genotype (*CC*). After adjusting with the multiple covariates, the death ratio remained approximately two times in heterozygous group (HR=2.18, 95% CI=1.04-4.58, p=**0.03**) (Figure 5.30 (b)). Mutant (*TT*) genotype for *ABCB1 $C^{3435}T$* showed very poor survival and high mortality rate as compared to the wild type genotype (*CC)* (MST=6.73 *vs.* 14.17, HR=2.10, 95% CI=1.21-3.65, log-rank-p=**0.007**). The multivariate regression model showed high death ratio in the lung cancer patients harboring mutant genotype (HR=1.95, 95% CI=0.99-3.86, p=**0.04**).

A

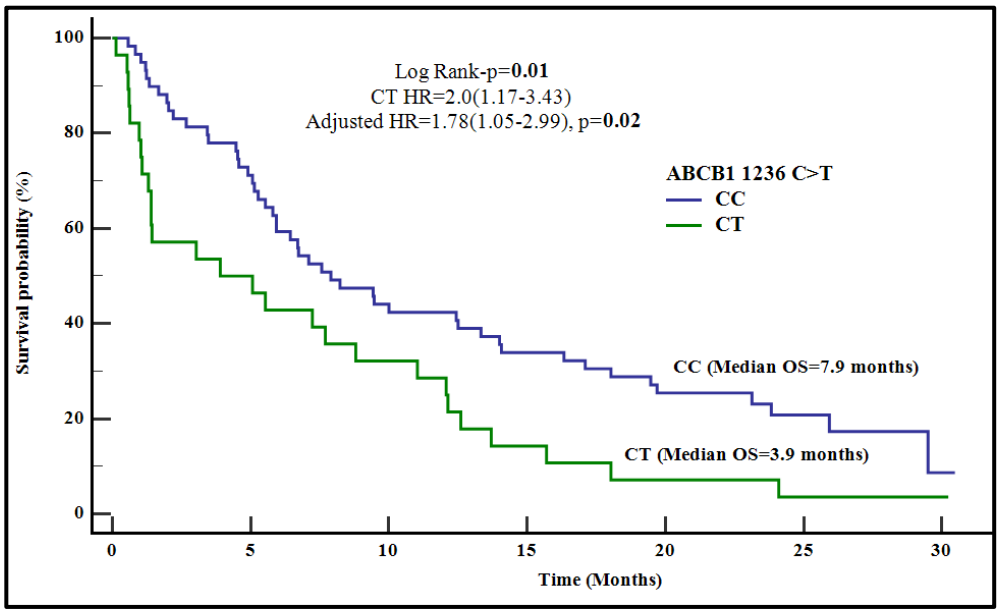


Figure 5.30 (A): Kaplan Meier curves illustrating the association between overall survival in different genotypes of *ABCB1 $C^{1236}T$* polymorphism in lung cancer patients undergoing cisplatin/ carboplatin and pemetrexed chemotherapy

B

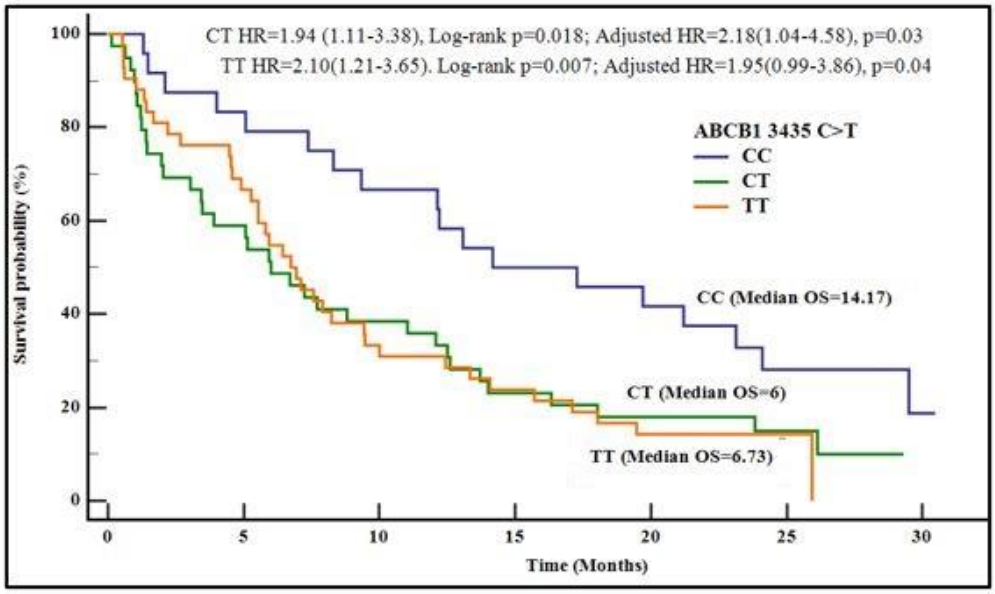


Figure 5.30 (B): Kaplan Meier curves illustrating the association between overall survival in different genotypes of *ABCB1 C^{3435}T* polymorphism in lung cancer patients undergoing cisplatin/carboplatin and pemetrexed chemotherapy. (Note: *CC* represents wild-type genotype, *CT* represents heterozygous genotype, and *TT* represents homozygous for mutant genotype)

Patients having *G^{2677}A* polymorphism undergoing cisplatin/carboplatin and pemetrexed therapy and harboring heterozygous genotype (*GA)* showed poor survival and high mortality rate as compared to the wild type genotype (*GG)* (MST=6.0 *vs.* 8.23, HR=1.49, 95% CI= 0.73-3.06, p=0.26). For *ABCB1 G^{2677}T* polymorphism, patients having mutant genotype (*TT)* showed reduced survival as compared to the patients harboring both wild-type alleles (*CC)* (MST= 7.1 *vs.* 8.8, HR=1.36, 95% CI=0.85-2.16, p=0.18). However, no association was found for either of the *ABCB1 G^{2677}A/T* polymorphism in predicting the prognosis in the lung cancer patients undergoing cisplatin/carboplatin and pemetrexed doublet chemotherapy.

Table 5.24 (c): Association of *ABCB1* SNPs on overall survival according to cisplatin/carboplatin + pemetrexed regimen

Cisplatin/carboplatin+pemetrexed								
Genotype rs1128503 ABCB1 (C1236T)	**CASES n (%), N=105**	**DEAD n (%), N=89**	**ALIVE n (%), N=16**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
Codominant model								
CC	59 (56.1)	48 (53.9)	11 (68.7)	7.9	-	1.0 (reference)	-	-
CT	28 (26.6)	27 (30.3)	1 (6.2)	3.9	**0.01**	2.0 (1.17-3.43)	1.78 (1.05-2.99)	**0.02**
TT	18 (17.1)	14 (15.7)	4 (25)	12.2	0.46	0.80 (0.45-1.42)	0.83 (0.41-1.64)	0.59
Dominant model								
CC	59 (56.1)	48 (53.9)	11 (68.7)	7.9	-	1.0 (reference)	-	-
CT+TT	46 (43.8)	41 (46.0)	5 (31.2)	7.37	0.24	1.28 (0.84-1.96)	1.25 (0.79-1.96)	0.32
Recessive model								
CC+CT	87 (82.8)	75 (84.2)	12 (75)	7.1	-	1.0 (reference)	-	-
TT	18 (17.1)	14 (15.7)	4 (25)	12.2	0.17	0.70 (0.421.16)	0.69 (0.36-1.32)	0.27
Genotype rs1045642 ABCB1 (C3435T)	**CASES n (%), N=105**	**DEAD n (%), N=89**	**ALIVE n (%), N=16**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
CC	24 (22.8)	18 (20.2)	6 (37.5)	14.17	-	1.0 (reference)	-	-
CT	39 (37.1)	34 (38.2)	5 (31.2)	6.0	**0.018**	1.94 (1.11-3.38)	2.18 (1.04-4.58)	**0.03**
TT	42 (40)	37 (41.5)	5 (31.2)	6.73	**0.007**	2.10 (1.21-3.65)	1.95 (0.99-3.86)	**0.04**
Dominant model								
CC	24 (22.8)	18 (20.2)	6 (37.5)	14.17	-	1.0 (reference)	-	-
CT+TT	81 (77.1)	71 (79.7)	10 (62.5)	6.7	**0.007**	1.86 (1.18-2.93)	2.04 (1.10-3.77)	**0.02**
Recessive model								
CC+CT	63 (60)	52 (58.4)	11 (68.7)	9.33	-	1.0 (reference)	-	-
TT	42 (40)	37 (41.5)	5 (31.2)	6.73	0.14	1.39 (0.89-2.18)	1.07 (0.68-1.69)	0.76
Genotype rs2032582 ABCB1 (G2677A)	**CASES n (%), N=105**	**DEAD n (%), N=89**	**ALIVE n (%), N=16**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GG	93 (88.5)	78 (87.6)	15 (93.7)	8.23	-	1.0 (reference)	-	-
GA	12 (11.4)	11 (12.3)	1 (6.2)	6.0	0.34	1.40 (0.69-2.87)	1.49 (0.73-3.06)	0.26
Genotype rs2032582 ABCB1 (G2677T)	**CASES n (%), N=105**	**DEAD n (%), N=89**	**ALIVE n (%), N=16**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GT	54 (51.4)	44 (49.4)	10 (62.5)	8.8	-	1.0 (reference)	-	-
TT	51 (48.5)	45 (50.5)	6 (37.5)	7.1	0.24	1.28 (0.83-1.95)	1.36 (0.85-2.16)	0.18

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, histology, KPS, ECOG

5.13.3.4 Association of ABCB1 polymorphisms on OS according to cisplatin/carboplatin and irinotecan regimen

As shown in table 5.24 (d), the lung carcinoma patients administered with cisplatin/carboplatin and irinotecan and having both copies of the mutant allele (*TT)* for *ABCB1 $C^{1236}T$* polymorphism showed poor survival as compared to the wild type genotype (*CC)* (MST=4.5 *vs.*10.47, HR=2.03, 95% CI=0.55-7.43, p=0.28). Lung cancer patients harboring one copy of mutant allele *(CT)* for the *ABCB1 $C^{3435}T$* polymorphism had lower survival than the wild-type genotype (MST= 7.47 *vs.* 7.67, p=0.46). The patients administered with cisplatin/carboplatin and irinotecan and having one

copy of mutant allele (*GA)* for *ABCB1* $G^{2677}A$ polymorphism showed substantially lower survival and high death ratio as compared to the subjects with wild genotype (*GG)* (MST= 4.53 *vs.* 10.57, HR=7.99log-rank-p=**0.009**). Upon multivariate analysis, the hazard rate increased to 5-fold in the heterozygous genotype (*GA)* (HR=5.0, 95% CI=1.55-16.65, p=**0.007**) (Figure 5.31). On the other hand, for *ABCB1* $G^{2677}T$ polymorphism, patients administered with cisplatin/carboplatin and irinotecan and having both copies of the mutant allele (*TT)* showed higher survival as compared to the patients harboring both wild type alleles (*CC)* (MST= 10.73 *vs.* 7.47, HR=0.63, 95% CI=0.34-1.19, p=0.16). However, no association was found between *ABCB1* $C^{1236}T$, $C^{3435}T$, and $G^{2677}T$ polymorphism in predicting the prognosis of lung cancer patients undergoing cisplatin/carboplatin and irinotecan doublet chemotherapy.

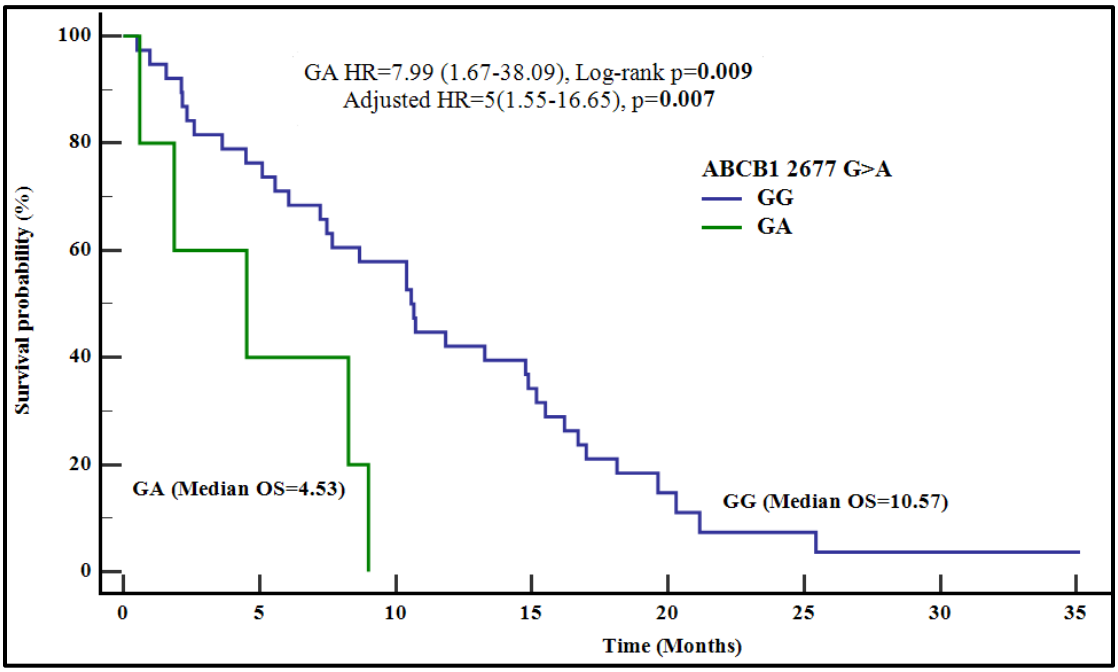


Figure 5.31: Kaplan Meier curves illustrating the association between overall survival in different genotypes of *ABCB1* $G^{2677}A$ polymorphism in lung cancer patients undergoing cisplatin/ carboplatin and irinotecan chemotherapy

Table 5.24 (d): Association of *ABCB1* SNPs on overall survival according to cisplatin/carboplatin + irinotecan regimen

Cisplatin/carboplatin + Irinotecan								
Genotype rs1128503 ABCB1 (C1236T)	**CASES n (%), N=43**	**DEAD n (%), N=40**	**ALIVE n (%), N=3**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
Codominant model								
CC	23 (53.4)	21 (52.5)	2 (66.6)	10.47	-	1.0 (reference)	-	-
CT	13 (30.2)	13 (32.5)	0 (0)	10.57	0.18	1.6 (0.78-3.64)	1.4 (0.49-4.14)	0.51
TT	7 (16.2)	6 (15)	1 (33.3)	4.5	0.78	1.14 (0.43-2.97)	2.03 (0.55-7.43)	0.28
Dominant model								
CC	23 (53.4)	21 (52.5)	2 (66.6)	10.4	-	1.0 (reference)	-	-
CT+TT	20 (46.5)	19 (47.5)	1 (33.3)	7.67	0.32	1.37 (0.72-2.61)	1.65 (0.66-4.11)	0.28
Recessive model								
CC+CT	36 (83.7)	34 (85)	2 (66.6)	10.4	-	1.0 (reference)	-	-
TT	7 (16.2)	6 (15)	1 (33.3)	4.5	0.9	1.0 (0.41-2.45)	1.41 (0.50-3.91)	0.50
Genotype rs1045642 ABCB1 (C3435T)	**CASES n (%), N=43**	**DEAD n (%), N=40**	**ALIVE n (%), N=3**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
CC	8 (18.6)	8 (20)	0 (0)	7.67	-	1.0 (reference)	-	-
CT	20 (46.5)	19 (47.5)	1 (33.3)	7.47	0.46	1.35 (0.60-3.04)	1.26 (0.45-3.50)	0.65
TT	15 (34.8)	13 (32.5)	2 (66.6)	10.57	0.9	1.03 (0.43-2.49)	1.16 (0.33-3.98)	0.8
Dominant model								
CC	8 (18.6)	8 (20)	0 (0)	7.67	-	1.0 (reference)	-	-
CT+TT	35 (81.3)	32 (80)	3 (100)	10.4	0.67	1.17 (0.55-2.50)	1.53 (0.54-4.35)	0.42
Recessive model								
CC+CT	28 (65.1)	27 (67.5)	1 (33.3)	7.67	-	1.0 (reference)	-	-
TT	15 (34.8)	13 (32.5)	2 (66.6)	10.57	0.5	0.81 (0.42-1.55)	1.32 (0.56-3.12)	0.52
Genotype rs2032582 ABCB1 (G2677A)	**CASES n (%), N=43**	**DEAD n (%), N=40**	**ALIVE n (%), N=3**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GG	38 (88.3)	35 (87.5)	3 (100)	10.57	-	1.0 (reference)	-	-
GA	5 (11.6)	5 (12.5)	0 (0)	4.53	**0.009**	7.99 (1.67-38.09)	5.0 (1.55-16.65)	**0.0071**
Genotype rs2032582 ABCB1 (G2677T)	**CASES n (%), N=43**	**DEAD n (%), N=40**	**ALIVE n (%), N=3**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GT	24 (55.8)	24 (60)	0 (0)	7.47	-	1.0 (reference)	-	-
TT	19 (44.1)	16 (40)	3 (100)	10.73	0.16	0.63 (0.34-1.19)	0.64 (0.31-1.34)	0.24

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, histology, KPS, ECOG

5.13.4 Association of ABCB1 polymorphism with gender and OS

In lung carcinoma patients, 85.05% of males and 82.1% of females were dead at the time of statistical analysis. Data for overall survival and all the *ABCB1* ($C^{1236}T$, $C^{3435}T$, $G^{2677}A$, $G^{2677}T$) gene polymorphisms are illustrated in Table 5.25. In *ABCB1* $C^{3435}T$ polymorphism, in the co-dominant model, higher survival was observed in the females harboring wild type (*CC*) genotype when compared with the mutant type (*TT*) (MST=14.17 *vs.* 8.23, HR=1.60, Log-rank-p=0.12). Though, after the cox regression model, the hazard ratio increased 3-times in the latter group (HR=3.17, 95% CI=1.10-9.11, p=**0.03**) (Figure 5.32). Even in the dominant model, the death ratio remained high in the combined heterozygous and mutant genotype (*CT+TT*) when compared with the wild-type genotype (*CC*) (HR=2.52, p=**0.02**).

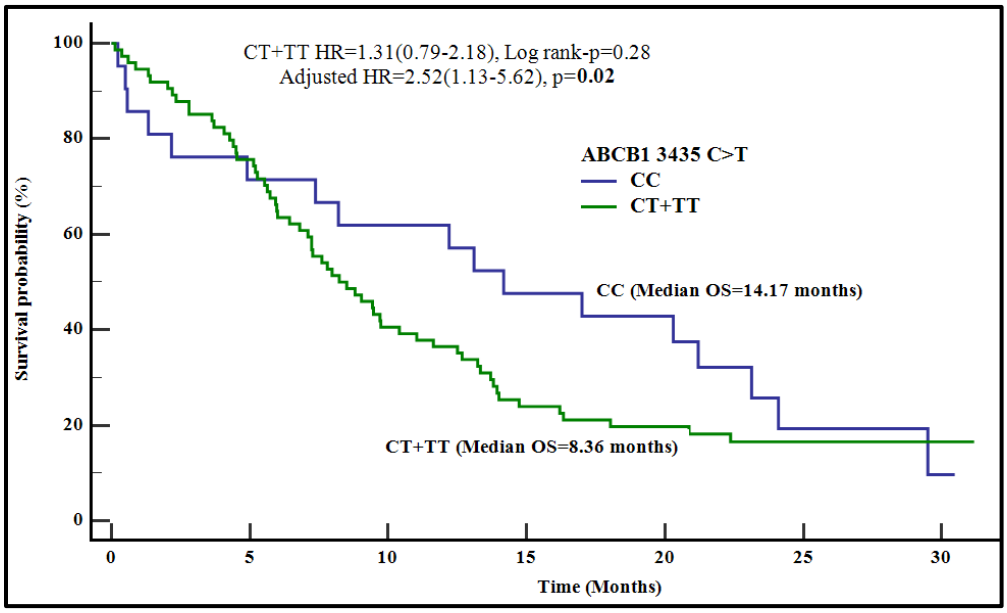


Figure 5.32: Kaplan Meier curves illustrating the association between overall survival in different genotypes of *ABCB1* $C^{3435}T$ polymorphism in females having lung cancer

Further, it was assessed that other polymorphisms in *ABCB1* ($C^{1236}T$, $G^{2677}A$, $G^{2677}T$) gene were not associated in predicting the prognosis in the females having lung cancer. Lack of association was found between overall survival in males and *ABCB1* ($C^{1236}T$, $C^{3435}T$, $G^{2677}A$, $G^{2677}T$) polymorphism.

Table 5.25: Association of *ABCB1 SNPs* on overall survival according to gender

Male								
Genotype rs1128503 ABCB1 (C1236T)	**CASES n (%), N=388**	**DEAD n (%), N=330**	**ALIVE n (%), N=58**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
Codominant model								
CC	216 (55.6)	184 (55.7)	32 (55.1)	8.57	-	1.0 (reference)	-	-
CT	117 (30.1)	103 (31.2)	14 (24.1)	7.5	0.31	1.13 (0.88-1.45)	1.35 (0.97-1.88)	0.06
TT	55 (14.1)	43 (13.03)	12 (20.6)	7.67	0.38	1.15 (0.83-1.59)	0.91 (0.58-1.43)	0.69
Dominant model								
CC	216 (55.6)	184 (55.7)	32 (55.1)	8.57	-	1.0 (reference)	-	-
CT+TT	172 (44.3)	146 (44.2)	26 (44.8)	7.57	0.73	1.03 (0.83-1.29)	1.17 (0.87-1.58)	0.27
Recessive model								
CC+CT	333 (85.8)	287 (86.9)	46 (79.3)	8.26	-	1.0 (reference)	-	-
TT	55 (14.1)	43 (13.03)	12 (20.6)	7.67	0.25	0.83 (0.61-1.13)	0.85 (0.55-1.30)	0.46
Genotype rs1045642 ABCB1 (C3435T)	**CASES n (%), N=388**	**DEAD n (%), N=330**	**ALIVE n (%), N=58**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
CC	72 (18.5)	59 (17.8)	13 (22.4)	7.67	-	1.0 (reference)	-	-
CT	158 (40.7)	138 (41.8)	20 (34.4)	6.73	0.26	1.18 (0.87-1.59)	1.37 (0.89-2.10)	0.14
TT	158 (40.7)	133 (40.3)	25 (43.1)	8.73	0.72	1.05 (0.77-1.43)	1.02 (0.66-1.59)	0.9
Dominant model								
CC	72 (18.5)	59 (17.8)	13 (22.4)	7.67	-	1.0 (reference)	-	-
CT+TT	316 (81.4)	271 (82.1)	45 (77.5)	8.26	0.41	1.12 (0.85-1.47)	1.16 (0.79-1.72)	0.43
Recessive model								
CC+CT	230 (59.2)	197 (59.6)	33 (56.8)	7.32	-	1.0 (reference)	-	-
TT	158 (40.7)	133 (40.3)	25 (43.1)	8.73	0.52	0.93 (0.74-1.16)	0.87 (0.64-1.18)	0.38
Genotype rs2032582 ABCB1 (G2677A)	**CASES n (%), N=388**	**DEAD n (%), N=330**	**ALIVE n (%), N=58**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GG	350 (90.2)	297 (90)	53 (91.3)	8.5	-	1.0 (reference)	-	-
GA	38 (9.7)	33 (10)	5 (8.6)	4.53	0.13	1.36 (0.90-2.04)	1.24 (0.78-1.97)	0.35
Genotype rs2032582 ABCB1 (G2677T)	**CASES n (%), N=388**	**DEAD n (%), N=330**	**ALIVE n (%), N=58**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GT	198 (51.03)	168 (50.9)	30 (51.7)	6.7	-	1.0 (reference)	-	-
TT	190 (48.9)	162 (49.09)	28 (48.2)	8.97	0.34	0.90 (0.72-1.11)	0.87 (0.64-1.17)	0.37
Female								
Genotype rs1128503 ABCB1 (C1236T)	**CASES n (%), N=95**	**DEAD n (%), N=78**	**ALIVE n (%), N=17**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***

Codominant model								
CC	52 (54.7)	42 (53.8)	10 (58.8)	8.23	-	1.0 (reference)	-	-
CT	27 (28.4)	23 (29.4)	4 (23.5)	9.73	0.76	1.08 (0.64-1.81)	0.98 (0.44-2.15)	0.9
TT	16 (16.8)	13 (16.6)	3 (17.6)	7.37	0.75	1.10 (0.58-2.10)	1.13 (0.43-2.96)	0.79
Dominant model								
CC	52 (54.7)	42 (53.8)	10 (58.8)	8.23	-	1.0 (reference)	-	-
CT+TT	43 (45.2)	36 (46.1)	7 (41.1)	9.73	0.71	1.08 (0.69-1.70)	1.04 (0.54-2.02)	0.8
Recessive model								
CC+CT	79 (83.1)	65 (83.3)	14 (82.3)	9.03	-	1.0 (reference)	-	-
TT	16 (16.8)	13 (16.6)	3 (17.6)	7.37	0.86	1.05 (0.57-1.93)	1.06 (0.46-2.44)	0.8
Genotype rs1045642 ABCB1 (C3435T)	**CASES n (%), N=95**	**DEAD n (%), N=78**	**ALIVE n (%), N=17**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
CC	21 (22.1)	17 (21.7)	4 (23.5)	14.17	-	1.0 (reference)	-	-
CT	41 (43.1)	33 (42.3)	8 (47.05)	8.5	0.49	1.22 (0.68-2.17)	2.06 (0.76-5.55)	0.15
TT	33 (34.7)	28 (35.8)	5 (29.4)	8.23	0.12	1.60 (0.87-2.94)	3.17 (1.10-9.11)	**0.03**
Dominant model								
CC	21 (22.1)	17 (21.7)	4 (23.5)	14.17	-	1.0 (reference)	-	-
CT+TT	74 (77.8)	61 (78.2)	13 (76.4)	8.36	0.28	1.31 (0.79-2.18)	2.52 (1.13-5.62)	**0.02**
Recessive model								
CC+CT	62 (65.2)	50 (64.1)	12 (70.5)	9.03	-	1.0 (reference)	-	-
TT	33 (34.7)	28 (35.8)	5 (29.4)	8.23	0.25	1.32 (0.81-2.16)	1.75 (0.89-3.41)	0.09
Genotype rs2032582 ABCB1 (G2677A)	**CASES n (%), N=95**	**DEAD n (%), N=78**	**ALIVE n (%), N=17**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GG	81 (85.2)	66 (84.6)	15 (88.2)	9.43	-	1.0 (reference)	-	-
GA	14 (14.7)	12 (15.3)	2 (11.7)	7.37	0.56	1.21 (0.62-2.34)	1.17 (0.49-2.79)	0.72
Genotype rs2032582 ABCB1 (G2677T)	**CASES n (%), N=95**	**DEAD n (%), N=78**	**ALIVE n (%), N=17**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GT	52 (54.7)	43 (55.1)	9 (52.9)	9.73	-	1.0 (reference)	-	-
TT	43 (45.2)	55 (70.5)	8 (47.05)	8.23	0.73	1.08 (0.68-1.69)	1.62 (0.87-3.02)	0.12

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, smoking, stage, regimen, histology, KPS, ECOG

5.13.5 Association of ABCB1 polymorphism with OS based on smoking status

In lung cancer patients, 86.6% of patient smokers were dead, while 13.3% of smokers were alive at the time of statistical analysis, as shown in table 5.26. For *ABCB1* $C^{1236}T$ polymorphism, in the case of smokers, our results revealed a trend in the survival of both heterozygous *(CT)* and mutant genotypes (*TT)*; however, the significance was not reached (MST=7.57, HR= 1.27, p=0.14 and MST=6.36, HR=0.92, p=0.72). Also, in case of $C^{3435}T$ polymorphism, our result did not show any association in predicting survival in lung cancer patients, as depicted in table 5.26. In the same line for *ABCB1* $G^{2677}A/T$, our results did not show any association in predicting prognosis, however, for $G^{2677}A$ polymorphism, a very drastic decrease in the overall survival was observed in the heterozygous genotype (GA) as compared to the wild type genotype (MST= 5.37 *vs.* 8.3 months, HR=1.47, 95% CI=0.93-2.35, p=0.09), but the outcomes were insignificant.

On evaluating the role of *ABCB1* polymorphisms in non-smokers with prognosis, the incidence of dead patients was 76%, whereas only 24% of non-smokers were alive after the follow-up. *ABCB1* ($C^{1236}T$, $C^{3435}T$, $G^{2677}A$, *and* $G^{2677}T$) polymorphism was not correlated in predicting prognosis in lung cancer patients who do not smoke as shown in table 5.26. Therefore, the polymorphisms of the *ABCB1* gene were not associated with prognosis in lung carcinoma patients in smokers and non-smokers. So, we conclude that smoking does not impact any role in predicting survival in lung cancer patients.

Table 5.26 Association of *ABCB1 SNPs* on overall survival according to smoking habits

Smokers								
Genotype rs1128503 ABCB1 (C1236T)	**CASES n (%), N=383**	**DEAD n (%), N=332**	**ALIVE n (%), N=51**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
Codominant model								
CC	216 (56.3)	187 (56.3)	29 (56.8)	8.37	-	1.0 (reference)	-	-
CT	113 (29.7)	101 (30.4)	12 (23.5)	7.57	0.46	1.09 (0.85-1.40)	1.27 (0.91-1.77)	0.14
TT	54 (14.2)	44 (13.2)	10 (19.6)	6.36	0.68	1.07 (0.77-1.48)	0.92 (0.59-1.44)	0.72
Dominant model								
CC	216 (56.3)	187 (56.3)	29 (56.8)	8.37	-	1.0 (reference)	-	-
CT+TT	167 (43.6)	145 (43.6)	22 (43.1)	7.23	0.71	1.04 (0.83-1.29)	1.12 (0.84-1.50)	0.42
Recessive model								
CC+CT	329 (85.9)	288 (86.7)	41 (80.3)	8.03	-	1.0 (reference)	-	-
TT	54 (14.09)	44 (13.2)	10 (19.6)	6.36	0.25	1.09 (0.80-1.49)	0.87 (0.57-1.33)	0.54
Genotype rs1045642 ABCB1 (C3435T)	**CASES n (%), N=383**	**DEAD n (%), N=332**	**ALIVE n (%), N=51**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
CC	71 (18.5)	61 (18.3)	10 (19.6)	7.67	-	1.0 (reference)	-	-
CT	158 (41.2)	139 (41.8)	19 (37.2)	7.17	0.37	1.14 (0.85-1.53)	1.26 (0.83-1.91)	0.26
TT	154 (40.2)	132 (39.7)	22 (43.1)	8.57	0.66	0.93 (0.68-1.26)	1.03 (0.68-1.57)	0.85
Dominant model								
CC	71 (18.5)	61 (18.3)	10 (19.6)	7.67	-	1.0 (reference)	-	
CT+TT	312 (81.4)	271 (81.6)	41 (80.3)	7.91	0.46	0.90 (0.68-1.18)	1.11 (0.76-1.60)	0.57
Recessive model								
CC+CT	229 (59.7)	200 (60.2)	29 (56.8)	7.23	-	1.0 (reference)	-	
TT	154 (40.2)	132 (39.7)	22 (43.1)	8.57	0.72	0.96 (0.77-1.19)	0.92 (0.68-1.24)	0.60
Genotype rs2032582 ABCB1 (G2677A)	**CASES n (%), N=383**	**DEAD n (%), N=332**	**ALIVE n (%), N=51**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GG	344 (89.8)	298 (89.7)	46 (90.1)	8.3	-	1.0 (reference)	-	-
GA	39 (10.1)	34 (10.2)	5 (9.8)	5.37	0.23	1.26 (0.85-1.86)	1.47 (0.93-2.35)	0.09
Genotype rs2032582 ABCB1 (G2677T)	**CASES n (%), N=383**	**DEAD n (%), N=332**	**ALIVE n (%), N=51**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GT	198 (51.6)	172 (51.8)	26 (50.9)	6.8	-	1.0 (reference)	-	-
TT	185 (48.3)	160 (18.07)	25 (49.01)	8.73	0.50	0.92 (0.74-1.15)	0.93 (0.70-1.25)	0.66

Non-Smokers

Genotype rs1128503 ABCB1 (C1236T)	CASES n (%), N=100	DEAD n (%), N=76	ALIVE n (%), N=24	Univariate analysis			Multivariate analysis	
				MST (months)	Log-rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
Codominant model								
CC	52 (52)	39 (51.31)	13 (54.16)	9.43	-	1.0 (reference)	-	-
CT	31 (31)	25 (32.89)	6 (25)	8.8	0.32	1.29 (0.77-2.18)	1.43 (0.67-3.06)	0.35
TT	17 (17)	12 (15.78)	5 (20.83)	14.17	0.70	0.88 (0.47-1.65)	1.02 (0.42-2.45)	0.9
Dominant model								
CC	52 (52)	39 (51.31)	13 (54.16)	9.43	-	1.0 (reference)	-	-
CT+TT	48 (48)	37 (48.68)	11(45.8)	9.73	0.62	1.12 (0.71-1.76)	1.12 (0.59-2.11)	0.71
Recessive model								
CC+CT	83 (83)	64 (84.21)	19 (79.16)	9.43	-	1.0 (reference)	-	-
TT	17 (17)	12 (15.78)	5 (20.83)	14.17	0.47	0.81 (0.45-1.44)	0.79 (0.35-1.79)	0.58
Genotype rs1045642 ABCB1 (C3435T)	**CASES n (%), N=100**	**DEAD n (%), N=76**	**ALIVE n (%), N=24**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
CC	22 (22)	15 (19.73)	7 (29.16)	14.16	-	1.0 (reference)	-	-
CT	41 (41)	32 (42.10)	9 (37.5)	8.5	0.36	1.31 (0.72-2.37)	1.29 (0.48-3.45)	0.61
TT	37 (37)	29 (38.15)	8 (33.33)	9.7	0.29	1.38 (0.75-2.52)	1.37 (0.53-3.53)	0.51
Dominant model								
CC	22 (22)	15 (19.73)	7 (29.16)	14.16	-	1.0 (reference)	-	-
CT+TT	78 (78)	61 (80.26)	17 (70.83)	8.8	0.28	1.32 (0.78-2.23)	1.52 (0.65-3.54)	0.33
Recessive model								
CC+CT	63 (63)	47 (61.84)	16 (66.66)	8.97	-	1.0 (reference)	-	-
TT	37 (37)	29 (38.15)	8 (33.33)	9.7	0.64	1.11 (0.69-1.79)	1.13 (0.60-2.14)	0.69
Genotype rs2032582 ABCB1 (G2677A)	**CASES n (%), N=100**	**DEAD n (%), N=76**	**ALIVE n (%), N=24**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GG	87 (87)	65 (85.52)	22 (91.66)	11.03	-	1.0 (reference)	-	-
GA	13 (13)	11 (14.47)	2 (8.33)	7.37	0.22	1.58 (0.75-3.32)	0.76 (0.33-1.72)	0.52
Genotype rs2032582 ABCB1 (G2677T)	**CASES n (%), N=100**	**DEAD n (%), N=76**	**ALIVE n (%), N=24**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GT	52 (52)	39 (51.31)	13 (54.16)	11.6	-	1.0 (reference)	-	-
TT	48 (48)	37 (48.68)	11 (45.83)	8.97	0.92	1.02 (0.65-1.60)	1.02 (0.49-2.09)	0.9

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, stage, regimen, histology, KPS, ECOG

5.13.6 Association of ABCB1 polymorphism with OS based on tumor stage

Lung cancer patients having *ABCB1* variations were stratified based on tumor stage to evaluate the overall survival, as illustrated in table 5.27. When patients were grouped based on stage III, 79.8% were dead, while 20.12% of subjects were alive for *ABCB1* $G^{2677}A$ polymorphisms. Our results showed poor survival in patients harboring at least one mutant allele (*GA)* as compared to the lung cancer patients carrying wild type (*GG)* genotype in stage III (MST= 3.87 *vs.* 9.27, HR=1.42, 95% CI=0.67-2.97, Log-rank p=0.34). However, after adjusting with multiple covariates such as age, gender, smoking, histology, regimen, and performance status, the hazard ratio increased to approximately 3-folds in the heterozygous genotype, and a significant association of $G^{2677}A$ was found in predicting prognosis in stage III lung cancer patients (HR=3.33; 95% CI=1.34-8.28, p=**0.009**) (Figure 5.33).

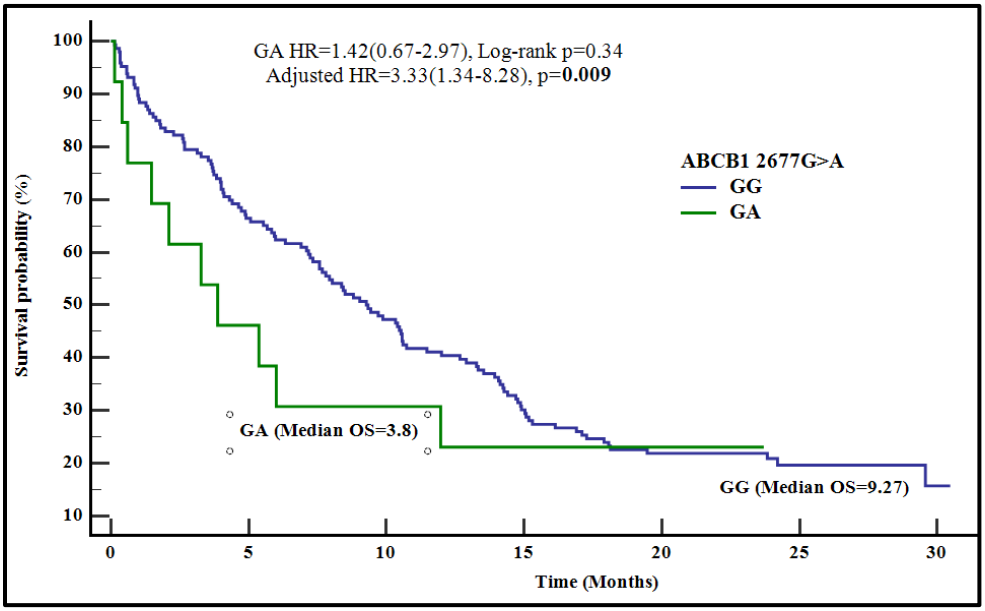


Figure 5.33: Kaplan Meier curves illustrating the association between overall survival in different genotypes of *ABCB1* $G^{2677}A$ polymorphism in stage III lung cancer patients

(Note: *GG* represents wild-type genotype, and *GA* represents the heterozygous genotype)

Upon evaluating the impact of *ABCB1* polymorphism on overall survival in stage IV lung cancer patients undergoing platinum-based doublet chemotherapy, our results showed that the incidence of alive patients at the time of statistical analysis was 11.59%. In comparison, 88.4% of patients in stage IV were dead (Table 5.27). For *ABCB1* $C^{1236}T$ polymorphism, 30% of patients had at least one copy of mutant allele (*CT)*, whereas 13.4% of subjects had both copies of variant alleles (*TT)*.

Our data revealed that patients harboring heterozygous genotype for *ABCB1 $C^{1236}T$* polymorphism had lower survival than those with wild-type genotype in stage IV lung cancer patients (MST= 5.9 *vs.* 7.9, HR=1.35, 95% CI=1.01-1.80), p=**0.04**). After multivariate analysis, the death ratio increased to 1.79 times in patients having at least one copy of mutant allele (*CT)* as compared to the patients with homozygous wild-type genotype (*CC)* (HR=1.79, 95% CI=1.22-2.62, p=**0.002**) (Figure 5.34). Also, on applying the dominant model, our results depicted marginal lower survival in the combined genotype (*CT+TT)* as compared to the wild type genotype (*CC)* (MST=7.5 *vs.* 7.6, HR=1.17, 95% CI=0.90-1.15, p=0.22). After adjusting with covariates such as age, gender, smoking status, histology, regimen, and performance status, our results indicated a significant association of *ABCB1 $C^{1236}T$* polymorphism in the prognosis of lung cancer patients (HR=1.43, 95% CI=1.02-1.99, p=**0.03**). However, the other polymorphisms of *ABCB1* (*$C^{3435}T$, $G^{2677}A/T$)* did not show any association in predicting prognosis in stage IV lung carcinoma patients.

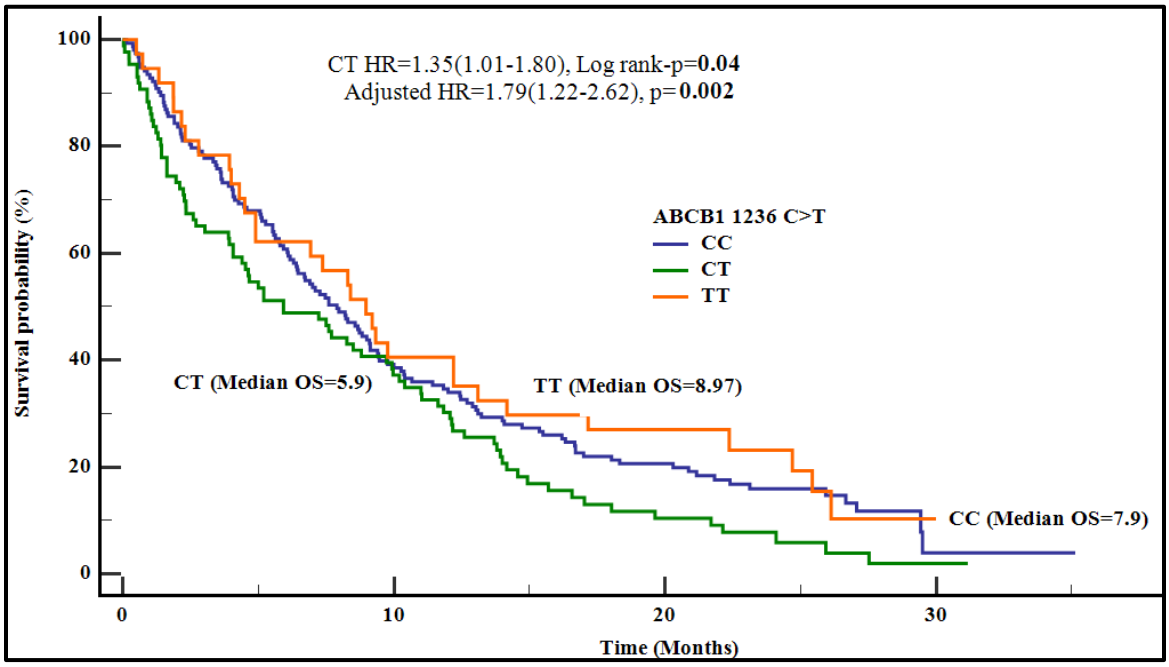


Figure 5.34: Kaplan Meier curves illustrating the association between overall survival in different genotypes of *ABCB1 $C^{1236}T$* polymorphism in stage IV lung cancer patients

(Note: *CC* represents wild-type genotype, *CT* represents heterozygous genotype, and TT represents mutant genotype)

Table 5.27: Association of *ABCB1 SNPs* on overall survival according to tumor stage

Stage III								
Genotype rs1128503 ABCB1 (C1236T)	**CASES n (%), N=159**	**DEAD n (%), N=127**	**ALIVE n (%), N=32**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
Codominant model								
CC	84 (52.83)	68 (53.54)	16 (50)	8.8	-	1.0 (reference)	-	-
CT	25 (15.72)	40 (31.49)	10 (31.25)	10.53	0.68	0.92 (0.62-1.35)	0.99 (0.55-1.79)	0.9
TT	50 (31.44)	19 (14.96)	6 (18.75)	4.7	0.81	1.06 (0.62-1.81)	1.04 (0.47-2.26)	0.9
Dominant model								
CC	84 (52.83)	68 (53.54)	16 (50)	8.8	-	1.0 (reference)	-	-
CT+TT	75 (47.16)	59 (46.45)	16 (50)	8.43	0.82	0.96 (0.67-1.36)	1.03 (0.60-1.72)	0.93
Recessive model								
CC+CT	109 (68.55)	108 (85.03)	26 (81.25)	9.33	-	1.0 (reference)	-	-
TT	50 (31.44)	19 (14.96)	6 (18.75)	4.73	0.67	1.11 (0.66-1.86)	0.95 (0.47-1.89)	0.8
Genotype rs1045642 ABCB1 (C3435T)	**CASES n (%), N=159**	**DEAD n (%), N=127**	**ALIVE n (%), N=32**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
CC	32 (20.12)	22 (17.32)	10 (31.25)	8.43	-	1.0 (reference)	-	-
CT	69 (43.39)	58 (45.66)	11 (34.37)	8.37	0.21	1.34 (0.83-2.14)	1.46 (0.77-2.74)	0.23
TT	58 (36.47)	47 (37.0)	11 (34.37)	9.43	0.37	1.25 (0.76-2.05)	1.15 (0.56-2.33)	0.69
Dominant model								
CC	32 (20.12)	22 (17.32)	10 (31.25)	8.43	-	1.0 (reference)	-	
CT+TT	127 (79.87)	105 (82.67)	22 (68.75)	8.8	0.22	1.30 (0.841.99)	1.27 (0.72-2.26)	0.40
Recessive model								
CC+CT	101 (63.52)	80 (62.99)	21 (65.62)	8.43		1.0 (reference)	-	
TT	58 (36.47)	47 (37.0)	11 (34.37)	9.43	0.9	1.02 (0.70-1.44)	0.79 (0.46-1.36)	0.40
Genotype rs2032582 ABCB1 (G2677A)	**CASES n (%), N=159**	**DEAD n (%), N=127**	**ALIVE n (%), N=32**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GG	146 (91.82)	117 (92.12)	29 (90.62)	9.27	-	1.0 (reference)	-	-
GA	13 (8.17)	10 (7.87)	3 (9.37)	3.87	0.34	1.42 (0.67-2.97)	3.33 (1.34-8.28)	**0.009**
Genotype rs2032582 ABCB1 (G2677T)	**CASES n (%), N=159**	**DEAD n (%), N=127**	**ALIVE n (%), N=32**				**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GT	83 (52.20)	64 (50.39)	19 (59.37)	9.03	-	1.0 (reference)	-	-
TT	76 (47.79)	63 (49.60)	13 (40.62)	8.5	0.50	1.12 (0.79-1.60)	1.16 (0.71-1.91)	0.53
Stage IV								
Genotype rs1128503 ABCB1 (C1236T)	**CASES n (%), N=276**	**DEAD n (%), N=244**	**ALIVE n (%), N=32**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***

Codominant model								
CC	153 (55.43)	132 (54.09)	21 (65.62)	7.9	-	1.0 (reference)	-	-
CT	86 (31.15)	81 (33.19)	5 (15.62)	5.9	**0.04**	1.35 (1.01-1.80)	1.79 (1.22-2.62)	**0.002**
TT	37 (13.40)	31 (12.70)	6 (18.75)	8.97	0.54	0.88 (0.60-1.29)	1.07 (0.65-1.76)	0.78
Dominant model								
CC	153 (55.43)	132 (54.09)	21 (65.62)	7.9	-	1.0 (reference)	-	-
CT+TT	123 (44.56)	112 (45.90)	11 (34.37)	7.5	0.22	1.17 (0.90-1.51)	1.43 (1.02-1.99)	**0.03**
Recessive model								
CC+CT	239 (86.59)	213 (87.29)	26 (81.25)	7.5	-	1.0 (reference)	-	-
TT	37 (13.40)	31 (12.70)	6 (18.75)	8.97	0.24	0.81 (0.57-1.15)	0.86 (0.54-1.38)	0.55
Genotype	**CASES**	**DEAD**	**ALIVE**	**Univariate analysis**			**Multivariate analysis**	
rs1045642 ABCB1 (C3435T)	**n (%), N=276**	**n (%), N=244**	**n (%), N=32**	**MST (months)**	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
CC	52 (18.84)	48 (19.67)	4 (12.5)	7.3		1.0 (reference)	-	-
CT	108 (39.13)	95 (38.93)	13 (40.62)	6.3	0.9	1.02 (0.71-1.42)	0.99 (0.6-1.60)	0.9
TT	116 (42.02)	101 (41.39)	15 (46.87)	8.26	0.89	1.02 (0.72-1.44)	1.14 (0.70-1.84)	0.58
Dominant model								
CC	52 (18.84)	48 (19.67)	4 (12.5)	7.3	-	1.0 (reference)	-	
CT+TT	224 (81.15)	196 (80.32)	28 (87.5)	7.6	0.9	0.99 (0.72-1.37)	1.05 (0.68-1.62)	0.81
Recessive model								
CC+CT	160 (57.97)	143 (58.60)	17 (53.12)	6.5	-	1.0 (reference)	-	-
TT	116 (42.02)	101 (41.39)	15 (46.87)	8.26	0.87	0.98 (0.75-1.26)	1.03 (0.74-1.44)	0.82
Genotype	**CASES**	**DEAD**	**ALIVE**	**Univariate analysis**			**Multivariate analysis**	
rs2032582 ABCB1 (G2677A)	**n (%), N=276**	**n (%), N=244**	**n (%), N=32**	**MST (months)**	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GG	240 (86.95)	211 (86.47)	29 (90.62)	7.93	-	1.0 (reference)	-	-
GA	36 (13.04)	33 (13.52)	3 (9.37)	4.9	0.26	1.25 (0.84-1.86)	0.94 (0.59-1.49)	0.81
Genotype	**CASES**	**DEAD**	**ALIVE**	**Univariate analysis**			**Multivariate analysis**	
rs2032582 ABCB1 (G2677T)	**n (%), N=276**	**n (%), N=244**	**n (%), N=32**	**MST (months)**	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GT	143 (51.81)	129 (52.86)	14 (43.75)	6.4	-	1.0 (reference)	-	-
TT	133 (48.18)	115 (47.13)	18 (56.25)	8.5	0.18	0.84 (0.65-1.08)	0.83 (0.59-1.17)	0.30

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, regimen, histology, KPS, ECOG

5.14 Impact of *ABCB1* polymorphisms on clinical outcomes in lung cancer patients

5.14.1 Association of ABCB1 polymorphisms with clinicopathological parameters

The present study investigated the impact of the *ABCB1* ($C^{1236}T$, $C^{3435}T$, $G^{2677}A$, and $G^{2677}T$) polymorphisms on various clinicopathological factors. Participants were divided into groups depending on their cancer stage (stage III *vs.* stage IV), tumor size (T3 *vs.* T4), lymph node invasion (Nx+N0+N1 *vs.* N2+N3+N4), and metastatic condition (M0 *vs.* M1) (Table 5.28).

The subjects were divided into stages III and IV based on the clinical stage. The genotypic frequency of all the polymorphisms has been evaluated. The incidence of patients in stage IV is 64.3%, whereas 35.6% of patients were in stage III of lung cancer. For *ABCB1* $C^{1236}T$, the prevalence of wild type genotype in stage IV (54.4%) is more than that of stage III (52.4%) as compared to the other genotypes, though in the case of *ABCB1* $C^{3435}T$ polymorphism, the frequency of wild type genotype (*CC)* is higher in stage III patients (20.3%) as compared to stage IV patients (18.8%). No significant odds were observed for both heterozygous (AOR=1.37, 95% CI=0.67-2.78, p=0.38) and mutant genotypes (AOR=1.85, 95% CI=0.87-3.91, p=0.10). Furthermore, no significant association was found between *ABCB1* polymorphisms ($C^{1236}T$, $C^{3435}T$, $G^{2677}A/T$) and tumor stage in all the models assessed.

Further, the patients were stratified based on direct tumor extension (T3 *vs.* T4). The frequency of each genotype is mentioned in table 5.28. For *ABCB1* $C^{1236}T$ polymorphism, it was observed that the frequency of homozygous variant allele with T3 extension was slightly higher than patients with T4 extension (17.3% *vs.* 15.2%). Also, no significant association of this polymorphism was found with direct tumor extension. For *ABCB1* $C^{3435}T$ polymorphism, 45.6% of patients with T4 had a higher risk than the 36.9% of patients with T3 tumor extension, though the results did not show any substantial differences (AOR=1.33, 95% CI=0.58-3.01, p=0.48). For *ABCB1* $G^{2677}A$, the prevalence of wild-type genotype was higher on T4 and T3 tumor extension (89% *vs.* 88%). In the case of polymorphism $G^{2677}T$, the frequency of patients with double variant alleles (*TT)* was slightly lower in T4 tumor extension than the T3 (46% *vs.* 47.8%). Also, odds of 1.10 was reported, but substantial significance was not achieved (AOR=1.10, 95% CI=0.60-2.02, p=0.73).

Our study also assessed *ABCB1* polymorphism in association with lymph node involvement. The patients were divided into two groups depending on lymph node invasion (Nx+N0+N1 *vs.* N2+N3+N4). In the case of $C^{1236}T$, when 31.7% of patients carrying at least one mutant allele in the N2+N3+N4 group were compared with 23.1% of subjects in Nx+N0+N1, high odds of 1.77 was noted in the former group (AOR=1.77, 95% CI=0.88-3.57, p=0.10). Similarly, in *ABCB1* $C^{3435}T$, patients harboring at least one mutant allele (*CT)* in the N2+N3+N4 showed a higher risk than patients with the Nx+N0+N1 (AOR=1.46, 955 CI=0.66-3.25, p=0.34), though substantial differences were not obtained. Lack of association was found in *ABCB1* $G^{2677}A/T$ polymorphism associated with lymph node invasion (Table 5.28).

Furthermore, the relationship of different *ABCB1* polymorphisms was evaluated with the metastatic status (M1 *vs.* Mo) illustrated in table 5.28. Patients with distant metastasis accounted for 56.03% of lung cancer patients, while 43.9% had no metastasis. In *ABCB1* $C^{3435}T$ and its association with metastasis, we observed that individuals harboring heterozygous (*CT*) (AOR=2.29, p=**0.01)** and mutant genotype *(TT)* (AOR=2.27, p=**0.01)** were more likely to manifest metastasis as compared to subjects carrying wild type genotype *(CC)*. Using the dominant model, after adjusting with covariates such as age, gender, smoking, and regimen, we observed more probable risk of manifesting metastasis in the combined genotypes (*CT +TT)* as compared to patients with wild type genotype *(CC)* (AOR=2.20, 95% CI=1.18-4.07; p=**0.01**)**.** None of the other *ABCB1* polymorphisms, such as $C^{1236}T$, $G^{2677}A$, and $G^{2677}T$, did show any association with the spread of lung carcinoma to the other organs.

Table 5.28: Relationship of different ABCB1 polymorphic genotypes with clinic-pathological parameters

CLINICAL STAGE					TUMOR SIZE				
Genotype rs1128503 ABCB1(C1236T)	**Stage III (162), n (%)**	**Stage IV (292), n (%)**	**AOR (95% CI) [a]**	***p*[b]**	**Genotype rs1128503 ABCB1(C1236T)**	**T3 (92), n (%)**	**T4 (302), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
CC	85(52.4)	159(54.4)	1.0 (reference)	-	CC	49(53.2)	164(54.3)	1.0 (reference)	-
CT	50(30.8)	92(31.5)	0.73(0.40-1.33)	0.30	CT	27(29.3)	92(30.4)	1.01(0.50-2.05)	0.9
TT	27(16.6)	41(14.04)	0.70(0.33-1.47)	0.35	TT	16(17.3)	46(15.2)	0.95(0.42-2.13)	0.9
CT+TT	67(41.3)	135(46.2)	0.71(0.42-1.22)	0.22	CT+TT	43(46.7)	138(45.6)	0.9(0.54-1.81)	0.9
Genotype rs1045642 ABCB1 (C3435T)	**Stage III (162), n (%)**	**Stage IV (292), n (%)**	**AOR (95% CI) [a]**	***p*[b]**	**Genotype rs1045642 ABCB1 (C3435T)**	**T3 (92), n (%)**	**T4 (302), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
CC	33(20.3)	55(18.8)	1.0 (reference)	-	CC	18(19.5)	57(18.8)	1.0 (reference)	-
CT	69(42.5)	116(39.7)	1.37(0.67-2.78)	0.38	CT	34(36.9)	132(45.6)	1.33(0.58-3.01)	0.48
TT	60(37.03)	121(41.4)	1.85(0.87-3.91)	0.10	TT	40(43.4)	113(37.4)	0.78(0.35-1.75)	0.55
CT+TT	129(79.6)	237(81.1)	1.54(0.80-2.95)	0.18	CT+TT	78(83.3)	245(81.1)	1.01(0.49-2.10)	0.9
Genotype rs2032582 ABCB1(G2677A)	**Stage III (162), n (%)**	**Stage IV (292), n (%)**	**AOR (95% CI) [a]**	***p*[b]**	**Genotype rs2032582 ABCB1(G2677A)**	**T3 (92), n (%)**	**T4 (302), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
GG	149(91.9)	255(87.3)	1.0 (reference)	-	GG	81(88.0)	269(89.0)	1.0 (reference)	-
GA	13(8.02)	37(12.6)	1.82(0.73-4.55)	0.19	GA	11(11.9)	33(10.9)	1.01(0.42-2.45)	0.96
Genotype rs2032582 ABCB1 (G2677T)	**Stage III (162), n (%)**	**Stage IV (292), n (%)**	**AOR (95% CI) [a]**	***p*[b]**	**Genotype rs2032582 ABCB1 (G2677T)**	**T3 (92), n (%)**	**T4 (302), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
GT	84(51.8)	154(52.7)	1.0 (reference)	-	GT	48(52.1)	163(53.9)	1.0 (reference)	-
TT	78(48.1)	138(47.2)	1.11(0.64-1.91)	0.69	TT	44(47.8)	139(46.0)	1.10(0.60-2.02)	0.73
LYMPH NODE INVASION					**METASTASIS**				
Genotype rs1128503 ABCB1 (C1236T)	**Nx+N0+N1 (108), n (%)**	**N2+N3+N4 (381), n (%)**	**AOR (95% CI) [a]**	***p*[b]**	**Genotype rs1128503 ABCB1(C1236T)**	**M0 (215), n (%)**	**M1 (274), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
CC	67(62.03)	199(52.2)	1.0 (reference)	-	CC	118(54.8)	148(54.01)	1.0 (reference)	-
CT	25(23.1)	121(31.7)	1.77(0.88-3.57)	0.10	CT	60(27.9)	86(31.3)	0.96(0.54-1.69)	0.89
TT	16(14.8)	61(16.01)	1.36(0.62-3.0)	0.43	TT	37(17.2)	40(14.5)	0.72(0.37-1.41)	0.35
CT+TT	41(37.9)	182(47.7)	1.59(0.89-2.85)	0.11	CT+TT	97(45.1)	126(45.9)	0.85(0.52-1.39)	0.54
Genotype rs1045642 ABCB1 (C3435T)	**Nx+N0+N1**	**N2+N3+N4 (381), n (%)**	**AOR (95% CI) [a]**	***p*[b]**	**Genotype rs1045642**	**M0**	**M1**	**AOR (95% CI) [a]**	***p*[b]**

	(108), n (%)				**ABCB1 (C3435T)**	**(215), n (%)**	**(274), n (%)**		
CC	20(18.5)	77(20.2)	1.0 (reference)	-	CC	47(21.8)	50(18.2)	1.0 (reference)	-
CT	41(37.9)	157(41.2)	1.46(0.66-3.25)	0.34	CT	86(40)	112(40.8)	2.29(1.14-4.59)	**0.01**
TT	47(43.5)	147(38.5)	0.86(0.41-1.81)	0.69	TT	82(38.1)	112(40.8)	2.27(1.15-4.50)	**0.01**
CT+TT	88(81.4)	304(79.7)	1.08(0.55-2.15)	0.80	CT+TT	168(78.1)	224(81.7)	2.20(1.18-4.07)	**0.01**
Genotype rs2032582 ABCB1(G2677A)	**Nx+N0+N1 (108), n (%)**	**N2+N3+N4 (381), n (%)**	**AOR (95% CI) [a]**	***p*[b]**	**Genotype rs2032582 ABCB1(G2677A)**	**M0 (215), n (%)**	**M1 (274), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
GG	98(90.7)	41(10.7)	1.0 (reference)	-	GG	18(8.3)	33(12.04)	1.0 (reference)	-
GA	10(9.2)	340(89.2)	0.88(0.37-2.09)	0.78	GA	197(91.6)	241(87.9)	1.62(0.74-3.54)	0.22
Genotype rs2032582 ABCB1(G2677T)	**Nx+N0+N1 (108), n (%)**	**N2+N3+N4 (381), n (%)**	**AOR (95% CI) [a]**	***p*[b]**	**Genotype rs2032582 ABCB1 (G2677T)**	**M0 (215), n (%)**	**M1 (274), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
GT	50(46.2)	204(53.5)	1.0 (reference)	-	GT	107(49.7)	147(53.6)	1.0 (reference)	-
TT	58(53.7)	177(46.4)	0.58(0.32-1.037)	0.06	TT	108(50.2)	127(46.3)	0.88(0.54-1.44)	0.61

a)-Adjusted Odds ratios, 95% confidence intervals, and corresponding p-values were calculated by logistic regression analysis after adjusting for age, gender, smoking, and regimen. (b)-Two-sided $\chi 2$ test for either genotype distribution or allelic frequencies between different clinicopathological parameters. The number in bold indicates the significant values in the table

5.14.2 Correlation of ABCB1 polymorphisms and their response toward chemotherapy

The relationship between *ABCB1* ($C^{1236}T$, $C^{3435}T$, $G^{2677}A$, and $G^{2677}T$) polymorphisms and the treatment outcomes of response rate was examined using univariate logistic regression. The odds ratio was adjusted with age, gender, regimen, and performance status parameters. Table 5.29 highlights the correlation between *ABCB1* polymorphism and chemotherapeutic response. People with stable and progressing diseases (*SD+PD)* were defined as poor responders, whereas those with partial or complete responses (*CR+PR)* were classed as good responders. A total of 260 patients' data was available for the objective response outcomes.

Table 5.29: Association of *ABCB1* SNPs and chemotherapeutic response

	Response of chemotherapy			
Genotype rs1128503 ABCB1 (C1236T)	**CR+PR (103), n (%)**	**SD+PD (157), n (%)**	**AOR (95% CI)** [a]	***p*** [b]
CC	57 (55.3)	92 (58.5)	1.0 (reference)	-
CT	27 (26.2)	42 (26.7)	1.57 (0.75-3.28)	0.22
TT	19 (18.4)	23 (14.6)	0.82 (0.36-1.87)	0.65
CT+TT	46 (44.6)	65 (41.4)	1.18 (0.64-2.15)	0.58
Genotype rs1045642 ABCB1 (C3435T)	**CR+PR (103), n (%)**	**SD+PD (157), n (%)**	**AOR (95% CI)** [a]	***p*** [b]
CC	22 (21.3)	27 (17.1)	1.0 (reference)	-
CT	45 (43.6)	63 (40.1)	1.09 (0.48-2.46)	0.82
TT	36 (34.9)	67 (42.6)	1.57 (0.71-3.48)	0.25
CT+TT	81 (78.6)	130 (82.8)	1.36 (0.67-2.78)	0.38
Genotype rs2032582 ABCB1 (G2677A)	**CR+PR (103), n (%)**	**SD+PD (157), n (%)**	**AOR (95% CI)** [a]	***p*** [b]
GG	92 (89.3)	144 (91.7)	1.0 (reference)	-
GA	11 (10.6)	13 (8.2)	0.81 (0.31-2.13)	0.68
Genotype rs2032582 ABCB1 (G2677T)	**CR+PR (103), n (%)**	**SD+PD (157), n (%)**	**AOR (95% CI)** [a]	***p*** [b]
GT	49 (47.5)	80 (50.9)	1.0 (reference)	-
TT	54 (52.4)	77 (49.0)	0.78 (0.43-1.43)	0.43

a)-Adjusted Odds ratios, 95% confidence intervals, and corresponding p-values were calculated by logistic regression analysis after adjusting for age, gender, smoking, and regimen. (b)-Two-sided $\chi 2$ test for either genotype distribution or allelic frequencies between the responders and non-responders. The number in bold indicates the significant values in the table

For *ABCB1* $C^{1236}T$ polymorphism, the 26.7% of poor responders with at least one mutant allele (*CT)* had an adjusted odd of 1.57 times compared to 26.2% of good responders (AOR=1.57, 95% CI=0.75-3.28, p=0.22), but no significant association was found. In the case of *ABCB1* $C^{3435}T$ polymorphism, the prevalence of inadequate responders was more in patients with mutant genotype (*TT)* as compared to the good responders (42.6% *vs.* 34.9%) with a risk of 1.57 times in the former group (AOR=1.57, 95% CI= 0.71-3.48, p=0.25). However, there was no correlation between the *ABCB1* polymorphism and the platinum-based chemotherapy treatment response.

5.15 Statistical analysis for polymorphic sites of *ABCC1* gene

5.15.1 Genotypic and allelic frequencies of ABCC1 polymorphic variants

The distribution of genotypes and alleles for *ABCC1* polymorphisms in lung carcinoma patients has been illustrated in Table 5.30. Among the patients for $G^{3173}A$ (rs41410450**)** polymorphism, 77.4% of patients harbored homozygous wild genotype *(CC)*, whereas 1.59% of patients had mutant genotype (*TT)*. The cases for the *ABCC1* $G^{3173}A$ polymorphism (N=502, $\chi 2$=0.08, df=1, p=0.76) followed Hardy Weinberg equilibrium (HWE). Minor allele frequency (MAF) was calculated to be 0.12 for the $G^{3173}A$ variant. Further, the genotypic frequencies of *ABCC1* $G^{2168}A$ (rs4148356) are depicted in table 5.30, and the results show that patients having wild-type genotype (*GG)* are over-represented as compared to the subjects having heterozygous or variant type genotype (*GA or AA)* in the sample population. The prevalence of wild-type genotype (*GG)* was highest, *i.e.*, 96.6%, while patients having at least a mutant allele was 3.38%. Also, while genotyping for $G^{2168}A$, no variant genotype (*AA)* was encountered in the sample population. (N=502, $\chi 2$=0.14, df=1, p=0.69).

Table 5.30: Genotypic and allelic distribution of *ABCC1* genetic polymorphism in lung cancer patients

Genotype rs41410450 *ABCC1* (*G3173A)*	**Cases (502) N (%)**	**$\chi 2$ (df)**	**p[a]-value**
GG	389 (77.4)		
GA	105 (20.9)		
AA	8 (1.59)	0.08 (1)	0.76
GA+AA	113 (22.5)		
G (allele)	883		
A (allele)	121		
MAF	0.12		
Genotype rs4148356 *ABCC1* (*G2168A)*	**Cases (502) N (%)**	**$\chi 2$ (df)**	**p[a]-value**
GG	485 (96.6)		
GA	17 (3.38)		
AA	0		
GA+AA	17 (3.38)	0.14 (1)	0.69
G (allele)	987		
A (allele)	17		
MAF	0.016		

5.16 Impact of *ABCC1* polymorphism on overall survival of lung carcinoma patients undergoing platinum-based doublet chemotherapy

5.16.1 Overall survival analysis for ABCC1 gene polymorphism

In the current study, we have evaluated the role of two polymorphisms of *ABCC1*, *i.e.*, $G^{3173}A$ and $G^{2168}A$, in correlation with overall survival in lung cancer patients. During analysis, 84.5% of patients were dead, while 15.5% were alive. For the *ABCC1* $G^{3173}A$ variant, our analysis depicted that in the co-dominant model, patients with both mutant alleles (*AA*) exhibited a significantly poor survival as compared to the patients with the wild-type genotype (*GG*) (MST =1.63 *vs.* 8.37, HR=4.15, 95% CI=1.40-12.2, Log Rank p=**0.009**) (Figure 5.35). After adjusting with different covariates such as age, gender, smoking, tumor stage, histology, performance status, and regimen, the hazards of 2.45 was observed in the people having both copies of the mutant allele (*AA)* (HR=2.45, 95% CI=1.19-5.04, p=**0.01**) (Table 5.31). As shown in table 5.31, even in the recessive model, where wild-type genotype and heterozygous genotype were combined and taken as reference, our results showed a high death ratio and substantial differences between the groups (MST=8.43 *vs.* 1.63, HR=4.51, 95% CI=1.50-13.55, Log-Rank p=**0.007**).

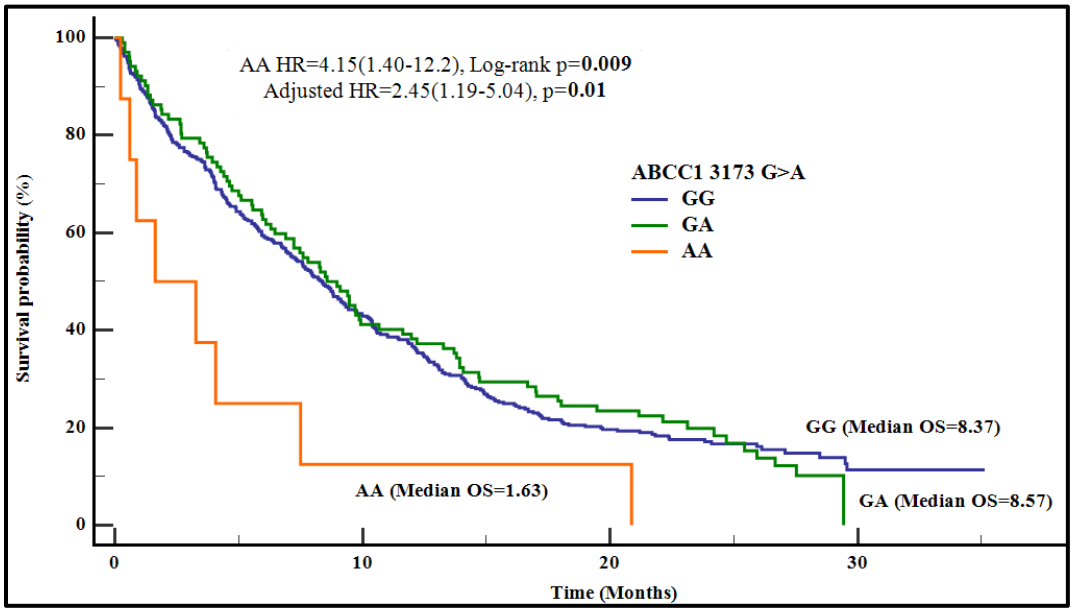


Figure 5.35: Kaplan Meier curves illustrating the association between overall survival in different genotypes of *ABCC1* $G^{3173}A$ polymorphism in lung cancer patients. (Note: *GG* represents wild-type genotype, *GA* represents heterozygous genotype, and *AA* represents homozygous for mutant genotype)

Table 5.31: Association of *ABCC1* polymorphism in predicting overall survival in lung cancer patients

Genotype rs41410450 ABCC1 (G3173A)	CASES n(%), N=483	DEAD n(%), N=408	ALIVE n(%), N=75	Univariate analysis			Multivariate analysis	
				MST (months)	Log-rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
Codominant model								
GG	373(77.2)	312(76.4)	61(81.3)	8.37	-	1.0 (reference)	-	-
GA	102(21.2)	88(21.5)	14(18.6)	8.57	0.81	0.97(0.76-1.23)	1.14(0.83-1.56)	0.41
AA	8(1.6)	8(1.9)	0(0)	1.63	**0.009**	4.15(1.40-12.2)	2.45(1.19-5.04)	**0.01**
Dominant model								
GG	373(77.2)	312(76.4)	61(81.3)	8.37	-	1.0 (reference)	-	-
GA+AA	110(22.7)	96(23.5)	14(18.6)	8.26	0.83	1.02(0.81-1.29)	1.16(0.85-1.58)	0.34
Recessive model								
GG+GA	475(98.3)	400(98.0)	75(100)	8.43	-	1.0 (reference)	-	-
AA	8(1.6)	8(1.9)	0(0)	1.63	**0.007**	4.51(1.50-13.55)	2.53(1.24-5.17)	**0.01**
Genotype rs4148356 ABCC1 (G2168A)	**CASES n(%), N=483**	**DEAD n(%), N=408**	**ALIVE n(%), N=75**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
GG	467(96.6)	395(96.8)	72(96)	8.40	-	1.0 (reference)	-	-
GA	16(3.3)	13(3.1)	3(4)	6.3	0.81	1.07(0.60-1.89)	1.51(0.78-2.90)	0.21

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, regimen, histology, KPS, ECOG

In the recessive model, after adjusting with covariates in cox-regression model, the death ratio remained approximately 2.5 times high in patients carrying *AA* genotype (HR=2.53, 95% CI=1.24-5.17, p=**0.01**). Besides gene polymorphism, the survival was correlated with tumor stage and ECOG of patients with lung cancer (Table 5.32). Hence, mutant *(AA)* genotype, ECOG (p=<**0.0001**), and tumor stage (p=**0.03**) in the *3173* site of *ABCC1* gene could be key factors for contributing to the development of lung cancer. Moreover, we found that age (P= 0.57), gender (P=0.44), smoking (P=0.05), KPS (P=0.83), and histology (P=0.14) did not correlate with OS. Additionally, in the case of *ABCC1* $G^{2168}A$, the patients having at least one mutant allele (*GA)* showed poor survival as compared to the patients having both homozygous ancestral allele *(CC)* (MST=6.3 *vs.* 8.40, HR=1.51, 95% CI=0.78-2.90, p=0.21), however, no association was found for *ABCC1* $G^{2168}A$ polymorphism with overall survival.

Table 5.32: Cox proportional hazards regression model analysis of overall survival in lung cancer subjects

N=483	b	SE	P-value	Adjusted HR (Exp b)	95% CI of Exp (b)
ABCC1_G3173A	0.9306	0.3636	**0.0105**	2.5361	1.2436 to 5.1718
Age	-0.003142	0.005676	0.5799	0.9969	0.9858 to 1.0080
Sex	0.1168	0.1546	0.4499	1.1239	0.8301 to 1.5218
Smoking	0.3241	0.1658	0.0506	1.3827	0.9991 to 1.9136
Stage	0.1974	0.09103	**0.0301**	1.2182	1.0191 to 1.4562
ECOG	0.5342	0.1086	**<0.0001**	1.7061	1.3791 to 2.1107
KPS	0.02397	0.1170	0.8377	1.0243	0.8143 to 1.2884
Histology	0.1099	0.07480	0.1418	1.1162	0.9639 to 1.2924

5.16.2 Association of ABCC1 polymorphism with OS in lung cancer histological subtypes

Different groups were divided based on histology to assess the importance of *ABCC1* polymorphic sites in the overall survival of lung cancer patients. Table 5.33 depicts the correlation of *ABCC1* variants on the prognosis of patients. In the case of *ABCC1* $G^{3173}A$, the frequency of patients having at least one copy of mutant allele *(GA)* was 17.2% for ADCC, 23.5% for SQCC, and 25.3% for SCLC histology. Our results showed that ADCC subjects harboring variant genotype (*AA)* showed poor survival and high death ratio of approximately 4.5 times as compared to the ADCC patients carrying wild type genotype (*GG)* (MST=3.27 *vs.* 8.5, HR=4.44, 95% CI=1.10-17.8, p=**0.03**). After applying the Cox regression model and adjusting with multiple covariates, the hazards ratio increased to 13.7 times in the variant genotype (*AA)* as compared to the wild type genotype (HR=13.7, 95% CI=2.70-70.12, p=**0.001**) (Figure 5.36). Similarly, patients with SQCC and harboring variant genotype showed poor survival than the wild type genotype (*GG)* (MST=1.63 *vs.* 7.20, HR=3.33), though a significant association was not found with the genotypes.

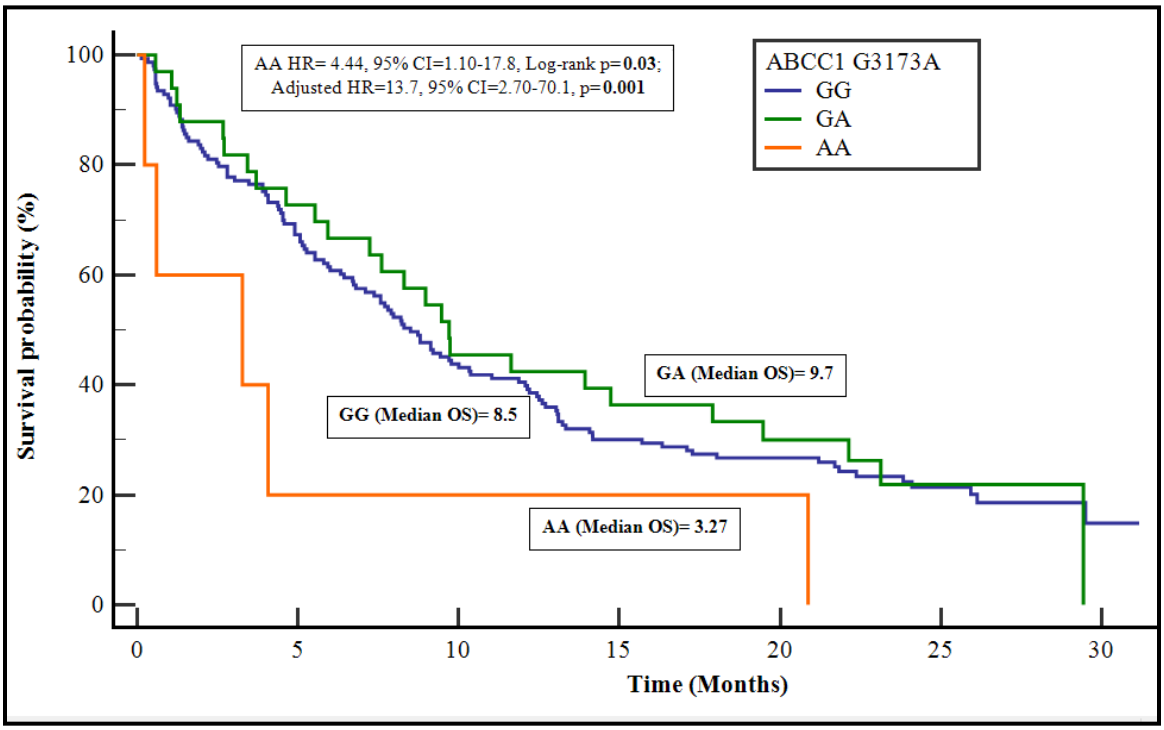


Figure 5.36: Kaplan Meier curves illustrating the association between overall survival in different genotypes of *ABCC1* $G^{3173}A$ polymorphism in ADCC patients. (Note: GG represents wild-type genotype, GA represents heterozygous genotype, and AA represents homozygous for mutant genotype)

In the case of the *ABCC1* $G^{2168}A$ variant, the percentage of patients having at least one mutant allele in ADCC, SQCC, and SCLC was 1.04%, 5.8%, and 1.3%, respectively. ADCC patients carrying heterozygous genotype (*GA)* had a low median survival time than the wild-type genotype (*GG),* but the results were not significant; also, very few patients harbored heterozygous genotype. In the case of SCLC, the patients carrying at least one variant allele (*GA)* had almost 3-times less MST than the wild-type genotype; also, the death ratio was three times (MST=3.63 *vs.* 9.7, HR=5.30, 95% CI=0.62-45.3, p=0.12). However, the hazard ratio retrieved from both univariate and multivariate analysis was not substantially correlated with these genotypes. Therefore, we conclude that this polymorphic site, when stratified based on histological subtypes such as ADCC, SQCC, and SCLC, did not act as an independent predictor of survival in lung cancer patients.

Table 5.33: Association of *ABCC1* SNPs on overall survival according to histology

ADCC								
Genotype rs41410450 ABCC1 (G3173A)	**CASES n (%), N=191**	**DEAD n (%), N=152**	**ALIVE n (%), N=39**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
GG	153 (80.1)	121 (79.6)	32 (82.0)	8.5	-	1.0 (reference)	-	-
GA	33 (17.2)	26 (17.1)	7 (17.9)	9.7	0.65	0.90 (0.60-1.37)	1.46 (0.83-2.58)	0.18
AA	5 (2.6)	5 (3.2)	0 (0)	3.27	**0.03**	4.44 (1.10-17.8)	13.7 (2.70-70.12)	**0.001**
GA+AA	38 (19.8)	31 (20.3)	7 (17.9)	8.97	0.94	1.01 (0.68-1.50)	1.61 (0.93-2.77)	0.08
Genotype rs4148356 ABCC1 (G2168A)	**CASES n (%), N=191**	**DEAD n (%), N=152**	**ALIVE n (%), N=39**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GG	189 (98.9)	150 (98.6)	39 (100)	8.73	-	1.0 (reference)	-	-
GA	2 (1.04)	2 (1.31)	0 (0)	0.57	0.27	3.11 (0.40-23.7)	1.87 (0.20-17.05)	0.57
SQCC								
Genotype rs41410450 ABCC1 (G3173A)	**CASES n (%), N=204**	**DEAD n (%), N=176**	**ALIVE n (%), N=28**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
GG	154 (75.4)	132 (75)	22 (78.5)	7.0	-	1.0 (reference)	-	-
GA	48 (23.5)	42 (23.8)	6 (21.4)	7.8	0.68	0.93 (0.66-1.31)	1.02 (0.62-1.65)	0.9
AA	2 (0.98)	2 (1.1)	0 (0)	1.63	0.27	3.11 (0.40-23.7)	0.19 (0.02-1.55)	0.12
GA+AA	50 (24.5)	44 (25)		7.5	0.79	0.95 (0.68-1.34)	0.90 (0.56-1.47)	0.69
Genotype rs4148356 ABCC1 (G2168A)	**CASES n (%), N=204**	**DEAD n (%), N=176**	**ALIVE n (%), N=28**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GG	192 (94.1)	166 (94.3)	26 (92.8)	7.17	-	1.0 (reference)	-	-
GA	12 (5.8)	10 (5.6)	2 (7.1)	6.3	0.9	0.97 (0.51-1.84)	1.87 (0.20-17.05)	0.57
SCLC								
Genotype rs41410450 ABCC1 (G3173A)	**CASES n (%), N=75**	**DEAD n (%), N=71**	**ALIVE n (%), N=4**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
GG	55 (73.3)	51 (71.8)	4 (100)	10.4	-	1.0 (reference)	-	-
GA	19 (25.3)	19 (26.7)	0 (0)	8.26	0.45	1.23 (0.70-2.15)	1.08 (0.52-2.22)	0.82
AA	1 (1.3)	1 (1.4)	0 (0)	-	-	-	-	-

GA+AA	20 (26.6)	20 (28.1)	0 (0)	7.47	0.35	1.29 (0.74-2.2)	1.08 (0.52-2.22)	0.82
Genotype	**CASES**	**DEAD**	**ALIVE**	**Univariate analysis**			**Multivariate analysis**	
rs4148356 ABCC1 (G2168A)	**n (%), N=75**	**n (%), N=71**	**n (%), N=4**	**MST (months)**	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GG	74 (98.6)	70 (98.5)	4 (100)	9.7	-	1.0 (reference)	-	-
GA	1 (1.3)	1 (1.4)	0 (0)	3.63	0.16	15.07 (0.34-663.7)	5.30 (0.62-45.3)	0.12

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, regimen, KPS, ECOG

5.16.3 Prognosis of lung cancer patients having ABCC1 polymorphism and treated with various chemotherapy regimens

Table 5.34 illustrates the role of the two polymorphisms of *ABCC1* on overall survival in lung cancer patients based on chemotherapy regimens. Chemotherapy consisted of four regimens in combination with platinum-based drugs such as cisplatin/carboplatin) which was administered to lung cancer subjects. The second-line chemotherapy drugs were used, such as paclitaxel, docetaxel, pemetrexed, and irinotecan.

As shown in table 5.34, lung cancer patients administered with cisplatin/carboplatin along with docetaxel showed reduced survival in the carrier alleles (*GA)* of $G^{3173}A$ polymorphism as compared to wild type genotype (*GG)* (MST=6.4 *vs.* 8.5). However, no association was found between the genotypes and prognosis. Similarly, a lack of association was found between $G^{2168}A$ polymorphism and the prognosis of patients undergoing platinum-based docetaxel therapy.

For the subjects undergoing cisplatin/carboplatin and paclitaxel regimen, the single allelic carrier (*GA*) for the *ABCC1* $G^{3173}A$ polymorphism reveals a higher survival than patients carrying wild type (*GG)* genotype (MST=6.9 *vs.* 5.67 months). The multivariate cox regression model showed a higher death rate (HR=1.32, 95% C.I.=0.74-2.35, p=0.33) for the heterozygous genotype, but it did not show statistical significance. In the same line, a lack of association was found between $G^{2168}A$ polymorphism and the overall survival in lung cancer patients undergoing cisplatin/carboplatin-based paclitaxel therapy.

When patients were stratified based on cisplatin/carboplatin and pemetrexed regimen for *ABCC1* $G^{3173}A$, our results showed that patients carrying heterozygous (*GA)* genotype had a shorter survival time as compared to the wild type genotype (*GG)* (MST =7.23 *vs.* 7.9, HR=1.45, p=0.23). However, $G^{2168}A$ polymorphism did not show any substantial difference (MST=6.93 *vs.* 7.7, HR=1.93, p=0.6) in predicting the prognosis in lung cancer patients undergoing cisplatin/carboplatin and pemetrexed regimen.

As shown in table 5.34, for the lung cancer patients undergoing cisplatin/carboplatin and irinotecan treatment, the heterozygous genotype (*GA*) for the *ABCC1* $G^{3173}A$ polymorphism exhibited a lower survival than patients harboring wild genotype (*GG)* (MST=7.47 *vs.* 10.4 months, log-rank p=0.71), but lacks statistical significance. In the case of $G^{2168}A$ polymorphism, the lung cancer patients carrying at least one variant type (*GA)* showed almost three times less

survival and a death ratio of 5.25 as compared to the wild type genotype (*GG)*, but a lack of association was found (MST=3.63 *vs.* 10.4, HR=5.5, p=0.13).

Table 5.34: Association of *ABCC1* SNPs on overall survival according to the regimen

Cisplatin/carboplatin+docetaxel								
Genotype rs41410450 ABCC1 (G3173A)	**CASES n (%), N=44**	**DEAD n (%), N=39**	**ALIVE n (%), N=5**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
GG	34 (77.2)	30 (76.9)	4 (80)	8.5	-	1.0 (reference)	-	-
GA	10 (22.7)	9 (23.07)	1 (20)	6.4	0.84	1.08 (0.50-2.32)	0.93 (0.39-2.24)	0.8
Genotype rs4148356 ABCC1 (G2168A)	**CASES n (%), N=44**	**DEAD n (%), N=39**	**ALIVE n (%), N=5**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
GG	43 (97.7)	38 (86.3)	5 (100)	8.5	-	1.0 (reference)	-	-
GA	1 (2.2)	1 (2.5)	0 (0)	11.63	0.80	0.80 (0.13-4.74)	2.39 (0.12-44.6)	0.55
Cisplatin/carboplatin+paclitaxel								
Genotype rs41410450 ABCC1 (G3173A)	**CASES n (%), N=85**	**DEAD n (%), N=72**	**ALIVE n (%), N=13**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
GG	60 (70.5)	50 (69.4)	10 (76.9)	5.67	-	1.0 (reference)	-	-
GA	23 (27.05)	20 (27.7)	3 (23.07)	6.9	0.9	0.97 (0.58-1.63)	1.32 (0.74-2.35)	0.33
AA	2 (2.3)	2 (2.7)	0	-	-	-	-	-
GA+AA	25 (29.4)	22 (30.5)	3 (23.07)	6.90	0.93	1.02 (0.61-1.69)	1.22 (0.70-2.12)	0.47
Genotype rs4148356 ABCC1 (G2168A)	**CASES n (%), N=85**	**DEAD n (%), N=72**	**ALIVE n (%), N=13**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
GG	77 (90.5)	65 (90.2)	12 (92.3)	6.9	-	1.0 (reference)	-	-
GA	8 (9.4)	7 (9.7)	1 (7.6)	6.1	0.79	1.11 (0.49-2.51)	1.26 (0.53-2.95)	0.59
Cisplatin/carboplatin+pemetrexed								
Genotype rs41410450 ABCC1 (G3173A)	**CASES n (%), N=105**	**DEAD n (%), N=89**	**ALIVE n (%), N=16**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
GG	89 (84.7)	74 (83.1)	15 (93.7)	7.9	-	1.0 (reference)	-	-
GA	15 (14.2)	14 (15.7)	1 (6.2)	7.23	0.45	1.26 (0.8-2.35)	1.45 (0.78-2.69)	0.23
AA	1 (0.95)	1 (1.1)	0 (0)	-	-	-	-	-
GA+AA	16 (10.3)	15 (16.8)	1 (6.25)	5.93	0.32	1.36 (0.73-2.52)	1.55 (0.85-2.82)	0.15
Genotype rs4148356 ABCC1 (G2168A)	**CASES n (%), N=105**	**DEAD n (%), N=89**	**ALIVE n (%), N=16**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
GG	104 (99.04)	88 (98.8)	16 (100)	7.7	-	1.0 (reference)	-	-
GA	1 (0.95)	1 (1.1)	0 (0)	6.93	0.60	1.93 (0.15-24.5)	1.14 (0.14-9.05)	0.89
Cisplatin/carboplatin+Irinotecan								
Genotype rs41410450 ABCC1 (G3173A)	**CASES n (%), N=43**	**DEAD n (%), N=40**	**ALIVE n (%), N=3**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
GG	30 (69.7)	27 (67.5)	3 (100)	10.4	-	1.0 (reference)	-	-
GA	13 (30.2)	13 (32.5)	0 (0)	7.47	0.71	1.13 (0.56-2.26)	1.04 (0.48-2.21)	0.9
Genotype rs4148356 ABCC1 (G2168A)	**CASES n (%), N=43**	**DEAD n (%), N=40**	**ALIVE n (%), N=3**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
GG	42 (97.6)	39 (97.5)	3 (100)	10.4	-	1.0 (reference)	-	-
GA	1 (2.3)	1 (2.5)	0 (0)	3.63	0.14	18.2 (0.37-890)	5.25 (0.58-47.02)	0.13

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, histology, KPS, ECOG

5.16.4 Association of ABCC1 polymorphism and overall survival based on gender

In lung carcinoma patients, 85.05% of males and 82.1% of females were dead at the time of statistical analysis, as shown in table 5.35. Our results revealed that the males harboring both copies of mutant alleles (*AA)* for *ABCC1* $G^{3173}A$ polymorphism had a higher death ratio of 4.75 as compared to the wild type genotype (*GG)* (MST=3.27 *vs.* 8.03, HR=4.75, 95% CI=0.98-22.9, p=0.05). Lack of association between overall survival in males and *ABCC1* $G^{2168}A$ gene polymorphisms is illustrated in table 5.35. On the contrary, in *ABCC1* $G^{3173}A$ polymorphism, in the co-dominant model, comparable survival was observed in the females harboring mutant genotype (*AA)* genotype, but a high death ratio was observed as compared with the wild type (*GG*) (MST=8.91 *vs.* 8.91, HR=4.62,95% CI= 0.96-22.2, Log-rank-p=0.05). In *ABCC1* $G^{2168}A$ polymorphism, in the co-dominant model, lower survival was observed in the females harboring heterozygous genotype (*GA)* genotype, and a high death ratio was observed as compared with the wild type (*GG*) (MST=1.33 *vs.* 9.03, HR=7.49, 95% CI= 1.04-53.8, Log-rank-p=**0.04**).

Table 5.35: Association of *ABCC1* SNPs on overall survival according to gender

Male								
Genotype rs41410450 ABCC1 (G3173A)	**CASES n (%), N=388**	**DEAD n (%), N=330**	**ALIVE n (%), N=58**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GG	305 (78.6)	258 (78.1)	47 (81.03)	8.03	-	1.0 (reference)	-	-
GA	79 (20.3)	68 (20.6)	11 (28.9)	8.5	0.64	0.93 (0.72-1.22)	1.06 (0.74-1.52)	0.71
AA	4 (1.03)	4 (1.2)	0 (0)	3.27	0.05	4.75 (0.98-22.9)	0.84 (0.20-3.58)	0.8
GA+AA	83 (21.39)	72 (21.81)	11 (28.9)	8.26	0.84	0.97 (0.75-1.26)	1.05 (0.74-1.50)	0.74
Genotype rs4148356 ABCC1 (G2168A)	**CASES n (%), N=388**	**DEAD n (%), N=330**	**ALIVE n (%), N=58**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GG	375 (96.6)	320 (96.9)	55 (94.8)	8.3	-	1.0 (reference)	-	-
GA	13 (3.3)	10 (3.03)	3 (5.1)	6.9	0.6	1.13 (0.62-2.05)	1.25 (0.60-2.61)	0.54
Female								
Genotype rs41410450 ABCC1 (G3173A)	**CASES n (%), N=95**	**DEAD n (%), N=78**	**ALIVE n (%), N=17**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
GG	68 (71.5)	54 (69.2)	14 (82.3)	8.91	-	1.0 (reference)	-	-
GA	23 (24.2)	20 (25.6)	3 (17.6)	9.7	0.64	1.13 (0.66-1.92)	1.06 (0.45-2.49)	0.8
AA	4 (4.2)	4 (5.1)	0 (0)	8.91	0.05	4.62 (0.96-22.2)	400.83 (17.9-8956)	**0.0002**
GA+AA				9.46	0.37	0.79 (0.47-1.31)	1.30 (0.58-2.93)	0.51
Genotype rs4148356 ABCC1 (G2168A)	**CASES n (%), N=95**	**DEAD n (%), N=78**	**ALIVE n (%), N=17**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
GG	92 (96.8)	75 (96.1)	17 (100)	9.03	-	1.0 (reference)	-	-
GA	3 (3.1)	3 (3.8)	0 (0)	1.33	**0.04**	7.49 (1.04-53.8)	1.63 (0.26-9.99)	0.59

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, smoking, stage, regimen, histology, KPS, ECOG

5.16.5 Association of ABCC1 polymorphism and overall survival based on tumor stage

To assess the role of tumor stage and *ABCC1* polymorphism in predicting the prognosis in lung cancer patients, we have evaluated the overall survival as shown in table 5.36. For $G^{3173}A$, the prevalence of wild-type genotype *(GG)* was higher in tumor stages III and IV (77.9% and 74.2%, respectively). In the case of $G^{2168}A$, patients in stages III and IV harboring at least one copy of mutant genotype *(GA)* showed reduced survival as compared to wild *(GG)* type genotype (MST=3.97 *vs.* 9.03, log-rank p=0.47 and MST=6.1 *vs.* 7.7, Log-rank-p=0.11), though the statistical significance could not be achieved. Therefore, we summarize that none of the *ABCC1* $G^{3173}A$ and $G^{2168}A$ polymorphism is associated with having an impact in predicting prognosis in stage III and IV lung cancer patients.

Table 5.36: Association of *ABCC1* polymorphism in predicting overall survival in lung cancer patients based on tumor stage

Stage III								
Genotype rs41410450 ABCC1 (G3173A)	**CASES n(%), N=159**	**DEAD n(%), N=127**	**ALIVE n(%), N=32**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
Codominant model								
GG	124(77.98)	97(76.37)	27(84.37)	9.3		1.0 (reference)	-	-
GA	32(20.12)	27(21.25)	5(15.62)	7.8	0.50	1.16(0.74-1.81)	1.13(0.57-2.26)	0.71
AA	3(1.88)	3(2.36)	0(0)	-	-	-	-	-
Dominant model								
GG	124(77.98)	97(76.37)	27(84.37)	9.3	-	1.0 (reference)	-	-
GA+AA	35(22.01)	30(23.62)	5(15.62)	7.23	0.26	1.28(0.82-1.98)	1.29(0.67-2.49)	0.43
Genotype rs4148356 ABCC1 (G2168A)	**CASES n(%), N=159**	**DEAD n(%), N=127**	**ALIVE n(%), N=32**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
GG	153(96.22)	122(96.06)	31(96.87)	9.03		1.0 (reference)	-	-
GA	6(3.77)	5(3.93)	1(3.12)	3.97	0.47	1.46(0.51-4.16)	1.12(0.33-3.81)	0.84
Stage IV								
Genotype rs41410450 ABCC1 (G3173A)	**CASES n(%), N=276**	**DEAD n(%), N=244**	**ALIVE n(%), N=32**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
Codominant model								
GG	205(74.27)	180(73.77)	25(78.12)	7.37		1.0 (reference)	-	-
GA	66((23.91)	59(24.18)	7(21.87)	8.57	0.30	0.86(0.64-1.14)	1.19(0.81-1.73)	0.36
AA	5(1.81)	5(2.04)	0(0)	4.07	0.31	1.76(0.58-5.32)	0.50(0.06-3.79)	0.50
Dominant model								
GG	205(74.27)	180(73.77)	25(78.12)	7.37	-	1.0(reference)	-	-
GA+AA	71(25.72)	64(26.22)	7(21.87)	8.3	0.41	0.89(0.67-1.17)	1.16(0.80-1.68)	0.43
Genotype rs4148356 ABCC1 (G2168A)	**CASES n(%), N=276**	**DEAD n(%), N=244**	**ALIVE n(%), N=32**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
GG	270(97.82)	238(97.54)	32(100)	7.7		1.0 (reference)	-	-
GA	6(2.17)	6(2.45)	0	6.1	0.11	2.40(0.79-7.25)	1.70(0.68-4.22)	0.24

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, regimen, histology, KPS, ECOG

5.16.6 Association of ABCC1 polymorphism and overall survival based on smoking status

To understand the interaction effect of genes and environment, the case group was stratified based on their smoking habits, *i.e.*, smokers and non-smokers (Table 5.37). In our case study, patients who were smokers and carrying variant genotype (*GA*) for $G^{3173}A$ polymorphism were shown to be less represented in both smokers and non-smokers (1.30% and 1.08%). When the co-dominant model was tested, we observed that smokers with lung carcinoma who harbored variant genotype *(AA)* had reduced survival as compared to the smokers with wild-type genotype (*GG)* for $G^{3173}A$ polymorphism (MST=3.27 *vs.* 7.95, HR=7.57, Log-rank p=**0.009)**. Furthermore, we did not find any association between $G^{2168}A$ polymorphism and smoking status as a parameter to impact survival in lung cancer patients.

Table 5.37: Association of *ABCC1* polymorphism in predicting overall survival in lung cancer patients based on smoking habits

Smokers								
Genotype rs41410450 ABCC1 (G3173A)	**CASES n(%), N=383**	**DEAD n(%), N=332**	**ALIVE n(%), N=51**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
Codominant model								
GG	300(78.32)	257(77.40)	43(84.31)	7.95	-	1.0 (reference)	-	-
GA	78(20.36)	70(21.08)	8(15.68)	8.26	0.89	0.98(0.75-1.27)	1.14(0.79-1.62)	0.47
AA	5(1.30)	5(1.50)	0	3.27	**0.009**	7.57(1.64-34.96)	0.89(0.21-3.79)	0.87
Dominant model								
GG	300(78.32)	257(77.40)	43(84.31)	7.95	-			
GA+AA	83(21.67)	75(22.59)	8(15.68)	7.5	0.82	1.02(0.79-1.33)	1.13(0.79-1.61)	0.47
Recessive model								
GG+GA	378(98.69)	327(98.49)	51(100)	7.97				
AA	5(1.30)	5(1.50)	0	3.27	**0.007**	8.17(1.74-38.3)	0.91(0.21-3.82)	0.89
Genotype rs4148356 ABCC1 (G2168A)	**CASES n(%), N=383**	**DEAD n(%), N=332**	**ALIVE n(%), N=51**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
GG	368(96.08)	320(96.38)	48(94.11)	6.3	-	1.0 (reference)	-	-
GA	15(3.91)	12(3.61)	3(5.88)	7.93	0.98	1.03(0.56-1.78)	1.37(0.68-2.75)	0.37
Non-smokers								
Genotype rs41410450 ABCC1 (G3173A)	**CASES n(%), N=100**	**DEAD n(%), N=76**	**ALIVE n(%), N=24**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
Codominant model								
GG	73(73))	55(72.36)	18(75)	9.43	-	1.0 (reference)	-	-
GA	24(24)	18(23.68)	6(25)	9.7	0.92	0.97(0.57-1.65)	1.38(0.63-2.99)	0.41
AA	3(3)	3(3.94)	0	0.60	0.11	4.13(0.70-24.28)	-	-
Dominant model								
GG	73(73)	55(72.36)	18(75)	9.43	-	1.0 (reference	-	-
GA+AA	27(27)	21(27.6)	6(25)	9.7	0.80	1.06(0.63-1.77)	1.48(0.70-3.12)	0.29
Genotype rs4148356 ABCC1 (G2168A)	**CASES n(%), N=100**	**DEAD n(%), N=76**	**ALIVE n(%), N=24**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
GG	99(99)	75(98.68)	24(100)	9.4		1.0 (reference)	-	-
GA	1(1)	1(1.31)	0(0)	11.63	0.79	0.73(0.07-7.07)	0.76(0.07-7.74)	0.82

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, stage, regimen, histology, KPS, ECOG

5.17 Role of *ABCC1* polymorphisms on clinical outcomes in lung cancer patients

5.17.1 Correlation of ABCC1 polymorphisms with clinicopathological parameters

The effect of *ABCC1* SNPs on the different clinicopathological parameters was categorized mainly into tumor stage III and IV, direct tumor extension into T3 and T4, lymph node extension Nx+N0+N1 N2+N3+N4 subgroups, and distant metastatic statuses such as M0 and M1 groups (Table 5.38). There was no association among the *ABCC1* polymorphisms in lung cancer in any co-dominant and dominant model regarding clinicopathological parameters.

For *ABCC1* $G^{3173}A$ polymorphism, the frequency of wild-type genotype *(GG)* is higher in stage III patients (78.3%) as compared to stage IV patients (75.1%). No significant risk was observed for both heterozygous *(GA)* (AOR=1.23, 95% CI=0.63-2.38, p=0.53) and mutant *(AA)* genotypes (AOR=0.40, 95% CI= 0.04-3.63, p=0.42). Furthermore, no risk was found between *ABCC1* $G^{2168}A$ polymorphism) and tumor stage. Further, the patients were stratified based on direct tumor extension (T3 *vs.* T4). The frequency of each genotype is mentioned in table 5.38. For *ABCC1* $G^{3173}A$ polymorphism, it was observed that the frequency of carriers with T3 extension was slightly higher than patients with T4 extension (27.1% *vs.* 20.5%). Also, no significant association of this polymorphism was found with direct tumor extension. For *ABCC1* $G^{2168}A$ polymorphism, the results did not show any substantial differences (AOR=1.13, 95% CI=0.29-4.43, p>0.05).

ABCC1 polymorphisms and its association with lymph node involvement was also studied. The patients were divided into two groups depending on lymph node invasion (Nx+N0+N1 *vs.* N2+N3+N4). In $G^{3173}A$ polymorphism, when 23.1% of patients in N2+N3+N4 carrying heterozygous *(GA)* genotype were compared with 20.4% of subjects in Nx+N0+N1, high odds of 1.47 was noted in the former group (AOR=1.47, 95% CI=0.69-3.11, p=0.31), but no associated risk was observed. Similarly, in *ABCC1* $G^{2168}A$, patients harboring at least one mutant allele *(GA)* in the N2+N3+N4 showed lower risk of having lymph node invasion than patients with the Nx+N0+N1, though substantial differences were not obtained. As shown in Table 5.38, none of the other *ABCC1* polymorphisms, $G^{3173}A$ *and* $G^{2168}A$, showed any association with lung carcinoma spread to the other organs.

Table 5.38: Relationship of different *ABCC1* polymorphic genotypes with clinic-pathological parameters

Clinical Stage					**Tumor Size**				
Genotype rs41410450 ABCC1 (G3173A)	**Stage III (162), n (%)**	**Stage IV (292), n (%)**	**AOR (95% CI)[a]**	***p*[b]**	**Genotype rs41410450 ABCC1 (G3173A)**	**T3 (92), n (%)**	**T4 (302), n (%)**	**AOR (95% CI)[a]**	***p*[b]**
GG	127 (78.3)	218 (75.1)	1.0 (Reference)	-	GG	66 (71.7)	235 (77.8)	1.0 (Reference)	-
GA	32 (19.7)	69 (23.6)	1.23 (0.63-2.38)	0.53	GA	25 (27.1)	62 (20.5)	0.82 (0.41-1.65)	0.58
AA	3 (1.85))	5 (1.71)	0.40 (0.04-3.63)	0.42	AA	1 (1.08)	5 (1.6)	-	-
GA+AA	35 (21.6)	74 (25.3)	1.12 (0.59-2.14)	0.7	GA+AA	26 (28.2)	67 (22.1)	0.89 (0.44-1.78)	0.74
Genotype rs4148356 ABCC1 (G2168A)	**Stage III (162), n (%)**	**Stage IV (292), n (%)**	**AOR (95% CI)[a]**	***p*[b]**	**Genotype rs4148356 ABCC1 (G2168A)**	**T3 (92), n (%)**	**T4 (302), n (%)**	**AOR (95% CI)[a]**	***p*[b]**
GG	156 (96.2)	285 (97.6)	1.0 (Reference)	-	GG	89 (96.7)	289 (95.6)	1.0 (Reference)	-
GA	6 (3.70)	7 (2.39)	0.85 (0.21-3.37)	0.8	GA	3 (3.2)	13 (4.30)	1.13 (0.29-4.43)	0.8
Lymph node invasion					**Metastasis**				
Genotype rs41410450 ABCC1 (G3173A)	**Nx+N0+N1 (108), n (%)**	**N2+N3+N4 (381), n (%)**	**AOR (95% CI)[a]**	***p*[b]**	**Genotype rs41410450 ABCC1 (G3173A)**	**M0 (215), n (%)**	**M1 (274), n (%)**	**AOR (95% CI)[a]**	***p*[b]**
GG	82 (75.9)	296 (77.6)	1.0 (Reference)	-	GG	169 (78.6)	209 (76.2)	1.0 (Reference)	-
GA	25 (23.1)	78 (20.4)	1.47 (0.69-3.11)	0.31	GA	43 (20)	60 (21.8)	0.90 (0.49-1.65)	0.75
AA	1 (0.92)	7 (1.83)	0.87 (0.08-8.61)	0.9	AA	3 (1.39)	5 (1.8)	0.21 (0.01-2.47)	0.21
GA+AA	26 (24.07)	85 (22.3)	1.38 (0.67-2.86)	0.37	GA+AA	46 (21.3)	65 (23.7)	0.87 (0.48-1.57)	0.64
Genotype rs4148356 ABCC1 (G2168A)	**Nx+N0+N1 (108), n (%)**	**N2+N3+N4 (381), n (%)**	**AOR (95% CI)[a]**	***p*[b]**	**Genotype rs4148356 ABCC1 (G2168A)**	**M0 (215), n (%)**	**M1 (274), n (%)**	**AOR (95% CI)[a]**	***p*[b]**
GG	102 (94.4)	370 (97.1)	1.0 (Reference)	-	GG	206 (95.8)	266(97.08)	1.0 (Reference)	-
GA	6 (5.5)	11 (2.8)	0.54 (0.15-1.89)	0.3	GA	9 (4.1)	8 (2.9)	1.09 (0.32-3.70)	0.88

a)-Adjusted Odds ratios, 95% confidence intervals, and corresponding p-values were calculated by logistic regression analysis after adjusting for age, gender, smoking, and regimen. (b)-Two-sided $\chi 2$ test for either genotype distribution or allelic frequencies between different clinical parameters. The number in bold indicates the significant values in the table

5.17.2 Correlation of ABCC1 polymorphisms with chemotherapeutic response

The association between *ABCC1* polymorphisms and the effectiveness result of response rate and clinical benefits from platinum-based doublet chemotherapy was evaluated by univariate logistic regression. The relation between $G^{3173}A$ and $G^{2168}A$ polymorphism is depicted in table 5.39. Patients with complete (CR) or partial responses (PR) were considered good responders, whereas those with stable (SD) and progressed disease (PD) were categorized as poor responders. Our results did not show any substantial difference in response to chemotherapy in lung cancer patients having *ABCC1* polymorphism. As a result, we concluded that none of the *ABCC1* polymorphisms did determine the chemotherapeutic response rate in a significant manner.

Table 5.39: Association of *ABCC1* SNPs and chemotherapeutic response

	Response of chemotherapy			
Genotype rs41410450 ABCC1 (G3173A)	**CR+PR (103), n (%)**	**SD+PD (157), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
GG	75(73)	121(77)	1.0(Reference)	
GA	28(27)	33(21)	0.61(0.30-1.24)	0.17
AA	0	3	-	-
GA+AA	28(27)	36(23)	0.64(0.32-1.29)	0.21
Genotype rs4148356 ABCC1 (G2168A)	**CR+PR (103), n (%)**	**SD+PD (157), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
GG	98(95.0)	152(97)	1.0(Reference)	-
GA	5(5.0)	5(3.0)	0.92(0.23-3.61)	0.9

a)-Adjusted Odds ratios, 95% confidence intervals, and corresponding p-values were calculated by logistic regression analysis after adjusting for age, gender, smoking, and regimen. (b)-Two-sided $\chi 2$ test for either genotype distribution or allelic frequencies between the responders and non-responders. The number in bold indicates the significant values in the table

5.18 Statistical analysis of *ABCC2* gene

5.18.1 Genotypic and allelic frequencies of *ABCC2* polymorphic variants

The distribution of genotypes and alleles for *ABCC2* polymorphisms in lung carcinoma patients has been depicted in Table 5.40. Genotyping a polymorphism of the *ABCC2* gene, *i.e., $G^{4544}A$* (rs8187710), was studied. Among the lung cancer patient, the prevalence of homozygous wild alleles *(GG)* was highly presented as compared to the carrier alleles (*GA)*; however, our study did not find any homozygous variant genotype in the sample population. 96.6% of patients harbored homozygous wild genotype *(CC)*, whereas 3.38% had at least one copy of the mutant genotype (*GA)*. The cases for the *ABCC2 $G^{4544}A$* polymorphism (N=502, $\chi 2$=0.14, df=1, p=0.69) followed Hardy Weinberg equilibrium. Minor allele frequency (MAF) was calculated to be 0.016 for the *$G^{4544}A$* variant.

Table 5.40: Genotypic and allelic distribution of *ABCC2* genetic polymorphism in lung cancer patients

Genotype rs8187710 ABCC2 (G4544A)	Cases (502) N (%)	$\chi 2$ (df)	p-value
GG	485 (96.6)		
GA	17 (3.38)		
G (allele)	970	0.14 (1)	0.69
A (allele)	34		
MAF	0.016		

5.18.2 Elucidation of overall survival (OS) of lung carcinoma patients with *ABCC2* polymorphism

5.18.2.1 Association of ABCC2 polymorphism with a prognosis based on different parameters such as histology and regimen

The overall survival was evaluated for 483 lung cancer patients, and their correlation with *ABCC2* polymorphism was assessed using the univariate Kaplan Meir, and multivariate cox regression method for the one genetic variant of *ABCC2 $G^{4544}A$* is illustrated in table 5.41. Lung carcinoma patients carrying at least one copy of mutant allele (*GA)* had almost 2-times less median survival time as compared to the patients harboring both copies of wild-type alleles (*GG)* (MST =8.4 *vs.* 4.73 months, HR=2.16, 95% CI= 1.14-4.09, p=**0.017** (Figure 5.37).

Table 5.41: Association of *ABCC2* genetic polymorphism in predicting prognosis in lung cancer patients when stratified based on different parameters

OVERALL SURVIVAL	**Overall survival**								
	Genotype rs8187710 ABCC2 (G4544A)	**CASES n(%), N=483**	**DEAD n(%), N=408**	**ALIVE n(%), N=75**	**Univariate analysis**			**Multivariate analysis**	
					MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
	GG	466(96.4)	391(95.8)	75(100)	8.4	-	1.0 (reference)	-	-
	GA	17(3.5)	17(4.1)	0(0)	4.37	**0.017**	2.16(1.14-4.09)	1.88(0.96-3.70)	**0.04**
HISTOLOGY	**ADCC**								
ADCC	**Genotype rs8187710 ABCC2 (G4544A)**	**CASES n(%), N=191**	**DEAD n(%), N=152**	**ALIVE n(%), N=39**	**Univariate analysis**			**Multivariate analysis**	
					MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
	GG	184(96.3)	145(95.3)	39(100)	8.8	-	1.0 (reference)	-	-
	GA	7(3.66)	7(4.6)	0(0)	4.37	**0.04**	3.03(1.02-8.97)	1.64(0.56-4.77)	0.35
SQCC	**SQCC**								
	Genotype rs8187710 ABCC2 (G4544A)	**CASES n(%), N=204**	**DEAD n(%), N=176**	**ALIVE n(%), N=28**	**Univariate analysis**			**Multivariate analysis**	
					MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
	GG	198(97.05)	170(96.5)	28(100)	7.1	-	1.0 (reference)	-	-
	GA	6(2.9)	6(3.4)	0(0)	9.03	0.3	1.55(0.63-3.82)	1.15(0.40-3.3)	0.78
SCLC	**SCLC**								
	Genotype rs8187710 ABCC2 (G4544A)	**CASES n(%), N=75**	**DEAD n(%), N=71**	**ALIVE n(%), N=4**	**Univariate analysis**			**Multivariate analysis**	
					MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
	GG	71(94.6)	67(94.3)	4(100)	9.93	-	1.0 (reference)	-	-
	GA	4(5.3)	4(5.6)	0(0)	4.0	0.20	2.92(0.5-15.1)	15.3(1.29-182.0)	**0.03**
REGIMEN	**Paclitaxel**								
Cisplatin/carboplatin and paclitaxel	**Genotype rs8187710 ABCC2 (G4544A)**	**CASES n(%), N=85**	**DEAD n(%), N=72**	**ALIVE n(%), N=13**	**Univariate analysis**			**Multivariate analysis**	
					MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
	GG	81(95.2)	68(94.4)	13(100)	6.3	-	1.0 (reference)	-	-
	GA	4(4.7)	4(5.5)	0(0)	4.3	0.58	1.38(0.43-4.36)	0.93(0.32-2.68)	0.89
Cisplatin/carboplatin and pemetrexed	**Pemetrexed**								
	Genotype rs8187710 ABCC2 (G4544A)	**CASES n(%), N=105**	**DEAD n(%), N=89**	**ALIVE n(%), N=16**	**Univariate analysis**			**Multivariate analysis**	
					MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
	GG	101(96.1)	85(95.5)	16(100)	7.9	-	1.0 (reference)	-	-
	GA	4(3.8)	4(4.4)	0(0)	1.3	**0.03**	5.96(1.15-30.8)	3.11(1.06-9.11)	**0.03**

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, regimen, histology, KPS, ECOG for overall survival; [a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, regimen, KPS, ECOG for histology; [a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, histology, KPS, ECOG for regimen.

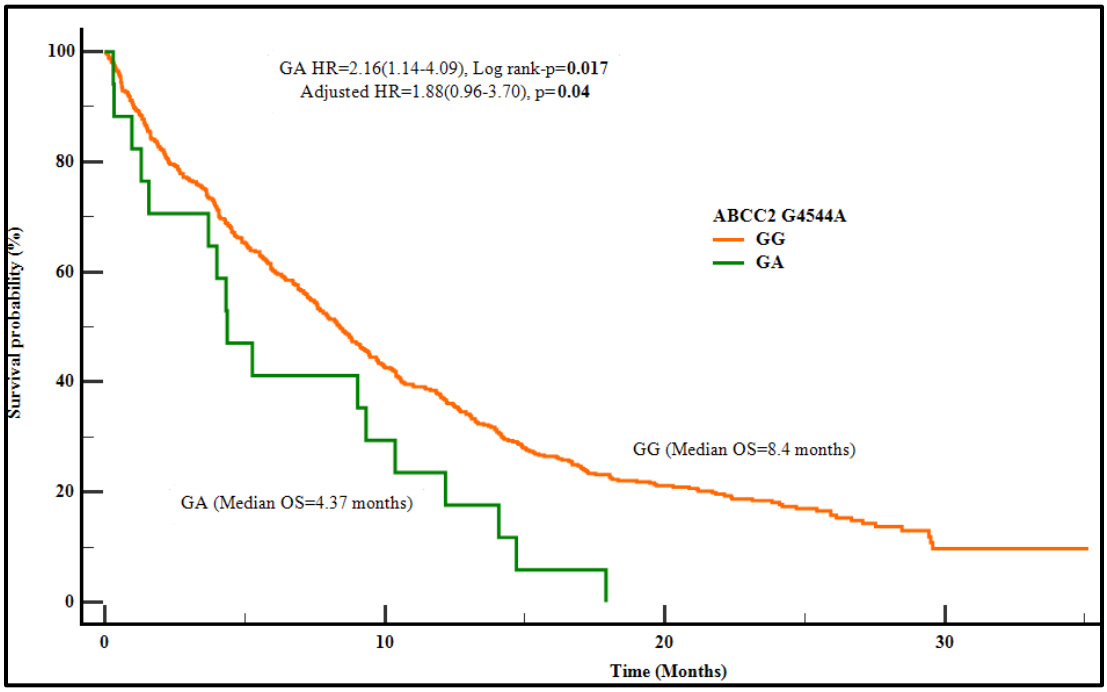


Figure 5.37: Kaplan Meier curves illustrating the association between overall survival in different genotypes of *ABCC2 G4544A* polymorphism in lung cancer patients. (Note: *GG* represents wild-type genotype, *GA* represents the heterozygous genotype

Multivariate cox proportional hazards analysis revealed that, besides gene polymorphism, the overall survival was correlated with ECOG (p<**0.0001**) of patients with lung cancer. It was also noted that the hazard ratio of patients carrying heterozygous *(GA)* genotype of *ABCC2* $G^{4544}A$ was 1.88 times higher than in patients carrying wild *(GG)* genotype (table 5.42) (HR=1.88, 95% CI=0.96-3.70, p=**0.04**). Hence, ECOG and heterozygous *(GA)* genotype in the *4544* site of *ABCC2* gene could be key factors for contributing to the development of lung cancer. Moreover, we found that age (P= 0.47), gender (P=0.16), smoking (P=0.66), stage (P=0.10), KPS (P=0.72), histology (P=0.68), and regimen (0.22) did not correlate with OS. Also, in our case samples, none of the patients carried homozygous variant genotype (*AA)*, so we could not evaluate the survival analysis for this genotype. Furthermore, it is pretty evident from our results that *ABCC2* $G^{4544}A$ is associated with prognostic significance in lung cancer patients.

Table 5.42: Cox proportional hazards regression model analysis of overall survival in lung cancer subjects

N=483	b	SE	P-value	Adjusted HR (Exp b)	95% CI of Exp (b)
ABCC2_G4544A	0.6350	0.3446	**0.044**	1.8870	0.9603 to 3.7080
Age	-0.005035	0.007062	0.4759	0.9950	0.9813 to 1.0088
Gender	0.2837	0.2056	0.1676	1.3281	0.8876 to 1.9872
Smoking	-0.09431	0.2152	0.6612	0.9100	0.5969 to 1.3874
Stage	0.1939	0.1212	0.1097	1.2140	0.9573 to 1.5396
ECOG	0.6447	0.1395	**<0.0001**	1.9054	1.4494 to 2.5047
KPS	0.04935	0.1417	0.7276	1.0506	0.7959 to 1.3868
Histology	0.04078	0.1002	0.6840	1.0416	0.8559 to 1.2676
Regimen	-0.08989	0.07332	0.2202	0.9140	0.7917 to 1.0553

Lung cancer patients having *ABCC2* $G^{4544}A$ polymorphism were stratified based on histology such as ADCC, SQCC, and SCLC to understand their impact on modulating survival in individuals. Patients having SCLC and carrying heterozygous genotype (*GA)* for $G^{4544}A$ polymorphism showed a poor survival time as compared to the wild type genotype (*GG)* (MST=4 *vs.* 9.93 months, HR=2.92, 95% CI= 0.5-15.1, Log-rank p=0.20), Upon adjusting with multiple covariates, the death ratio increased in the patients having heterozygous genotype (*GA)* (HR=15.3, p=**0.03**). Also, our data showed survival differences in the genotypes having $G^{4544}A$ and possessing ADCC and SQCC, but substantial significance was not achieved.

Lung carcinoma patients were administered with cisplatin/carboplatin-based doublet chemotherapeutic regimen as second-line therapy. Therefore, we have evaluated the modulating effect of the *ABCC2* $G^{4544}A$ variant and its correlation with prognosis in lung cancer patients. The outcomes of the *ABCC2* $G^{4544}A$ variant as per cisplatin/carboplatin and paclitaxel regimen have been illustrated in table 5.41. For the *ABCC2* $G^{4544}A$ variant, the incidence of patients harboring carrier genotype (*GA)* was substantially more minor than the wild type genotype (4.7% *vs.* 95.2%). Therefore, subjects carrying at least one variant allele (*GA)* for $G^{4544}A$ showed poor survival and high death ratio as compared to the wild type genotype (*GG)* (MST=4.3 *vs.* 6.3 months, HR=1.38, 95% CI=0.43-4.36, p=0.5), however, no substantial differences in overall survival between both the groups were observed. On the contrary, the patients administered with cisplatin/carboplatin along with pemetrexed and harboring heterozygous genotype for *ABCC2* $G^{4544}A$ variant exhibited

a poor median survival than those carrying the wild type genotype (MST=1.3 *vs.* 7.9 months; HR= 5.96, 95% CI= 1.15-30.8, Log-rank p=**0.03**). In the cox hazards model, after adjusting with different covariates, the death ratio of 3.11 was observed in the patients carrying heterozygous genotype (HR=3.11, 95% CI=1.06-9.11, p=**0.03**) (Figure 5.38). Therefore, our results showed shown a strong association between *ABCC2* polymorphism in modulating survival in lung cancer patients undergoing platinum-based pemetrexed chemotherapy.

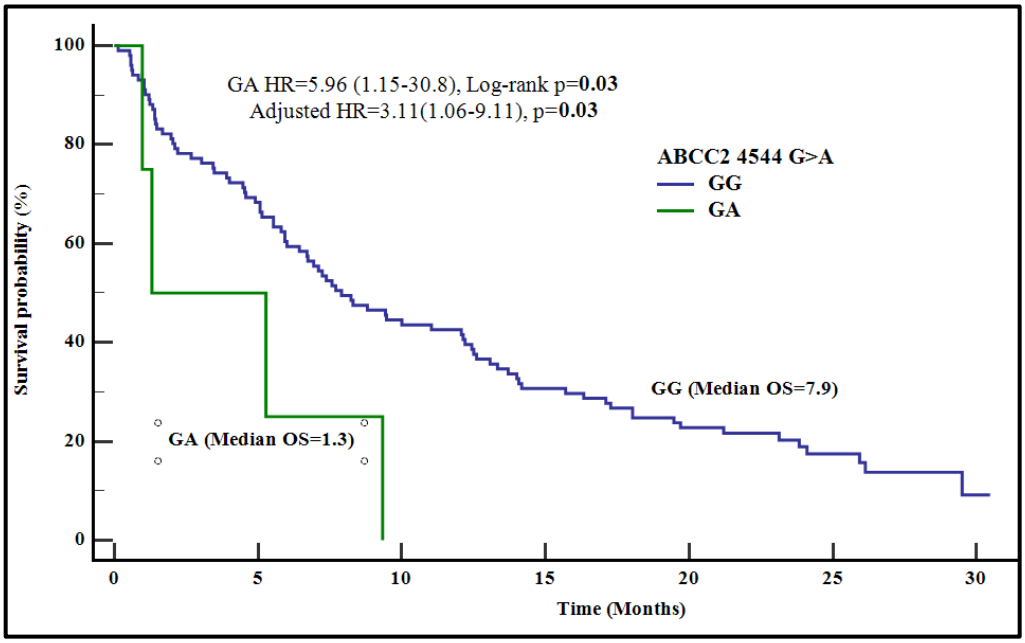


Figure 5.38: Kaplan Meier curves illustrating the association between overall survival in different genotypes of *ABCC2 G4544A* polymorphism in lung cancer patients undergoing cisplatin/carboplatin and pemetrexed therapy. (Note: *GG* represents wild-type genotype, *GA* represents the heterozygous genotype

5.18.2.2 Association of ABCC2 polymorphism with a prognosis based on gender, smoking habits, and tumor stage

To assess the effect of *ABCC2* $G^{4544}A$ polymorphism in predicting overall survival, the lung cancer patients were stratified based on gender, smoking status, and tumor stage. When we evaluated the patients based on gender, we noted that the majority of males were dead at the time of analysis and only 14.9% of males were alive, and among that also none of the lung cancer patients having heterozygous genotype (*GA)* were observed to be alive (Table 5.43). Our results depicted reduced

survival in males having lung cancer and harboring at least one mutant allele (*GA)* for *ABCC2* $G^{4544}A$ as compared to the wild-type genotype (*GG)* (MST=4.33 *vs.* 8.26, HR=1.94, 95% CI=0.95-3.85, p=0.05). Furthermore, a similar trend of reduced survival was also observed in females having heterozygous genotype *(GA)* for *ABCC2* $G^{4544}A$ in lung cancer patients; however, no significant association was observed among the genotypes in predicting overall survival in female lung cancer patients as compared to the wild type genotype (*GG)* (MST=5.27 *vs.* 9.46, HR=4.62, 95% CI=0.75-28.32, p=0.09).

The interaction effect of the *ABCC2* gene and environment was also studied, and patients were bifurcated based on their smoking habits, *i.e.,* smokers and non-smokers (Table 5.43). Our results depicted that patients who were smokers and carrying at least one variant allele (*GA*) for $G^{4544}A$ polymorphism were shown to be less represented in both smokers and non-smokers (3.13% and 5%). We observed that smokers with lung carcinoma who harbored heterozygous genotype (*GA)* had reduced survival as compared to the smokers with wild-type genotype (*GG)* for $G^{4544}A$ polymorphism (MST=4.37 *vs.* 7.93, HR=1.61, Log-rank p=0.17). Nevertheless, we did not find any association between $G^{4544}A$ polymorphism and smokers predicting survival in lung cancer patients. Similarly, non-smokers having heterozygous genotype (*GA)* have poor survival and high death ratio as compared to the wild type genotype (*GG)* (MST=3.7 *vs.* 9.73 months, HR=9.44, 95% CI=1.93-46.23, p=**0.005**). Even after adjusting with multiple covariates such as age, gender, stage, histology, regimen, and performance status, the death ratio remained high in the former group compared to the latter (HR=5.73, 95% CI=1.49-21.93, p=**0.017**).

As shown in table 5.43, in the case of $G^{4544}A$, patients in stages III and IV harboring at least one copy of mutant genotype (*GA)* showed better survival as compared to wild (*GG)* type genotype (MST=9.03 *vs.* 8.5, log-rank p=0.37), though the statistical significance could not be achieved. Also, our results showed poor survival in stage IV lung cancer patients having heterozygous genotype (*GA)* as compared to the wild type genotype (*GG)* (MST=5.27 *vs.* 7.6, p=0.17). Therefore, we summarize that *ABCC2* $G^{4544}A$ polymorphism was not associated with having an impact on predicting prognosis in stage III and IV lung cancer patients.

Table 5.43: Association of *ABCC2* genetic polymorphism in predicting prognosis in lung cancer patients when stratified based on gender, smoking habits, and tumor stage

Parameter	Gender								
Male	**Genotype rs8187710 ABCC2(G4544A)**	**CASES n(%), N=388**	**DEAD n(%), N=330**	**ALIVE n(%), N=58**	**Univariate analysis**			**Multivariate analysis**	
					MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
	GG	375(96.64)	317(96.06)	58(100)	8.26	-	1.0(reference)	-	-
	GA	13(3.35)	13(3.93)	0	4.33	0.05	1.94(0.98-3.85)	1.76(0.86-3.63)	0.12
Female	**Genotype rs8187710 ABCC2(G4544A)**	**CASES n(%), N=95**	**DEAD n(%), N=78**	**ALIVE n(%), N=17**	**Univariate analysis**			**Multivariate analysis**	
					MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
	GG	91(95.78)	74(94.87)	17(100)	9.46		1.0(reference)	-	-
	GA	4(4.25)	4(5.12)	0	5.27	0.09	4.62(0.75-28.32)	3.76(0.43-32.29)	0.22
Smoking status	**Smoking status**								
Smokers	**Genotype rs8187710 ABCC2(G4544A)**	**CASES n(%), N=383**	**DEAD n(%), N=332**	**ALIVE n(%), N=51**	**Univariate analysis**			**Multivariate analysis**	
					MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
	GG	371(96.86)	320(96.38)	51(100)	7.93	-	1.0(reference)	-	-
	GA	12(3.13)	12(3.61)	0	4.37	0.17	1.61(0.80-3.22)	1.35(0.59-3.10)	0.46
Non-smokers	**Non-smokers**								
	Genotype rs8187710 ABCC2(G4544A)	**CASES n(%), N=100**	**DEAD n(%), N=76**	**ALIVE n(%), N=24**	**Univariate analysis**			**Multivariate analysis**	
					MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
	GG	95(95)	71(93.42)	24(100)	9.73	-	1.0(reference)	-	-
	GA	5(5)	5(6.57)	0	3.7	**0.005**	9.44(1.93-46.2)	5.73(1.49-21.93)	**0.017**
Tumor stage	**Stage III**								
Stage III	**Genotype rs8187710 ABCC2(G4544A)**	**CASES n(%), N=159**	**DEAD n(%), N=127**	**ALIVE n(%), N=32**	**Univariate analysis**			**Multivariate analysis**	
					MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
	GG	152(95.59)	120(94.48)	32(100)	8.5	-	1.0(reference)	-	-
	GA	7(4.40)	7(5.51)	0	9.03	0.37	1.50(0.61-3.67)	1.06(0.25-4.55)	0.9
Stage IV	**Stage IV**								
	Genotype rs8187710 ABCC2(G4544A)	**CASES n(%), N=276**	**DEAD n(%), N=244**	**ALIVE n(%), N=32**	**Univariate analysis**			**Multivariate analysis**	
					MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
	GG	269(97.46)	237(97.1)	32(100)	7.6	-	1.0(reference)	-	-
	GA	7(2.53)	7(2.86)	0	5.27	0.17	1.95(0.74-5.12)	1.38(0.56-3.36)	0.47

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, smoking, stage, regimen, histology, KPS, ECOG for gender; [a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, stage, regimen, histology, KPS, ECOG for smoking habits and [a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, smoking, sex, regimen, histology, KPS, ECOG for tumor stage

5.18.3 Role of ABCC2 polymorphism on clinical outcomes in lung cancer patients

The result of *ABCC2* polymorphism on the different clinical-pathological outcomes was characterized mainly by tumor stage (stage III and IV), direct tumor extension (T3 and T4), lymph node extension (Nx+N0+N1 and N2+N3+N4), and on distant metastatic status (M0 and M1) (Table 5.44). Our results demonstrated that individuals having *ABCC2* $G^{4544}A$ polymorphism have a higher frequency of wild-type genotype (*GG)* stage IV patients (97.6%) as compared to stage III patients (95.6%). Statistical analysis showed lack of association when the heterozygous genotype (*GA)* for *ABCC2* $G^{4544}A$ of stage IV was compared with stage III (AOR=1.14, 95% CI=0.20-6.3, p=0.8). Further, when the patients were stratified based on primary tumor extension (T3 *vs.* T4), our data for $G^{4544}A$ did not show any risk when carriers (*GA)* of the T4 subgroup were compared with the T3 subgroup (AOR=0.35, 95% CI=0.08-1.40, p=0.13).

Additionally, the correlation of *ABCC2* polymorphism with lymph node involvement was also studied. The patients were stratified into two groups depending on the lymph node invasion (Nx+N0+N1 *vs.* N2+N3+N4). When 3.4% of patients in N2+N3+N4 carrying heterozygous genotype were compared with 2.7% of subjects in Nx+N0+N1, odds of 1.08 was noted in the former group (AOR=1.08, 95% CI=0.22-5.30), though substantial differences were not obtained. When the patients were stratified based on distant metastasis, our results showed an odd of 1.17 when lung cancer patients with heterozygous genotype and metastasis were compared with those with no metastasis (AOR=1.17, 95% CI=0.24-5.59, p=0.8). However, we did not find a significant association between $G^{4544}A$ polymorphism and the spread of lung carcinoma to the other organs. Therefore, we conclude no association between the *ABCC2* polymorphism in lung cancer concerning clinicopathological parameters.

The correlation between *ABCC2* $G^{4544}A$ polymorphism and the efficacy of response rate along with clinical benefits from platinum-based doublet chemotherapy was evaluated by univariate logistic regression. The relation of $G^{4544}A$ polymorphism is depicted in table 5.44. Lung cancer patients with complete or partial responses were considered good responders, while those with stable and progressed disease was poor. For *ABCC2* $G^{4544}A$ polymorphism, poor responders with at least one mutant allele (*CT)* had an adjusted odd of 1.42 times good responders (AOR=1.42, 95% CI=0.24-8.32, p=0.69), but no significant association was found. Our results did not show any substantial difference in response to chemotherapy in lung cancer patients having *ABCC2*

G^{4544}*A* polymorphism. As a result, we conclude that *ABCC2 G*^{4544}*A* polymorphisms did determine the chemotherapeutic response rate in a significant manner.

Table 5.44: Association of *ABCC2 SNPs* and clinical outcomes

CLINICAL STAGE				
Genotype rs8187710 ABCC2 (G4544A)	**Stage III (162), n (%)**	**Stage IV (292), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
GG	155 (95.6)	285 (97.6)	1.0 (Reference)	
GA	7 (4.32)	7 (4.32)	1.14 (0.20-6.3)	0.8
TUMOR SIZE				
Genotype rs8187710 ABCC2 (G4544A)	**T3 (92), n (%)**	**T4 (302), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
GG	85 (92.3)	294 (97.3)	1.0 (Reference)	-
GA	7 (7.6)	8 (2.6)	0.35 (0.08-1.40)	0.13
LYMPH NODE INVASION				
Genotype rs8187710 ABCC2 (G4544A)	**Nx+N0+N1 (108), n (%)**	**N2+N3+N4 (381), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
GG	105 (97.2)	368 (96.5)	1.0 (Reference)	-
GA	3 (2.7)	13 (3.4)	1.08 (0.22-5.30)	0.9
METASTASIS				
Genotype rs8187710 ABCC2 (G4544A)	**M0 (215), n (%)**	**M1 (274), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
GG	208 (96.7)	265 (96.7)	1.0 (Reference)	-
GA	7 (3.2)	9 (3.2)	1.17 (0.24-5.59)	0.83
RESPONSE TO CHEMOTHERAPY				
Genotype rs8187710 ABCC2 (G4544A)	**CR+PR (102), n (%)**	**SD+PD (156), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
GG	100 (98.0)	150 (96.1)	1.0 (Reference)	-
GA	2 (1.96)	6 (3.84)	1.42 (0.24-8.32)	0.69

a)-Adjusted Odds ratios, 95% confidence intervals, and corresponding p-values were calculated by logistic regression analysis after adjusting for age, gender, smoking, and regimen. (b)- Two-sided $\chi 2$ test for either genotype distribution or allelic frequencies between different clinical parameters. The number in bold indicates the significant values in the table

5.19 Statistical analysis of *ABCG2* gene

5.19.1 Genotypic and allelic frequencies of *ABCG2* polymorphic variants

Table 5.45 depicts the genotypic and allelic frequency distribution for the *ABCG2* polymorphic variant, $C^{421}A$ (rs2231142). The genotypic distribution among cases (n=502, $\chi 2$=2.40, df=1, p=0.12) were consistent with Hardy Weinberg equilibrium. The prevalence of heterozygous genotype (*CA)* was less frequent in the case group as compared to the wild type (*CC)* genotype (12.94% *vs.* 87.05%). Also, lung cancer patients did not have the variant genotype (*AA)* for *ABCG2* $C^{421}A$ polymorphism in lung carcinoma patients. The MAF was calculated to be 0.06 for the case group having $C^{421}A$ variation.

Table 5.45 Genotypic and allelic distribution of *ABCG2* genetic polymorphism in lung cancer patients

Genotype rs2231142 ABCG2 (C421A)	Cases (502) N (%)	$\chi 2$ (df)	p[a]-value
CC	437 (87.05)		
CA	65 (12.94)		
C (allele)	939	2.40 (1)	0.12
A (allele)	65		
MAF	0.06		

5.19.2 Elucidation of overall survival of lung carcinoma patients with *ABCG2* variation

5.19.2.1 Association of ABCG2 polymorphism with a prognosis based on different parameters

The overall survival of 502 lung cancer patients was evaluated in table 5.46. Kaplan Meir curves and log-rank test approach were used for univariate analysis to understand the correlation of *ABCG2* towards prognosis in lung carcinoma patients. In the *ABCG2* $C^{421}A$ polymorphism, patients having heterozygous genotype (*CA)* showed lower survival time as compared to the subjects with wild type genotype (*CC)* (MST=7.67 *vs.* 8.37 months). We did not observe any substantial differences between $C^{421}A$ and OS. We could not evaluate the role of the variant genotype of $C^{421}A$ concerning prognosis as in our sample population, none of the patients had variant alleles (*AA).*

Table 5.46: Association of *ABCG2* genetic polymorphism in predicting prognosis in lung cancer patients when stratified based on different parameters

OVERALL SURVIVAL	**Overall survival**								
	Genotype rs2231142 ABCG2 (C421A)	**CASES n (%), N=483**	**DEAD n (%), N=408**	**ALIVE n (%), N=75**	**Univariate analysis**			**Multivariate analysis**	
					MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
	CC	419 (86.7)	353 (86.5)	66 (88)	8.37	-	1.0 (reference)	-	-
	CA	64 (13.2)	55 (13.4)	9 (12)	7.67	0.87	1.02 (0.76-1.36)	0.94 (0.64-1.38)	0.77
GENDER	**Genotype rs2231142 ABCG2 (C421A)**	**CASES n (%), N=388**	**DEAD n (%), N=330**	**ALIVE n (%), N=58**	**Univariate analysis**			**Multivariate analysis**	
					MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Male	CC	331 (85.3)	280 (84.8)	51 (87.9)	8.3	-	1.0 (reference)	-	-
	CA	57 (14.6)	50 (15.1)	7 (12.06)	6.7	0.59	1.08 (0.79-1.48)	0.92 (0.62-1.37)	0.7
Female	**Genotype rs2231142 ABCG2 (C421A)**	**CASES n (%), N=95**	**DEAD n (%), N=78**	**ALIVE n (%), N=17**	**Univariate analysis**			**Multivariate analysis**	
					MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
	CC	88 (92.6)	73 (93.5)	15 (88.2)	8.5	-	1.0 (reference)	-	-
	CA	7 (7.3)	5 (6.4)	2 (11.7)	13.9	0.28	0.66 (0.31-1.40)	0.59 (0.15-2.25)	0.44
HISTOLOGY	**ADCC**								
ADCC	**Genotype rs2231142 ABCG2 (C421A)**	**CASES n (%), N=191**	**DEAD n (%), N=152**	**ALIVE n (%), N=39**	**Univariate analysis**			**Multivariate analysis**	
					MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
	CC	166 (86.9)	132 (86.8)	34 (87.1)	8.50	-	1.0 (reference)	-	-
	CA	25 (13.08)	20 (13.1)	5 (12.8)	9.13	0.77	0.93 (0.59-1.48)	0.81 (0.44-1.49)	0.51
SQCC	**SQCC**								
	Genotype rs2231142 ABCG2 (C421A)	**CASES n (%), N=204**	**DEAD n (%), N=176**	**ALIVE n (%), N=28**	**Univariate analysis**			**Multivariate analysis**	
					MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
	CC	174 (85.2)	150 (85.2)	24 (85.7)	7.27	-	1.0 (reference)	-	-
	CA	30 (14.7)	26 (14.7)	4 (14.2)	5.37	0.7	1.06 (0.69-1.62)	0.93 (0.48-1.79)	0.84
SCLC	**SCLC**								
	Genotype rs2231142 ABCG2 (C421A)	**CASES n (%), N=75**	**DEAD n (%), N=71**	**ALIVE n (%), N=4**	**Univariate analysis**			**Multivariate analysis**	
					MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
	CC	66 (88)	62 (87)	4 (100)	9.4	-	1.0 (reference)	-	-
	CA	9 (12)	9 (13)	0 (0)	9.93	0.58	1.232 (0.57-2.64)	1.65 (0.60-4.51)	0.32

REGIMEN	Cisplatin/carboplatin and docetaxel								
Cisplatin/carboplatin and docetaxel	**Genotype rs2231142 ABCG2 (C421A)**	**CASES n (%), N=44**	**DEAD n (%), N=39**	**ALIVE n (%), N=5**	**Univariate analysis**			**Multivariate analysis**	
					MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
	CC	38 (86.3)	34 (87.1)	4 (80)	8.5	-	1.0 (reference)	-	-
	CA	6 (13.6)	5 (12.8)	1 (20)	6.46	0.9	1.06 (0.40-2.78)	1.71 (0.62-4.68)	0.29
Cisplatin/carboplatin and paclitaxel	**Cisplatin/carboplatin and paclitaxel**								
	Genotype rs2231142 ABCG2 (C421A)	**CASES n (%), N=85**	**DEAD n (%), N=72**	**ALIVE n (%), N=13**	**Univariate analysis**			**Multivariate analysis**	
					MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
	CC	74 (87.05)	63 (87.5)	11 (84.6)	5.8	-	1.0 (reference)	-	-
	CA	11 (12.9)	9 (12.5)	2 (15.3)	8.5	0.54	0.81 (0.42-1.55)	0.81 (0.38-1.71)	0.58
Cisplatin/carboplatin and pemetrexed	**Cisplatin/carboplatin and pemetrexed**								
	Genotype rs2231142 ABCG2 (C421A)	**CASES n (%), N=105**	**DEAD n (%), N=89**	**ALIVE n (%), N=16**	**Univariate analysis**			**Multivariate analysis**	
					MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
	CC	91 (86.6)	78 (87.6)	13 (81.2)	7.7	-	1.0 (reference)	-	-
	CA	14 (13.3)	11 (12.3)	3 (18.7)	6.7	0.52	0.82 (0.45-1.48)	0.65 (0.33-1.28)	0.21
Cisplatin/carboplatin and irinotecan	**Cisplatin/carboplatin and irinotecan**								
	Genotype rs2231142 ABCG2 (C421A)	**CASES n (%), N=43**	**DEAD n (%), N=40**	**ALIVE n (%), N=3**	**Univariate analysis**			**Multivariate analysis**	
					MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
	CC	35 (81.3)	32 (80)	3 (100)	10.4	-	1.0 (reference)	-	-
	CA	8 (18.6)	8 (20)	0 (0)	7.67	0.69	1.17 (0.51-2.67)	1.75 (0.66-4.59)	0.25
SMOKING STATUS	**SMOKING STATUS**								
Smokers	**Genotype rs2231142 ABCG2 (C421A)**	**CASES n (%), N=383**	**DEAD n (%), N=332**	**ALIVE n (%), N=51**	**Univariate analysis**			**Multivariate analysis**	
					MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
	CC	330 (86.16)	286 (86.14)	44 (86.27)	7.93	-	1.0 (reference)	-	-
	CA	53 (13.83)	46 (13.85)	7 (1.96)	6.7	0.84	1.03 (0.75-1.41)	0.94 (0.63-1.42)	0.79
Non- Smokers	**Genotype rs2231142 ABCG2 (C421A)**	**CASES n (%), N=100**	**DEAD n (%), N=76**	**ALIVE n (%), N=24**	**Univariate analysis**			**Multivariate analysis**	
					MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
	CC	89 (89)	67 (88.15)	22 (91.66)	9.43	-	1.0 (reference)	-	-
	CA	11 (11)	9 (11.84)	2 (8.33)	13.03	0.87	0.94 (0.47-1.87)	0.32 (0.08-1.22)	0.09

TUMOR STAGE

Stage III	**Genotype rs2231142 ABCG2 (C421A)**	**CASES n (%), N=159**	**DEAD n (%), N=127**	**ALIVE n (%), N=32**	**Univariate analysis**			**Multivariate analysis**	
					MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
	CC	132 (83.01)	104 (81.88)	28 (87.5)	8.8				
	CA	27 (16.98)	23 (18.11)	4 (12.5)	8.5	0.62	1.12 (0.70-1.79)	1.01 (052-1.93)	0.9
Stage IV	**Genotype rs2231142 ABCG2 (C421A)**	**CASES n (%), N=276**	**DEAD n (%), N=244**	**ALIVE n (%), N=32**	**Univariate analysis**			**Multivariate analysis**	
					MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
	CC	242 (87.68)	214 (87.7)	28 (87.5)	7.65	-	1.0 (reference)	-	-
	CA	34 (12.31)	30 (12.29)	4 (12.5)	6.46	0.78	1.05 (0.72-1.53)	0.93 (0.57-1.54)	0.80

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, regimen, histology, KPS, ECOG for overall survival; [a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, regimen, KPS, ECOG for histology; [a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, histology, KPS, ECOG for regimen. [a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, smoking, stage, regimen, histology, KPS, ECOG for gender; [a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, stage, regimen, histology, KPS, ECOG for smoking habits and [a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, smoking, sex, regimen, histology, KPS, ECOG for tumor stage

Overall survival in *ABCG2* $C^{421}A$ polymorphism and their correlation with different histological subtypes of lung cancer are depicted in Table 5.46. In $C^{421}A$, the percentage of heterozygous patients were 13.08%, 14.7%, and 12% among ADCC, SQCC, and SCLC subtypes. ADCC patients having carrier (*CA)* genotype presented an improved OS as compared to wild genotype (*CC)* (9.13 *vs.* 8.50 months; p=0.51). On the opposing side, the median survival time of SQCC patients carrying heterozygous *(CA)* genotype was 5.37 months, which was lesser than 7.27 months in individuals with wild (*CC*) genotype. However, the p-values obtained after the univariate and multivariate analysis were not suggestively associated with the genotype. Simultaneously, SCLC patients with at least one variant allele (*CA)* showed somewhat better survival than the wild type alleles (*CC)* (MST=9.93 *vs.* 9.4 months, p=0.32). Therefore, we conclude that *ABCG2* $C^{421}A$ polymorphic site did not play any role in predicting prognosis in SCLC, ADCC, and SQCC tumor subtypes.

Table 5.46 illustrates the effect of $C^{421}A$ of *ABCG2* on prognosis after bifurcating based on chemotherapeutic regimens utilized to treat lung cancer. The four prior regimens, such as docetaxel, paclitaxel, pemetrexed, and irinotecan, were given to different patients. These four regimens were given along with cisplatin/carboplatin, which was common to all the patients. The univariate and multivariate analysis evaluated lung cancer patients' median survival time and hazard rate. As shown in Table 5.46, patients administered with cisplatin/carboplatin in addition to docetaxel and having heterozygous genotype (*CA)* had lower survival as compared to the wild-type genotype (*CC)* (MST=6.46 *vs.* 8.5, p=0.29). For the subjects receiving paclitaxel along with cis/carboplatin, the single allelic carrier (*CA*) for the *ABCG2* $C^{421}A$ polymorphism displays an improved survival than patients carrying wild type (*CC)* genotype (8.5 *vs.* 5.8 months, log-rank p=0.54). A median survival time of 6.7 months was noted for the lung cancer subjects harboring the heterozygous genotype (*CA*) for *ABCG2* $C^{421}A$ polymorphism and administered with pemetrexed and cisplatin/carboplatin regimen, whereas wild type genotype showed the MST of 7.7 months (MST=6.7 *vs.* 7.7 months, p=0.21). For *ABCG2* $C^{421}A$ polymorphism, patients receiving irinotecan and cisplatin/carboplatin had a reduced survival time than the wild genotype (MST=7.67 *vs.* 10.4 months p=0.25). Therefore, our results show that *ABCG2* $C^{421}A$ polymorphism was not associated with predicting overall survival for subjects administered cisplatin/carboplatin and docetaxel, paclitaxel, and irinotecan.

The effect of the *ABCG2* gene was studied, and patients were bifurcated based on their smoking habits, *i.e.,* smokers and non-smokers (Table 5.46). Our results showed that patients who were smokers and carrying heterozygous genotype (*CA*) for $C^{421}A$ polymorphism were less prevalent in both smokers and non-smokers (13.8% and 11%). We noted that smokers having lung carcinoma who had at least one variant allele (*CA)* had reduced survival as compared to the smokers with wild-type genotype (*CC)* for $C^{421}A$ polymorphism (MST=6.7 *vs.* 7.93, HR=1.03, Log-rank p=0.84). On the contrary, non-smokers having heterozygous genotype (*CA)* have improved survival and low death ratio as compared to the wild type genotype (*CC)* (MST=13.03 *vs.* 9.43 months, p=0.09). Nevertheless, we did not find any association between *ABCG2* $C^{421}A$ polymorphism and smokers/non-smokers in predicting survival in lung cancer patients.

As depicted in table 5.46, in the case of $C^{421}A$, 16.9% of patients had single carrier genotype and were detected in stage III lung cancer, while 12.31% of patients harboring heterozygous genotype had stage IV lung cancer. Patients with at least one variant allele (*CA)* in stage III showed marginal lower MST as compared to wild (*CC)* type genotype (MST=8.5 *vs.* 8.8, log-rank p=0.62), though the statistical significance could not be achieved. Also, our results showed poor survival in stage IV lung cancer patients having heterozygous genotype (*CA)* as compared to the wild type genotype (*CC)* (MST=6.46 *vs.* 7.65, Adjusted-HR= 0.93, p=0.78). Therefore, we summarize that *ABCG2* $C^{421}A$ polymorphism is not associated with predicting prognosis in stage III and IV lung cancer patients.

5.19.3 Role of ABCG2 polymorphism on clinical outcomes in lung cancer patients

The association of *ABCG2* $C^{421}A$ and clinicopathological parameters and lung cancer patients were bifurcated based on tumor stage (III and IV), direct tumor extension (T3 and T4), and lymph node involvement (Nx+N0+N1 and N2+N3+N4), and metastasis status (negative and positive). Table 5.47 depicts the genotypic frequencies and correlation of *ABCG2* polymorphism with various clinicopathological parameters. When stage III patients were compared with stage IV individuals, overall *ABCG2* $C^{421}A$ polymorphic site did not show any substantial difference in genotypic frequencies between the two groups. Our results confirmed a lack of risk when the heterozygous genotype of *ABCG2* $C^{421}A$ of stage IV was compared with stage III (AOR=0.7, 95% CI=0.37-1.62, p=0.5).

Further, when the patients were divided based on primary tumor extension (T3 *vs.* T4), a higher prevalence of lung cancer subjects was found in the T4 subgroup (302), while the T3 category had only 92 patients. Our data for $C^{421}A$ did not show any risk when carriers (*GA)* of the T4 subgroup were compared with the T3 subgroup (AOR=1.30, 95% CI=0.52-3.22, p=0.56). Similarly, the *ABCG2* $C^{421}A$ polymorphism was also studied in association with lymph node involvement and metastatic spread of lung cancer. Therefore, we summarize no association among the *ABCG2* $C^{421}A$ polymorphism in lung cancer concerning clinicopathological parameters.

The lung cancer patients were investigated for the response rate to the chemotherapeutic regimen associated with their genotypes for *ABCG2* variant, as depicted in table 5.47. Complete and partial responders (CR+PR) were categorized, whereas progressive and stable disease (PD+SD) were characterized as non-responders. Confounding factors such as age, gender, smoking status, and regimen were used to adjust the odds ratio. Our results depict lack of significant correlation between *ABCG2* $C^{421}A$ variants and clinical response in lung carcinoma patients.

Table 5.47: Association of *ABCG2 SNPs* and clinical outcomes

CLINICAL STAGE				
Genotype rs2231142 ABCG2 (C421A)	**Stage III (162), n (%)**	**Stage IV (292), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
CC	135 (83.3)	257 (88.0)	1.0 (Reference)	-
CA	27 (16.8)	35 (11.9)	0.7 (0.37-1.62)	0.5
TUMOR SIZE				
Genotype rs2231142 ABCG2 (C421A)	**T3 (92), n (%)**	**T4 (302), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
CC	83 (90.2)	258 (85.4)	1.0 (Reference)	-
CA	9 (9.7)	44 (14.5)	1.30 (0.52-3.22)	0.56
LYMPH NODE INVASION				
Genotype rs2231142 ABCG2 (C421A)	**Nx+N0+N1 (108), n (%)**	**N2+N3+N4 (381), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
CC	89 (82.4)	336 (88.1)	1.0 (Reference)	-
CA	19 (17.5)	45 (11.8)	0.89 (0.39-2.02)	0.79
METASTASIS				
Genotype rs2231142 ABCG2 (C421A)	**M0 (215), n (%)**	**M1 (274), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
CC	183 (85.1)	242 (88.3)	1.0 (Reference)	-
CA	32 (14.8)	32 (11.6)	1.01 (0.50-2.05)	0.9
Response of chemotherapy				
Genotype rs2231142 ABCG2 (C421A)	**CR+PR (102), n (%)**	**SD+PD (156), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
CC	86 (84.31)	136 (87.1)	1.0 (Reference)	
CA	16 (15.6)	20 (12.8)	0.82 (0.35-1.92)	0.65

a)-Adjusted Odds ratios, 95% confidence intervals, and corresponding p-values were calculated by logistic regression analysis after adjusting for age, gender, smoking, and regimen. (b)- Two-sided $\chi 2$ test for either genotype distribution or allelic frequencies between different clinical parameters. The number in bold indicates the significant values in the table

5.20. Association of ABC transporter genes (*ABCB1, ABCC1, ABCC2,* and *ABCG2*) and toxicity

The current research's objective was to investigate the onset of toxicity in lung cancer patients who received platinum-based doublet chemotherapy with paclitaxel, pemetrexed, docetaxel, and irinotecan. The toxicity data for 407 of the 502 subjects in the study was available. We used three potential analytic strategies to see how *ABC* variants affected different toxicity levels. In the first analysis, individuals with any toxicity grade (grades 1-4) were compared to those with no toxicity (grade 0); in the second and third assessments, patients with intermediate/severe toxicity (grades 2-4) were compared to those with no/low-grade (grade 0-1) toxicity, and high-grade toxicity (grades 3-4) *vs.* no/low/intermediate toxicity (grades 0-2) to see whether the ABC polymorphisms, *i.e., ABCB1* ($C^{1236}T$, $C^{3435}T$, *and* $G^{2677}T/A$), *ABCC1* ($G^{3173}A$ *and* $G^{2168}A$), *ABCC2* ($G^{4544}A$), *and ABCG2* ($C^{421}A$) were associated to severe toxicity. Cases were then classified into different categories of toxicity in this manner.

5.20.1 Association between ABC polymorphisms and hematological toxicity

All chemotherapy-related adverse events (AEs) were recorded for each treatment cycle. We assessed leukopenia, anemia, thrombocytopenia, and absolute neutropenia in the hematological toxicity. In anemia, among 407 patients, 67.8% of individuals had grade1-2 toxicity, and 7.12% of patients had grade 3-4 toxicity, while 25% showed no toxicity (grade 0). Among 405 patients, 10.8% showed low/intermediate toxicity (grade 1-2) in leukopenia, while 2.22% of patients had high toxicity (grade 3-4). In the case of thrombocytopenia, 13.5% of patients had grade 1-2 toxicity, while only one patient showed grade 3-4 toxicity. Among 366 patients, 6.55% of individuals showed grade 1-2 neutropenia, whereas 2.45% had severe neutropenia (grade 3-4) (Table 5.48).

Table 5.48: Hematological and nephrotoxicity with CTC grades in lung cancer patients

Toxicity	Adverse events	n	Toxicity profiles		
			Grade 0, n (%)	Grade 1-2, n (%)	Grade 3-4, n (%)
Hematological toxicity	Anemia	407	102 (25.0)	276 (67.8)	29 (7.12)
	Leukopenia	405	352 (86.9)	44 (10.8)	9 (2.22)
	Thrombocytopenia	399	344 (86.2)	54 (13.5)	1 (0.25)
	Neutropenia	366	333 (90.9)	24 (6.55)	9 (2.45)
Nephrotoxicity	GFR	397	58 (14.6)	322 (81.1)	17 (4.28)

Upon assessing the effect of different *ABC* polymorphisms, our results showed that 21.2% of patients harbored a single copy of mutant allele (*GA)* for *ABCC1* $G^{3173}A$ polymorphism. Table 5.49a shows that when patients with low/intermediate/severe toxicity (grade 1-4) were compared with no toxicity grade (grade 0), then patients harboring heterozygous *(GA)* genotype had an increased risk of developing leukopenia as compared to the wild type *(GG)* genotype of *ABCC1* $G^{3173}A$ polymorphism (OR=1.88, 95% CI=1.0-3.56, p=**0.04**) (Table 5.49a). However, when adjusting with different covariates such as age, gender, chemotherapy regimen, and performance status, the increased risk of developing leukopenia in heterozygous genotype *(GA)* was consistent; however, substantial differences were not achieved (AOR=1.72, 95% CI=0.75-3.90, p=0.19). None of the other *ABCB1* ($C^{1236}T$, $C^{3435}T$, and $G^{2677}T/A$), *ABCC1* ($G^{2168}A$), *ABCC2* ($G^{4544}A$), and *ABCG2* ($C^{421}A$) were associated with the risk of developing leukopenia.

Table 5.49 (a): Leukopenia-Haematological toxicity (grade 0 *vs.* 1-4, grade 0-1 *vs.* 2-4, and grade 0-2 *vs.* 3-4) associated with *ABCB1, ABCC1, ABCC2*, and *ABCG2* polymorphism

Leukopenia												
Genotype rs1128503 ABCB1 (C1236T)	**Grade of toxicity 0, n(%) N=352**	**Grade of toxicity 1-4, n(%) N=53**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs41410450 ABCC1 (G3173A)**	**Grade of toxicity 0, n(%) N=352**	**Grade of toxicity 1-4, n(%) N=53**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	196(55.6)	27(50.9)	1.00 (Reference)		1.00 (Reference)		GG	276(78.4)	36(67.9)	1.00 (Reference)	-	1.00 (Reference)	-
CT	101(28.6)	20(37.7)	1.43(0.78-2.68)	0.2	0.96(0.4-2.13)	0.9	GA	69(19.6)	17(32.0)	1.88(1.0-3.56)	**0.04**	1.72(0.75-3.90)	0.19
TT	55((15.6)	6(11.3)	0.79(0.31-2.01)	0.6	0.31(0.08-1.14)	0.07	AA	7(1.98)	0(0)	-	-	-	-
CT+TT	156(44.3)	26(49.0)	1.20(0.67-2.15)	0.5	0.6(0.33-1.42)	0.31	GA+AA	76(21.59)	17(32.0)	1.71(0.91-3.22)	0.09	1.61(0.71-3.62)	0.25
Genotype rs1045642 ABCB1 (C3435T)	**Grade of toxicity 0, n(%) N=352**	**Grade of toxicity 1-4, n(%) N=53**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs4148356 ABCC1 (G2168A)**	**Grade of toxicity 0, n(%) N=352**	**Grade of toxicity 1-4, n(%) N=53**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	65(18.4)	8(15.0)	1.00 (Reference)	-	1.00 (Reference)	-							
CT	143(40.6)	27(50.9)	1.53(0.66-3.55)	0.3	1.94(0.70-5.39)	0.2	GG	339(96.3)	51(96.2)	1.00 (Reference)		1.00 (Reference)	
TT	144(40.9)	18(33.9)	1.01(0.42-2.4)	0.9	1.49(0.52-4.29)	0.4	GA	13(3.6)	2(3.7)	1.02(0.22-4.66)	0.9	0.67(0.08-5.68)	0.71
CT+TT	287(81.5)	45(84.9)	1.27(0.57-2.83)	0.5	1.66(0.63-4.32)	0.29							
Genotype rs2032582 ABCB1 (G2677A)	**Grade of toxicity 0, n(%) N=352**	**Grade of toxicity 1-4, n(%) N=53**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs8187710 ABCC2 (G4544A)**	**Grade of toxicity 0, n(%) N=352**	**Grade of toxicity 1-4, n(%) N=53**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GG	318(90.3)	46(86.7)	1.00 (Reference)	-	1.00 (Reference)	-	GG	341(96.8)	51(96.2)	1.00 (Reference)		1.00 (Reference)	
GA	34(9.65)	7(13.2)	1.42(0.59-3.39)	0.42	1.83(0.65-5.12)	0.24	GA	11(3.1)	2(3.7)	1.21(0.26-5.64)	0.8	1.86(0.33-10.2)	0.47
Genotype rs2032582 ABCB1 (G2677T)	**Grade of toxicity 0, n(%) N=352**	**Grade of toxicity 1-4, n(%) N=53**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs2231142 ABCG2 (C421A)**	**Grade of toxicity 0, n(%) N=352**	**Grade of toxicity 1-4, n(%) N=53**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GT	176(50)	32(60.3)	1.00 (Reference)	-	1.00 (Reference)	-	CC	300(85.2)	48(90.5)	1.00 (Reference)		1.00 (Reference)	
TT	176(50)	21(39.6)	0.65(0.36-1.18)	0.16	0.82(0.40-1.67)	0.58	CA	52(14.7)	5(9.4)	0.60(0.22-1.58)	0.3	0.82(0.28-2.35)	0.71
Genotype rs1128503 ABCB1 (C1236T)	**Grade of toxicity 0-1, n(%) N=389**	**Grade of toxicity 2-4, n(%) N=16**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs2032582 ABCB1 (G2677T)**	**Grade of toxicity 0-1, n(%) N=389**	**Grade of toxicity 2-4, n(%) N=16**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	217(55.7)	6(37.5)	1.00 (Reference)		1.00 (Reference)								
CT	115(29.5)	6(37.5)	1.88(0.59-5.98)	0.2	1.13(0.25-4.94)	0.8	GT	198(50.8)	10(62.5)	1.00 (Reference)		1.00 (Reference)	
TT	57(14.6)	4(25)	2.53(0.69-9.29)	0.15	1.15(0.20-6.66)	0.8	TT	191(49.1)	6(37.5)	0.62(0.22-1.74)	0.36	1.27(0.34-4.67)	0.71
CT+TT	172(44.2)	10(62.5)	2.10(0.74-5.9)	1.16	0.32-4.22)	0.8							
Genotype rs1045642 ABCB1 (C3435T)	**Grade of toxicity 0-1, n(%) N=389**	**Grade of toxicity 2-4, n(%) N=16**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs8187710 ABCC2 (G4544A)**	**Grade of toxicity 0-1, n(%) N=389**	**Grade of toxicity 2-4, n(%) N=16**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	71(18.2)	2(12.5)	1.00 (Reference)		1.00 (Reference)		GG	377(96.9)	15(93.7)	1.00 (Reference)		1.00 (Reference)	
CT	163(41.9)	7(43.7)	1.52(0.30-7.52)	0.6	1.03(0.14-7.40)	0.9	GA	12(3.0)	1(6.2)	2.09(0.25-17.1)	0.4	4.43(0.43-45.6)	0.21
TT	155(39.8)	7(43.7)	1.60(0.32-7.91)	0.5	2.04(0.33-12.2)	0.4							

CT+TT	318(81.7)	14(87.5)	1.56(0.34-7.03)	0.56	1.28(0.25-6.50)	0.76							
Genotype rs2032582 ABCB1 (G2677A)	**Grade of toxicity 0-1, n(%) N=389**	**Grade of toxicity 2-4, n(%) N=16**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs2231142 ABCG2 (C421A)**	**Grade of toxicity 0-1, n(%) N=389**	**Grade of toxicity 2-4, n(%) N=16**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GG	350(89.9)	14(87.5)	1.00 (Reference)	-	1.00 (Reference)		CC	55(14.1)	2(12.5)	1.00 (Reference)		1.00 (Reference)	
GA	39(10.0)	2(12.5)	1.28(0.28-5.8)	0.7	1.98(0.37-10.5)	0.42	CA	334(85.8)	14(87.5)	0.86(0.19-3.92)	0.8	1.63(0.31-8.46)	0.55

OR[1] Crude odds ratio, 95% CI: 95% confidence interval, **AOR[2]**Adjusted Odds ratio data were calculated by unconditional logistic regression and adjusted for age, gender, performance status, and regimen. A p-value < 0.05 was considered statistically significant

A total of 407 patients' toxicity data was available in the case of anemia (Table 5.49 (b)). 25.1% of individuals were classified in grade 0 toxicity, and 74.9% of patients were categorized in low/intermediate/severe toxicity (grade 1-4) (table 5.49 b). As observed for *ABCC1* $G^{3173}A$ polymorphism, 18.6% of patients harbored single copy mutant allele (*GA)*. Individuals with the heterozygous (*GA)* genotype had an increased risk of developing anemia (OR=1.24, 95% CI=0.70-2.19; p=0.45) when compared to the patients with a wild type genotype (*GG)*. After adjusting with different confounding, factors like gender, age, performance status, and regimen, 2.7 folds increased risk was observed in heterozygous genotype (*GA)* as compared to wild-type genotype (*GG)* (AOR=2.70, 95% CI=1.06-6.88; p=**0.03**). In the case of the dominant model, increased risk in combined genotype (*GA+AA)* for *ABCC1* $G^{3173}A$ polymorphism was noted with the odds of 2.85 as compared to the wild type (*GG)* (AOR=2.85, 95% CI=1.12-7.23, p=**0.02**). For *ABCC2* $G^{4544}A$ polymorphism, when individuals with severe toxicity (grades 3-4) were compared to no/low-grade (grade 0-2) toxicity, our results showed that 7.1% of individuals had grade 3-4 anemia. Individuals harboring one copy of the mutant (*GA)* allele showed an increased risk of developing anemia (OR=4.24, 95% CI=1.10-16.38; p=**0.03**) as compared to patients having both copies of the wild type allele (*GG)*. When adjusted with various confounding factors such as age, regimen, performance status, and gender, the heterozygous (*GA)* genotype showed a 5.63-fold increased risk of developing anemia as compared to the wild type (*GG)* genotype (AOR=5.63, 95% CI=1.13-28.04, p=**0.03**). However, we did not find any correlation between the *ABC* polymorphisms and other hematological toxicities such as neutropenia (table 5.49 c) and thrombocytopenia (Table 5.49 d).

Table 5.49(b): Anaemia-Haematological toxicity (grade 0 *vs.* 1-4, grade 0-1 *vs.* 2-4, and grade 0-2 *vs.* 3-4) associated with *ABCB1, ABCC1, ABCC2*, and *ABCG2* polymorphism

Anaemia												
Genotype rs1128503 ABCB1 (C1236T)	**Grade of toxicity 0, n (%) N=102**	**Grade of toxicity 1-4, n (%) N=305**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**	**Genotype rs41410450 ABCC1 (G3173A)**	**Grade of toxicity 0, n (%) N=102**	**Grade of toxicity 1-4, n (%) N=305**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
CC	56 (54.9)	169 (55.4)	1.00 (Reference)		1.00 (Reference)		GG	83 (81.3)	232 (76.0)	1.00 (Reference)	-	1.00 (Reference)	
CT	31 (30.3)	90 (29.5)	0.96 (0.57-1.59)	0.8	1.37 (0.65-2.85)	0.39	GA	19 (18.6)	66 (21.6)	1.24 (0.70-2.19)	0.45	2.70 (1.06-6.88)	**0.03**
TT	15 (14.7)	46 (15.0)	1.01 (0.52-1.95)	0.9	0.91 (0.39-2.09)	0.8	AA	0 (0)	7 (2.2)	-	-	-	-
CT+TT	46 (45)	136 (44.5)	0.97 (0.62-1.53)	0.9	1.15 (0.62-2.13)	0.64	GA+AA	19 (18.6)	73 (23.9)	1.37 (0.78-2.41)	0.26	2.85 (1.12-7.23)	**0.02**
Genotype rs1045642 ABCB1(C3435 T)	**Grade of toxicity 0, n (%) N=102**	**Grade of toxicity 1-4, n (%) N=305**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**	**Genotype rs4148356 ABCC1 (G2168A)**	**Grade of toxicity 0, n (%) N=102**	**Grade of toxicity 1-4, n (%) N=305**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
CC	15 (14.7)	59 (19.3)	1.00 (Reference)		1.00 (Reference)								
CT	47 (46.0)	122 (40)	0.65 (0.34-1.27)	0.21	0.89 (0.39-2.0)	0.78	GG	98 (96.0)	294 (96.3)	1.00 (Reference)		1.00 (Reference)	
TT	40 (39.2)	124 (40.6)	0.78 (0.40-1.53)	0.48	1.41 (0.603.30)	0.41	GA	4 (3.9)	11 (3.6)	0.91 (0.28-2.94)	0.8	0.61 (0.14-2.53)	0.5
CT+TT	87 (85.2)	246 (80.6)	0.71 (0.38-1.33)	0.29	1.12 (0.53-2.36)	0.75							
Genotype rs2032582 ABCB1 (G2677A)	**Grade of toxicity 0, n (%) N=102**	**Grade of toxicity 1-4, n (%) N=305**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**	**Genotype rs8187710 ABCC2 (G4544A)**	**Grade of toxicity 0, n (%) N=102**	**Grade of toxicity 1-4, n (%) N=305**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
GG	95 (93.1)	270 (88.5)	1.00 (Reference)		1.00 (Reference)		GG	100 (98.0)	291 (95.4)	1.00 (Reference)		1.00 (Reference)	
GA	7 (6.8)	35 (11.4)	1.75 (0.75-4.09)	0.18	2.59 (0.73-9.12)	0.13	GA	2 (1.9)	11 (3.6)	1.89 (0.41-8.67)	0.41	2.57 (0.29-22.3)	0.38
Genotype rs2032582 ABCB1(G2677 T)	**Grade of toxicity 0, n (%) N=102**	**Grade of toxicity 1-4, n (%) N=305**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**	**Genotype rs2231142 ABCG2 (C421A)**	**Grade of toxicity 0, n (%) N=102**	**Grade of toxicity 1-4, n (%) N=305**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
GT	48 (47.0)	159 (52.1)	1.00 (Reference)		1.00 (Reference)	-	CC	14 (13.7)	44 (14.4)	1.00 (Reference)		1.00 (Reference)	
TT	54 (52.9)	146 (47.8)	0.81 (0.52-1.27)	0.37	1.22 (0.66-2.27)	0.51	CA	88 (86.2)	261 (85.5)	1.05 (0.55-2.02)	0.8	1.56 (0.60-4.05)	0.35
Genotype rs1128503 ABCB1 (C1236T)	**Grade of toxicity 0-1, n (%) N=247**	**Grade of toxicity 2-4, n (%) N=160**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**	**Genotype rs41410450 ABCC1 (G3173A)**	**Grade of toxicity 0-1, n (%) N=247**	**Grade of toxicity 2-4, n (%) N=160**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
CC	140 (56.6)	85 (53.1)	1.00 (Reference)		1.00 (Reference)		GG	190 (76.9)	125 (78.1)	1.00 (Reference)		1.00 (Reference)	
CT	72 (29.1)	49 (30.6)	1.12 (0.71-1.76)	0.62	1.25 (0.70-2.23)	0.43	GA	53 (21.4)	32 (20)	0.91 (0.56-1.50)	0.7	0.73 (0.39-1.39)	0.34
TT	35 (14.1)	26 (16.2)	1.22 (0.68-2.17)	0.49	1.12 (0.55-2.28)	0.74	AA	4 (1.6)	3 (1.8)	1.14 (0.25-2.18)	0.8	3.7 (0.38-37.0)	0.25
CT+TT	107 (43.3)	75 (46.8)	1.15 (0.77-1.72)	0.48	1.20 (0.73-1.98)	0.46	GA+AA	57 (23.0)	35 (21.8)	0.93 (0.57-1.50)	0.7	0.78 (0.42-1.45)	0.43
Genotype rs1045642 ABCB1(C3435 T)	**Grade of toxicity 0-1, n (%) N=247**	**Grade of toxicity 2-4, n (%) N=160**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**	**Genotype rs4148356 ABCC1 (G2168A)**	**Grade of toxicity 0-1, n (%) N=247**	**Grade of toxicity 2-4, n (%) N=160**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
CC	44 (17.8)	30 (18.7)	1.00 (Reference)		1.00 (Reference)								

CT	108 (43.7)	61 (38.1)	0.82 (0.47-1.45)	0.51	0.76 (0.38-1.48)	0.42	GG	236 (95.5)	156 (97.5)	1.00 (Reference)		1.00 (Reference)	
TT	95 (38.4)	69 (43.1)	1.06 (0.60-1.86)	0.82	0.98 (0.50-1.92)	0.9	GA	11 (4.4)	4 (2.5)	0.55 (0.17-1.75)	0.55	0.45 (0.11-1.77)	0.25
CT+TT	203 (82.1)	130 (81.2)	0.93 (0.56-1.56)	0.81	0.87 (0.48-1.61)	0.67							
Genotype rs2032582 ABCB1(G2677 A)	**Grade of toxicity 0-1, n (%) N=247**	**Grade of toxicity 2-4, n (%) N=160**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**	**Genotype rs8187710 ABCC2 (G4544A)**	**Grade of toxicity 0-1, n (%) N=247**	**Grade of toxicity 2-4, n (%) N=160**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
GG	26 (10.5)	16 (10)	1.00 (Reference)		1.00 (Reference)		GG	242 (97.9)	152 (95)	1.00(Reference)		1.00(Reference)	
GA	221 (89.4)	144 (90)	0.94 (0.48-1.82)	0.8	1.16 (0.52-2.55)	0.7	GA	5 (2.0)	8 (5)	2.54 (0.81-7.93)	0.10	4.04 (0.8-20.83)	0.09
Genotype rs2032582 ABCB1(G2677 T)	**Grade of toxicity 0-1, n (%) N=247**	**Grade of toxicity 2-4, n (%) N=160**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**	**Genotype rs2231142 ABCG2 (C421A)**	**Grade of toxicity 0-1, n (%) N=247**	**Grade of toxicity 2-4, n (%) N=160**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
GT	127 (51.4)	80 (50)	1.00 (Reference)		1.00 (Reference)		CC	34 (13.7)	24 (15)	1.00 (Reference)		1.00 (Reference)	
TT	120 (48.5)	80 (50)	1.05 (0.71-1.57)	0.7	1.22 (0.67-1.85)	0.65	CA	213 (86.2)	136 (85)	1.10 (0.62-1.94)	0.72	1.12 (0.55-2.27)	0.7
Genotype rs1128503 ABCB1 (C1236T)	**Grade of toxicity 0-2, n (%) N=378**	**Grade of toxicity 3-4, n (%) N=29**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**	**Genotype rs2032582 ABCB1 (G2677T)**	**Grade of toxicity 0-2, n (%) N=378**	**Grade of toxicity 3-4, n (%) N=29**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
CC	210 (55.5)	15 (51.7)	1.00 (Reference)		1.00 (Reference)								
CT	112 (29.6)	9 (31.0)	1.12 (0.47-2.65)	0.78	0.91 (0.31-2.62)	0.87	GT	191 (50.5)	16 (55.1)	1.00 (Reference)	-	1.00 (Reference)	-
TT	56 (14.8)	5 (17.2)	1.25 (0.43-3.58)	0.67	0.87 (0.22-3.37)	0.84	TT	187 (49.4)	13 (44.8)	0.82 (0.38-1.77)	0.63	0.65 (0.25-1.69)	0.38
CT+TT	168 (44.4)	14 (48.2)	1.16 (0.54-2.48)	0.6	0.91 (0.35-2.30)	0.8							
Genotype rs1045642 ABCB1(C3435 T)	**Grade of toxicity 0-2, n (%) N=378**	**Grade of toxicity 3-4, n (%) N=29**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**	**Genotype rs41410450 ABCC1 (G3173A)**	**Grade of toxicity 0-2, n (%) N=378**	**Grade of toxicity 3-4, n (%) N=29**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
CC	68 (17.9)	6 (20.6)	1.00 (Reference)		1.00 (Reference)		GG	290 (76.7)	25 (86.2)	1.00 (Reference)	-	1.00 (Reference)	-
CT	161 (42.5)	8 (27.5)	0.56 (0.18-1.68)	0.30	0.98 (0.26-3.6)	0.9	GA	81 (21.4)	4 (13.7)	0.57 (0.19-1.69)	0.31	0.20 (0.02-1.57)	0.12
TT	149 (39.4)	15 (51.7)	1.14 (0.42-3.06)	0.79	1.47 (0.4-5.30)	0.5	AA	7 (1.8)	0 (0)	-	-	-	-
CT+TT	310 (82.0)	23 (79.3)	0.84 (0.32-2.14)	0.71	1.18 (0.37-3.77)	0.7	GA+AA	88 (23.2)	4 (13.7)	0.52 (0.17-1.55)	0.24	0.18 (0.02-1.44)	0.10
Genotype rs2032582 ABCB1 (G2677A)	**Grade of toxicity 0-2, n (%) N=378**	**Grade of toxicity 3-4, n (%) N=29**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**	**Genotype rs8187710 ABCC2 (G4544A)**	**Grade of toxicity 0-2, n (%) N=378**	**Grade of toxicity 3-4, n (%) N=29**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
GG	39 (10.3)	3 (10.3)	1.00 (Reference)		1.00 (Reference)		GG	368 (97.3)	26 (89.6)	1.00 (Reference)		1.00 (Reference)	
GA	339 (89.6)	26 (89.6)	1.03 (0.29-3.46)	0.9	1.06 (0.27-4.09)	0.9	GA	10 (2.6)	3 (10.3)	4.24 (1.1-16.38)	**0.03**	5.63 (1.1-28.04)	**0.03**

Genotype rs2231142 ABCG2(C421A)	Grade of toxicity 0-2, n (%) N=378	Grade of toxicity 3-4, n (%) N=29	OR[1] (95% CI)	P	AOR[2] (95% CI)	P
CC	54 (14.2)	4 (13.7)	1.00 (Reference)		1.00 (Reference)	
CA	324 (85.7)	25 (86.2)	0.96 (0.322.86)	0.9	1.67(0.50-5.56)	0.39

OR[1] Crude odds ratio, 95% CI: 95% confidence interval, **AOR[2]**Adjusted Odds ratio data were calculated by unconditional logistic regression and adjusted for age, gender, performance status, and regimen. A p-value < 0.05 was considered statistically significant

Table 5.49 (c): Neutropenia-Haematological toxicity (grade 0 *vs.* 1-4, grade 0-1 *vs.* 2-4) associated with *ABCB1, ABCC1, ABCC2,* and *ABCG2* polymorphism

Neutropenia												
Genotype rs1128503 ABCB1 (C1236T)	**Grade of toxicity 0, n(%) N=333**	**Grade of toxicity 1-4, n(%) N=33**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs41410450 ABCC1 (G3173A)**	**Grade of toxicity 0, n(%) N=333**	**Grade of toxicity 1-4, n(%) N=33**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	180(54.0)	19(57.5)	1.00 (Reference)		1.00 (Reference)		GG	262(78.6)	25(75.7)	1.00 (Reference)	-	1.00 (Reference)	
CT	101(30.3)	10(30.3)	0.93(0.42-2.09)	0.8	0.55(0.18-1.62)	0.28	GA	65(19.5)	8(24.2)	1.28(0.55-2.99)	0.55	1.13(0.38-3.34)	0.8
TT	52(15.6)	4(12.1)	0.72(0.23-2.23)	0.58	0.33(0.06-1.57)	0.16	AA	6(1.8)	0(0)	-	-	-	-
CT+TT	153(45.9)	14(42.4)	0.86(0.42-1.78)	0.69	0.46(0.17-1.20)	0.11	GA+AA	71(21.3)	8(24.2)	1.18(0.51-2.73)	0.69	1.06(0.36-3.11)	0.9
Genotype rs1045642 ABCB1 (C3435T)	**Grade of toxicity 0, n(%) N=333**	**Grade of toxicity 1-4, n(%) N=33**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs4148356 ABCC1 (G2168A)**	**Grade of toxicity 0, n(%) N=333**	**Grade of toxicity 1-4, n(%) N=33**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	62(18.6)	4(12.1)	1.00 (Reference)		1.00 (Reference)								
CT	144(43.2)	13(39.3)	1.39(0.43-4.46)	0.57	0.92(0.24-3.44)	0.9	GG	321(96.3)	31(93.9)	1.00 (Reference)		1.00 (Reference)	
TT	127(38.1)	16(48.4)	1.95(0.62-6.08)	0.24	1.86(0.52-6.59)	0.33	GA	12(3.6)	2(6.0)	1.72(0.36-8.06)	0.48	1.27(0.14-10.8)	0.8
CT+TT	271(81.3)	29(87.8)	1.65(0.56-4.89)	0.35	1.24(0.39-3.98)	0.7							
Genotype rs2032582 ABCB1 (G2677A)	**Grade of toxicity 0, n(%) N=333**	**Grade of toxicity 1-4, n(%) N=33**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs8187710 ABCC2(G 4544A)**	**Grade of toxicity 0, n(%) N=333**	**Grade of toxicity 1-4, n(%) N=33**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GG	33(9.9)	3(9.0)	1.00 (Reference)		1.00 (Reference)		GG	324(97.2)	32(96.9)	1.00 (Reference)	-	1.00 (Reference)	-
GA	300(90.0)	30(90.9)	0.90(0.26-3.14)	0.8	0.91(0.19-4.26)	0.9	GA	9(2.7)	1(3.0)	1.12(0.13-9.16)	0.9	1.62(0.17-15.11)	0.66
Genotype rs2032582 ABCB1 (G2677T)	**Grade of toxicity 0, n(%) N=333**	**Grade of toxicity 1-4, n(%) N=33**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs2231142 ABCG2 (C421A)**	**Grade of toxicity 0, n(%) N=333**	**Grade of toxicity 1-4, n(%) N=33**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GT	172(51.6)	19(57.5)	1.00 (Reference)		1.00 (Reference)	-	CC	285(85.5)	28(84.8)	1.00 (Reference)		1.00 (Reference)	
TT	161(48.3)	14(42.4)	0.78(0.38-1.62)	0.51	1.14(0.46-2.81)	0.76	CA	48(14.4)	5(15.1)	1.06(0.39-2.88)	0.9	0.96(0.26-3.54)	0.9
Genotype rs1128503 ABCB1 (C1236T)	**Grade of toxicity 0-1, n(%) N=349**	**Grade of toxicity 2-4, n(%) N=17**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs41410450 ABCC1 (G3173A)**	**Grade of toxicity 0-1, n(%) N=349**	**Grade of toxicity 2-4, n(%) N=17**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	189(54.1)	10(58.8)	1.00 (Reference)		1.00 (Reference)		GG	274(78.5)	13(76.4)	1.00 (Reference)		1.00 (Reference)	
CT	107(30.6)	4(23.5)	0.70(0.21-2.30)	0.56	0.52(0.13-2.07)	0.36	GA	69(19.7)	4(23.5)	1.22(0.38-3.86)	0.73	0.66(0.13-3.20)	0.61
TT	53(15.1)	3(17.6)	1.06(0.28-4.02)	0.9	0.57(0.11-2.92)	0.5	AA	6(1.7)	0(0)	-	-	-	-
CT+TT	160(45.8)	7(41.1)	1.23(0.45-3.34)	0.67	0.55(0.17-1.73)	0.30	GA+AA	75(21.4)	4(23.5)	1.12(0.35-3.54)	0.84	0.63(0.13-3.03)	0.56
Genotype rs1045642 ABCB1 (C3435T)	**Grade of toxicity 0-1, n(%) N=349**	**Grade of toxicity 2-4, n(%) N=17**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs8187710 ABCC2 (G4544A)**	**Grade of toxicity 0-1, n(%) N=349**	**Grade of toxicity 2-4, n(%) N=17**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	63(18.0)	3(17.6)	1.00 (Reference)		1.00 (Reference)		GG	340(97.4)	16(94.1)	1.00 (Reference)		1.00 (Reference)	
CT	152(43.5)	5(29.4)	0.69(0.16-2.97)	0.61	0.64(0.12-3.29)	0.59	GA	9(2.5)	1(5.8)	2.36(0.28-19.78)	0.42	2.53(0.26-24.65)	0.42
TT	134(38.3)	9(52.9)	1.41(0.39-5.38)	0.6	1.53(0.35-6.65)	0.56							

CT+TT	286(81.9)	14(82.3)	1.02(0.28-3.68)	0.9	0.96(0.25-3.70)	0.9							
Genotype rs2032582 ABCB1 (G2677A)	**Grade of toxicity 0-1, n(%) N=349**	**Grade of toxicity 2-4, n(%) N=17**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs2231142 ABCG2 (C421A)**	**Grade of toxicity 0-1, n(%) N=349**	**Grade of toxicity 2-4, n(%) N=17**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GG	315(90.2)	15(88.2)	1.00 (Reference)	-	1.00 (Reference)		CC	298(85.3)	15(88.2)	1.00 (Reference)		1.00 (Reference)	
GA	34(9.7)	2(11.7)	1.23(0.27-5.63)	0.78	1.41(0.28-7.13)	0.67	CA	51(14.6)	2(11.7)	0.77(0.17-3.50)	0.7	0.94(0.19-4.57)	0.9

Genotype rs2032582 ABCB1 (G2677T)	**Grade of toxicity 0-1, n(%) N=349**	**Grade of toxicity 2-4, n(%) N=17**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GT	183(52.4)	8(47.0)	1.00 (Reference)	-	1.00 (Reference)	
TT	166(47.5)	9(52.9)	1.24(0.46-3.28)	0.66	1.63(0.53-5.0)	0.39

OR[1] Crude odds ratio, 95% CI: 95% confidence interval, **AOR[2]**Adjusted Odds ratio data were calculated by unconditional logistic regression and adjusted for age, gender, performance status, and regimen. A p-value < 0.05 was considered statistically significant

Table 5.49 (d): Thrombocytopenia-Haematological toxicity (grade 0 *vs.* 1-4) associated with *ABCB1, ABCC1, ABCC2*, and *ABCG2* polymorphism

					Thrombocytopenia							
Genotype rs1128503 ABCB1 (C1236T)	**Grade of toxicity 0, n(%) N=344**	**Grade of toxicity 1-4, n(%) N=55**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs41410450 ABCC1 (G3173A)**	**Grade of toxicity 0, n(%) N=344**	**Grade of toxicity 1-4, n(%) N=55**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	190(55.2)	31(56.3)	1.00 (Reference)		1.00 (Reference)		GG	263(76.4)	43(78.1)	1.00 (Reference)	-	1.00 (Reference)	
CT	100(29.0)	18(32.7)	1.10(0.58-2.06)	0.7	1.18(0.54-2.55)	0.66	GA	74(21.5)	12(21.8)	0.99(0.49-1.97)	0.9	1.29(0.55-2.98)	0.55
TT	54(15.6)	6(10.9)	0.68(0.27-1.71)	0.41	0.45(0.12-1.60)	0.21	AA	7(2.0)	0(0)	-	-	-	-
CT+TT			0.95(0.53-1.69)	0.87	0.89(0.43-1.81)	0.75	GA+AA	81(23.5)	12(21.8)	0.90(0.45-1.80)	0.7	1.20(0.52-2.76)	0.6
Genotype rs1045642 ABCB1 (C3435T)	**Grade of toxicity 0, n(%) N=344**	**Grade of toxicity 1-4, n(%) N=55**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs4148356 ABCC1 (G2168A)**	**Grade of toxicity 0, n(%) N=344**	**Grade of toxicity 1-4, n(%) N=55**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	63(18.3)	9(16.3)	1.00 (Reference)		1.00 (Reference)		GG	331(96.2)	54(98.1)	1.00 (Reference)		1.00 (Reference)	
CT	139(40.4)	27(49.0)	1.35(0.60-3.05)	0.45	1.45(0.55-3.80)	0.44	GA	13(3.7)	1(1.8)	0.47(0.06-3.67)	0.47	0.58(0.07-4.84)	0.6
TT	142(41.2)	19(34.5)	0.93(0.40-2.18)	0.87	1.17(0.40-3.38)	0.7							
CT+TT	281(81.6)	46(83.6)	1.14(0.53-2.46)	0.72	1.23(0.50-3.03)	0.64							
Genotype rs2032582 ABCB1 (G2677A)	**Grade of toxicity 0, n(%) N=344**	**Grade of toxicity 1-4, n(%) N=55**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs8187710 ABCC2 (G4544A)**	**Grade of toxicity 0, n(%) N=344**	**Grade of toxicity 1-4, n(%) N=55**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GG	307(89.2)	51(92.7)	1.00 (Reference)		1.00 (Reference)		GG	332(96.5)	54(98.1)	1.00 (Reference)	-	1.00 (Reference)	-
GA	37(10.7)	4(7.2)	0.65(0.22-1.90)	0.43	0.39(0.08-1.78)	0.22	GA	12(3.4)	1(1.8)	0.51(0.06-4.02)	0.52	-	-
Genotype rs2032582 ABCB1 (G2677T)	**Grade of toxicity 0, n(%) N=344**	**Grade of toxicity 1-4, n(%) N=55**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs2231142 ABCG2 (C421A)**	**Grade of toxicity 0, n(%) N=344**	**Grade of toxicity 1-4, n(%) N=55**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GT	176(51.1)	29(52.7)	1.00 (Reference)		1.00 (Reference)	-	CC	295(85.7)	47(85.4)	1.00 (Reference)		1.00 (Reference)	
TT	168(48.8)	26(47.2)	0.93(0.53-1.66)	0.8	1.0(0.492.05)	0.9	CA	49(14.2)	8(14.5)	1.02(0.45-2.29)	0.9	1.32(0.52-3.33)	0.54

OR[1] Crude odds ratio, 95% CI: 95% confidence interval, **AOR[2]**Adjusted Odds ratio data were calculated by unconditional logistic regression and adjusted for age, gender, performance status, and regimen. A p-value < 0.05 was considered statistically significant

5.20.2 Association between ABC polymorphisms and nephrological toxicity

Chemotherapeutic drugs are excreted through the kidneys and are nephrotoxic; thus, renal functions are monitored during chemotherapy. Data of 397 patients was available to evaluate the nephrotoxicity. Among 397 patients, 81.1% were categorized as low/intermediate toxicity, whereas 4.28% of patients were classified in severe toxicity grade, and the other 14.6% showed no nephrotoxicity (Table 5.50). We have evaluated chemotherapy-induced nephrotoxicity by comparing intermediate/severe toxicity (grades 3-5) versus no/low grade (grades 1-2) toxicity. We compared low/intermediate/severe (grades 2-5) toxicity to no grade (grades 1) toxicity in chemotherapy-induced nephrotoxicity. As illustrated in table 5.50, lung cancer patients with heterozygous (*GA)* genotype for *ABCB1* $G^{2677}A$ polymorphism showed a lower incidence of developing nephrotoxicity when compared to individuals with wild-type genotype *(GG)* (OR=0.37, 95% CI=0.17-0.77; p=**0.008**). For *ABCC1* $G^{3173}A$ polymorphism, 85.3% of patients were categorized as low/intermediate and severe toxicity, whereas 14.6% showed no renal toxicity. In the dominant model, the combined heterozygous and mutant genotype (*GA+AA)* were associated with a reduced risk of nephrotoxicity with the odds of 0.54 as compared to the wild-type genotype (*GG)* (OR=0.54, 95% CI=0.29-0.99, p=**0.04**). We did not find any correlation between the A*BCB1* ($C^{1236}T$, $C^{3435}T$, *and* $G^{2677}T$), *ABCC1* ($G^{2168}A$), *ABCC2* ($G^{4544}A$), and *ABCG2* ($C^{421}A$) polymorphisms and nephrotoxicity related adverse events due to chemotherapy **(**Table 5.50).

Table 5.50: Nephrological toxicity (grade 0 *vs.* 1-4 and grade 0-1 *vs.* 2-4) associated with *ABCB1, ABCC1, ABCC2,* and *ABCG2* polymorphism

Nephrotoxicity												
Genotype rs1128503 ABCB1 (C1236T)	**Grade of toxicity 1-2, n(%) N=204**	**Grade of toxicity 3-5, n(%) N=193**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs41410450 ABCC1 (G3173A)**	**Grade of toxicity 1-2, n(%) N=204**	**Grade of toxicity 3-5, n(%) N=193**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	109(53.4)	106(54.9)	1.00 (Reference)	-	1.00 (Reference)	-	GG	158(77.4)	149(77.2)	1.00 (Reference)		1.00 (Reference)	
CT	66(32.3)	55(28.4)	0.85(0.54-1.33)	0.49	0.72(0.38-1.35)	0.3	GA	40(19.6)	43(22.2)	1.13(0.70-1.85)	0.59	1.18(0.6-2.31)	0.62
TT	29(14.2)	32(16.5)	1.13(0.64-2.0)	0.6	0.98(0.45-2.13)	0.96	AA	6(2.9)	1(0.5)	0.17(0.02-1.48)	0.11	0.30(0.02-4.06)	0.37
CT+TT	95(46.5)	87(45.0)	0.94(0.63-1.39)	0.76	0.80(0.46-1.38)	0.43	GA+AA	46(22.5)	44(22.7)	1.01(0.63-1.62)	0.9	1.12(0.58-2.18)	0.72
Genotype rs1045642 ABCB1 (C3435T)	**Grade of toxicity 1-2, n(%) N=204**	**Grade of toxicity 3-5, n(%) N=193**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs4148356 ABCC1 (G2168A)**	**Grade of toxicity 1-2, n(%) N=204**	**Grade of toxicity 3-5, n(%) N=193**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	37(18.1)	36(18.6)	1.00 (Reference)		1.00 (Reference)		GG	198(97.0)	184(95.3)	1.00 (Reference)	-	1.00 (Reference)	-
CT	79(38.7)	87(45.0)	1.13(0.62-1.96)	0.65	1.18(0.55-2.54)	0.65	GA	6(2.9)	9(4.6)	1.61(0.56-4.62)	0.37	1.62(0.37-6.93)	0.51
TT	88(43.1)	70(36.2)	0.81(0.46-1.42)	0.47	0.77(0.37-1.57)	0.47							
CT+TT	167(81.8)	157(81.3)	0.96(0.58-1.60)	0.89	0.95(0.48-1.85)	0.88							
Genotype rs2032582 ABCB1 (G2677A)	**Grade of toxicity 1-2, n(%) N=204**	**Grade of toxicity 3-5, n(%) N=193**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs8187710 ABCC2(G 4544A)**	**Grade of toxicity 1-2, n(%) N=204**	**Grade of toxicity 3-5, n(%) N=193**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GG	182(89.2)	173(89.6)	1.00 (Reference)	-	1.00 (Reference)		GG	197(96.5)	188(97.4)	1.00 (Reference)	-	1.00 (Reference)	-
GA	22(10.7)	20(10.3)	0.95(0.50-1.81)	0.8	1.04(0.44-2.43)	0.9	GA	7(3.4)	5(2.5)	0.74(0.23-2.39)	0.62	0.90(0.17-4.62)	0.9
Genotype rs2032582 ABCB1 (G2677T)	**Grade of toxicity 1-2, n(%) N=204**	**Grade of toxicity 3-5, n(%) N=193**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs2231142 ABCG2 (C421A)**	**Grade of toxicity 1-2, n(%) N=204**	**Grade of toxicity 3-5, n(%) N=193**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GT	100(49.0)	105(54.4)	1.00 (Reference)		1.00 (Reference)		CC	182(89.2)	161(83.4)	1.00 (Reference)	-	1.00 (Reference)	-
TT	104(50.9)	88(45.5)	1.24(0.83-1.84)	0.28	0.82(0.47-1.42)	0.48	CA	22(10.7)	32(16.5)	1.64(0.91-2.94)	0.09	1.83(0.82-4.06)	0.13
Genotype rs1128503 ABCB1 (C1236T)	**Grade of toxicity 1, n(%) N=58**	**Grade of toxicity 2-5, n(%) N=339**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs41410450 ABCC1 (G3173A)**	**Grade of toxicity 1, n(%) N=58**	**Grade of toxicity 2-5, n(%) N=339**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	31(53.4)	184(54.2)	1.00 (Reference)		1.00 (Reference)		GG	39(67.2)	268(79.0)	1.00 (Reference)	-	1.00 (Reference)	
CT	15(25.8)	106(31.2)	1.19(0.61-2.30)	0.6	1.84(0.68-4.99)	0.22	GA	17(29.3)	66(19.4)	0.56(0.30-1.06)	0.07	0.52(0.22-1.22)	0.13
TT	12(20.6)	49(14.4)	0.68(0.32-1.43)	0.31	0.79(0.29-2.15)	0.65	AA	2(3.4)	5(1.4)	0.36(0.06-1.94)	0.23	0.38(0.03-4.17)	0.43
CT+TT	27(46.5)	155(45.7)	0.96(0.55-1.69)	0.9	1.28(0.59-2.79)	0.5	GA+AA	19(32.7)	71(20.9)	0.54(0.29-0.99)	**0.04**	0.56(0.24-1.32)	0.18
Genotype rs1045642 ABCB1 (C3435T)	**Grade of toxicity 1, n(%) N=58**	**Grade of toxicity 2-5, n(%) N=339**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs8187710 ABCC2(G 4544A)**	**Grade of toxicity 1, n(%) N=58**	**Grade of toxicity 2-5, n(%) N=339**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	11(18.9)	62(18.2)	1.00 (Reference)		1.00 (Reference)		GG	56(96.5)	329(97.0)	1.00 (Reference)	-	1.00 (Reference)	-
CT	22(37.9)	144(42.4)	1.16(0.53-2.54)	0.7	1.33(0.45-3.90)	0.59	GA	2(3.4)	10(2.9)	0.85(0.18-3.98)	0.83	1.77(0.17-17.7)	0.62

TT	25(43.1)	133(39.2)	0.94(0.43-2.03)	0.8	0.86(0.31-2.32)	0.76							
CT+TT	47(81.0)	277(81.7)	1.04(0.51-2.13)	0.9	1.0(0.39-2.56)	0.9							
Genotype rs2032582 ABCB1 (G2677A)	**Grade of toxicity 1, n(%) N=58**	**Grade of toxicity 2-5, n(%) N=339**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs2231142 ABCG2 (C421A)**	**Grade of toxicity 1, n(%) N=58**	**Grade of toxicity 2-5, n(%) N=339**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GG	46(79.3)	309(91.1)	1.00 (Reference)		1.00 (Reference)		CC	53(91.3)	290(85.5)	1.00 (Reference)		1.00 (Reference)	
GA	12(20.6)	30(8.8)	0.37(0.17-0.77)	**0.008**	0.41(0.15-1.11)	0.07	CA	5(8.6)	49(14.4)	1.79(0.68-4.7)	0.23	1.35(0.37-4.85)	0.64

Genotype rs2032582 ABCB1 (G2677T)	**Grade of toxicity 1, n(%) N=58**	**Grade of toxicity 2-5, n(%) N=339**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GT	26(44.8)	179(52.8)	1.00 (Reference)	0.26	1.00 (Reference)	-
TT	32(55.1)	160(47.1)	1.37(0.78-2.40)		0.65(0.29-1.41)	0.27

OR[1] Crude odds ratio, 95% CI: 95% confidence interval, **AOR[2]**Adjusted Odds ratio data were calculated by unconditional logistic regression and adjusted for age, gender, performance status, and regimen. A p-value < 0.05 was considered statistically significant

5.20.3 Association between ABC polymorphisms and gastrointestinal toxicity

In the gastrointestinal (GI) toxicity, we assessed diarrhea, constipation, anorexia, and nausea/ vomiting. Among 135 patients, 45.9% of individuals had grade 1 toxicity, and 54.0% of patients had grade 2-4 toxicity. In constipation, among 99 patients, 30.3% showed grade 1 toxicity, while 69.6% patients had low/intermediate/high toxicity (grade 2-4). In the case of anorexia, 5.78% of patients had grade-1 toxicity, while 94.2% of patients showed grade 2-4 toxicity. Among 118 patients, 45.7% of individuals did not suffer from nausea/vomiting, whereas 54.2% experienced grade 2-4 toxicity (Table 5.51). Upon evaluating the effect of different *ABC* polymorphisms, our results showed that 8.88% of patients harbored a single copy of mutant allele (*CA)* for *ABCG2* $C^{421}A$ polymorphism. Table 5.52 shows that when patients with low/intermediate/ severe toxicity (Grade 2-4) were compared with no toxicity grade (grade 1), then patients harboring heterozygous (*CA)* genotype had a lower incidence of having diarrhea as compared to the wild type (*CC)* genotype of *ABCG2* $C^{421}A$ polymorphism (OR=0.25, 95% CI=0.06-0.97, p=**0.04**).

In diarrhea, when the comparison was made between intermediate/severe toxicity (grades 3-4) versus no/low grade (grade 1-2) toxicity, our results for *ABCC1* $G^{3173}A$ polymorphism showed that patients with one copy of mutant allele (*GA)* were associated with high odds of having diarrhea (OR=1.42, 95% CI=0.55-3.65, p=0.4). When adjusted with different covariates such as age, regimen, gender, and performance status, a 2.78-fold increased risk of having diarrhea was observed in the heterozygous genotype (*GA)* compared to the wild type genotype (AOR=2.78, 95% CI=0.94-8.21, p=**0.04**). Similarly, in the dominant model, the increased risk was noted in the combined (*GA+AA*) genotype as compared to the wild type (*GG)* genotype (AOR=2.82, 95% CI=0.99-8.04, p=**0.04**).

Table 5.51: Gastrointestinal toxicity with CTC grades in lung cancer patients

Toxicity	Adverse events	n	Toxicity profiles	
			Grade 1, n (%)	Grade 2-4, n (%)
Gastrointestinal toxicity	Diarrhea	135	62 (45.9)	73 (54.0)
	Constipation	99	30 (30.3)	69 (69.6)
	Anorexia	121	7 (5.78)	114 (94.2)
	Nausea-vomiting	118	54 (45.7)	64 (54.2)

Anorexia can be caused by cancer, chemotherapy, radiation, or several other factors and is a typical side effect of cancer treatment. Chemotherapy and radiation therapy have several adverse effects that can cause anorexia and weight loss, including nausea and vomiting. In the case of anorexia, when patients with grade-1 toxicity were compared with individuals with grade 2-4 toxicity for *ABCC1 $G^{3173}A$* polymorphism, a lower incidence of anorexia was observed in patients with one copy of mutant allele (*GA)* as compared to patients having both copies wild-type alleles (*GG)* (AOR= 0.20; 95%CI=0.04-0.97, p=**0.04)** (Table 5.52). In case of nausea and vomiting, patients harboring heterozygous genotype (*CT)* for *ABCB1 $C^{1236}T$* variant were associated with a reduced risk of having nausea/vomiting as compared to the wild type (*CC)* genotype (OR=0.35, 95% CI=0.15-0.81, p=**0.01**). Upon adjusting with different covariates, the lower incidence of having nausea/vomiting was consistent in the heterozygous genotype (*CT)* as compared to the wild type (*CC)* genotype (AOR=0.30, 95% CI=0.11-0.79, p=**0.015**). A decrease of having nausea/vomiting was also observed in the mutant genotype (*TT)* as compared to the wild type (*CC)* (AOR=0.23, 95% CI=0.06-0.81, p=**0.02**). Similarly, in the dominant model, the combined genotype (*CT+TT)* showed a lower incidence of nausea as compared to the wild-type genotype (*CC)* (AOR=0.27, 95% CI=0.11-0.66, p=**0.003**), as shown in Table 5.52**.**

Additionally, we assessed GI toxicity (constipation) by comparing individuals with no toxicity (grade 1) grade to patients with intermediate-high toxicity (grade 2). Lung carcinoma patients with the heterozygous genotype (*CT)* for *ABCB1 $C^{3435}T$* variant had a lower incidence of constipation when compared to the individuals harboring wild-type genotype (*CC)* (OR=0.27, 95% CI=0.06-1.12, p=0.07). The reduced risk was consistent when we adjusted with different covariates such as age, gender, performance status, and regimen in the heterozygous (*CT)* genotype as compared with the wild type (*CC)* genotype (AOR=0.17, 95% CI=0.03-0.97, p=**0.04**) as shown in Table 5.52. We did not find any correlation between other ABC polymorphisms and gastrointestinal-related adverse events due to chemotherapy**.**

Table 5.52: Gastrointestinal toxicity (grade 0 *vs.* 1-4 and grade 0-1 *vs.* 2-4) associated with *ABCB1, ABCC1, ABCC2,* and *ABCG2* polymorphism

Diarrhoea												
Genotype rs1128503 ABCB1 (C1236T)	**Grade of toxicity 1, n(%) N=62**	**Grade of toxicity 2-4, n(%) N=73**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs41410450 ABCC1 (G3173A)**	**Grade of toxicity 1, n(%) N=62**	**Grade of toxicity 2-4, n(%) N=73**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	33(53.2)	39(53.4)	1.00 (Reference)		1.00 (Reference)		GG	49(79.0)	51(69.8)	1.00 (Reference)	-	1.00 (Reference)	
CT	13(20.9)	24(32.8)	1.56(0.68-3.54)	0.28	2.13(0.74-6.13)	0.16	GA	13(20.9)	19(26.0)	1.40(0.62-3.14)	0.4	1.61(0.59-4.38)	0.34
TT	16(25.8)	10(13.6)	0.52(0.21-1.32)	0.17	0.55(0.19-1.62)	0.28	AA	0(0)	3(4.1)	-	-	-	-
CT+TT	29(46.7)	34(46.5)	0.99(0.50-1.95)	0.9	1.13(0.49-2.58)	0.76	GA+AA	13(20.9)	22(30.1)	1.62(0.73-3.58)	0.22	1.82(0.68-4.86)	0.23
Genotype rs1045642 ABCB1 (C3435T)	**Grade of toxicity 1, n(%) N=62**	**Grade of toxicity 2-4, n(%) N=73**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs8187710 ABCC2 (G4544A)**	**Grade of toxicity 1, n(%) N=62**	**Grade of toxicity 2-4, n(%) N=73**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	16(25.8)	14(19.1)	1.00 (Reference)		1.00 (Reference)		GG	60(96.7)	71(97.2)	1.00 (Reference)	-	1.00 (Reference)	-
CT	21(33.8)	32(43.8)	1.74(0.70-4.30)	0.22	1.76(0.53-5.83)	0.34	GA	2(3.2)	2(2.7)	0.84(0.11-6.18)	0.8	0.37(0.03-4.42)	0.43
TT	25(40.3)	27(36.9)	1.23(0.50-3.03)	0.64	1.12(0.39-3.22)	0.82							
CT+TT	46(74.1)	59(80.8)	1.46(0.64-3.30)	0.35	1.40(0.55-3.60)	0.47							
Genotype rs2032582 ABCB1 (G2677A)	**Grade of toxicity 1, n(%) N=62**	**Grade of toxicity 2-4, n(%) N=73**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs2231142 ABCG2 (C421A)**	**Grade of toxicity 1, n(%) N=62**	**Grade of toxicity 2-4, n(%) N=73**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GG	5(8.0)	10(13.6)	1.00 (Reference)		1.00 (Reference)		CC	53(85.4)	70(95.8)	1.00 (Reference)	-	1.00 (Reference)	-
GA	57(91.9)	63(86.3)	1.80(0.58-5.61)	0.3	1.26(0.33-4.80)	0.7	CA	9(14.5)	3(4.1)	0.25(0.06-0.97)	**0.04**	0.25(0.05-1.10)	0.06
Genotype rs2032582 ABCB1 (G2677T)	**Grade of toxicity 1, n(%) N=62**	**Grade of toxicity 2-4, n(%) N=73**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs1128503 ABCB1 (C1236T)**	**Grade of toxicity 1-2, n(%) N=106**	**Grade of toxicity 3-4, n(%) N=29**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
							CC	54(50.9)	18(62.0)	1.00 (Reference)	-	1.00 (Reference)	-
GT	27(43.5)	39(53.4)	1.00 (Reference)		1.00 (Reference)	-	CT	30(28.3)	7(24.1)	0.70(0.26-1.86)	0.47	1.17(0.36-3.77)	0.78
TT	35(56.4)	34(46.5)	0.67(0.34-1.32)	0.25	0.47(0.20-1.11)	0.08	TT	22(20.7)	4(13.7)	0.54(0.16-1.79)	0.31	0.83(0.23-3.03)	0.78
							CT+TT	52(49.0)	11(37.9)	0.63(0.27-1.47)	0.28	0.99(0.36-2.66)	0.9
Genotype rs1045642 ABCB1 (C3435T)	**Grade of toxicity 1-2, n(%) N=106**	**Grade of toxicity 3-4, n(%) N=29**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs41410450 ABCC1 (G3173A)**	**Grade of toxicity 1-2, n(%) N=106**	**Grade of toxicity 3-4, n(%) N=29**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	22(20.7)	8(27.5)	1.00 (Reference)		1.00 (Reference)		GG	81(76.4)	19(65.5)	1.00 (Reference)		1.00 (Reference)	
CT	43(40.5)	10(34.4)	0.63(0.22-1.85)	0.40	0.57(0.14-2.24)	0.42	GA	24(22.6)	8(27.5)	1.42(0.55-3.65)	0.46	2.78(0.94-8.21)	**0.04**
TT	41(38.6)	11(37.9)	0.73(0.25-2.10)	0.56	0.53(0.15-1.81)	0.31	AA	1(0.9)	2(6.8)	-	-	-	-
CT+TT	84(79.2)	21(72.4)	0.68(0.26-1.75)	0.43	0.580.19-1.74)	0.33	GA+AA	25(23.5)	10(34.4)	1.70(0.70-4.14)	0.23	2.82(0.99-8.04)	**0.04**
Genotype rs2032582 ABCB1 (G2677A)	**Grade of toxicity 1-2, n(%) N=106**	**Grade of toxicity 3-4, n(%) N=29**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs4148356 ABCC1 (G2168A)**	**Grade of toxicity 1-2, n(%) N=106**	**Grade of toxicity 3-4, n(%) N=29**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GG	97(91.5)	23(79.3)	1.00 (Reference)	-	1.00 (Reference)		GG	103(97.1)	28(96.5)	1.00 (Reference)	-	1.00 (Reference)	-

GA	9(8.4)	6(20.6)	2.81(0.90-8.69)	0.07	2.20(0.55-8.85)	0.26	GA	3(2.8)	1(3.4)	1.22(0.12-12.2)	0.8	1.32(0.10-15.92)	0.82
Genotype rs2032582 ABCB1 (G2677T)	**Grade of toxicity 1-2, n(%) N=106**	**Grade of toxicity 3-4, n(%) N=29**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs8187710 ABCC2 (G4544A)**	**Grade of toxicity 1-2, n(%) N=106**	**Grade of toxicity 3-4, n(%) N=29**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GT	53(50)	13(44.8)	1.00 (Reference)		1.00 (Reference)		GG	103(97.1)	28(96.5)	1.00 (Reference)	-	1.00 (Reference)	-
TT	53(50)	16(55.1)	1.23(0.53-2.80)	0.62	1.02(0.38-2.73)	0.9	GA	3(2.8)	1(3.4)	1.22(0.12-12.2)	0.86	10.1(0.26-380.8)	0.21
Genotype rs2231142 ABCG2 (C421A)		**Grade of toxicity 1-2, n(%) N=106**		**Grade of toxicity 3-4, n(%) N=29**		**OR[1] (95% CI)**		**P**		**AOR[2](95% CI)**		**P**	
CC		95(89.6)		28(96.5)		1.00 (Reference)		-		1.00 (Reference)		-	
CA		11(10.3)		1(3.4)		0.30(0.03-2.49)		0.27		0.26(0.03-2.24)		0.22	
Anorexia													
Genotype rs1128503 ABCB1 (C1236T)	**Grade of toxicity 1, n(%) N=7**	**Grade of toxicity 2-3, n(%) N=114**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs2032582 ABCB1 (G2677T)**	**Grade of toxicity 1, n(%) N=7**	**Grade of toxicity 2-3, n(%) N=114**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	6(85.7)	66(57.8)	1.00 (Reference)	-	1.00 (Reference)	-	GT	2(28.5)	46(40.3)	1.00 (Reference)		1.00 (Reference)	-
CT	0(0)	34(29.8)	-	-	-	-	TT	5(71.4)	68(59.6)	0.59(0.11-3.17)	0.54	0.28(0.02-2.83)	0.28
TT	1(14.2)	14(12.2)	1.27(0.14-11.41)	0.8	1.01(0.09-10.9)	0.9							
CT+TT	1(14.2)	48(42.1)	4.36(0.50-37.4)	0.17	3.80(0.39-36.4)	0.24							
Genotype rs1045642 ABCB1 (C3435T)	**Grade of toxicity 1, n(%) N=7**	**Grade of toxicity 2-3, n(%) N=114**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs41410450 ABCC1 (G3173A)**	**Grade of toxicity 1, n(%) N=7**	**Grade of toxicity 2-3, n(%) N=114**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	1(14.2)	17(14.9)	1.00 (Reference)		1.00 (Reference)		GG	3(42.8)	87(76.3)	1.00 (Reference)	-	1.00 (Reference)	
CT	1(14.2)	44(38.5)	2.58(0.15-43.7)	0.5	1.61(0.04-61.8)	0.7	GA	4(57.1)	24(21.0)	0.20(0.04-0.98)	**0.04**	0.43(0.06-2.94)	0.39
TT	5(71.4)	53(46.4)	0.62(0.06-5.71)	0.67	0.92(0.07-10.89)	0.9	AA	0(0)	3(2.6)	-	-	-	-
CT+TT	6(85.7)	97(85.0)	0.95(0.10-8.40)	0.9	1.28(0.12-13.8)	0.8	GA+AA	4(57.1)	27(23.6)	0.23(0.04-1.10)	0.06	0.46(0.06-3.07)	0.42
Genotype rs2032582 ABCB1 (G2677A)	**Grade of toxicity 1, n(%) N=7**	**Grade of toxicity 2-3, n(%) N=114**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs2231142 ABCG2 (C421A)**	**Grade of toxicity 1, n(%) N=7**	**Grade of toxicity 2-3, n(%) N=114**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GG	6(85.7)	107(93.8)	1.00 (Reference)		1.00 (Reference)		CC	6(85.7)	105(92.1)	1.00 (Reference)	-	1.00 (Reference)	-
GA	1(14.2)	7(6.1)	0.39(0.04-3.72)	0.4	0.24(0.01-3.08)	0.27	CA	1(14.2)	9(7.8)	0.51(0.05-4.75)	0.55	0.45(0.04-5.0)	0.5
Constipation													
Genotype rs1128503 ABCB1 (C1236T)	**Grade of toxicity 1, n(%) N=30**	**Grade of toxicity 2, n(%) N=69**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs41410450 ABCC1 (G3173A)**	**Grade of toxicity 1, n(%) N=30**	**Grade of toxicity 2, n(%) N=69**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	18(60)	37(53.6)	1.00 (Reference)	-	1.00 (Reference)	-	GG	22(73.3)	50(72.4)	1.00 (Reference)		1.00 (Reference)	
CT	6(20)	19(27.5)	1.54(0.52-4.52)	0.43	1.71(0.43-6.73)	0.43	GA	8(26.6)	16(23.1)	0.8(0.32-2.35)	0.79	1.03(0.31-3.38)	0.9
TT	6(20)	13(18.8)	1.05(0.34-3.22)	0.9	0.88(0.22-3.40)	0.85	AA	0(0)	3(4.3)	-	-	-	-
CT+TT	12(40)	32(46.3)	1.29(0.54-3.09)	0.5	1.26(0.44-3.60)	0.66	GA+AA	8(26.6)	19(27.5)	1.04(0.39-2.74)	0.9	1.09(0.3-3.53)	0.8

Genotype rs1045642 ABCB1 (C3435T)	**Grade of toxicity 1, n(%) N=30**	**Grade of toxicity 2, n(%) N=69**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs8187710 ABCC2(G4 544A)**	**Grade of toxicity 1, n(%) N=30**	**Grade of toxicity 2, n(%) N=69**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	3(10)	14(20.2)	1.00 (Reference)		1.00 (Reference)								
CT	17(56.6)	22(31.8)	0.27(0.06-1.12)	0.07	0.17(0.03-0.97)	**0.04**	GG	29(96.6)	68(98.5)	1.00 (Reference)	-	1.00 (Reference)	-
TT	10(33.3)	33(47.8)	0.70(0.16-2.9)	0.63	0.61(0.10-3.63)	0.58	GA	1(3.3)	1(1.4)	0.42(0.02-7.05)	0.5	0.48(0.02-8.56)	0.62
CT+TT	27(90)	55(79.7)	0.43(0.11-1.64)	0.22	0.39(0.09-1.70)	0.21							
Genotype rs2032582 ABCB1 (G2677A)	**Grade of toxicity 1, n(%) N=30**	**Grade of toxicity 2, n(%) N=69**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs2231142 ABCG2 (C421A)**	**Grade of toxicity 1, n(%) N=30**	**Grade of toxicity 2, n(%) N=69**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GG	28(93.3)	64(92.7)	1.00 (Reference)	-	1.00 (Reference)	-	CC	24(80)	58(84.0)	1.00 (Reference)	-	1.00 (Reference)	-
GA	2(6.6)	5(7.2)	1.09(0.2-5.98)	0.9	1.98(0.19-20.0)	0.56	CA	6(20)	11(15.9)	0.75(0.25-2.2)	0.62	0.51(0.13-2.0)	0.33

Genotype rs2032582 ABCB1 (G2677T)	**Grade of toxicity 1, n(%) N=30**	**Grade of toxicity 2, n(%) N=69**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GT	16(53.3)	34(49.2)	1.00 (Reference)	-	1.00 (Reference)	-
TT	14(46.6)	35(50.7)	1.17(0.49-2.77)	0.7	0.98(0.34-2.76)	0.9

Nausea-Vomiting

Genotype rs1128503 ABCB1 (C1236T)	**Grade of toxicity 1, n(%) N=54**	**Grade of toxicity 2-3, n(%) N=64**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs2032582 ABCB1 (G2677T)**	**Grade of toxicity 1, n(%) N=54**	**Grade of toxicity 2-3, n(%) N=64**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	20(37.0)	41(64.0)	1.00 (Reference)	-	1.00 (Reference)	-							
CT	22(40.7)	16(25)	0.35(0.15-0.81)	**0.01**	0.30(0.11-0.79)	**0.015**	GT	28(51.8)	29(45.3)	1.00 (Reference)		1.00 (Reference)	
TT	12(22..2)	7(10.9)	0.28(0.09-0.83)	**0.02**	0.23(0.06-0.81)	**0.02**	TT	26(48.1)	35(54.6)	1.29(0.62-2.68)	0.47	1.23(0.51-2.95)	0.63
CT+TT	34(62.9)	23(35.9)	0.33(0.15-0.70)	**0.003**	0.27(0.11-0.66)	**0.0039**							
Genotype rs1045642 ABCB1 (C3435T)	**Grade of toxicity 1, n(%) N=54**	**Grade of toxicity 2-3, n(%) N=64**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs41410450 ABCC1 (G3173A)**	**Grade of toxicity 1, n(%) N=54**	**Grade of toxicity 2-3, n(%) N=64**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	12(22.2)	9(14.0)	1.00 (Reference)	-	1.00 (Reference)	-	GG	41(75.9)	44(68.7)	1.00 (Reference)		1.00 (Reference)	
CT	22(40.7)	25(39.0)	1.51(0.53-4.27)	0.43	1.24(0.34-4.51)	0.74	GA	12(22.2)	19(29.6)	1.47(0.63-3.41)	0.36	1.44(0.56-3.73)	0.44
TT	20(37.0)	30(46.8)	2.0(0.71-5.61)	0.18	2.58(0.76-8.73)	0.12	AA	1(1.8)	1(1.5)	0.93(0.05-15.38)	0.9	0.82(0.04-15.41)	0.89
CT+TT	42(77.7)	55(85.9)	1.74(0.67-4.52)	0.25	1.60(0.55-4.63)	0.37	GA+AA	13(24.0)	20(31.2)	1.43(0.63-3.24)	0.38	1.33(0.52-3.3)	0.54
Genotype rs2032582 ABCB1 (G2677A)	**Grade of toxicity 1, n(%) N=54**	**Grade of toxicity 2-3, n(%) N=64**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs2231142 ABCG2 (C421A)**	**Grade of toxicity 1, n(%) N=54**	**Grade of toxicity 2-3, n(%) N=64**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GG	51(94.4)	56(87.5)	1.00 (Reference)	-	1.00 (Reference)		CC	45(83.3)	59(92.1)	1.00 (Reference)	-	1.00 (Reference)	-
GA	3(5.5)	8(12.5)	2.42(0.61-9.65)	0.2	2.06(0.46-9.27)	0.34	CA	9(16.6)	5(7.8)	0.42(0.13-1.35)	0.14	0.62(0.18-2.18)	0.46

OR[1] Crude odds ratio, 95% CI: 95% confidence interval, **AOR[2]**Adjusted Odds ratio data were calculated by unconditional logistic regression and adjusted for age, gender, performance status, and regimen. A p-value < 0.05 was considered statistically significant

5.20.4 Association between ABC polymorphisms and hepatotoxicity

An unanticipated reaction typically causes chemotherapy-induced hepatotoxicity. The enzyme levels of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), and alkaline phosphatase (ALP) are the primary components of the liver function test (LFT). On categorizing patients based on SGOT levels, 74 patients were classified in grades 1-2, while only one patient was categorized in grades 3-4, while 242 patients were classified in grades 0 (Table 5.53). The analysis of SGOT to *ABCC1* $G^{3173}A$ showed statistical differences. Our results revealed an increased risk of liver injury in the heterozygous genotype of the *ABCC1* $G^{3173}A$ variant compared to the wild-type genotype (OR=2.06, 95% CI=1.11-3.81, p=**0.02**). In the dominant model, the combined genotype (*GA+AA*) showed a high risk of liver damage as compared to the individuals having wild-type genotype (*GG)* (OR=1.86, 95% CI=1.03-3.3, p=**0.03**) as shown in Table 5.54.

Table 5.53: Hepatotoxicity with CTC grades in lung cancer patients

Toxicity	Adverse events	n	Toxicity profiles		
	SGOT	317	**Grade 0, n (%)**	**Grade 1-2, n (%)**	**Grade 3-4, n (%)**
Hepatotoxicity	SGPT	317	180 (56.7)	132 (41.6)	5 (1.57)
	ALP	307	153 (49.8)	144 (46.9)	10 (3.25)

On categorizing patients based on SGPT levels for *ABCB1* $C^{1236}T$ polymorphism, our results demonstrated an increased risk of liver injury in patients harboring heterozygous genotype (*CT)* as compared to the wild type genotype (*CC)* (OR=1.85, 95% CI=1.11-3.08, p=**0.01**). Upon adjusting with different covariates, the heterozygous genotype *(CT)* showed an increased risk of 1.93-fold as compared to the wild-type genotype (*CC)* (AOR=1.93, 95% CI=0.98-3.081, p=**0.04**). For *ABCB1* $G^{2677}A$ polymorphism, mutant genotype (*AA)* was associated with a lower incidence of liver toxicity as compared to the heterozygous genotype (*GA)* (OR=0.61, 95% CI=0.39-0.96, p=**0.03**). Upon adjusting with various covariates such as age, gender, performance status, and regimen, the mutant genotype was still associated with reduced risk compared to the wild-type genotype (*GG)* (AOR=0.46, 95% CI=0.25-0.85, p=**0.01**) as shown in Table 5.54. We did not find any correlation between other ABC polymorphisms and hepatic-related adverse events due to chemotherapy**.**

Table 5.54: Hepato-toxicity (grade 0 *vs.* 1-4 and grade 0-1 *vs.* 2-4) associated with *ABCB1, ABCC1, ABCC2*, and *ABCG2* polymorphism

AST

Genotype rs1128503 ABCB1 (C1236T)	**Grade of toxicity 0, n(%) N=242**	**Grade of toxicity 1-3, n(%) N=75**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs41410450 ABCC1 (G3173A)**	**Grade of toxicity 0, n(%) N=242**	**Grade of toxicity 1-3, n(%) N=75**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	136(56.1)	42(56)	1.00 (Reference)	-	1.00 (Reference)	-	GG	198(81.8)	53(70.6)	1.00 (Reference)		1.00 (Reference)	
CT	68(28.0)	24(32)	1.14(0.64-2.04)	0.65	1.18(0.54-2.57)	0.66	GA	38(15.7)	21(28)	2.06(1.11-3.81)	**0.02**	1.76(0.76-4.07)	0.18
TT	38(15.7)	9(12)	0.76(0.34-1.7)	0.51	1.35(0.51-3.58)	0.53	AA	6(2.4)	1(1.3)	0.62(0.07-5.28)	0.6	1.77(0.12-24.49)	0.66
CT+TT	106(43.8)	33(44)	1.0(0.59-1.69)	0.9	1.24(0.63-2.47)	0.52	GA+AA	44(18.1)	22(29.3)	1.86(1.03-3.3)	**0.03**	1.78(0.79-4.02)	0.16
Genotype rs1045642 ABCB1 (C3435T)	**Grade of toxicity 0, n(%) N=242**	**Grade of toxicity 1-3, n(%) N=75**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs4148356 ABCC1 (G2168A)**	**Grade of toxicity 0, n(%) N=242**	**Grade of toxicity 1-3, n(%) N=75**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	38(15.7)	17(22.6)	1.00 (Reference)		1.00 (Reference)		GG	232(95.8)	72(96)	1.00 (Reference)	-	1.00 (Reference)	-
CT	101(41.7)	33(44)	0.73(0.36-1.46)	0.37	1.01(0.41-2.46)	0.9	GA	10(4.1)	3(4)	0.9(0.25-3.60)	0.9	1.28(0.30-5.40)	0.7
TT	103(42.5)	25(33.3)	0.54(0.26-1.11)	0.09	0.43(0.15-1.20)	0.10							
CT+TT	204(84.2)	58(77.3)	0.63(0.33-1.20)	0.16	0.73(0.32-1.67)	0.46							
Genotype rs2032582 ABCB1 (G2677A)	**Grade of toxicity 0, n(%) N=242**	**Grade of toxicity 1-3, n(%) N=75**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs8187710 ABCC2 (G4544A)**	**Grade of toxicity 0, n(%) N=242**	**Grade of toxicity 1-3, n(%) N=75**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GG	219(90.4)	65(86.6)	1.00 (Reference)	-	1.00 (Reference)	-	GG	235(97.1)	72(96)	1.00 (Reference)	-	1.00 (Reference)	-
GA	23(9.5)	10(13.3)	1.46(0.66-3.23)	0.34	2.10(0.78-5.62)	0.13	GA	7(2.8)	3(4)	1.39(0.35-5.5)	0.6	1.72(0.28-10.36)	0.54
Genotype rs2032582 ABCB1 (G2677T)	**Grade of toxicity 0, n(%) N=242**	**Grade of toxicity 1-3, n(%) N=75**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs2231142 ABCG2 (C421A)**	**Grade of toxicity 0, n(%) N=242**	**Grade of toxicity 1-3, n(%) N=75**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GT	123(50.8)	45(60)	1.00 (Reference)	-	1.00 (Reference)	-	CC	208(85.9)	64(85.3)	1.00 (Reference)	-	1.00 (Reference)	-
TT	119(49.1)	30(40)	0.68(0.40-1.16)	0.16	0.42(0.20-0.87)	**0.02**	CA	34(14.0)	11(14.6)	1.05(0.50-2.19)	0.8	1.08(0.41-2.82)	0.86

ALT

Genotype rs1128503 ABCB1 (C1236T)	**Grade of toxicity 0, n(%) N=180**	**Grade of toxicity 1-3, n(%) N=137**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs41410450 ABCC1 (G3173A)**	**Grade of toxicity 0, n(%) N=180**	**Grade of toxicity 1-3, n(%) N=137**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	107(59.4)	70(51.0)	1.00 (Reference)	-	1.00 (Reference)	-	GG	144(80)	106(77.3)	1.00 (Reference)		1.00 (Reference)	
CT	42(23.3)	51(37.2)	1.85(1.11-3.08)	**0.01**	1.93(0.98-3.81)	**0.04**	GA	32(17.7)	28(20.4)	1.18(0.67-2.09)	0.54	0.89(0.41-1.93)	0.78
TT	31(17.2)	16(11.6)	0.78(0.40-1.54)	0.49	0.83(0.34-2.03)	0.69	AA	4(2.2)	3(2.1)	1.01(0.22-4.64)	0.9	0.66(0.05-8.09)	0.74
CT+TT	73(40.5)	67(48.9)	1.40(0.89-2.19)	0.13	1.46(0.8-2.65)	0.20	GA+AA	36(20)	31(22.6)	1.16(0.68-2.0)	0.57	0.88(0.41-1.85)	0.74
Genotype rs1045642 ABCB1 (C3435T)	**Grade of toxicity 0, n(%) N=180**	**Grade of toxicity 1-3, n(%) N=137**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs4148356 ABCC1 (G2168A)**	**Grade of toxicity 0, n(%) N=180**	**Grade of toxicity 1-3, n(%) N=137**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	28(15.5)	27(19.7)	1.00 (Reference)	-	1.00 (Reference)	-	GG	173(96.1)	130(94.8)	1.00 (Reference)	-	1.00 (Reference)	-
CT	70(38.8)	64(46.7)	0.94(0.5-1.77)	0.86	1.04(0.46-2.33)	0.9	GA	7(3.8)	7(5.1)	1.33(0.45-3.88)	0.60	1.98(0.52-7.44)	0.31

TT	82(45.5)	46(33.5)	0.58(0.30-1.10)	0.09	0.46(0.19-1.10)	0.08							
CT+TT	152(84.4)	110(80.2)	0.75(0.41-1.34)	0.33	0.75(0.36-1.58)	0.46							
Genotype rs2032582 ABCB1 (G2677A)	**Grade of toxicity 0, n(%) N=180**	**Grade of toxicity 1-3, n(%) N=137**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs2231142 ABCG2 (C421A)**	**Grade of toxicity 0, n(%) N=180**	**Grade of toxicity 1-3, n(%) N=137**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GG	157(87.2)	127(92.7)	1.00 (Reference)	-	1.00 (Reference)	-	CC	158(87.7)	114(83.2)	1.00 (Reference)	-	1.00 (Reference)	-
GA	23(12.7)	10(7.2)	0.53(0.24-1.17)	0.11	0.46(0.17-1.26)	0.13	CA	22(12.2)	23(16.7)	1.44(0.77-2.76)	0.25	1.16(0.50-2.68)	0.72
Genotype rs2032582 ABCB1 (G2677T)		**Grade of toxicity 0, n(%) N=180**		**Grade of toxicity 1-3, n(%) N=137**		**OR[1] (95% CI)**		**P**		**AOR[2](95% CI)**		**P**	
GT		86(47.7)		82(59.8)		1.00 (Reference)		-		1.00 (Reference)		-	
TT		94(52.2)		55(40.1)		0.61(0.39-0.96)		**0.03**		0.46(0.25-0.85)		**0.01**	
ALP													
Genotype rs1128503 ABCB1 (C1236T)	**Grade of toxicity 0, n(%) N=153**	**Grade of toxicity 1-3, n(%) N=154**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs41410450 ABCC1 (G3173A)**	**Grade of toxicity 0, n(%) N=153**	**Grade of toxicity 1-3, n(%) N=154**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	94(61.4)	76(49.3)	1.00 (Reference)	-	1.00 (Reference)	-	GG	126(82.3)	117(75.9)	1.00 (Reference)		1.00 (Reference)	
CT	36(23.5)	54(35.0)	1.85(1.10-3.11)	**0.01**	1.93(0.98-3.83)	**0.04**	GA	25(16.3)	32(20.7)	1.37(0.77-2.46)	0.27	1.24(0.58-2.66)	0.56
TT	23(15.0)	24(15.5)	1.29(0.67-2.46)	0.43	1.37(0.59-3.16)	0.45	AA	2(1.3)	5(3.2)	2.69(0.51-14.14)	0.24	2.80(0.21-36.19)	0.42
CT+TT	59(38.5)	78(50.6)	1.63(1.03-2.57)	**0.03**	1.67(0.92-3.02)	0.08	GA+AA	27(17.6)	37(24.0)	1.47(0.84-2.57)	0.17	1.32(0.63-2.77)	0.45
Genotype rs1045642 ABCB1 (C3435T)	**Grade of toxicity 0, n(%) N=153**	**Grade of toxicity 1-3, n(%) N=154**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs4148356 ABCC1 (G2168A)**	**Grade of toxicity 0, n(%) N=153**	**Grade of toxicity 1-3, n(%) N=154**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
CC	30(19.6)	23(14.9)	1.00 (Reference)		1.00 (Reference)		GG	148(96.7)	146(94.8)	1.00 (Reference)	-	1.00 (Reference)	-
CT	58(37.9)	73(47.4)	1.64(0.86-3.12)	0.13	1.37(0.59-3.18)	0.46	GA	5(3.2)	8(5.1)	1.62(0.51-5.07)	0.40	1.38(0.35-5.40)	0.63
TT	65(42.4)	58(37.6)	1.16(0.60-2.22)	0.64	1.15(0.50-2.62)	0.72							
CT+TT	123(80.3)	131(85.0)	1.38(0.76-2.52)	0.28	1.27(0.60-2.67)	0.52							
Genotype rs2032582 ABCB1 (G2677A)	**Grade of toxicity 0, n(%) N=153**	**Grade of toxicity 1-3, n(%) N=154**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs8187710 ABCC2(G4544A)**	**Grade of toxicity 0, n(%) N=153**	**Grade of toxicity 1-3, n(%) N=154**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GG	139(90.8)	135(87.6)	1.00 (Reference)	-	1.00 (Reference)	-	GG	149(97.3)	150(97.4)	1.00 (Reference)	-	1.00 (Reference)	-
GA	14(9.1)	19(12.3)	1.39(0.67-2.89)	0.36	1.58(0.64-3.90)	0.31	GA	4(2.6)	4(2.5)	0.9(0.24-4.04)	0.9	2.16(0.37-12.32)	0.3
Genotype rs2032582 ABCB1 (G2677T)	**Grade of toxicity 0, n(%) N=153**	**Grade of toxicity 1-3, n(%) N=154**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs2231142 ABCG2 (C421A)**	**Grade of toxicity 0, n(%) N=153**	**Grade of toxicity 1-3, n(%) N=154**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GT	72(47.0)	91(59.0)	1.00 (Reference)		1.00 (Reference)		CC	132(86.2)	131(85.0)	1.00 (Reference)	-	1.00 (Reference)	-
TT	81(52.9)	63(40.9)	0.70(0.44-1.09)	0.11	0.59(0.331.08)	0.09	CA	21(13.7)	23(14.9)	1.10(0.58-2.09)	0.76	0.91(0.39-2.13)	0.83

OR[1] Crude odds ratio, 95% CI: 95% confidence interval, **AOR[2]**Adjusted Odds ratio data were calculated by unconditional logistic regression and adjusted for age, gender, performance status, and regimen. A p-value < 0.05 was considered statistically significant

5.21 Solute carrier (SLC) transporter genes

5.21.1 Genotyping of SLC19A1 gene

SLC19A1 is highly polymorphic in humans and genotyping of $G^{80}A$ (rs1051266) polymorphism has been investigated in the current study. In the *SLC19A1* $G^{80}A$ polymorphism, the amplified product of 230 bp was observed and resolved on 2% agarose gel electrophoresis. The amplified products were digested with enzyme *Hha I* and resolved on 6% native-polyacrylamide gel electrophoresis, as shown in figure 5.39. The gel was stained with ethidium bromide bands to observe the restriction pattern. The wild type allele (*G)* had restriction sites for *Hha I* and produced fragments of 125bp, 68bp, and 37bp, whereas mutant allele (*AA)* had a single site for *Hha I*, so two fragments of 162bp and 68bp were observed, whereas in case of heterozygous alleles (*GA)*, bands of 162bp, 125bp,68bp, and 37 bp was recorded.

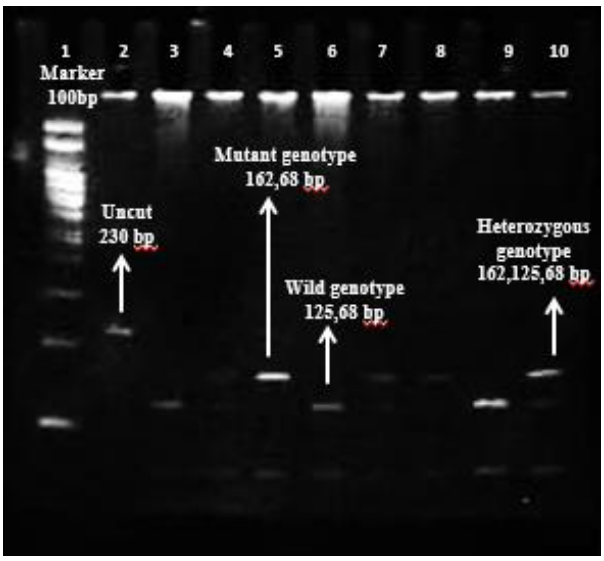


Figure 5.39: Restriction digestion pattern of *SLC19A1* $G^{80}A$ polymorphism, Wild type (*GG*): 125,68, 37 bp (the 37bp was too small to resolve accurately); Mutant genotype (*AA)*: 162, 68 bp; Heterozygous genotype (*GA)*: 162,125,68,37 bp. Lane 1: marker, Lane 2: Amplified PCR product, Lane 3,6,9:*GG* genotype; Lane 4,7,10: *GA* genotype; Lane 5,8: *AA* genotype

5.21.2 Genotyping of SLCO1B1 gene

For the *SLCO1B1* gene, genotyping of two polymorphisms ($A^{388}G$ *and* $T^{521}C$) was done. In the case of $A^{388}G$ polymorphism, the amplified product of 214 bp was observed. The PCR amplicon

was digested with *Taq I* and was separated on 6% polyacrylamide gel and stained with ethidium bromide. The wild-type alleles (*AA)* had a restriction site for *Taq I* and two bands of 151 and 63bp, whereas bands of 128, 63, and 23bp was retrieved for variant alleles (*GG)*, and all the band of 151, 128, 63, and 23 bp were retrieved in case of heterozygous alleles (*AG)*. Figure 5.40 (A) depicted the digested products of $A^{388}G$ polymorphism which were separated on 6% polyacrylamide gel electrophoresis and stained with ethidium bromide.

SLCO1B1 $T^{521}C$ polymorphism was also studied, and PCR product of 209 bp was checked on 2.0% agarose gel and then digested with 5U of *Hha I* restriction enzyme (New England Biolabs) at 37˚C. Digested fragments were separated on 6% Native- polyacrylamide gel electrophoresis and were later stained with the help of ethidium bromide and visualized under a UV transilluminator (Figure 5.40 (B)). The wild type alleles (*TT)* produced a single band of 209bp, variant alleles (*CC)* were identified by bands of 189 and 20 bp, and heterozygous displayed all the three bands of 209, 189, and 20bp.

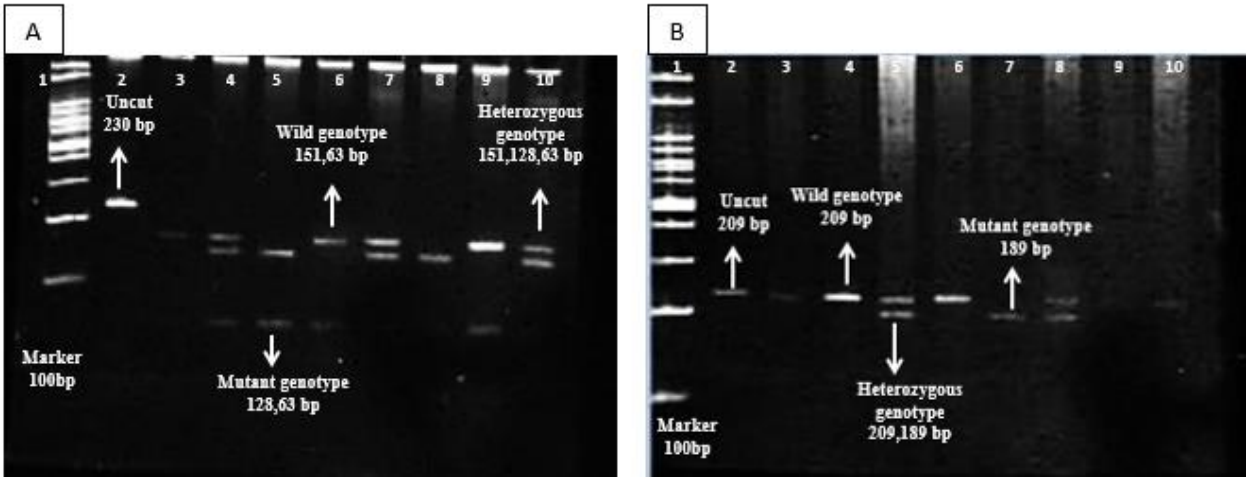


Figure 5.40: Restriction digestion pattern of *SLCO1B1* polymorphisms (A) 6% Native-PAGE of PCR products for $A^{388}G$ polymorphism, Wild type (*AA*): 151, 63 bp; Mutant genotype (*GG)*: 128, 63, 23 bp (the 23 bp was too small to resolve accurately); Heterozygous genotype (*AG)*: 151,128,63,23 bp. Lane 1: marker, Lane 2: Amplified PCR product, Lane 3,6,9:*AA* genotype; Lane 4,7,10: *AG* genotype; Lane 5,8: *GG* genotype. (B) 6% Native-PAGE of PCR products for $T^{521}C$ polymorphism, Wild type (*TT*): 209 bp; Mutant genotype (*CC)*: 189 and 20 bp (the 20bp was too small to resolve accurately); Heterozygous genotype (*TC)*: 209, 189, and 20 bp. Lane 1: marker, Lane 2: Amplified PCR product, Lane 3,4,6,9,10:*TT* genotype; Lane 5,8: *TC* genotype; Lane 7: *GG* genotype.

5.21.3 Genotyping of SLCO1B3 gene

For the *SLCO1B3* gene, one polymorphism, $A^{1683\text{-}5676}G$, was studied, and the substitution was of *A* to *G* nucleotide. The PCR product of 286 bp for *SLCO1B3* $A^{1683\text{-}5676}G$ was digested with 5U of *RsaI* restriction enzyme (New England Biolabs, Ispwich, MA) at 37^0C. The digested sample was resolved on 6% native polyacrylamide gel stained in ethidium bromide and was visualized under UV-transilluminator. For $A^{1683\text{-}5676}G$, wild-type produced two bands of 262 and 24 bp (24 bp was too small to resolve accurately), while mutant-type genotype generated three bands of 155, 107, and 24 bp, and heterozygous genotype showed all the above bands (262, 155, 107, and 24 bp) as shown in figure 5.41.

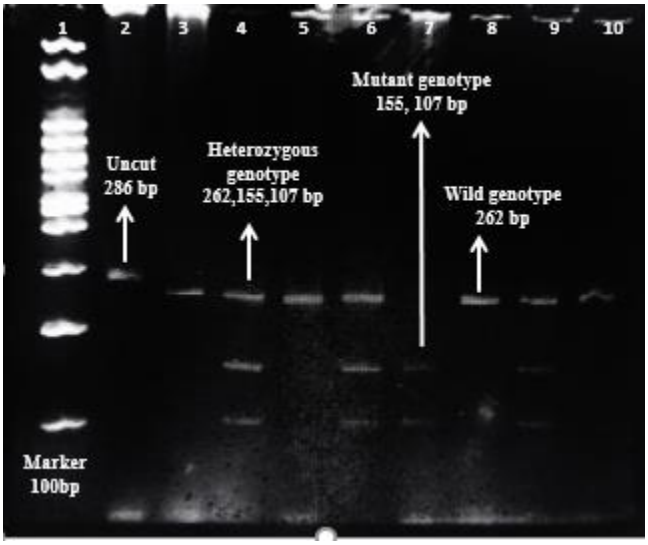


Figure 5.41: Restriction digestion pattern of *SLCO1B3* $A^{1683\text{-}5676}G$ polymorphism, Wild type (*AA*): 262 bp; Mutant genotype (*GG)*: 155, 107, 24 bp (the 24 bp was too small to resolve accurately); Heterozygous genotype (*AG)*: 262,155,107,24 bp. Lane 1: marker, Lane 2: Amplified PCR product, Lane 3,5,8,10: *AA* genotype; Lane 4,6,9: *AG* genotype; Lane 7: *GG* genotype.

5.22 Patients characteristics and clinical predictors for *SLC* gene

Demographic characteristics of the case study group consisted of gender, age, smoking status, tumor size, node, metastasis (TNM) staging, pack years, and various other clinical factors that are illustrated in Table 5.55. At the time of diagnosis, the lung cancer patients had an average age of 60.10 years (29–86). Among 610 patients, 496 (81.3%) males and 114 (18.6%) females were present in the case group. Smokers accounted for 79.6% of the group, more significant than non-

smokers (20.3%). Smokers had an average pack-year of 25.58±31.88. TNM staging was used to classify the patients: 0.81% had stage I lung cancer, 3.11% had stage II lung cancer, 27.5% had stage III lung cancer, and 63.1% had stage IV lung carcinoma. The lung cancer patients T3 and T4 exhibited high frequencies of 20% and 56.8%, whereas subjects with T1 and T2 tumor size constituted 5.90% and 8.8%, respectively. TNM data revealed that 44.5% of patients had no metastatic involvement (M0), while 50.6% had distant metastases (M1). Regarding lymph node invasion, 12.9% of lung carcinoma subjects had no lymph node involvement, while N1, N2, N3, and N4 lymph node invasion accounted for 8.3, 41.8, 31.9, and 0.16% of all cases, respectively. Histologically, squamous cell carcinoma (SQCC) patients made up 40.8% of lung cancer diagnoses, adenocarcinoma (ADCC) patients made up 40.66%, and small-cell lung cancer (SCLC) patients made up 17.3%. Karnofsky's performance status (KPS) and the Eastern Cooperative Oncology Group (ECOG), which indicated the participants' performance status, were used to classify the cases. There were 168 individuals (27.5%) with KPS scores of 100-90, 314 (51.4%) with KPS scores of 80-70, and 106 people (17.3%) with KPS scores of less than 60 in our study. As per the ECOG score, 43.7% (267) of the people had ECOG scores ranging from 0 to 1, 38.1% (233) had ECOG 2, and 14.5% (89%) of the people had ECOG scores of 3-4. Furthermore, 440 patients received cisplatin/carboplatin-based therapy, with 130 patients receiving pemetrexed, 71 patients receiving irinotecan, 87 patients receiving paclitaxel, 70 patients receiving docetaxel, 29 patients receiving gemcitabine, 15 patients receiving gefitinib, 17 patients receiving etoposide, and 21 patients receiving other regimens.

Table 5.55: Patient characteristics among case group recruited to study SLC polymorphisms

Variable	*SLC* SNPs			
	Total (N=610)	**Cases, n (%)**	**Cases, n (%)**	**Total (N)**	**Cases, n (%)**
Gender	610		**Lymph Node**	610	
Male		496 (81.3)	N0		79 (12.9)
Female		114 (18.6)	N1		51 (8.3)
Age	603	60±10.05	N2		255 (41.8)
		29-86	N3		195 (31.9)
Smoking status	609		N4		1 (0.16)
Smokers		485 (79.6)	Unknown		29 (4.75)
Non-smokers		124 (20.3)	**Performance status**	610	
Pack years	600		KPS (below 60)		106 (17.3)
Mean ± SD		25.58±31.88	KPS (70-80)		314 (51.4)
Overall survival	593		KPS (90-100)		168 (27.5)
Dead		493 (83.1)	Unknown		22 (3.6)
Alive		100 (16.8)	ECOG (0-1)		267 (43.7)
TNM staging	610		ECOG (2)		233 (38.1)
I		5 (0.81)	ECOG (3-4)		89 (14.5)
II		19 (3.11)	Unknown		21 (3.4)
III		168 (27.5)	**Histological types**	600	
IV		385 (63.1)	SQCC		245 (40.8)
Unclassified		33 (5.40)	ADCC		244 (40.6)
			SCLC		104 (17.3)
			Others		7 (1.17)
Tumor Size	610		**Chemotherapy regimen**	440	
Tx		21 (3.44)	Pemetrexed cis/carboplatin		130 (29.54)
T1		36 (5.90)	Irinotecan cis/carboplatin		71 (16.13)
T2		54 (8.8)	Docetaxel cis/carboplatin		70 (15.9)
T3		122 (20)	Paclitaxel cis/carboplatin		87 (19.77)
T4		347 (56.8)	Gemcitabine cis/carboplatin		29 (6.59)
Unknown		30 (4.91)	Gefitinib		15 (3.40)
Metastasis	610		Etoposide		17 (3.86)
M0		272 (44.5)	Others		21 (4.77)
M1		309 (50.6)	**Chemotherapy response**	315	
Unknown		29 (4.75)	CR/PR		133 (42.2)
			SD/PD		182 (57.7)

5.23 Statistical analysis of *SLC19A1* gene

5.23.1 Genotypic and genetic equilibrium for SLC19A1 variants

The distribution of genotypes and alleles for *ABCB1* polymorphisms in lung cancer patients has been illustrated in Table 5.56. For *SLC19A1* $G^{80}A$ polymorphism, 195 (31.9%) patients were homozygous for the wild type (*GG)* genotype, and 131 (21.4%) patients were homozygous for the variant (*AA)* genotype, whereas 284 (46.5%) patients were heterozygous (*GA*)**.** MAF was calculated to be 0.44 for *SLC19A1* $G^{80}A$ polymorphism. For *SLC19A1* $G^{80}A$ polymorphism, the genotypic and allelic frequency of case subjects were consistent with Hardy-Weinberg equilibrium with a p-value of 0.14 and $\chi 2$=2.08.

Table 5.56: Genotypic and allelic distribution of *SLC19A1* genetic polymorphism in lung cancer patients

Genotype rs1051266 SLC19A1 (G80A)	No. patients (%)	$\chi 2$	P-value
GG	195 (31.9)		
GA	284 (46.5)		
AA	131 (21.4)	2.08	0.14
G (allele)	674		
A (allele)	546		
MAF	0.44		

5.24 Elucidation of overall survival analysis of *SLC19A1*

5.24.1 Association of SLC19A1 $G^{80}A$ polymorphism in predicting prognosis in LC patients

Survival analysis for 593 lung cancer samples and its association with the *SLC19A1* polymorphism has been studied and depicted in table 5.57. After three years of follow-up, 83.13% of patients were dead and 16.8% alive. After adjusting for gender, smoking status, age, tumor stage, histology, regimen, ECOG, and KPS, the association of the *SLC19A1* SNP with the lung carcinoma survival was evaluated using both univariate and multivariate analysis. In our study, we have applied four different models (*i.e.*, co-dominant, dominant, recessive, and additive) to find the association of OS in lung carcinoma patients regarding *SLC19A1* $G^{80}A$ polymorphism. The results have been presented as the best-fitted model, as shown in Table 5.57.

Lung carcinoma patients who were harboring mutant genotype (*AA*) for *SLC19A1* $G^{80}A$ polymorphism had a higher median survival time (MST) as compared to the subjects carrying wild type (*GG*) genotype (MST=9.33 *vs.* 8.23, p=0.4), though the lack of association was noted. The majority of the lung cancer patients (46.54%) had heterozygous genotype (*GA)* and showed reduced survival as compared to the wild-type genotype (*GG)* (MST=7.27 *vs.* 8.23, HR= 1.10, 95% CI= 0.90-1.34, p=0.33). The *SLC19A1* $G^{80}A$ polymorphism did not show any significant difference in survival in any of the models used. Therefore, we summarize that *SLC19A1* $G^{80}A$ polymorphism did not play any role in predicting overall survival in lung cancer patients.

In lung carcinoma patients, 83.2% (404) of males and 82.4% (89) of females were dead. Males having *SLC19A1* $G^{80}A$ polymorphism and possessing heterozygous genotype (*GA*) showed reduced survival as compared to the subjects harboring wild-type alleles (*GG*) (MST=7.13 *vs.* 7.9 months; HR= 1.1; log-rank p=0.38). After using multivariate analysis and adjusting for factors such as age, smoking, stage, histology, KPS, and ECOG, the death ratio increased to 1.26 times in heterozygous genotype (*GA*) as compared to the wild type (*GG*) genotype (HR=1.26, 95% CI=0.99-1.61; p=0.05) as shown in Table 5.57. However, we did not find any significant association of *SLC19A1* $G^{80}A$ polymorphism with overall survival when variant genotype (*AA)* in females was compared to the wild-type genotype (*GG)* (MST=12.2 *vs.* 8.8 months, HR=0.70, 95% CI=0.70, p=0.35).

Table 5.57: Correlation of *SLC19A1* polymorphism in predicting overall survival in lung cancer patients

Overall Survival								
Genotype rs1051266 SLC19A1 (G80A)	**CASES n (%), N=593**	**DEAD n (%), N=493**	**ALIVE n (%), N=100**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
Codominant model								
GG	194 (32.71)	161 (32.65)	33 (33)	8.23	-	1.0 (reference)	-	-
GA	276 (46.54)	231 (46.85)	45 (45)	7.27	0.33	1.10 (0.90-1.34)	1.11 (0.85-1.45)	0.42
AA	123 (20.74)	101 (20.48)	22 (22)	9.33	0.55	0.92 (0.72-1.18)	0.87 (0.61-1.23)	0.44
Dominant model								
GG	194 (32.71)	161 (32.65)	33 (33)	8.23	-	1.0 (reference)	-	-
GA+AA	399 (67.28)	332 (67.34)	67 (67)	7.93	0.64	1.04 (0.86-1.26)	1.00 (0.78-1.29)	0.94
Recessive model								
GG+GA	470 (79)	392 (79.51)	78 (78)	7.6	-	1.0 (reference)	-	-
AA	123 (21)	101 (20.48)	22 (22)	9.33	0.23	0.88 (0.71-1.08)	0.79 (0.58-1.08)	0.14
Additive model								
GG+AA	317 (53.45)	262 (53.14)	55 (55)	8.8	-	1.0 (reference)	-	-
GA	276 (46.54)	231 (46.85)	45 (45)	7.27	0.15	1.13 (0.95-1.36)	1.16 (0.92-1.48)	0.20
Overall Survival on the basis of gender: Male								
Genotype rs1051266 SLC19A1 (G80A)	**CASES n (%), N=485**	**DEAD n (%), N= 404**	**ALIVE n (%), N=81**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
Codominant model								
GG	155 (31.95)	129 (31.93)	26 (32.09)	7.9	-	1.0 (reference)	-	-
GA	226 (46.59)	190 (47.02)	36 (44.44)	7.13	0.38	1.1 (0.88-1.37)	1.26 (0.99-1.61)	0.05
AA	104 (21.44)	85 (21.03)	19 (23.45)	9.33	0.54	0.91 (0.70-1.20)	0.97 (0.72-1.30)	0.84
Dominant model								
GG	155 (31.95)	129 (31.93)	26 (32.09)	7.9	-	1.0 (reference)	-	-
GA+AA	330 (68.04)	275 (68.06)	55 (67.90)	7.8	0.70	1.04 (0.84-1.28)	1.15 (0.92-1.45)	0.20
Recessive model								
GG+GA	381 (78.55)	319 (78.96)	62 (76.54)	7.47	-	1.0 (reference)	-	-
AA	104 (21.44)	85 (21.03)	19 (23.45)	9.33	0.24	0.87 (0.69-1.09)	0.84 (0.65-1.08)	0.19
Overall Survival on the basis of Gender: Female								
Genotype rs1051266 SLC19A1 (G80A)	**CASES n (%), N=108**	**DEAD n (%), N= 89**	**ALIVE n (%), N=19**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
Codominant model								
GG	39 (36.11)	32 (35.95)	7 (36.84)	8.8	-	1.0 (reference)	-	-
GA	50 (46.29)	41 (46.06)	9 (47.36)	7.6	0.73	1.08 (0.68-1.71)	0.92 (0.54-1.56)	0.76
AA	19 (17.59)	16 (17.97)	3 (15.78)	12.2	0.85	0.94 (0.52-1.71)	0.70 (0.33-1.47)	0.35
Dominant model								
GG	39 (36.11)	32 (35.95)	7 (36.84)	8.8	-	1.0 (reference)	-	-
GA+AA	69 (63.88)	57 (64.04)	12 (63.15)	7.97	0.84	1.04 (0.67-1.60)	0.84 (0.51-1.37)	0.49
Recessive model								
GG+GA	89 (82.40)	73 (82.02)	16 (84.21)	8.2	-	1.0 (reference)	-	-
AA	19 (17.59)	16 (17.97)	3 (15.78)	12.2	0.74	0.91 (0.54-1.55)	0.75 (0.40-1.38)	0.36

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, histology, regimen, KPS, and ECOG for overall survival

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, smoking, stage, histology, regimen KPS, and ECOG for prognosis based on gender

5.24.2 Association of SLC19A1 polymorphism based on histological examination

We further assessed the role of *SLC19A1* $G^{80}A$ variants in predicting median survival of lung carcinoma patients based on histological subtypes. Among 577 lung cancer patients, 42.7% had SQCC, 40.03% had ADCC, and 17.85% were detected with SCLC. On analysis, we noted that ADCC patients harboring mutant genotype (*AA*) showed better survival outcomes as compared to the ADCC subjects with wild-type genotype (*GG*) for *SLC19A1* $G^{80}A$ (MST=9.4 *vs.* 8.8, Log-rank p=0.2) as shown in Table 5.58. After applying the cox regression model, there was a decreased hazard ratio in lung cancer patients with mutant genotype (HR=0.60, 95% CI=0.36-1.00, p**=0.04**), as shown in figure 5.42.

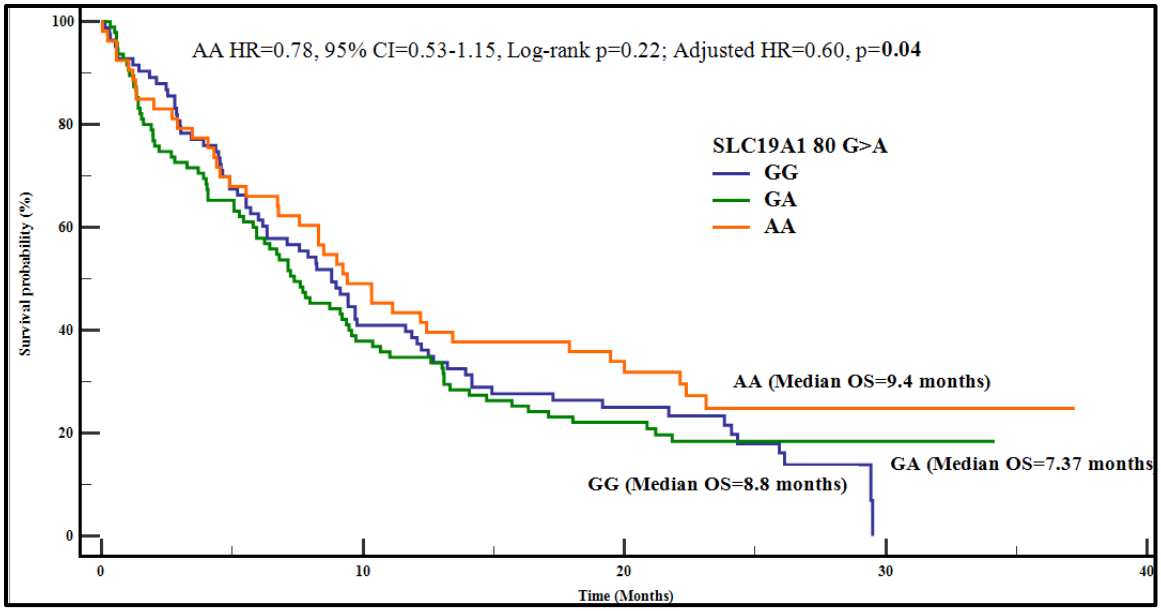


Figure 5.42: Kaplan Meier curves illustrating the association between overall survival in different genotypes of *SLC19A1* ($G^{80}A$) polymorphism in ADCC patients. (Note: *GG* represents wild-type genotype, *GA* represents heterozygous genotype, and *AA* represents homozygous for mutant genotype)

Also, on analyzing the association of *SLC19A1* $G^{80}A$ polymorphism with SCLC histology, our results in the co-dominant model depicted increased survival in the patients harboring mutant genotype (*AA*) as compared to the SCLC subjects having wild type genotype (*GG*) (MST=9.6 months *vs.* 7.6 months, HR= 0.75, 95% CI= 0.41-1.35, p=0.34). After multivariate analysis, such patients had a low death ratio than SCLC subjects carrying the wild genotype (*GG*) (HR=0.22, 95%CI=0.05-0.93, **p=0.04**), as shown in Table 5.58 and Figure 5.43. On applying the recessive model, similar results were also obtained in which SCLC patients with mutant genotype had better survival than the combined wild and heterozygous genotype (MST= 9.6 *vs.* 7.6 months HR=0.30, 95%CI=0.11-0.84, **p=0.02)**. Likewise, patients having SQCC histology showed increased survival difference in the patients having homozygous variant alleles (*AA)* as compared to the homozygous wild type alleles (*GG)* (MST=8.73 *vs.* 7.5, HR=1.28, 95% CI=0.65-2.50, p=0.46), however association was not found among these genotypes and overall survival based on SQCC histology.

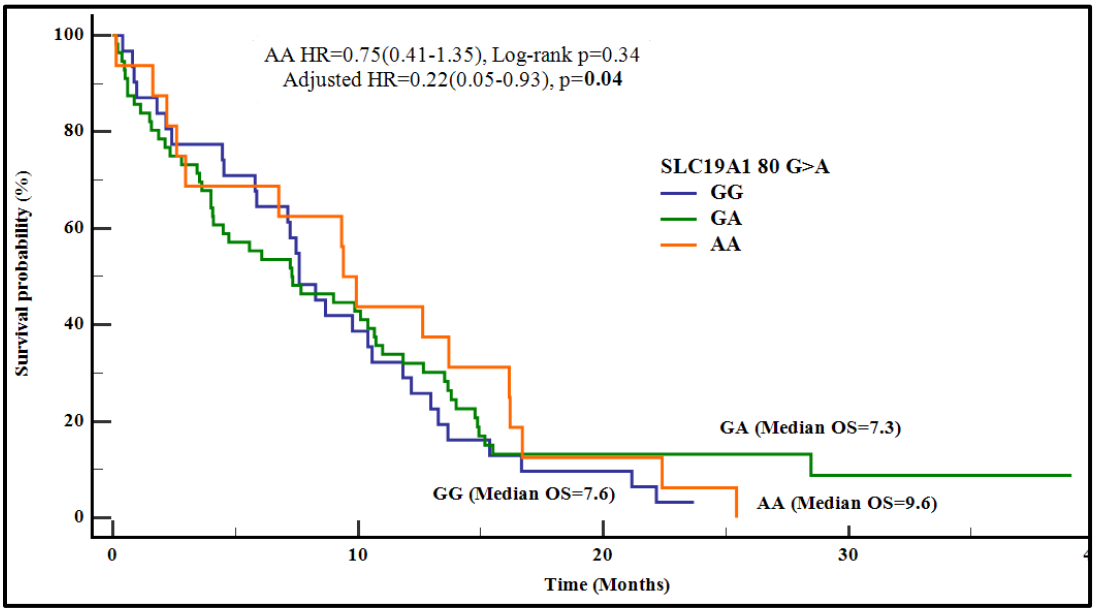


Figure 5.43: Kaplan Meier curves illustrating the association between overall survival in different genotypes of *SLC19A1* ($G^{80}A$) polymorphism in SCLC patients. (Note: *GG* represents wild-type genotype, *GA* represents heterozygous genotype, and *AA* represents homozygous for mutant genotype)

Table 5.58: Correlation of *SLC19A1* polymorphism based on histological examination

Genotype rs1051266 SLC19A1 (G80A)	CASES n (%), N=231	DEAD n (%), N=186	ALIVE n (%), N=45	Univariate analysis			Multivariate analysis	
				MST (months)	Log-rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
Histology-ADCC								
Codominant model								
GG	83 (35.93)	70 (37.63)	13 (28.88)	8.8	-	1.0 (reference)	-	-
GA	95 (41.12)	77 (41.39)	18 (0.4)	7.37	0.82	1.03 (0.74-1.43)	0.91 (0.58-1.44)	0.71
AA	53 (22.94)	39 (20.96)	14 (31.11)	9.4	0.22	0.78 (0.53-1.15)	0.60 (0.36-1.00)	**0.04**
Dominant model								
GG	83 (35.93)	70 (37.63)	13 (28.88)	8.8	-	1.0 (reference)	-	-
GA+AA	148 (64.06)	116 (62.36)	32 (71.11)	8.3	0.69	0.94 (0.69-1.27)	0.76 (0.52-1.12)	0.18
Recessive model								
GG+GA	178 (77.05)	147 (79.03)	31 (68.88)	7.9	-	1.0 (reference)	-	-
AA	53 (22.94)	39 (20.96)	14 (31.11)	9.4	0.16	0.78 (0.56-1.09)	0.70 (0.44-1.10)	0.13
SCLC								
Genotype rs1051266 SLC19A1 (G80A)	**CASES n (%), N=103**	**DEAD n (%), N=95**	**ALIVE n (%), N=8**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
Codominant model								
GG	31 (30.09)	30 (31.57)	1 (12.5)	7.6	-	1.0 (reference)	-	-
GA	56 (54.36)	49 (51.57)	7 (87.5)	7.3	0.62	1.11 (0.70-1.78)	0.87 (0.52-1.45)	0.61
AA	16 (15.53)	16 (16.84)	-	9.6	0.34	0.75 (0.41-1.35)	0.22 (0.05-0.93)	**0.04**
Dominant model								
GG	31 (30.09)	30 (31.57)	1 (12.5)	7.6	-	1.0 (reference)	-	-
GA+AA	72 (69.90)	65 (68.42)	7 (87.5)	9	0.47	0.85 (0.54-1.33)	0.56 (0.30-1.04)	0.07
Recessive model								
GG+GA	87 (84.46)	79 (83.15)	8 (100)	7.6	-	1.0 (reference)	-	-
AA	16 (15.53)	16 (16.84)	-	9.6	0.69	0.89 (0.53-1.51)	0.30 (0.11-0.84)	**0.02**
SQCC								
Genotype rs1051266 SLC19A1 (G80A)	**CASES n (%), N=243**	**DEAD n (%), N=201**	**ALIVE n (%), N=42**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
Codominant model								
GG	73 (30.04)	55 (27.36)	18 (42.85)	7.5	-	1.0 (reference)	-	-
GA	119 (48.97)	102 (50.74)	17 (40.47)	7.1	0.14	1.26 (0.92-1.74)	1.56 (0.96-2.55)	0.07
AA	51 (20.98)	44 (21.89)	7 (16.66)	8.73	0.50	0.87 (0.58-1.30)	1.28 (0.65-2.50)	0.46
Dominant model								
GG	73 (30.04)	55 (27.36)	18 (42.85)	7.5	-	1.0 (reference)	-	-
GA+AA	170 (69.95)	146 (72.63)	24 (57.14)	7.33	0.17	1.23 (0.91-1.65)	1.53 (0.96-2.42)	0.06
Recessive model								
GG+GA	192 (79.01)	157 (78.10)	35 (83.33)	7.1	-	1.0 (reference)	-	-
AA	51 (20.98)	44 (21.89)	7 (16.66)	8.73	0.90	0.98 (0.70-1.36)	0.95 (0.58-1.58)	0.87

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, regimen, KPS, ECOG

5.24.3 Correlation of SLC19A1 polymorphism with chemotherapy regimens and overall survival

The lung carcinoma patients in the study were administered platinum-based chemotherapy along with paclitaxel, docetaxel, pemetrexed, and irinotecan. So, we chose to investigate the modifying effects of the *SLC* polymorphism and its relationship to chemotherapy, and overall patient survival, as shown in Table 5.59. Chemotherapy was divided into four regimens, each given to different patient groups. All lung malignancy patients received platinum-based therapy (carboplatin/ cisplatin) and a second-line chemotherapy treatment such as docetaxel, pemetrexed, paclitaxel, or irinotecan. Our results demonstrated that LC patients administered with cisplatin/carboplatin along with docetaxel did not show any significant association when patients with at least one mutant allele (*GA)* were compared with the individuals having wild type genotype (*GG)* (MST=9.73 *vs.* 9.96 months, p=0.44).

Additionally, LC patients undergoing cisplatin/carboplatin and paclitaxel showed 2-times better survival in the patients having mutant genotype (*AA)* as compared to the wild type genotype (*GG)*, though substantial differences were not achieved (MST=9.1 *vs.* 4.9 months, HR=0.65, p=0.34). Along the same lines, patients undergoing pemetrexed (MST=10.3 *vs.* 8.8, HR=0.77, p=0.40) or irinotecan (MST=16.7 *vs.* 7.47, HR=0.49, p=0.23) treatment along with cisplatin/carboplatin did not show any association in predicting overall survival in lung cancer patients; however, better survival was observed in both regimens in the mutant genotype as compared to the wild type genotype as shown in table 5.59. Therefore, we summarize that *SLC19A1* $G^{80}A$ polymorphism was not correlated with predicting prognosis in lung cancer patients who underwent platinum-based doublet chemotherapy.

Table 5.59: Association of *SLC19A1* SNP on overall survival according to chemotherapeutic regimen

Regimen-Docetaxel cisplatin/carboplatin

Genotype rs1051266 SLC19A1 (G80A)	CASES n (%), N=69	DEAD n (%), N= 56	ALIVE n (%), N=13	Univariate analysis			Multivariate analysis	
				MST (months)	Log-rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
Codominant model								
GG	22 (31.88)	17 (30.35)	5 (38.46)	9.66	-	1.0 (reference)	-	-
GA	30 (43.47)	25 (44.64)	5 (38.46)	9.73	0.69	1.12 (0.61-2.08)	1.34 (0.62-2.89)	0.44
AA	17 (24.63)	14 (25)	3 (23.07)	8.5	0.78	1.10 (0.54-2.26)	1.05 (0.48-2.31)	0.88
Dominant model								
GG	22 (31.88)	17 (30.35)	5 (38.46)	9.66	-	1.0 (reference)	-	-
GA+AA	47 (68.11)	39 (69.64)	8 (61.53)	8.57	0.70	1.11 (0.63-1.95)	1.21 (0.64-2.28)	0.55
Recessive model								
GG+GA	52 (75.36)	42 (75)	10 (76.92)	9.13	-	1.0 (reference)	-	-
AA	17 (24.63)	14 (25)	3 (23.07)	8.5	0.9	1.0 (0.54-1.84)	0.87 (0.45-1.79)	0.70

Regimen-Paclitaxel cisplatin/carboplatin

Genotype rs1051266 SLC19A1 (G80A)	CASES n (%), N=86	DEAD n (%), N= 73	ALIVE n (%), N=13	Univariate analysis			Multivariate analysis	
				MST (months)	Log-rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
Codominant model								
GG	29 (33.72)	24 (32.87)	5 (38.46)	4.9	-	1.0 (reference)	-	-
GA	42 (48.83)	35 (47.94)	7 (53.84)	6.1	0.95	0.98 (0.58-1.66)	1.25 (0.69-2.27)	0.45
AA	15 (17.44)	14 (19.17)	1 (7.69)	9.1	0.77	0.90 (0.46-1.75)	0.65 (0.27-1.55)	0.34
Dominant model								
GG	29 (33.72)	24 (32.87)	5 (38.46)	4.9	-	1.0 (reference)	-	-
GA+AA	57 (66.27)	49 (67.12)	8 (61.53)	7.97	0.9	0.97 (0.59-1.58)	1.17 (0.66-2.07)	0.57
Recessive model								
GG+GA	71 (82.55)	59 (80.82)	12 (92.30)	5.63	-	1.0 (reference)	-	-
AA	15 (17.44)	14 (19.17)	1 (7.69)	9.1	0.67	0.86 (0.50-1.55)	0.79 (0.42-1.51)	0.49

Regimen-Pemetrexed cisplatin/carboplatin

Genotype rs1051266 SLC19A1 (G80A)	CASES n (%), N=122	DEAD n (%), N=101	ALIVE n (%), N=21	Univariate analysis			Multivariate analysis	
				MST (months)	Log-rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
Codominant model								
GG	45 (36.88)	38 (37.62)	7 (33.33)	8.8		1.0 (reference)	-	-
GA	50 (40.98)	43 (42.57)	7 (33.33)	6.4	0.31	1.25 (0.80-1.94)	0.94 (0.58-1.54)	0.83
AA	27 (22.13)	20 (19.80)	7 (33.33)	10.3	0.5	0.85 (0.50-1.45)	0.77 (0.42-1.41)	0.40
Dominant model								
GG	45 (36.88)	38 (37.62)	7 (33.33)	8.8	-	1.0 (reference)	-	-
GA+AA	77 (63.11)	63 (62.37)	14 (66.66)	10.3	0.67	1.09 (0.73-1.62)	0.90 (0.58-1.40)	0.6
Recessive model								
GG+GA	95 (77.86)	81 (80.19)	14 (66.66)	7.57	-	1.0 (reference)	-	-
AA	27 (22.13)	20 (19.80)	7 (33.33)	10.3	0.28	0.77 (0.49-1.22)	0.79 (0.47-1.34)	0.40

Regimen-Irinotecan cisplatin/carboplatin

Genotype rs1051266 SLC19A1 (G80A)	CASES n (%), N=70	DEAD n (%), N=62	ALIVE n (%), N=8	Univariate analysis			Multivariate analysis	
				MST (months)	Log-rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
Codominant model								
GG	23 (32.85)	22 (35.48)	1 (12.5)	7.47	-	1.0 (reference)	-	-
GA	38 (54.28)	33 (53.22)	5 (62.5)	7.67	0.72	0.90 (0.52-1.56)	0.82 (0.43-1.59)	0.5
AA	9 (12.85)	7 (11.29)	2 (25)	16.7	0.10	0.51 (0.24-1.09)	0.49 (0.15-1.55)	0.23
Dominant model								
GG	23 (32.85)	22 (35.48)	1 (12.5)	7.47	-	1.0 (reference)	-	
GA+AA	47 (67.14)	40 (64.51)	7 (87.5)	9.86	0.3	0.78 (0.45-1.34)	0.70 (0.38-1.28)	0.25
Recessive model								
GG+GA	61 (87.14)	55 (88.70)	6 (75)	7.47	-	1.0 (reference)	-	-
AA	9 (12.85)	7 (11.29)	2 (25)	16.7	0.08	0.56 (0.29-1.07)	0.43 (0.16-1.13)	0.09

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, histology, KPS, ECOG

5.24.4 Relationship of SLC19A1 polymorphism with prognosis based on smoking habits

In the present study, we have assessed the role of *SLC19A1* $G^{80}A$ polymorphism in modulating prognosis when stratified based on smokers and non-smokers, as depicted in table 5.60. According to our analysis, 84.81% of smokers were dead, while only 15.18% were alive. For $G^{80}A$ polymorphism, reduced survival was observed in the heterozygous (*GA)* (MST=7.13 *vs.* 7.47, Log-rank p=0.23) genotype as compared to the wild type genotype (*GG)*; however, the data lacked statistical significance. In the case of non-smoker lung cancer patients, 46.15% of individuals harbored at least one copy of mutant allele (*GA),* while 33.33% of patients had wild-type genotype (*GG)*. Patients with heterozygous genotype had poor survival compared to the wild-type genotype, but a lack of association between $G^{80}A$ and overall survival was noted (MST=7.8 *vs.* 9.7, HR=1.47, p=0.26). We did not find any correlation between *SLC19A1* polymorphism in predicting prognosis in lung carcinoma patients based on smoking status.

Table 5.60: Association of *SLC19A1* SNP on overall survival according to smoking habits, tumor stage, and performance status

Smokers								
Genotype rs1051266 SLC19A1 (G80A)	**CASES n (%), N=474**	**DEAD n (%), N= 402**	**ALIVE n (%), N=72**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
Codominant model								
GG	153 (32.27)	127 (31.59)	26 (36.11)	7.47	-	1.0 (reference)	-	-
GA	222 (46.83)	191 (47.51)	31 (43.05)	7.13	0.23	1.14 (0.91-1.43)	1.10 (0.81-1.50)	0.52
AA	99 (20.88)	84 (20.89)	15 (20.83)	9.10	0.76	0.95 (0.72-1.26)	0.90 (0.60-1.35)	0.63
Dominant model								
GG	153 (32.27)	127 (31.59)	26 (36.11)	7.47	-	1.0 (reference)	-	-
GA+AA	321 (67.72)	275 (68.40)	46 (63.88)	7.67	0.45	1.08 (0.88-1.33)	1.01 (0.76-1.34)	0.93
Recessive model								
GG+GA	375 (79.11)	318 (79.10)	57 (79.16)	7.23	-	1.0 (reference)	-	-
AA	99 (20.88)	84 (20.89)	15 (20.83)	9.10	0.31	0.88 (0.70-1.11)	0.84 (0.59-1.19)	033
Non-Smokers								
Genotype rs1051266 SLC19A1 (G80A)	**CASES n (%), N=117**	**DEAD n (%), N= 90**	**ALIVE n (%), N=27**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
Codominant model								
GG	39 (33.33)	33 (36.66)	6 (22.22)	9.7	-	1.0 (reference)	-	-
GA	54 (46.15)	40 (54.44)	14 (51.85)	7.8	0.69	0.91 (0.57-1.44)	1.47 (0.74-2.90)	0.26
AA	24 (20.51)	17 (18.88)	7 (25.92)	12.2	0.37	0.77 (0.43-1.36)	0.61 (0.24-1.56)	0.30
Dominant model								
GG	39 (33.33)	33 (36.66)	6 (22.22)	9.7	-	1.0 (reference)	-	-
GA+AA	78 (66.66)	57 (63.33)	21 (77.77)	9.4	0.51	0.86 (0.55-1.33)	1.23 (0.65-2.31)	0.51
Recessive model								
GG+GA	93 (79.48)	73 (81.11)	20 (74.07)	9.43	-	1.0 (reference)	-	-
AA	24 (20.51)	17 (18.88)	7 (25.92)	12.2	0.47	0.83 (0.50-1.37)	0.63 (0.30-1.31)	0.22

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, stage histology, regimen, KPS, and ECOG

5.24.5 Relationship of SLC19A1 polymorphism with prognosis based on tumor size

To understand the prognostic impact of the cancer stage in lung carcinoma patients, we have stratified the patients based on their respective tumor stages III and IV, as shown in table 5.61. In stage III lung cancer patients, the prevalence of at least one mutant allele (*GA)* was predominant as compared to wild (*GG)* and mutant genotype (*AA)* (51.52%, 26.67%, and 21.82%, respectively). Patients in stage III harboring mutant genotype (*AA)* had better survival as compared to the individual having wild type genotype (*GG)*, though the substantial significance between the genotypes was not reached (MST=10.3 *vs.* 8.37, HR=0.51, p=0.12). In the case of *SLC19A1* $G^{80}A$ polymorphism, in the co-dominant model, the stage IV patients having mutant genotype (*AA)* showed better survival outcome as compared to the wild genotype (*GG)* (MST=10.3 *vs.* 8.37, HR=0.87, 95% CI=0.22-1.19, p=0.12). However, we did not find any association of $G^{80}A$ polymorphism in predicting overall survival in stage III and IV lung carcinoma patients.

Table 5.61: Association of *SLC19A1* polymorphism with overall survival based on tumor size

Stage III

Genotype rs1051266 SLC19A1 (G80A)	CASES n (%), N=165	DEAD n (%), N=132	ALIVE n (%), N=33	Univariate analysis			Multivariate analysis	
				MST (months)	Log-rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
Codominant model								
GG	44 (26.67)	34 (25.76)	10 (30.30)	8.37	-	1.0 (reference)	-	-
GA	85 (51.52)	71 (53.79)	14 (42.42)	8.5	0.67	1.08 (0.72-1.63)	0.88 (0.51-1.53)	0.66
AA	36 (21.82)	27 (20.45)	9 (27.27)	10.3	0.58	0.87 (0.52-1.44)	0.51 (0.22-1.19)	0.12
Dominant model								
GG	44 (26.67)	34 (25.76)	10 (30.30)	8.37	-	1.0 (reference)	-	-
GA+AA	121 (73.3))	98 (74.24)	23 (69.69)	9.27	0.93	1.01 (0.68-1.49)	0.71 (0.42-1.18)	0.19
Recessive model								
GG+GA	129 (78.1)	105 (94.6)	24 (72.72)	8.5	-	1.0 (reference)	-	-
AA	36 (21.82)	27 (20.45)	9 (27.27)	10.33	0.34	0.82 (0.55-1.23)	0.56 (0.26-1.17)	0.12

Stage IV

Genotype rs1051266 SLC19A1 (G80A)	CASES n (%), N=371	DEAD n (%), N=318	ALIVE n (%), N=53	Univariate analysis			Multivariate analysis	
				MST (months)	Log-rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
Codominant model								
GG	128 (34.50)	110 (34.59)	18 (33.96)	7.6	-	1.0 (reference)	-	-
GA	169 (45.55)	144 (45.28)	25 (47.17)	6.43	0.25	1.15 (0.90-1.47)	1.29 (0.93-1.78)	0.12
AA	74 (19.95)	64 (20.13)	10 (18.87)	9.0	0.80	0.96 (0.70-1.30)	1.09 (0.73-1.65)	0.65
Dominant model								
GG	128 (34.50)	110 (34.59)	18 (33.96)	7.6	-	1.0 (reference)	-	-
GA+AA	243 (65.4)	208 (65.4)	35 (66.0)	7.23	0.45	1.09 (0.86-1.37)	1.18 (0.88-1.60)	0.25
Recessive model								
GG+GA	297 (79.24)	254 (79.87)	43 (81.13)	7.13	-	1.0 (reference)	-	-
AA	74 (19.95)	64 (20.13)	10 (18.87)	9.0	0.40	0.89 (0.68-1.16)	0.88 (0.62-1.27)	0.52

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, histology, regimen, KPS, and ECOG

5.24.6 Relationship of SLC19A1 polymorphism with prognosis based on ECOG performance status

The impact of ECOG-PS in association with *SLC19A1* polymorphism to predict survival in lung cancer patients was also assessed; the individuals were categorized based on ECOG-PS scores 0,1 (suitable/restricted performance status), and ECOG-PS scores 2,3,4 (limited/bad performance status) as shown in table 5.62. For ECOG-PS 0,1, 27.90% of patients had wild-type genotype (*GG)*, 51.55% of patients had heterozygous genotype (*GA)*, and 20.54% of patients had mutant genotype (*AA)*. When the co-dominant model was applied, the mutant genotype (*AA)* showed better survival as compared to the wild-type genotype (*GG)* (MST=12.2 *vs.* 11.87, HR=1.08, p=0.77). In the recessive model, patients with ECOG-PS 2,3,4 having $G^{80}A$ polymorphism showed better survival in mutant genotype (*AA)* as compared to the combined wild type and heterozygous genotype (*GG+GA)* (MST=6.8 *vs.* 5.93, HR=0.82, p=0.17). After adjusting with different covariates, the patients carrying mutant genotype *(AA)* had low hazard ratio as compared to the reference (*GG+GA)* genotype (HR=0.60, p=**0.02)**.

Table 5.62: Association of *SLC19A1* polymorphism with overall survival based on ECOG-PS

ECOG 0,1								
Genotype rs1051266 SLC19A1 (G80A)	**CASES n (%), N=258**	**DEAD n (%), N=193**	**ALIVE n (%), N=65**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
Codominant model								
GG	72 (27.90)	54 (27.97)	18 (27.69)	11.87	-	1.0 (reference)	-	-
GA	133 (51.55)	99 (51.29)	34 (52.30)	10.57	0.53	1.1 (0.79-1.53)	1.28 (0.80-2.06)	0.29
AA	53 (20.54)	40 (20.72)	13 (20)	12.2	0.81	0.95 (0.63-1.43)	1.08 (0.61-1.94)	0.77
Dominant model								
GG	72 (27.90)	54 (27.97)	18 (27.69)	11.87	-	1.0 (reference)	-	-
GA+AA	186 (72.09)	139 (72.02)	47 (72.30)	10.6	0.70	1.06 (0.77-1.44)	1.25 (0.81-1.92)	0.30
Recessive model								
GG+GA	205 (79.4)	153 (79.27)	52 (80.0)	11.0	-	1.0 (reference)	-	-
AA	53 (20.54)	40 (20.72)	13 (20.0)	12.2	0.50	0.89 (0.63-1.24)	1.08 (0.67-1.73)	0.74
ECOG 2,3,4								
Genotype rs1051266 SLC19A1 (G80A)	**CASES n (%), N=315**	**DEAD n (%), N=284**	**ALIVE n (%), N=31**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
Codominant model								
GG	113 (35.87)	102 (35.91)	11 (35.48)	6.3	-	1.0 (reference)	-	-
GA	136 (43.17)	125 (44.01)	11 (35.48)	5.27	0.20	1.18 (0.91-1.53)	1.11 (0.79-1.58)	0.52
AA	66 (20.95)	57 (20.07)	9 (29.03)	6.8	0.48	0.89 (0.64-1.23)	0.70 (0.43-1.13)	0.15
Dominant model								
GG	113 (35.87)	102 (35.91)	11 (35.48)	6.3	-	1.0 (reference)	-	-
GA+AA	202 (64.12)	182 (64.08)	20 (64.51)	5.8	0.57	1.07 (0.84-1.36)	0.91 (0.66-1.25)	0.57
Recessive model								
GG+GA	249 (79.04)	227 (79.92)	22 (70.96)	5.93	-	1.0 (reference)	-	-
AA	66 (20.95)	57 (20.07)	9 (29.03)	6.86	0.17	0.82 (0.62-1.08)	0.61 (0.39-0.94)	**0.02**

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, histology, regimen, and KPS.

5.24.7 Association of SLC19A1 polymorphism and clinic-pathological parameters

In order to assess the correlation between *SLC19A1* variants and the clinic-pathological parameters, the patients were bifurcated based on cancer stage, lymph node involvement, direct tumor extension, and metastasis, as shown in Table 5.63. The lung cancer patients were divided into stages III and IV based on the clinical stage. The genotypic frequency of all the polymorphisms has been evaluated. Among 553 patients, the incidence of patients in stage IV was 69.62% (385/553), whereas 30.37% of patients were in stage III of lung cancer. For *SLC19A1* $G^{80}A$ polymorphism, the prevalence of mutant type genotype in stage IV (20.77%) was less than that of stage III (22.61%). Our data showed that LC patients in stage IV with mutant genotype *(AA)* had higher odds of having lung cancer than stage III patients (AOR=1.28, 95% CI=0.61-2.70, p=0.50). However, no significant association was found between $G^{80}A$ polymorphisms and tumor stage.

Further, the patients were stratified based on direct tumor extension (T3 *vs.* T4). The frequency of each genotype is mentioned in table 5.63. 18.7% of lung cancer patients diagnosed with T4 tumor extension carried mutant alleles for the *SLC19A1* $G^{80}A$ genotype compared to 30.3% of patients in T3 tumor extension**.** A decreased odd of developing T4 were observed in mutant genotype (*AA*) compared to the patients categorized in T3 tumor extension (AOR=0.42, 95% CI=0.2-0.89; p=**0.02**). Furthermore, we did not observe any significant association between lymph node invasion and metastasis with any genotype of $G^{80}A$ polymorphic variants**.**

Correlation between *SLC19A1* $G^{80}A$ polymorphism and the effectiveness of response rate and clinical benefits were also studied using univariate logistic regression. Table 5.63 shows the relationship between *SLC19A1* polymorphism and treatment responses. The individuals showing stable and progressive disease were categorized as non-responders (SD+PD), whereas subjects demonstrating complete or partial response (CR+PR) were classified as good responders. The outcomes did not show any statistical significance between the *SLC19A1* $G^{80}A$ polymorphism and platinum-based chemotherapeutic treatment response.

Table 5.63: Association of *SLC19A1* SNPs and chemotherapeutic response

CLINICAL STAGE				
Genotype rs1051266 SLC19A1 (G80A)	**Stage III (168), n (%)**	**Stage IV (385), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
GG	45 (26.78)	128 (33.24)	1.0 (Reference)	
GA	85 (50.59)	177 (45.97)	1.14 (0.64-2.03)	0.64
AA	38 (22.61)	80 (20.77)	1.28 (0.61-2.70)	0.50
GA+AA	123 (73.21)	257 (66.75)	1.19 (0.69-2.04)	0.52
TUMOR SIZE				
Genotype rs1051266 SLC19A1 (G80A)	**T3 (122), n (%)**	**T4 (347), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
GG	35 (28.68)	101 (29.10)	1.0 (Reference)	
GA	50 (40.98)	181 (52.16)	1.19 (0.62-2.29)	0.58
AA	37 (30.32)	65 (18.73)	0.42 (0.20-0.89)	**0.02**
GA+AA	87 (71.31)	246 (70.89)	0.86 (0.48-1.55)	0.62
LYMPH NODE INVASION				
Genotype rs1051266 SLC19A1 (G80A)	**Nx+N0+N1 (130), n (%)**	**N2+N3+N4 (451), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
GG	40 (30.76)	142 (31.48)	1.0 (Reference)	
GA	63 (48.46)	212 (47.00)	0.82 (0.45-1.49)	0.52
AA	27 (20.76)	97 (21.50)	1.07 (0.50-2.30)	0.8
GA+AA	90 (69.23)	309 (68.51)	0.89 (0.51-1.55)	0.69
METASTASIS				
Genotype rs1051266 SLC19A1 (G80A)	**M0 (272), n (%)**	**M1 (309), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
GG	73 (26.83)	109 (35.27)	1.0 (Reference)	
GA	142 (52.20)	133 (43.04)	0.69 (0.41-1.17)	0.17
AA	57 (20.95)	67 (21.68)	1.30 (0.68-2.49)	0.41
GA+AA	199 (73.16)	200 (64.72)	0.84 (0.52-1.37)	0.50
	Response of chemotherapy		**AOR (95% CI) [a]**	***p*[b]**
Genotype rs1051266 SLC19A1 (G80A)	**CR+PR (133), n (%)**	**SD+PD (182), n (%)**		
GG	42 (31.57)	54 (29.67)	1.0 (Reference)	
GA	70 (52.63)	78 (42.85)	0.79 (0.43-1.46)	0.45
AA	21 (15.78)	50 (27.47)	1.57 (0.76-3.23)	0.21
GA+AA	91 (68.42)	128 (70.32)	0.9 (0.57-1.74)	0.9

a)-Adjusted Odds ratios, 95% confidence intervals, and corresponding *p*-values were calculated by logistic regression analysis after adjusting for age, gender, regimen, and smoking. (b)-Two-sided $\chi 2$ test for either genotype distribution or allelic frequencies between different clinical-pathological parameters. The number in bold indicates the significant values in the table

5.25 Statistical analysis of *SLCO1B1* variants

5.25.1 Genotypic and allelic distribution of SLCO1B1 variants

For the *SLCO1B1* gene, we have studied two common non-synonymous single-nucleotide polymorphisms, $A^{388}G$ and $T^{521}C$. In the case of *SLCO1B1* $A^{388}G$ polymorphism, 152 (24.9%) patients were harboring homozygous *AA* genotype, 297 (48.6%) were heterozygous *AG* genotype, and 161 (26.3%) were homozygote *GG* variant, whereas, for *SLCO1B1* $T^{521}C$, 563 (92.2%) patients were homozygous for *TT* genotype, 45 (7.3%) patients harbored heterozygous (*TC)* genotype and 2 (0.32%) individuals had homozygous (*CC)* genotype. The genotypic frequencies of *SLCO1B1* $A^{388}G$ ($\chi2$=0.41, df=2; p=0.52) and *SLCO1B1* $T^{521}C$ polymorphism ($\chi2$=1.13, df=2; p=0.28) is depicted in table 5.64. The MAF for *SLCO1B1* $A^{388}G$ and $T^{521}C$ was calculated to be 0.50 and 0.04, respectively. Thus, genotypic distribution for these two polymorphic sites followed the Hardy-Weinberg equilibrium with a p-value> 0.05**.** So, these samples could be a Mendelian population in a state of genetic equilibrium.

Table 5.64: Genotypic and allelic distribution of *SLCO1B1* variants

Genotype rs2306283 SLCO1B1 (A388G)	**No. patients (%) N=610**	**$\chi2$**	**P-value**
AA	152 (24.9)		
AG	297 (48.6)	0.41	0.52
GG	161 (26.3)		
AG+GG	458 (75.0)		
A (allele)	601		
G (allele)	619		
MAF	0.50		
Genotype rs4149056 SLCO1B1 (T521C)	**No. patients (%) N=610**	**$\chi2$**	**P-value**
TT	563 (92.2)		
TC	45 (7.3)	1.13	0.28
CC	2 (0.32)		
TC+CC	47 (7.7)		
T (allele)	1171		
C (allele)	49		
MAF	0.04		

5.26 Elucidation of *SLCO1B1* polymorphism in predicting overall survival in LC patients

5.26.1 Correlation of SLCO1B1 polymorphisms with prognosis

In the current study, the correlation of the *SLCO1B1* polymorphisms with overall survival in 593 lung cancer samples was investigated. The analysis used four alternative models (co-dominant, dominant, recessive, and additive) to determine the relationship between *SLCO1B1* polymorphism and OS in lung cancer patients. As demonstrated in Table 5.65, the findings were presented as the best-fitted model. For *SLCO1B1* $A^{388}G$, in the co-dominant model, we observed that the prevalence of the wild-type genotype (*AA)* was higher as compared to the mutant-type genotype (*GG)* (26.30% *vs.* 24.78%). There was no difference in MST's of patients carrying mutant (*GG*) and wild-type (*AA*) genotype (MST=7.8 *vs.* 7.9; Log-rank p=0.41; HR=1.10). After applying the Cox regression model, the death ratio increased to 1.40-times in patients carrying mutant (*GG*) alleles when compared with patients carrying wild (*AA*) allele (HR=1.40, 95% CI=1.0-1.97; p=**0.04**), as shown in figure 5.44. For *SLCO1B1* $T^{521}C$ polymorphism, there was a high incidence of wild-type genotype (*TT)* and a very low prevalence of mutant genotype (*CC)* (92.41% *vs.* 0.33%). We observed lower survival in the lung cancer patients who had mutant genotype (*CC*) as compared to the patients carrying wild-type genotype (*TT*) (MST=5.16 versus 7.97; p=0.80). However, no significant differences in terms of survival were observed for the *SLCO1B1* $T^{521}C$ variant.

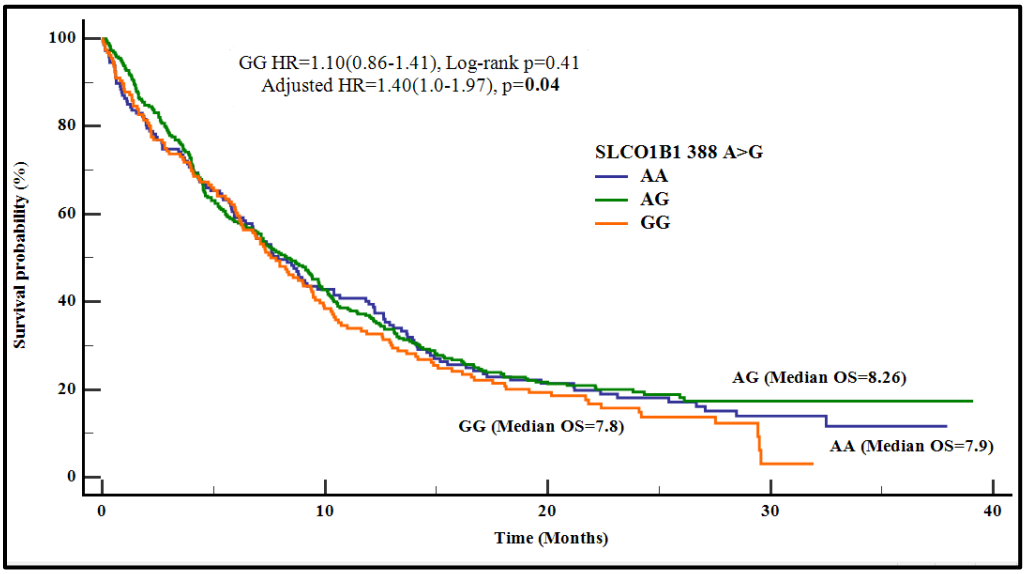


Figure 5.44: Kaplan Meier curves illustrating the association between overall survival in different genotypes of *SLCO1B1* ($A^{388}G$) polymorphism in lung cancer patients. (Note: AA represents wild-type genotype, AG represents heterozygous genotype, and GG represents homozygous for mutant genotype)

Table 5.65: Association of *SLCO1B1* polymorphism and overall survival in lung cancer patients

Genotype rs2306283 SLCO1B1 (A388G)	CASES n (%), N=593	DEAD n (%), N=493	ALIVE n (%), N=100	Univariate analysis			Multivariate analysis	
				MST (months)	Log rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*P*
Codominant model								
AA	147 (24.78)	124 (25.15)	23 (23)	7.9	-	1.0 (reference)	-	
AG	290 (48.90)	234 (47.46)	56 (56)	8.26	0.67	0.95 (0.76-1.18)	1.18 (0.88-1.59)	0.26
GG	156 (26.30)	135 (27.38)	21 (21)	7.8	0.41	1.10 (0.86-1.41)	1.40 (1.0-1.97)	**0.04**
Dominant model								
AA	147 (24.78)	124 (25.15)	23 (23)	7.9	-	1.0 (reference)	-	
AG+GG	446 (75.21)	369 (74.84)	77 (77)	7.97	0.9	1.00 (0.81-1.22)	1.27 (0.96-1.69)	0.08
Recessive model								
AA+AG	437 (73.69)	358 (72.61)	79 (79)	8.26	-	1.0 (reference)	-	
GG	156 (26.30)	135 (27.38)	21 (21)	7.8	0.24	1.12 (0.92-1.38)	1.23 (0.95-1.60)	0.11
Additive model								
AA+GG	303 (51.09)	259 (52.53)	44 (44)	7.8	-	1.0 (reference)	-	
AG	290 (48.90)	234 (47.46)	56 (56)	8.26	0.31	0.91 (0.76-1.08)	1.02 (0.80-1.29)	0.8
Genotype rs4149056 SLCO1B1 (T521C)	**CASES n (%), N=593**	**DEAD n (%), N=493**	**ALIVE n (%), N=100**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
TT	548 (92.41)	453 (91.88)	95 (95)	7.97	-	1.0 (reference)		
TC	43 (7.25)	38 (7.70)	5 (5)	7.47	0.57	1.10 (0.78-1.55)	0.95 (0.61-1.48)	0.84
CC	2 (0.33)	2 (0.40)	0	5.16	0.98	0.98 (0.24-3.93)	0.77 (0.10-5.69)	0.80
Dominant model								
TT	548 (92.41)	453 (91.88)	95 (95)	7.97	-	-	-	-
TC+CC	45 (7.58)	40 (8.11)	5 (5)	7.47	0.58	1.09 (0.78-1.53)	0.94 (0.61-1.44)	0.79

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, regimen, KPS, ECOG

5.26.2 Association of SLCO1B1 polymorphism with histology and overall survival

For two *SLCO1B1* polymorphisms, we assessed the relevance of predicting overall survival in lung cancer patients with different histological subtypes. The data is depicted in Table 5.66. For $A^{388}G$, the incidence of subjects carrying homozygous mutant genotype *(GG)* was 24.67%, 26.74%, and 29.12% in lung cancer patients diagnosed with ADCC, SQCC, and SCLC histological subtype, respectively. ADCC subjects having mutant alleles (*GG)* had better survival than the patients with wild-type alleles (MST=8.3 *vs.* 7.9). After multivariate analysis, no statistical significance was found even though ADCC patients had a high death ratio (HR=1.34, 95% CI=0.73-2.44, p=0.33), as shown in table 5.66. However, SQCC patients with mutant genotype for $A^{388}G$ polymorphism showed reduced survival in the recessive model compared to the survival of combined wild and heterozygous genotype (*AA+AG)* (MST=6.8 *vs.* 7.5, HR=1.13, Log-rank p=0.42). Though, after multivariate analysis, the death ratio increased to 1.54 times, and borderline significance was noted (HR=1.54, 95% CI=0.99-2.39, p=0.05). In SCLC patients with $A^{388}G$ polymorphism, the patients with mutant genotype had reduced survival compared to the wild-type

genotype. However, a lack of association among the groups was found (MST=9 *vs.* 11.83, HR = 1.53, 9% CI= 0.89-2.64, p=0.10).

In the case of $T^{521}C$ polymorphism, the number of mutants (*CC)* were less represented, but the prevalence of heterozygous genotype (*TC)* was 7.79%, 5.34%, and 11.65% in ADCC, SQCC, and SCLC, respectively, as shown in table 5.66. In ADCC patients, no mutant (*CC)* genotype was present, and the heterozygous genotype (*TC)* showed better survival as compared to the wild-type genotype (*TT)* (MST=9 *vs.* 8.3, Log-rank p=0.47). In SQCC patients, the combined heterozygous and mutant genotype *(TC+CC*) in the dominant model showed reduced survival compared to the wild-type genotype (MST=5.16 *vs.* 7.33, HR=1.58, 95% CI=0.85-2.96, Log-rank p=0.14). The difference in MST pertains to decreased death ratio (HR=1.30, 95% CI=0.59-2.89, p=0.5) but lacked statistical difference. In SCLC patients, none of the LC individuals harbored mutant genotype. The SCLC patients with $T^{521}C$ polymorphism showed reduced survival in individuals harboring at least one mutant genotype (*TC)* as compared to the wild type genotype (*TT)* (MST=7.23 *vs.* 8.26 months, HR= 0.65, p=0.42). Therefore, we summarize no significant correlation between $T^{521}C$ polymorphism and prognosis among all histological subtypes in lung cancer patients.

Table 5.66: Association of *SLCO1B1* polymorphisms and prognosis in lung cancer patients based on histological subtypes

Genotype rs2306283 SLCO1B1(A388G)	CASES n (%), N=231	DEAD n (%), N=186	ALIVE n (%), N=45	Univariate analysis			Multivariate analysis	
				MST (months)	Log rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*P*
Histology- ADCC								
Codominant model								
AA	58 (25.01)	47 (25.26)	11 (24.44)	7.9	-	1.0 (reference)	-	
AG	116 (50.21)	92 (49.46)	24 (53.33)	9.13	0.81	0.95 (0.67-1.36)	1.21 (0.72-2.04)	0.45
GG	57 (24.67)	47 (25.26)	10 (22.22)	8.3	0.95	0.98 (0.65-1.48)	1.34 (0.73-2.44)	0.33
Dominant model								
AA	58 (25.01)	47 (25.26)	11 (24.44)	7.9	-	1.0 (reference)	-	
AG+GG	173 (74.89)	139 (74.73)	34 (75.55)	9	0.83	0.96 (0.69-1.34)	1.27 (0.78-2.05)	0.32
Recessive model								
AA+AG	174 (75.32)	139 (74.73)	35 (77.77)	8.5	-	1.0 (reference)	-	
GG	57 (24.67)	47 (25.26)	10 (22.22)	8.3	0.90	1.02 (0.73-1.42)	1.08 (0.70-1.66)	0.71
Genotype rs4149056 SLCO1B1 (T521C)	**CASES n (%), N=231**	**DEAD n (%), N=186**	**ALIVE n (%), N=45**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
TT	213 (92.20)	172 (92.47)	41 (91.11)	8.3	-	1.0 (reference)		
TC	18 (7.79)	14 (7.52)	4 (8.88)	9	0.47	0.83 (0.50-1.37)	0.77 (0.41-1.46)	0.44
SQCC								
Genotype rs2306283 SLCO1B1(A388G)	**CASES n (%), N=243**	**DEAD n (%), N=201**	**ALIVE n (%), N=42**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
AA	59 (24.27)	50 (24.87)	9 (21.42)	6.73	-	1.0 (reference)	-	
AG	119 (48.97)	95 (47.26)	24 (57.14)	8.43	0.33	0.84 (0.59-1.19)	1.06 (0.65-1.74)	0.80
GG	65 (26.74)	56 (27.86)	9 (21.42)	6.86	0.79	1.05 (0.71-1.54)	1.68 (0.93-3.03)	0.08
Dominant model								
AA	59 (24.27)	50 (24.87)	9 (21.42)	6.73	-	1.0 (reference)	-	
AG+GG	184 (75.72)	151 (75.12)	33 (78.57)	7.8	0.56	0.90 (0.65-1.26)	1.26 (0.80-1.98)	0.31
Recessive model								
AA+AG	178 (73.25)	145 (72.13)	33 (78.57)	7.5	-	1.0 (reference)	-	
GG	65 (26.74)	56 (27.86)	9 (21.42)	6.8	0.42	1.13 (0.82-1.56)	1.54 (0.99-2.39)	0.05
Genotype rs4149056 SLCO1B1 (T521C)	**CASES n (%), N=243**	**DEAD n (%), N=201**	**ALIVE n (%), N=42**	**Univariate analysis**				
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
TT	228 (93.82)	186 (92.53)	42 (100)	7.33	-	1.0 (reference)	-	-
TC	13 (5.34)	13 (6.46)	0	4.56	0.10	1.79 (0.89-3.59)	1.40 (0.59-3.27)	0.43
CC	2 (0.82)	2 (0.99)	0	-	-	-	-	-
Dominant model								
TT	228 (93.82)	186 (92.53)	42 (100)	7.33	-	1.0 (reference)	-	-
TC+CC	15 (6.17)	15 (7.46)	0	5.16	0.14	1.58 (0.85-2.96)	1.30 (0.59-2.89)	0.50
SCLC								
Genotype rs2306283 SLCO1B1 (A388G)	**CASES n (%), N=103**	**DEAD n (%), N= 95**	**ALIVE n (%), N=8**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
AA	26 (25.24)	24 (25.26)	2 (25)	11.83	-	1.0 (reference)	-	
AG	47 (45.63)	42 (44.21)	5 (62.5)	7.23	0.35	1.26 (0.77-2.06)	1.47 (0.69-3.14)	0.31

GG	30 (29.12)	29 (30.52)	1 (12.5)	9	0.10	1.53 (0.89-2.64)	1.51 (0.70-3.25)	0.28
Dominant model								
AA	26 (25.24)	24 (25.26)	2 (25)	11.83	-	1.0 (reference)	-	
AG+GG	77 (74.75)	71 (74.73)	6 (75)	7.33	0.20	1.32 (0.85-2.05)	1.35 (0.72-2.54)	0.34
Recessive model								
AA+AG	73 (70.87)	66 (69.47)	7 (87.5)	7.67	-	1.0 (reference)	-	
GG	30 (29.12)	29 (30.52)	1 (12.5)	9	0.31	0.79 (0.49-1.25)	1.26 (0.67-2.36)	0.46
Genotype rs4149056 SLCO1B1 (T521C)	**CASES n (%), N=103**	**DEAD n (%), N= 95**	**ALIVE n (%), N=8**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
TT	91 (88.34)	84 (88.42)	7 (87.5)	8.26	-	1.0 (reference)	-	-
TC	12 (11.65)	11 (11.57)	1 (12.5)	7.23	0.83	1.07 (0.56-2.04)	0.65 (0.23-1.84)	0.42

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, regimen, KPS, ECOG

5.26.3 Association of SLCO1B1 variants with OS in LC patients treated with different chemotherapeutic regimens

The prognosis of patients treated with various chemotherapeutic regimens and their correlation with the *SLCO1B1* polymorphic variant has been depicted in Table 5.67. The patients were administered cisplatin/carboplatin and either of the four regimens (docetaxel, paclitaxel, pemetrexed, and irinotecan). In the case of $A^{388}G$, increased survival was found in patients administered with cisplatin and docetaxel and harboring a mutant genotype as compared to the wild genotype (MST=9.9 *vs.* 8.8 months, HR=0.74, 95% CI=0.36-1.54, p=0.43). After multivariate analysis, a 2-times increased death ratio was observed in the mutant genotype compared to the wild genotype (HR=2.0, 95% CI=0.87-4.6, p=0.10). Similarly, patients administered with cisplatin along with paclitaxel and harboring mutant genotype (*GG)* for $A^{388}G$ polymorphism showed approximately 2-times less survival in the combined heterozygous and mutant (*AG+GG)* genotype as compared to the wild type genotype (MST=4.4 *vs.* 7.9 months, HR=1.69, p=0.09). Additionally, the patients undergoing platinum-based pemetrexed chemotherapy and having at least one mutant allele (*AG)* had 2-times reduced survival as compared to the wild type allele (*AA)* (MST=6.73 *vs.* 12.3, HR=1.27, Log-rank p=0.31). There was no significant correlation between cisplatin/carboplatin + irinotecan regimen and overall survival in LC patients with $A^{388}G$ polymorphism.

For $T^{521}C$ polymorphism, the number of patients with the mutant genotype (*CC)* in the present study was less for this particular variant. The frequency of heterozygous genotype (*TC)*

subjects treated with cisplatin/carboplatin and four regimens (docetaxel, paclitaxel, pemetrexed, and irinotecan) was 7.24%, 4.65%, 8.19%, and 8.57%, respectively. Patients treated with cisplatin/carboplatin docetaxel regimen and having heterozygous (*TC)* genotype showed reduced survival as compared to the wild type genotype (*TT)* (MST=4.56 *vs.* 8.8 months, HR=2.32, 95% CI=0.83-6.46, p=0.10). The case patients treated with cis/carboplatin-paclitaxel regimen and having at least one copy of mutant allele *(TC)* showed better survival than the irinotecan regimen (MST=9.05 *vs.* 6.1 months, HR=1.62, p=0.36). Subjects undergoing cis/carboplatin and pemetrexed regimen showed reduced survival in heterozygous genotype (*TC)* as compared to the wild type genotype (MST=6.7 *vs.* 7.9 months, p=0.34). In the same line, the heterozygous genotype (*TC)* in patients undergoing cis/carboplatin irinotecan regimen showed reduced survival compared to the reference genotype (MST=7.47 *vs.* 8.26, Log-rank p=0.74). However, the data showed a lack of association between treatment regimen and overall survival in LC patients.

Table 5.67: Association of *SLCO1B1* SNPs on overall survival according to chemotherapeutic regimen

Regimen-Docetaxel cisplatin/carboplatin								
Genotype rs2306283 SLCO1B1 (A388G)	**CASES n (%), N=69**	**DEAD n (%), N= 56**	**ALIVE n (%), N=13**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
AA	18 (26.08)	12 (21.42)	6 (46.15)	8.8	-	1.0 (reference)	-	
AG	21 (30.43)	26 (46.42)	4 (30.76)	8.4	0.19	1.54 (0.80-2.95)	1.98 (0.91-4.32)	0.08
GG	30 (43.47)	18 (32.14)	3 (23.07)	9.9	0.43	0.74 (0.36-1.54)	2.0 (0.87-4.6)	0.10
Dominant model								
AA	18 (26.08)	12 (21.42)	6 (46.15)	8.8	-	-	-	-
AG+GG	51 (73.91)	44 (78.57)	7 (53.84)	8.73	0.21	1.44 (0.80-2.59)	1.91 (0.96-3.79)	0.06
Recessive model								
AA+AG	39 (56.52)	38 (67.85)	10 (76.92)	8.73	-	-	-	-
GG	30 (43.47)	18 (32.14)	3 (23.07)	9.97	0.85	0.95 (0.54-1.66)	1.20 (0.65-2.19)	0.55
Genotype rs4149056 SLCO1B1 (T521C)	**CASES n (%), N=69**	**DEAD n (%), N= 56**	**ALIVE n (%), N=13**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
TT	63 (91.30)	50 (89.28)	13 (100)	8.8	-	1.0 (reference)	-	-
TC	5 (7.24)	5 (8.92)	0	4.56	0.29	1.83 (0.58-5.71)	2.32 (0.83-6.46)	0.10
CC	1 (1.44)	1 (1.78)	0	-	-	-	-	-
Dominant model								
TT	63 (91.30)	50 (89.28)	16	8.8	-	1.0 (reference)	-	-
TC+CC	6 (8.69)	6 (10.71)	0	4.5	0.52	1.36 (0.52-3.52)	1.64 (0.65-4.13)	0.29
Regimen-Paclitaxel cisplatin/carboplatin								
Genotype rs2306283 SLCO1B1 (A388G)	**CASES n (%), N=86**	**DEAD n (%), N= 73**	**ALIVE n (%), N=13**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
AA	23 (26.74)	20 (27.39)	3 (23.07)	8.3	-	1.0 (reference)	-	
AG	45 (52.32)	37 (50.68)	8 (61.53)	7.5	0.75	0.91 (0.52-1.59)	1.12 (0.62-2.03)	0.69
GG	18 (20.93)	16 (21.91)	2 (15.38)	4.4	0.48	1.27 (0.64-2.51)	1.76 (0.80-3.85)	0.15
Dominant model								
AA	23 (26.74)	20 (27.39)	3 (23.07)	8.3	-	1.0 (reference)	-	-
AG+GG	63 (73.25)	53 (72.60)	10 (76.92)	6.3	0.9	0.9 (0.59-1.67)	1.23 (0.71-2.14)	0.45
Recessive model								
AA+AG	68 (79.06)	57 (78.08)	11 (84.61)	7.9	-			
GG	18 (20.93)	16 (21.91)	2 (15.38)	4.4	0.24	1.43 (0.77-2.6)	1.69 (0.91-3.12)	0.09
Genotype rs4149056 SLCO1B1 (T521C)	**CASES n (%), N=86**	**DEAD n (%), N= 73**	**ALIVE n (%), N=13**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
TT	82 (95.34)	69 (94.52)	13 (100)	6.1	-	1.0 (reference)	-	-
TC	4 (4.65)	4 (5.47)	0	9.05	0.72	1.21 (0.40-3.65)	1.62 (0.574.63)	0.36
Regimen-Pemetrexed cisplatin/carboplatin								
Genotype rs2306283 SLCO1B1 (A388G)	**CASES n (%), N=122**	**DEAD n (%), N= 101**	**ALIVE n (%), N=21**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
AA	29 (23.77)	23 (22.77)	6 (28.57)	12.3	-	1.0 (reference)	-	
AG	60 (49.18)	51 (50.49)	9 (42.85)	6.73	0.31	1.27 (0.79-2.05)	1.03 (0.59-1.82)	0.89
GG	33 (27.04)	27 (26.73)	6 (28.57)	8.3	0.7	1.11 (0.63-1.94)	1.05 (0.54-2.06)	0.87
Dominant model								
AA	29 (23.77)	23 (22.77)	6 (28.57)	12.3	-	1.0 (reference)	-	
AG+GG	93 (76.22)	78 (77.22)	15 (71.42)	7.7	0.40	1.20 (0.77-1.88)	1.09 (0.64-1.86)	0.72
Recessive model								
AA+AG	89 (72.95)	74 (73.26)	15 (71.42)	7.7	-	1.0 (reference)	-	-
GG	33 (27.04)	27 (26.73)	6 (28.57)	8.3	0.84	0.95 (0.61-1.48)	1.02 (0.64-1.64)	0.9

Genotype rs4149056 SLCO1B1 (T521C)	CASES n (%), N=122	DEAD n (%), N= 101	ALIVE n (%), N=21	Univariate analysis			Multivariate analysis	
				MST (months)	Log rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*P*
TT	112(91.80)	93 (92.07)	19 (90.47)	7.9	-	1.0 (reference)	-	-
TC	10 (8.19)	8 (7.92)	2 (9.52)	6.7	0.72	0.88 (0.44-1.75)	0.69 (0.33-1.46)	0.34
Regimen-Irinotecan cisplatin/carboplatin								
Genotype rs2306283 SLCO1B1 (A388G)	**CASES n (%), N=70**	**DEAD n (%), N= 62**	**ALIVE n (%), N=8**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
AA	17 (24.28)	17 (27.41)	0	10.4	-	1.0 (reference)	-	
AG	30 (42.85)	23 (37.09)	7 (87.5)	8.67	0.37	0.74 (0.38-1.42)	0.93 (0.43-2.02)	0.87
GG	23 (32.85)	22 (35.48)	1 (12.5)	7.47	0.30	1.36 (0.72-2.55)	1.64 (0.76-3.53)	0.20
Dominant model								
AA	17 (24.28)	17 (27.41)	0	10.4	-	1.0 (reference)	-	-
AG+GG	53 (75.71)	45 (72.58)	8 (100)	8.26	0.78	0.92 (0.52-1.63)	1.12 (0.59-2.11)	0.71
Recessive model								
AA+AG	47 (67.14)	40 (64.51)	7 (87.5)	8.67				
GG	23 (32.85)	22 (35.58)	1 (12.5)	7.47	0.18	1.46 (0.83-2.57)	1.45 (0.79-2.66)	0.22
Genotype rs4149056 SLCO1B1 (T521C)	**CASES n (%), N=70**	**DEAD n (%), N= 62**	**ALIVE n (%), N=8**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
TT	64 (91.42)	57 (91.93)	7 (87.5)	8.26	-	1.0 (reference)	-	-
TC	6 (8.57)	5 (8.06)	1 (12.5)	7.47	0.74	0.86 (0.36-2.05)	0.80 (0.29-2.2)	0.67

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, histology, KPS, ECOG

5.26.4 Association of SLCO1B1 polymorphisms with gender and OS

In lung carcinoma patients, 83.2% (404) of males and 82.4% (89) of females were dead during the follow-up period (Table 5.68). For *SLCO1B1A*^{388}*G* polymorphism, in the co-dominant model, lower survival was observed in the males having mutant genotype (*GG*) as compared to the subjects harboring wild-type genotype (*AA*) (MST=7.57 *vs.* 7.7 months). After adjusting with multiple covariates, the death ratio of 1.35 was observed in the former genotype (HR=1.35, 95% CI=1.01-1.81; p=**0.04**), as shown in Figure 5.45. Additionally, males harboring heterozygous genotype (*TC)* for *T*^{521}*C* polymorphism showed reduced survival as compared to the wild-type genotype (*CC)* (MST=7.47 *vs.* 7.9 months, Log-rank p=0.44), though no association of *T*^{521}*C* polymorphism was found in predicting overall survival in males having lung cancer.

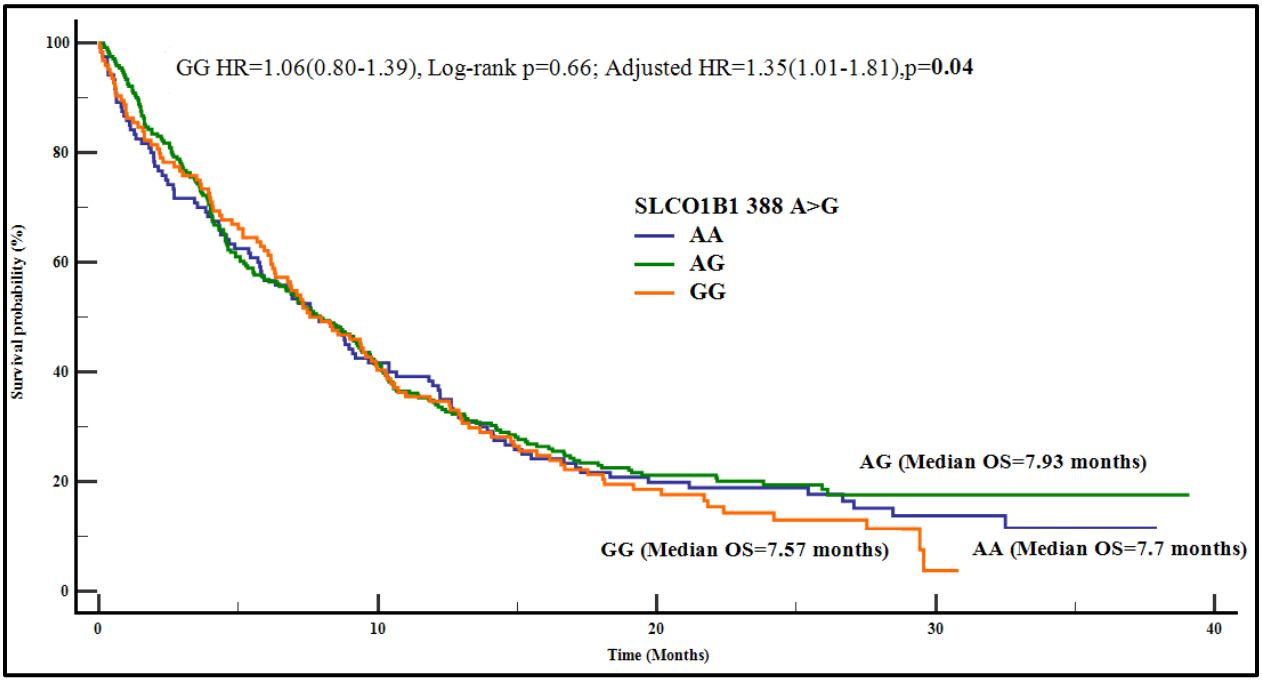


Figure 5.45: Kaplan Meier curves illustrating the association between overall survival in different genotypes of *SLCO1B1* ($A^{388}G$) polymorphism in male lung cancer patients. (Note: AA represents wild-type genotype, AG represents heterozygous genotype, and GG represents homozygous for mutant genotype)

When the female patients having $A^{388}G$ polymorphism were statistically analyzed, our data showed that females harboring mutant genotype (*GG)* had reduced survival as compared to the females having wild-type genotype (*AA)* (MST=7.1 *vs.* 8.5, Log-rank p=0.48). Overall, we did not find any significant association between *SLCO1B1* $A^{388}G$ and $T^{521}C$ polymorphisms in females and overall survival in lung carcinoma patients.

Table 5.68: Association of *SLCO1B1* SNPs on overall survival based on gender

GENDER-MALE								
Genotype rs2306283 SLCO1B1 (A388G)	**CASES n (%), N=485**	**DEAD n (%), N= 404**	**ALIVE n (%), N=81**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
AA	120 (24.74)	102 (25.24)	18 (22.22)	7.7	-	1.0 (reference)	-	
AG	241 (49.69)	194 (48.01)	47 (58.02)	7.93	0.59	0.93 (0.73-1.19)	1.03 (0.80-1.33)	0.80
GG	124 (25.56)	108 (26.73)	16 (19.75)	7.57	0.66	1.06 (0.80-1.39)	1.35 (1.01-1.81)	**0.04**
Dominant model								
AA	120 (24.74)	102 (25.24)	18 (22.22)	7.7	-	-	-	-
AG+GG	365 (75.25)	302 (74.75)	63 (77.77)	7.93	0.82	0.97 (0.77-1.22)	1.11 (0.87-1.41)	0.37
Recessive model								
AA+AG	361 (74.43)	296 (73.26)	65 (80.24)	7.9	-	-	-	-
GG	124 (25.56)	108 (26.73)	16 (19.75)	7.5	0.46	1.08 (0.86-1.36)	1.24 (0.98-1.56)	0.06
Genotype rs4149056 SLCO1B1 (T521C)	**CASES n (%), N=485**	**DEAD n (%), N= 404**	**ALIVE n (%), N=81**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
TT	448 (92.37)	371 (91.83)	77 (95.06)	7.9	-	1.0 (reference)	-	-
TC	35 (7.21)	31 (7.67)	4 (4.93)	7.47	0.44	1.16 (0.78-1.71)	1.17 (0.80-1.70)	0.39
CC	2 (0.41)	2 (0.49)	0	-	-	-	-	-
Dominant model								
TT	448 (92.37)	371 (91.83)	77 (95.06)	7.9	-	1.0 (reference)	-	-
TC+CC	37 (7.62)	33 (8.1)	4 (4.93)	7.47	0.47	1.14 (0.78-1.67)	1.16 (0.80-1.67)	0.42
GENDER-FEMALE								
Genotype rs2306283 SLCO1B1 (A388G)	**CASES n (%), N=108**	**DEAD n (%), N= 89**	**ALIVE n (%), N=19**	**Univariate analysis**		**Multivariate analysis**		
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
AA	27 (25)	22 (24.71)	5 (26.31)	8.5	-	1.0 (reference)	-	
AG	49 (45.37)	40 (44.94)	9 (47.36)	9.7	0.85	1.04 (0.62-1.76)	0.89 (0.50-1.58)	0.71
GG	32 (29.62)	27 (30.33)	5 (26.31)	7.1	0.48	1.22 (0.69-2.1)	1.16 (0.58-2.28)	0.66
Dominant model								
AA	27 (25)	22 (24.71)	5 (26.31)	8.5	-	-	-	-
AG+GG	81 (75)	67 (75.28)	14 (73.68)	8.23	0.65	1.11 (0.69-1.78)	1.07 (0.63-1.82)	0.78
Recessive model								
AA+AG	76 (70.37)	62 (69.66)	14 (73.68)	9.7	-	-	-	-
GG	32 (29.62)	27 (30.33)	5 (26.31)	7.1	0.25	1.32 (0.81-2.13)	1.30 (0.80-2.13)	0.28
Genotype rs4149056 SLCO1B1 (T521C)	**CASES n (%), N=108**	**DEAD n (%), N= 89**	**ALIVE n (%), N=19**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
TT	100 (92.59)	82 (92.13)	18 (94.73)	8.23	-	1.0 (reference)	-	-
TC	8 (7.40)	7 (7.86)	1 (5.26)	7.23	0.77	0.89 (0.42-1.88)	0.74 (0.31-1.78)	0.51

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, smoking, stage, histology, KPS, ECOG

5.26.5 Association of SLCO1B1 polymorphisms with a prognosis based on tumor stage

Lung cancer patients with *SLCO1B1* variations were stratified based on tumor stage to evaluate the overall survival, as illustrated in table 5.69. When patients were grouped based on stage III, 80% of patients were dead while 20% of subjects were alive. For *SLCO1B1* $A^{388}G$ polymorphisms, our results showed two-fold better survival time in patients harboring at least one mutant allele

(*AG)* as compared to the lung cancer patients carrying wild type (*AA)* genotype in stage III (MST= 10.57 *vs.* 5.83, HR=0.49, 95% CI=0.31-0.79, Log-rank p=**0.003**). However, after adjusting with multiple covariates such as age, gender, smoking, histology, regimen, and performance status, the death ratio of 0.50 was noted in the heterozygous genotype, and a significant association of $A^{388}G$ was found in predicting prognosis in stage III lung cancer patients (HR=0.50; 95% CI=0.26-0.97, p=**0.04**) (Figure 5.46).

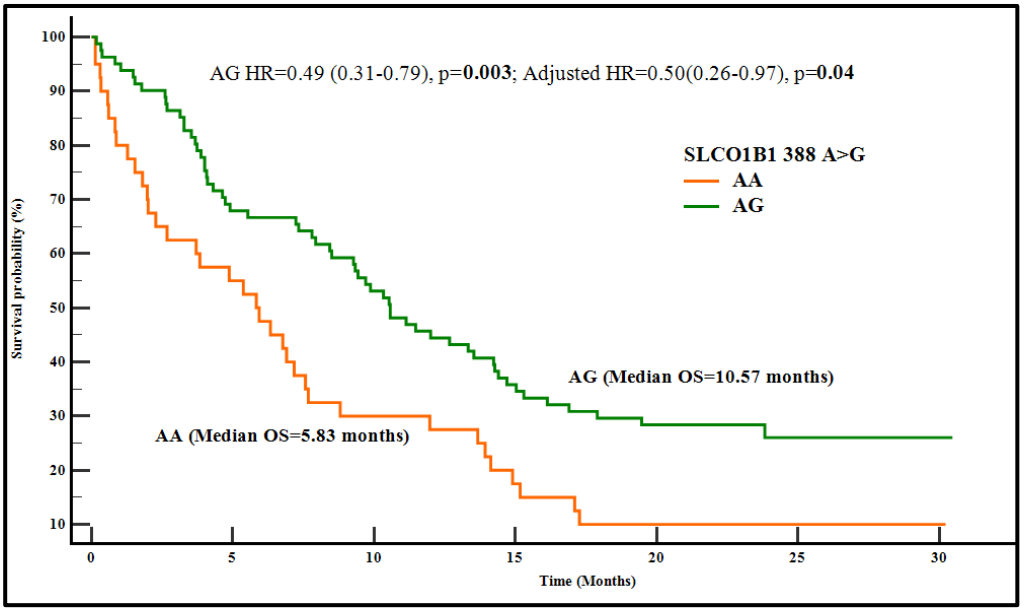


Figure 5.46: Kaplan Meier curves illustrating the association between overall survival in different genotypes of *SLCO1B1* ($A^{388}G$) polymorphism in stage III lung cancer patients. (Note: AA represents wild-type genotype, AG represents heterozygous genotype, and GG represents homozygous for mutant genotype)

Upon evaluating the impact of *SLCO1B1* polymorphism on overall survival in stage IV lung cancer patients undergoing platinum-based doublet chemotherapy, our results showed that the incidence of alive patients was 14.28%, while 85.71% of patients in stage IV were dead (Table 5.69). For *SLCO1B1* $A^{388}G$ polymorphism, 49.32% of patients had at least one copy of mutant allele (*AG),* whereas 26.41% of subjects had both copies of variant alleles (*GG)*. Our data revealed that patients harboring heterozygous genotype *(AG)* for *SLCO1B1* $A^{388}G$ polymorphism had lower survival than those with wild-type genotype *(AA)* in stage IV lung cancer patients (MST= 7.0 *vs.* 8.97, HR=1.21, 95% CI=0.93-1.59, p=0.15). After multivariate analysis, the death ratio increased to 1.48 times in patients having at least one copy of mutant allele (*AG)* as compared to the patients with

homozygous wild-type genotype (*AA)* (HR=1.48, 95% CI=1.02-2.15, p=**0.03**). Also, the patients with mutant genotype (*GG)* depicted lower survival as compared to the wild type genotype (*AA)* (MST=6.86 *vs.* 8.97, HR=1.38, 95% CI=1.01-1.89, p=**0.03**). After adjusting with covariates such as age, gender, smoking status, histology, regimen, and performance status, our results indicated a significant association of *SLCO1B1* $A^{388}G$ polymorphism in the prognosis of lung cancer patients (HR=1.79, 95% CI=1.15-2.76, p=**0.008**) (Figure 5.47). However, the other polymorphism of *SLCO1B1* $T^{521}C$ did not show any association in predicting prognosis in stage III and IV lung carcinoma patients.

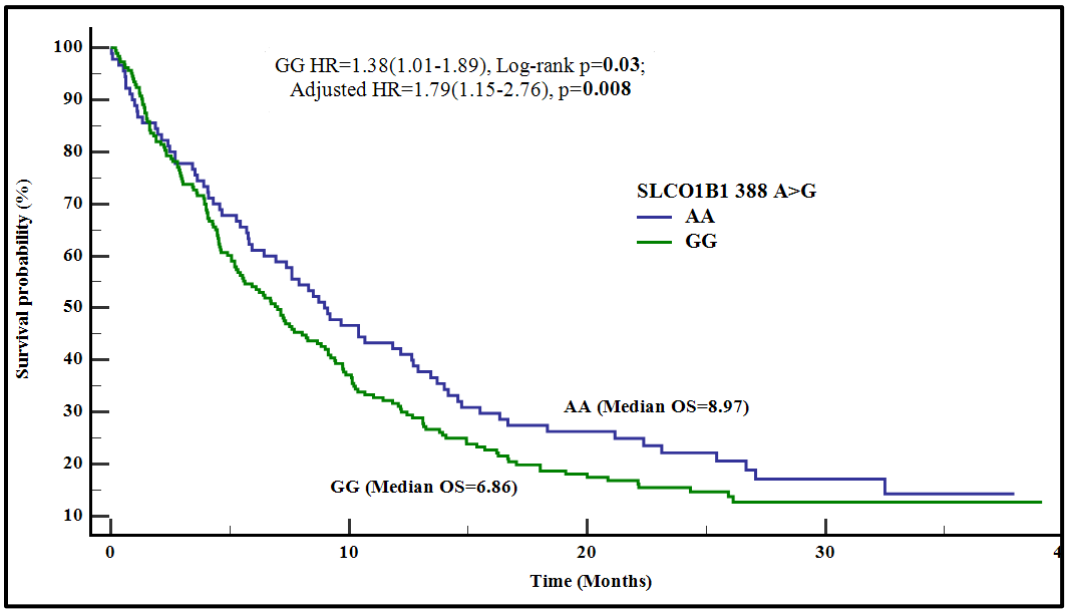


Figure 5.47: Kaplan Meier curves illustrating the association between overall survival in different genotypes of *SLCO1B1* ($A^{388}G$) polymorphism in stage IV lung cancer patients. (Note: AA represents wild-type genotype, AG represents heterozygous genotype, and GG represents homozygous for mutant genotype)

Table 5.69: Association of *SLCO1B1* SNPs on overall survival based on tumor stage

Stage III								
Genotype rs2306283 SLCO1B1 (A388G)	**CASES n (%), N=165**	**DEAD n (%), N= 132**	**ALIVE n (%), N=33**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
AA	40 (24.24)	36 (27.27)	4 (12.12)	5.83	-	1.0 (reference)	-	-
AG	81 (49.09)	59 (44.69)	22 (66.66)	10.57	**0.003**	0.49 (0.31-0.79)	0.50 (0.26-0.97)	**0.04**
GG	44 (26.66)	37 (28.03)	7 (21.21)	9.03	0.21	0.74 (0.46-1.18)	0.89 (0.41-1.91)	0.76
Dominant model								
AA	40 (24.24)	36 (27.27)	4 (12.12)	5.83	-	1.0 (reference)	-	-
AG+GG	125 (75.75)	96 (72.72)	29 (87.87)	10.47	**0.01**	0.56 (0.36-0.87)	0.66 (0.36-1.19)	0.16
Recessive model								
AA+AG	121 (73.33)	95 (71.96)	26 (78.78)	8.8	-	1.0 (reference)	-	-
GG	44 (26.66)	37 (28.03)	7 (21.21)	9.03	0.61	1.10 (0.74-1.63)	1.26 (0.72-2.21)	0.40
Genotype rs4149056 SLCO1B1 (T521C)	**CASES n (%), N=165**	**DEAD n (%), N= 132**	**ALIVE n (%), N=33**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
TT	152 (92.12)	121 (91.66)	31 (93.93)	8.8	-	1.0 (reference)	-	-
TC	13 (7.87)	11 (8.33)	2 (6.06)	10.47	0.84	1.06 (0.562.01)	0.98 (0.37-2.59)	0.9
Stage IV								
Genotype rs2306283 SLCO1B1 (A388G)	**CASES n (%), N=371**	**DEAD n (%), N= 318**	**ALIVE n (%), N=53**	**Univariate analysis**		**Multivariate analysis**		
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
AA	90 (24.24)	73 (22.95)	17 (32.07)	8.97	-	1.0 (reference)	-	-
AG	183 (49.32)	156 (49.05)	27 (50.94)	7.0	0.15	1.21 (0.93-1.59)	1.48 (1.02-2.15)	**0.03**
GG	98 (26.41)	89 (27.98)	9 (16.98)	6.86	**0.03**	1.38 (1.01-1.89)	1.79 (1.15-2.76)	**0.008**
Dominant model								
AA	90 (24.24)	73 (22.95)	17 (32.07)	8.97	-	1.0 (reference)	-	-
AG+GG	281 (75.74)	245 (77.04)	36 (67.92)	7.0	0.06	1.25 (0.98-1.60)	1.59 (1.13-2.25)	**0.007**
Recessive model								
AA+AG	273 (7358)	229 (72.01)	44 (83.01)	7.6	-	1.0 (reference)	-	-
GG	98 (26.41)	89 (27.98)	9 (16.98)	6.86	0.16	1.19 (0.92-1.54)	1.21 (0.88-1.67)	0.21
Genotype rs4149056 SLCO1B1 (T521C)	**CASES n (%), N=371**	**DEAD n (%), N= 318**	**ALIVE n (%), N=53**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
TT	340 (91.64)	290 (91.19)	50 (94.33)	7.3	-	1.0 (reference)	-	-
TC	29 (7.81)	26 (8.17)	3 (5.66)	7.47	0.81	1.05 (0.69-1.58)	0.99 (0.60-1.65)	0.9
CC	2 (0.53)	2 (0.62)	0 (0)	5.16	0.91	0.93 (0.24-3.58)	0.85 (0.31-2.32)	0.75

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, smoking, regimen, histology, KPS, ECOG

5.26.6 Association of SLCO1B1 polymorphisms with a prognosis based on smoking habits

In the present study, the lung cancer patients having *SLCO1B1* polymorphism were stratified based on their smoking habits. Most lung cancer patients were smokers, and 84.81% of smokers were dead at analysis. Table 5.70 depicts that smokers having mutant genotype (*GG)* for $A^{388}G$ had low marginal survival as compared to the wild type genotype (*AA)* (MST=7.23 *vs.* 7.57, HR=1.14, 95% CI=0.87-1.50, Log-rank p=0.33). After multivariate analysis, the death ratio increased to 1.50-times in the mutant genotype (HR=1.50, 95% CI=1.02-2.20, p=**0.03**). However, non-smokers having *SLCO1B1* $A^{388}G$ polymorphism showed a lack of association as a significant p-value was not observed. So, our results for $A^{388}G$ polymorphism revealed that smoking is an essential parameter in predicting prognosis in lung cancer patients.

Lung cancer patients having *SLCO1B1* $T^{521}C$ polymorphism showed that the majority of the smokers had wild-type genotype (*TT)* and the prevalence of heterozygous (*TC)* and mutant genotype (*CC)* was less in these groups (92.40%, 7.17%, and 0.42%, respectively). Smokers having heterozygous (*TT)* (MST=7.23 *vs.* 7.57, Log-rank p=0.49) and mutant genotype (*CC)* (MST=5.16 *vs.* 7.57, Log-rank p=0.90) showed reduced survival as compared to the wild type genotype (*TT)*. However, substantial differences among the groups were not reached. So, we summarize that *SLCO1B1* $T^{521}C$ polymorphism is not correlated in predicting overall survival in both smokers and non-smokers.

Table 5.70: Association of *SLCO1B1* SNPs on overall survival based on smoking habits

Smokers								
Genotype rs2306283 SLCO1B1 (A388G)	**CASES n (%), N=474**	**DEAD n (%), N= 402**	**ALIVE n (%), N=72**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
AA	116 (24.47)	100 (24.87)	16 (22.22)	7.57	-	1.0 (reference)	-	-
AG	235 (49.57)	192 (47.76)	43 (59.72)	7.8	0.70	0.95 (0.74-1.21)	1.24 (0.88-1.75)	0.20
GG	123 (25.94)	110 (27.36)	13 (18.05)	7.23	0.33	1.14 (0.87-1.50)	1.50 (1.02-2.20)	**0.03**
Dominant model								
AA	116 (24.47)	100 (24.87)	16 (22.22)	7.57	-	1.0 (reference)	-	-
AG+GG	358 (75.52)	302 (75.12)	56 (77.77)	7.47	0.90	1.01 (0.80-1.27)	1.35 (0.98-1.85)	0.06
Recessive model								
AA+AG	351 (74.05)	292 (72.63)	59 (81.94)	7.67	-	1.0 (reference)	-	-
GG	123 (25.94)	110 (27.36)	13 (18.05)	7.23	0.19	1.16 (0.92-1.45)	1.31 (0.98-1.76)	0.06
Genotype rs4149056 SLCO1B1 (T521C)	**CASES n (%), N=474**	**DEAD n (%), N= 402**	**ALIVE n (%), N=72**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
TT	438 (92.40)	370 (92.03)	68 (94.44)	7.57	-	1.0 (reference)	-	-
TC	34 (7.17)	30 (7.46)	4 (5.55)	7.23	0.49	1.14 (0.77-1.70)	1.06 (0.64-1.74)	0.81
CC	2 (0.42)	2 (0.49)	0	5.16	0.91	0.92 (0.24-3.54)	0.99 (0.36-2.72)	0.9
Dominant model								
TT	438 (92.40)	370 (92.03)	68 (94.44)	7.57	-	1.0 (reference)	-	-
TC+CC	36 (7.59)	32 (7.96)	4 (5.55)	7.23	0.52	1.13 (0.77-1.65)	1.05 (0.64-1.70)	0.83
Non-smokers								
Genotype rs2306283 SLCO1B1 (A388G)	**CASES n (%), N=118**	**DEAD n (%), N= 90**	**ALIVE n (%), N=28**	**Univariate analysis**		**Multivariate analysis**		
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
AA	30 (25.42)	23 (25.55)	7 (25)	8.8	-	1.0 (reference)	-	-
AG	55 (46.61)	42 (46.66)	13 (46.42)	11.03	0.76	0.92 (0.55-1.54)	0.95 (0.41-2.17)	0.90
GG	33 (27.96)	25 (27.77)	8 (28.57)	9.4	0.95	0.98 (0.55-1.73)	0.87 (0.34-2.22)	0.77
Dominant model								
AA	30 (25.42)	23 (25.55)	7 (25)	8.8	-	1.0 (reference)	-	-
AG+GG	88 (74.57)	67 (74.44)	21 (75)	9.7	0.88	0.96 (0.59-1.55)	1.04 (0.51-2.15)	0.89
Recessive model								
AA+AG	85 (72.03)	65 (72.22)	20 (71.42)	9.7	-	1.0 (reference)	-	-
GG	33 (27.96)	25 (27.77)	8 (28.57)	9.4	0.94	1.01 (0.63-1.61)	0.95 (0.50-1.81)	0.89
Genotype rs4149056 SLCO1B1 (T521C)	**CASES n (%), N=118**	**DEAD n (%), N= 90**	**ALIVE n (%), N=28**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
TT	109 (92.37)	82 (91.11)	27 (96.42)	9.46	-	1.0 (reference)	-	-
TC	9 (7.62)	8 (8.88)	1 (3.57)	18.03	0.9	1.1 (0.48-2.09)	0.77 (0.28-2.12)	0.62

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, regimen, stage, histology, KPS, ECOG

5.26.7 Association of SLCO1B1 genotype with tumor characteristics

In order to assess the correlation between *SLCO1B1* variants and the clinic-pathological parameters, the patients were bifurcated based on cancer stage (stage III *vs.* stage IV), lymph node involvement (Nx+N0+N1 *vs.* N2+N3+N4), primary tumor extension (T3 *vs.* T4), and metastasis (positive *vs.* negative) as shown in Table 5.71. In the case of the clinical-stage, carriers in stage IV with $A^{388}G$ polymorphism had higher odds of having lung cancer than the carriers of the stage III group (AOR=1.15, p=0.65), though data lacked significance. Also, our results for $T^{521}C$ polymorphism did not show any substantial risk in tumor stage IV and stage III (AOR=1.39, p=0.50).

Similarly, when the comparison was made between the patients with T3 and T4 size in *SLCO1B1* $T^{521}C$, a decreased risk of lung carcinoma was observed in the heterozygous *(TC)* genotype T4 as compared to the patients with T3 tumor extension (AOR=0.29, 95% CI=0.11-0.77, p=**0.01)**. Using the dominant model, a decreased-odds of developing T4 was noted in combined two risk genotypes (*TC+CC)* when compared to the patients categorized in T3 tumor extension (AOR=0.34, 95% CI=0.13-0.88; p=**0.02).** Therefore, our results show that these genotypes did not affect the tumor size and progression. Furthermore, no significant association was found between *SLCO1B1* $A^{388}G$ and $T^{521}C$ polymorphisms and lymph node invasion in all the models assessed. Lack of correlation was observed between *SLCO1B1* $A^{388}G$ and $T^{521}C$ polymorphism and its association with metastasis.

Table 5.71: Association of *SLCO1B1* polymorphism based on clinicopathological parameters

Clinical stage					***Tumor Size***				
Genotype rs2306283 SLCO1B1 (A388G)	**Stage III (168), n (%)**	**Stage IV (385), n (%)**	**AOR (95% CI) [a]**	***p*[b]**	**Genotype rs2306283 SLCO1B1 (A388G)**	**T3 (122), n (%)**	**T4 (347), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
AA	43(25.59)	92(23.89)	1.0(Reference)		AA	31(25.40)	80(23.05)	1.0(Reference)	
AG	81(48.21)	190(49.35)	1.15(0.61-2.14)	0.65	AG	61(50)	164(47.26)	0.74(0.37-1.46)	0.39
GG	44(26.19)	103(26.75)	0.97(0.49-1.92)	0.9	GG	30(24.59)	103(29.68)	1.15(0.52-2.53)	0.72
AG+GG	125(74.40)	293(76.10)	1.07(0.6-1.91)	0.8	AG+GG	91(74.59)	267(76.94)	0.86(0.45-1.64)	0.66
Genotype rs4149056 SLCO1B1(T521C)	**Stage III (168), n (%)**	**Stage IV (385), n (%)**	**AOR (95% CI) [a]**	***p*[b]**	**Genotype rs4149056 SLCO1B1(T521C)**	**T3 (122), n (%)**	**T4 (347), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
TT	156(92.85)	353(91.68)	1.0(Reference)		TT	109(89.34)	323(93.08)	1.0(Reference)	
TC	12(7.14)	30(7.79)	1.39(0.51-3.75)	0.5	TC	12(9.83)	23(6.62)	0.29(0.11-0.77)	**0.01**
CC	0	2(0.51)	-	-	CC	1(0.81)	1(0.28)	-	-
TC+CC	12(7.14)	32(8.31)	1.52(0.56-4.08)	0.40	TC+CC	13(10.65)	24(6.91)	0.34(0.13-0.88)	**0.02**
Lymph node Invasion					***Metastasis***				
Genotype rs2306283 SLCO1B1 (A388G)	**Nx+N0+N1 (130), n (%)**	**N2+N3+N4 (451), n (%)**	**AOR (95% CI) [a]**	***p*[b]**	**Genotype rs2306283 SLCO1B1 (A388G)**	**M0 (272), n (%)**	**M1 (309), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
AA	35(26.92)	109(24.16)	1.0(Reference)		AA	74(27.20)	70(22.65)	1.0(Reference)	
AG	57(43.84)	225(49.88)	1.12(0.60-2.12)	0.70	AG	126(46.32)	156(50.48)	1.58(0.90-2.75)	0.10
GG	38(29.23)	117(25.94)	1.05(0.52-2.13)	0.87	GG	72(26.47)	83(26.86)	1.24(0.67-2.31)	0.48
AG+GG	95(73.07)	342(75.83)	1.11(0.62-1.99)	0.71	AG+GG	198(72.79)	239(77.34)	1.46(0.86-2.48)	0.15
Genotype rs4149056 SLCO1B1(T521C)	**Nx+N0+N1 (130), n (%)**	**N2+N3+N4 (451), n (%)**	**AOR (95% CI) [a]**	***p*[b]**	**Genotype rs4149056 SLCO1B1(T521C)**	**M0 (272), n (%)**	**M1 (309), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
TT	117(90)	419(92.90)	1.0(Reference)		TT	253(93.01)	283(91.58)	1.0(Reference)	
TC	11(8.46)	32(7.09)	0.56(0.23-1.36)	0.20	TC	17(6.25)	26(8.41)	1.68(0.69-4.03)	0.24
CC	2(1.53)	0	-	-	CC	2(0.73)	0	-	-
TC+CC	13(10)	32(7.09)	0.50(0.21-1.18)	0.11	TC+CC	19(6.98)	26(8.41)	1.57(0.66-3.71)	0.30

a)-Adjusted Odds ratios, 95% confidence intervals, and corresponding *p*-values were calculated by logistic regression analysis after adjusting for age, gender, smoking, and regimen. (b)-Two-sided $\chi 2$ test for either genotype distribution or allelic frequencies between the responders and non-responders. The number in bold indicates the significant values in the table

5.26.8 Association of SLCO1B1 polymorphism and chemotherapy response

Correlation between *SLCO1B1* polymorphism and the effectiveness of response rate and clinical benefits were studied using univariate logistic regression. Table 5.72 shows the relationship between *SLCO1B1* polymorphism and treatment responses. The individuals showing stable and progressive disease were categorized as non-responders (SD+PD), whereas subjects demonstrating complete or partial response were classified as good responders (CR+PR). According to the chemotherapeutic response to platinum-based doublet therapy, our outcomes showed no association between *SLCO1B1* $A^{388}G$ and $T^{521}C$ polymorphisms. So, we summarize that none of the *SLCO1B1* polymorphisms might predict chemotherapeutic response or clinical benefit in a significant way.

Table 5.72: Association of *SLCO1B1* SNPs and chemotherapeutic response

	Response to chemotherapy			
Genotype rs2306283 SLCO1B1 (A388G)	**CR+PR (133), n (%)**	**SD+PD (182), n (%)**	**AOR (95% CI)** [a]	***p*** [b]
AA	33 (24.81)	45 (24.72)	1.0 (Reference)	-
AG	65 (48.87)	94 (51.64)	1.07 (0.57-2.03)	0.81
GG	35 (26.31)	43 (23.62)	0.75 (0.36-1.58)	0.45
AG+GG	100 (75.18)	137 (75.27)	0.94 (0.52-1.70)	0.84
Genotype rs4149056 SLCO1B1 (T521C)	**CR+PR (133), n (%)**	**SD+PD (182), n (%)**	**AOR (95% CI)** [a]	***p*** [b]
TT	125 (93.98)	166 (91.20)	1.0 (Reference)	
TC	8 (6.01)	15 (8.24)	1.47 (0.55-3.91)	0.43
CC	0	1 (0.54)	-	-
TC+CC	8 (6.01)	16 (8.79)	1.58 (0.60-4.17)	0.34

a)-Adjusted Odds ratios, 95% confidence intervals, and corresponding *p*-values were calculated by logistic regression analysis after adjusting for age, gender, smoking, and regimen. (b)-Two-sided $\chi 2$ test for either genotype distribution or allelic frequencies between the responders and non-responders. The number in bold indicates the significant values in the table

5.27. Statistical analysis for the polymorphic site of the *SLCO1B3* gene

5.27.1 Genotypic and allelic frequencies of SLCO1B3 polymorphic variant

For the *SLCO1B3* gene, one polymorphic variant $A^{1683\text{-}5676}G$ was studied; genotypic analysis showed that 545 (89.34%) patients were harboring homozygous wild (*AA)* genotype, 64 (10.4%) patients had heterozygous (*AG)* genotype and only 1 (0.16%) individual had homozygote mutant (*GG)* genotype. The genotypic frequencies for *SLCO1B1* $A^{1683\text{-}5676}G$ are depicted in **table 5.73.** The genotypic distribution for this polymorphic site followed the Hardy-Weinberg equilibrium with a p-value> 0.05, and the minor allele frequency of the $A^{1683\text{-}5676}G$ variant was 0.50. So, these samples could be a Mendelian population in a state of genetic equilibrium.

Table 5.73: Genotypic and allelic distribution of *SLCO1B3* variants

Genotype rs11045585 SLCO1B3 (1683-5676A>G)	No. patients (%) N=610	$\chi 2$	P-value
AA	545 (89.34)		
AG	64 (10.4)	0.38	0.53
GG	1 (0.16)		
AG+GG	65 (10.6)		
A (allele)	1154		
G (allele)	66		
MAF	0.05		

5.28 Association of *SLCO1B3* polymorphism in predicting prognosis based on different parameters

The correlation of the *SLCO1B3* polymorphisms with the overall survival of 593 lung cancer samples was assessed using both univariate and multivariate analysis after adjusting for smoking status, gender, age, tumor stage, regimen, ECOG, KPS, and histology (Table 5.74). For the *SLCO1B3* $A^{1683\text{-}5676}G$ variant, the patients having heterozygous allele (*AG*) showed lower median survival in lung carcinoma patients as compared to the individuals harboring wild type (*AA*) genotype (MST=7.6 *vs.* 7.97; p=0.58). However, only one patient harbored a mutant genotype, so the survival differences were not calculated for this group**.** None of the other models showed any prognostic significance for $A^{1683\text{-}5676}G$ polymorphism for overall survival in lung cancer patients.

The lung cancer patients were stratified based on histology, and the analysis showed that most of the patients had SQCC compared to ADCC and SCLC. The LC patients having ADCC had lower median survival in the individuals having at least one mutant allele (*AG)* as compared to the wild type genotype (*AA)* (MST=5.53 *vs.* 8.8 months, p=0.35). In the same line, SQCC patients also showed reduced survival in individuals harboring heterozygous genotype compared to the wild type genotype (MST=5.76 *vs.* 7.47, p=0.73), though the results were not significant. On the contrary, the patients with SCLC showed better survival than LC patients with heterozygous genotype than the reference genotype, but a lack of association was noted between the two groups (MST=11 *vs.* 7.47, p=0.17). Therefore, we summarize that *SLCO1B3* $A^{1683\text{-}5676}G$ polymorphism was not associated with predicting overall survival in lung cancer patients having ADCC, SQCC, and SCLC.

The lung carcinoma patients in the study were administered platinum-based chemotherapy along with paclitaxel, docetaxel, pemetrexed, and irinotecan. So, we chose to investigate the modifying effects of the *SLCO1B3* $A^{1683\text{-}5676}G$ polymorphism and its relationship to chemotherapy, and overall patient survival, as shown in Table 5.74. Chemotherapy was divided into four regimens, each given to different patient groups. All lung malignancy patients received platinum-based therapy (carboplatin/cisplatin) and a second-line chemotherapy treatment such as docetaxel, pemetrexed, paclitaxel, or irinotecan. For *SLCO1B3* $A^{1683\text{-}5676}G$ polymorphism, our results showed that patients who were given carboplatin/cisplatin along with the docetaxel and carrying heterozygous alleles (*AG*) showed inferior survival outcomes as compared to the patients having wild type genotype (*AA*) (MST=2.9 months *vs.* 9.6 months, Log-Rank p=**0.006,** HR=14.01). Upon adjusting with multivariate factors such as age, gender, smoking, stage, histology, and performance status, we observed a high hazard or death ratio in the patients having heterozygous genotype (*AG*) as compared to the subjects harboring wild type genotype (*AA)* (HR=3.84, 95% CI=1.16-12.72; **p=0.02**, Figure 5.48) as shown in Table 5.74. On the contrary, patients undergoing cis/carboplatin and paclitaxel treatment regimen showed 2-times better survival in the heterozygous genotype (*AG)* as compared to the reference genotype (*AA)* (MST=10.47 *vs.* 5.83, p=0.18), though the substantial significance could not be achieved. The patients undergoing cis/carboplatin and pemetrexed or irinotecan treatment did not show any correlation in predicting prognosis in lung cancer patients.

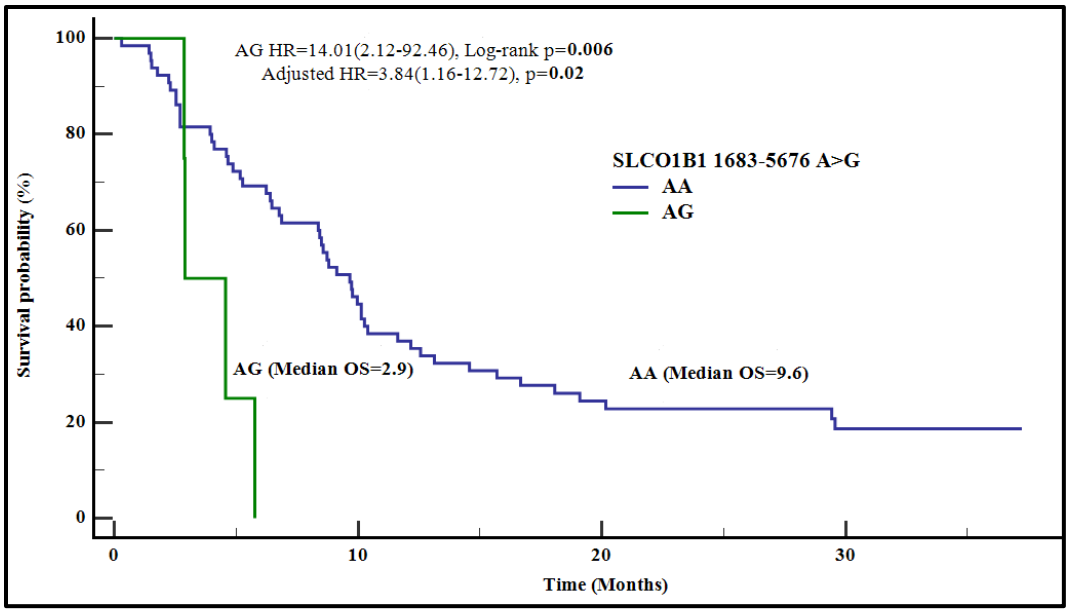


Figure 5.48: Kaplan Meier curves illustrating the association between overall survival in different genotypes of *SLCO1B3* (*$A^{1683\text{-}5676}G$*) polymorphism in patients administered with docetaxel cisplatin/carboplatin. (Note: AA represents wild-type genotype and AG represents heterozygous genotype)

To assess the effect of *SLCO1B3* *$A^{1683\text{-}5676}G$* polymorphism in predicting overall survival, the lung cancer patients were stratified based on gender. Our results showed that most males were dead at the analysis time, and only 16.70% of males were alive. Table 5.74 depicts reduced survival in males having lung cancer and harboring at least one mutant allele (*AG)* for *SLCO1B3* *$A^{1683\text{-}5676}G$* as compared to the wild-type genotype (*AA)* (MST=7.23 *vs.* 7.9, HR=1.10, 95% CI=0.79-1.53, p=0.54). On the contrary, better survival was observed in lung cancer patients in females with heterozygous genotype *(AG)* for *SLCO1B3* *$A^{1683\text{-}5676}G$*. However, no significant association was observed among the genotypes in predicting overall survival in female lung cancer patients compared to the wild-type genotype (*AA)* (MST=9.43 *vs.* 8.2, HR=0.67, 95% CI=3.23-1.39, p=0.29). Therefore, our data for *SLCO1B3* *$A^{1683\text{-}5676}G$* polymorphism did not show any association between overall survival and gender in LC patients.

Table 5.74: Association of *SLCO1B3* polymorphism in predicting prognosis based on various parameters

Overall Survival

Genotype rs11045585 SLCO1B3 (1683-5676A>G)	CASES n (%), N=593	DEAD n (%), N=493	ALIVE n (%), N=100	Univariate analysis			Multivariate analysis	
				MST (months)	Log rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*P*
Codominant model								
AA	529 (89.20)	437 (88.64)	92 (92)	7.97	-	-	-	-
AG	63 (10.62)	56 (11.35)	7 (7)	7.6	0.52	1.09 (0.82-1.46)	0.89 (0.61-1.31)	0.58
GG	1 (0.16)	0	1 (1)	-	-	-	-	-
Dominant model				7.97	-	1.0 (reference)	-	
AA	529 (89.20)	437 (88.64)	92 (92)	7.6	0.67	1.06 (0.79-1.41)	0.86 (0.58-1.26)	0.44
AG+GG	64 (10.79)	56 (11.35)	8 (8)					
Additive model								
AA+GG	530 (89.37)	437 (88.64)	93 (93)	8.02	-	1.0 (reference)	-	-
AG	63 (10.62)	56 (11.35)	7 (7)	7.6	0.50	1.10 (0.82-1.47)	0.90 (0.61-1.33)	0.62

Histology-ADCC

Genotype rs11045585 SLCO1B3 (1683-5676A>G)	CASES n (%), N=231	DEAD n (%), N=186	ALIVE n (%), N= 45	Univariate analysis			Multivariate analysis	
				MST (months)	Log rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*P*
Codominant model								
AA	195 (84.41)	156 (83.87)	39 (86.66)	8.8	-	-	-	-
AG	35 (15.15)	30 (16.12)	5 (11.11)	5.53	0.35	1.22 (0.80-1.85)	0.93 (0.55-1.57)	0.80
GG	1 (0.43)	0	1 (2.22)	-	-	-	-	-
Dominant model								
AA	195 (84.41)	156 (83.87)	39 (86.66)	8.8	-	1.0 (reference)	-	-
AG+GG	36 (15.58)	30 (16.12)	6 (13.33)	5.53	0.50	1.14 (0.76-1.73	0.87 (0.52-1.46)	0.61

Histology-SQCC

Genotype rs11045585 SLCO1B3 (1683-5676A>G)	CASES n (%), N=243	DEAD n (%), N= 201	ALIVE n (%), N= 42	Univariate analysis			Multivariate analysis	
				MST (months)	Log rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*P*
AA	228 (93.82)	188 (93.53)	40 (95.23)	7.33	-	1.0 (reference)	-	-
AG	15 (6.17)	13 (6.46)	2 (4.76)	5.76	0.90	1.03 (0.58-1.82)	0.84 (0.33-2.13)	0.73

Histology-SCLC

Genotype rs11045585 SLCO1B3 (1683-5676A>G)	CASES n (%), N=103	DEAD n (%), N= 95	ALIVE n (%), N= 8	Univariate analysis			Multivariate analysis	
				MST (months)	Log rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*P*
AA	92 (89.32)	84 (88.42)	8 (100)	7.47	-	1.0 (reference)	-	-
AG	11 (10.67)	11 (11.57)	0	11	0.53	0.83 (0.46-1.49)	0.48 (0.17-1.35)	0.17

Regimen-Docetaxel cisplatin/carboplatin

Genotype rs11045585 SLCO1B3 (1683-5676A>G)	CASES n (%), N=69	DEAD n (%), N= 56	ALIVE n (%), N= 13	Univariate analysis			Multivariate analysis	
				MST (months)	Log rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*P*
AA	65 (94.20)	52 (92.85)	13 (100)	9.6	-	1.0 (reference)	-	-
AG	4 (5.79)	4 (7.14)	0	2.9	**0.006**	14.01 (2.12-92.46)	3.84 (1.16-12.72)	**0.02**

Regimen-Paclitaxel cisplatin/carboplatin

Genotype rs11045585 SLCO1B3	CASES n (%), N=86	DEAD n (%), N= 73	ALIVE n (%), N= 13	Univariate analysis			Multivariate analysis	
				MST (months)	Log rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*P*

(1683-5676A>G)								
AA	79 (91.86)	68 (93.15)	11 (84.61)	5.83	-	1.0 (reference)	-	-
AG	7 (8.13)	5 (6.84)	2 (15.38)	10.47	0.18	0.61 (0.30-1.26)	0.44 (0.16-1.19)	0.11
Regimen-Pemetrexed cisplatin/carboplatin								
Genotype rs11045585 SLCO1B3 (1683-5676A>G)	**CASES n (%), N=122**	**DEAD n (%), N= 101**	**ALIVE n (%), N= 21**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
AA	101 (82.78)	83 (82.17)	18 (85.71)	7.7	-	1.0 (reference)	-	-
AG	20 (16.39)	18 (17.82)	2 (9.52)	9.43	0.67	1.12 (0.65-1.90)	0.84 (0.47-1.51)	0.57
GG	1 (0.81)	-	1 (4.76)	-	-	-	-	-
Regimen-Irinotecan cisplatin/carboplatin								
Genotype rs11045585 SLCO1B3 (1683-5676A>G)	**CASES n (%), N=70**	**DEAD n (%), N= 62**	**ALIVE n (%), N= 8**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
AA	62 (88.57)	54 (87.09)	8 (100)	7.67	-	1.0 (reference)	-	-
AG	8 (11.42)	8 (12.90)	0	8.26	0.89	0.94 (0.45-1.97)	0.50 (0.18-1.38)	0.18
Gender- male								
Genotype rs11045585 SLCO1B3 (1683-5676A>G)	**CASES n (%), N=485**	**DEAD n (%), N= 404**	**ALIVE n (%), N=81**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
AA	435 (89.69)	360 (89.10)	75 (92.59)	7.9	-	1.0 (reference)	-	-
AG	50 (10.30)	44 (10.89)	6 (7.40)	7.23	0.54	1.10 (0.79-1.53)	1.07 (0.76-1.50)	0.67
Gender-Female								
Genotype rs11045585 SLCO1B3 (1683-5676A>G)	**CASES n (%), N=108**	**DEAD n (%), N= 89**	**ALIVE n (%), N=19**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***P***
Codominant model								
AA	94 (87.03)	77 (86.51)	17 (89.47)	8.2	-	1.0 (reference)	-	-
AG	13 (12.03)	12 (13.48)	1 (5.26)	9.43	0.76	1.10 (0.58-2.07)	0.67 (3.23-1.39)	0.29
GG	1 (0.92)	0	1 (5.26)	-	-	-	-	-

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, regimen, histology, KPS, ECOG for overall survival; [a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, regimen, KPS, ECOG for histology; [a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, sex, smoking, stage, histology, KPS, ECOG for regimen. [a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, smoking, stage, regimen, histology, KPS, ECOG for gender

5.28.1 Association of SLCO1B3 polymorphism with clinical outcomes in lung carcinoma patients

The impact of *SLCO1B3* $A^{1683\text{-}5676}G$ polymorphism on the different clinical-pathological outcomes was characterized mainly by tumor stage (Stage III and IV), direct tumor extension (T3 and T4), lymph node extension (Nx+N0+N1 and N2+N3+N4), and on distant metastatic status (M0 and M1) (Table 5.75). Our results demonstrated that individuals having *SLCO1B3* $A^{1683\text{-}5676}G$ polymorphism have a higher frequency of wild-type genotype (*AA)* stage IV patients (90.64%) as compared to stage III patients (88.09%). No risk was found when heterozygous genotype for *SLCO1B3* $A^{1683\text{-}5676}G$ of stage IV was compared with stage III (AOR=0.74, 95% CI=0.33-1.67, p=0.47). Additionally, only one patient harbored mutant genotype in stage IV for *SLCO1B3* $A^{1683\text{-}5676}G$, so the risk could not be evaluated for this group. Furthermore, when the patients were stratified based on direct tumor extension (T3 *vs.* T4), our data for *SLCO1B3* $A^{1683\text{-}5676}G$ did not show any risk when carriers (*AG)* of the T4 subgroup were compared with carriers of the T3 subgroup (AOR=1.41, 95% CI=0.54-3.69, p=0.47).

Moreover, upon evaluating the correlation of *SLCO1B3* $A^{1683\text{-}5676}G$ polymorphism with lymph node involvement, lack of association was found. Also, we did not find any significant association between *SLCO1B3* $A^{1683\text{-}5676}G$ polymorphism and spread of lung carcinoma to the other organs. Therefore, we report no association among the *SLCO1B3* $A^{1683\text{-}5676}G$ polymorphism in lung cancer concerning clinicopathological parameters.

The correlation between *SLCO1B3* $A^{1683\text{-}5676}G$ polymorphism and the efficacy of response rate and clinical benefits from platinum-based doublet chemotherapy was evaluated by logistic regression. The relation of *SLCO1B3* $A^{1683\text{-}5676}G$ polymorphism is depicted in table 5.75. Lung cancer patients with complete (CR) or partial responses (PR) were considered good responders, while those with stable (SD) and progressed disease (PD) were considered as poor responders. Our results did not show any substantial difference in response to chemotherapy in lung cancer patients having *SLCO1B3* $A^{1683\text{-}5676}G$ polymorphism.

Table 5.75: Association of SLCO1B3 polymorphism based on clinical outcomes

Tumor stage				
Genotype rs11045585 SLCO1B3 (1683-5676A>G)	**Stage III (168), n (%)**	**Stage IV (385), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
AA	148 (88.09)	349 (90.64)	1.0 (Reference)	
AG	20 (11.90)	35 (9.09)	0.74 (0.33-1.67)	0.47
GG	0	1 (0.25)	-	-
Tumor Size				
Genotype rs11045585 SLCO1B3 (1683-5676A>G)	**T3 (122), n (%)**	**T4 (347), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
AA	111 (90.98)	312 (89.91)	1.0 (Reference)	
AG	11 (9.01)	34 (9.79)	1.41 (0.54-3.69)	0.47
GG	0	1 (0.28)	-	-
AG+GG	11 (9.01)	35 (10.08)	1.46 (0.56-3.82)	0.43
Lymph Node Invasion				
Genotype rs11045585 SLCO1B3 (1683-5676A>G)	**Nx+N0+N1 (130), n (%)**	**N2+N3+N4 (451), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
AA	114 (87.69)	405 (89.80)	1.0 (Reference)	
AG	16 (12.30)	45 (9.97)	0.91 (0.41-2.03)	0.82
GG	0	1 (0.22)	-	-
AG+GG	16 (12.30)	46 (10.19)	0.94 (0.42-2.09)	0.88
Metastasis				
Genotype rs11045585 SLCO1B3 (1683-5676A>G)	**M0 (272), n (%)**	**M1 (309), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
AA	245 (90.07)	274 (88.67)	1.0 (Reference)	
AG	27 (9.92)	34 (11.00)	0.89 (0.44-1.83)	0.77
GG	0	1 (0.32)	-	-
AG+GG	27 (9.92)	35 (11.32)	0.92 (0.45-1.87)	0.82
Response to chemotherapy				
Genotype rs11045585 SLCO1B3 (1683-5676A>G)	**CR+PR (133), n (%)**	**SD+PD (182), n (%)**	**AOR (95% CI) [a]**	***p*[b]**
AA	120 (90.22)	156 (85.71)	1.0 (Reference)	
AG	13 (9.77)	26 (14.28)	1.70 (0.81-3.53)	0.15

a)-Adjusted Odds ratios, 95% confidence intervals, and corresponding *p*-values were calculated by logistic regression analysis after adjusting for age, gender, smoking, and regimen. (b)-Two-sided $\chi 2$ test for either genotype distribution or allelic frequencies between clinic-pathological features. The number in bold indicates the significant values in the table

5.29 Elucidation of different genotypic combinations of *SLC19A1, SLCO1B1, and SLCO1B3* genes with the prognosis of lung carcinoma patients

Multiple interaction analyses involving various genes implicated in SLC transporters add a new dimension to assessing the high-order parametric interactions within them and their impact on the overall survival of lung cancer patients. The recursive partitioning strategy was adopted to generate a survival tree in this study, demonstrating various SLC SNPs' roles in prognosis. Figure 5.49 shows the tree generated by the STREE software. The log-rank approach was used to generate the tree out of all the methods available. There was a total of four-terminal nodes found. *SLC19A1G*^{80}*A* was the most crucial factor as it is a root node influencing the prognosis of patients. Terminal node 5, with the highest MST, was used as a reference node (13.1 months). In addition, each node's HR is mentioned. The survival curves and the MST associated with each terminal node are illustrated in Figure 5.50. It shows a difference between the median survival time of different nodes and the reference node (log-rank p 0.18), though a significant correlation was not observed. The findings of the Cox regression analysis for every terminal node are summarized in Table 5.76. Significant differences were observed in the median survival times when terminal node four was compared to the reference node (4.8 months *vs.* 13.1 months; HR=2.24, 95% CI= 0.99-5.02; p=0.05**).** After adjusting with different covariates, including age, gender, stage, smoking, histology, regimen, ECOG, and KPS, the death risk was 1.94 times compared to the reference node (HR=1.94; p=**0.002**). A similar trend was noted in all the other nodes; however, significant differences were not achieved.

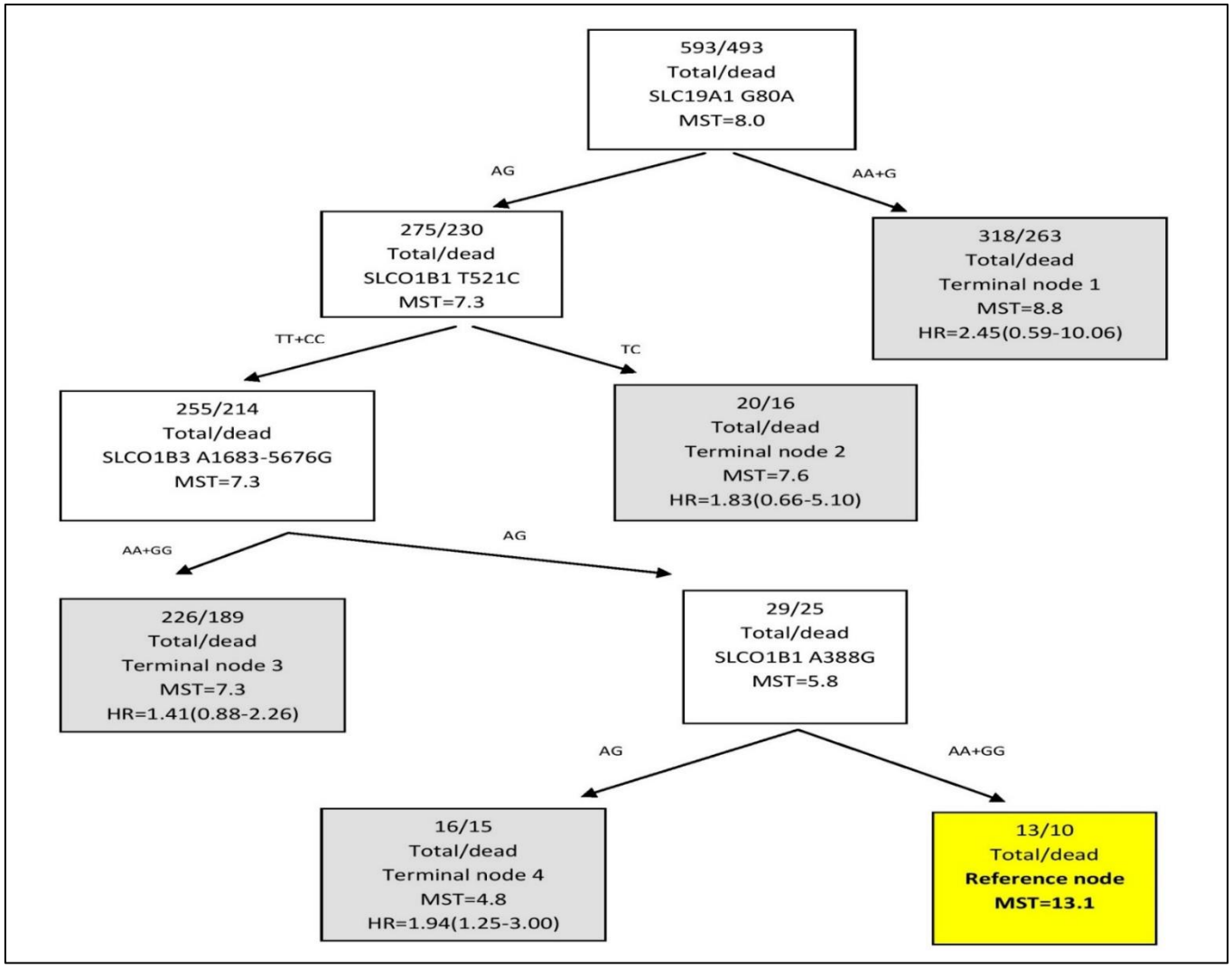


Figure 5.49: Recursive partitioning method (STREE) explaining the correlation of *SLC* SNPs with overall survival in lung carcinoma patients

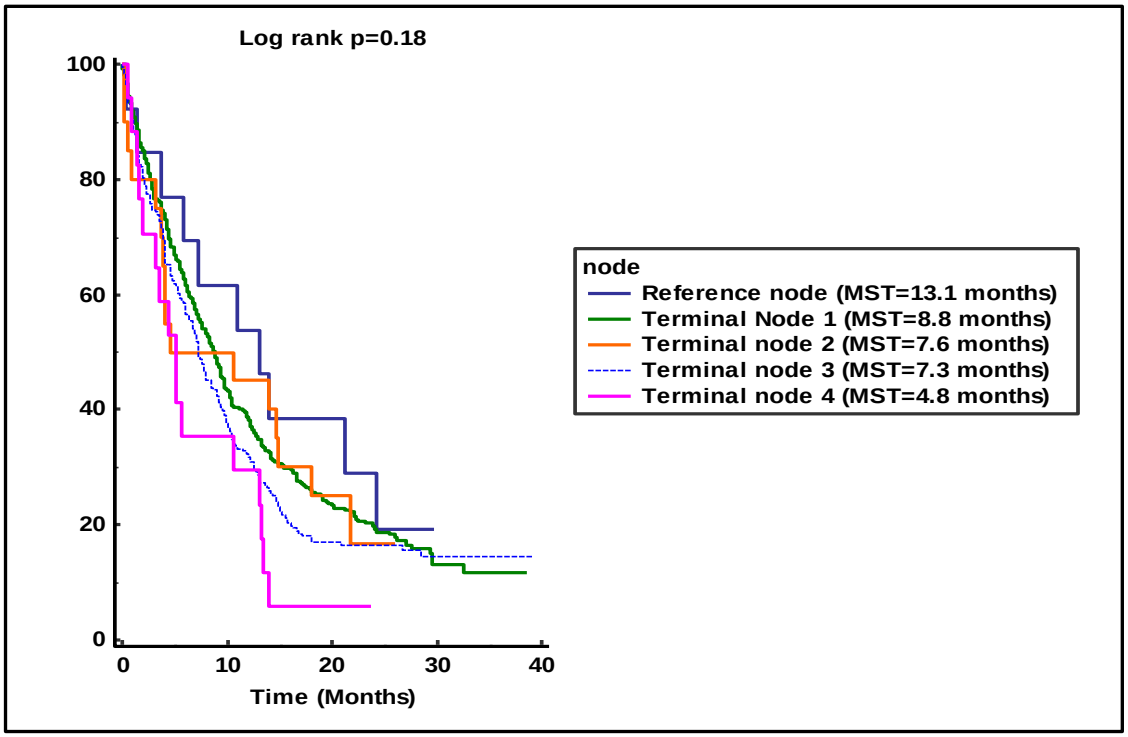


Figure 5.50: Survival according to different nodes for SLC genes identified by survival tree analysis.

HR, hazard ratio; MST, median survival time

Table 5.76: Overall survival in *SLC* SNPs by applying STREE

Terminal nodes	Dead	Alive	Median OS (months)	HR[a] (95% CI)	Log P	HR[b] (95% CI)	P-value
Terminal node 1 *SLC19A1* $G^{80}A$ (*W+H*)	263	55	8.8	1.24 (0.70-2.19)	0.44	2.45 (0.59-10.06)	0.21
Terminal node 2 *SLC19A1* $G^{80}A$ (*H*), *SLCO1B1* $T^{521}C$ (*H*)	16	4	7.6	1.30 (0.59-2.85)	0.50	1.83 (0.66-5.10)	0.24
Terminal node 3 *SLC19A1* $G^{80}A$ (*H*), *SLCO1B1* $T^{521}C$ (*W+M*), *SLCO1B3* $A^{1683\text{-}5676}G$ (*W+M*)	189	37	7.3	1.37 (0.80-2.36)	0.24	1.41 (0.88-2.26)	0.15
Terminal node 4 *SLC19A1* $G^{80}A$ (*H*), *SLCO1B1* $T^{521}C$ (*W+M*), *SLCO1B3* $A^{1683\text{-}5676}G$ (*H*), *SLCO1B1* *A388G* (*H*)	15	1	4.8	2.24 (0.99-5.02)	0.05	1.94 (1.25-3.00)	**0.002**
Terminal node 5 (Reference node) *SLC19A1* $G^{80}A$ (*H*), *SLCO1B1* $T^{521}C$ (*W+M*), *SLCO1B3* $A^{1683\text{-}5676}G$ (*H*), *SLCO1B1* $A^{388}G$ (*W+M*)	10	3	13.1	1.00 (reference)	-	1.00 (reference)	-

[a]Unadjusted Hazards ratio for Kaplan Meier analysis, [b]hazards ratio for age, gender, smoking, stage, histology, KPS, ECOG, and regimen

5.30 Association of SLC transporter genes (*SLC19A1, SLCO1B1,* and *SLCO1B3*) and toxicity

One of the current research goals was to examine the emergence of toxicity in lung carcinoma patients given platinum-based doublet chemotherapy with docetaxel, pemetrexed, irinotecan, and paclitaxel. The odds ratio (OR) and 95 % confidence interval (CI) for obtaining different toxicity grades following therapy in participants with each genotype were calculated using univariate logistic regression analysis. The adjusted ORs were also evaluated using a multivariate regression model with the toxicity grades as the dependent variable. Age, gender, regimen, and performance status, were all included in the multivariate model and were evaluated for their interactions or confounding effects. We utilized three possible analysis methods to see how SLC polymorphisms affected different toxicity levels. We compared patients with any toxicity grade (grades 1-4) to no toxicity (grade 0) in the initial assessment, individuals with intermediate/severe toxicity (grades 2-4) to those with no/low grade (grade 0-1) toxicity were compared in the second analysis and the third assessment, and high-grade toxicity (grades 3-4) *vs.* no/low/intermediate toxicity (grades 0-2) to see if the SLC polymorphisms were linked to severe toxicity. In this manner, cases were categorized into different toxicity levels.

5.30.1 Correlation of SLC gene polymorphisms and hematological toxicity

All chemotherapy-related adverse effects (AEs) were recorded for each treatment cycle. We evaluated anemia, leukopenia, absolute neutropenia, and thrombocytopenia in hematological toxicities. A total of 406 patients' toxicity data was available (Table 5.77).

Table 5.77: Hematological with CTC grades in lung cancer patients

Toxicity	Adverse events	n	Toxicity profiles		
			Grade 0, n (%)	Grade 1-2, n (%)	Grade 3-4, n (%)
Hematological toxicity	Anemia	406	102 (25.1)	274 (66.9)	30 (7.38)
	Leukopenia	404	349 (86.3)	45 (11.1)	10 (2.47)
	Thrombocytopenia	398	342 (85.9)	55 (13.8)	1 (0.25)
	Neutropenia	367	333 (90.7)	24 (6.53)	10 (2.72)

Upon assessing the effect of different SLC polymorphisms on toxicity, our results showed lack of substantial differences between *SLC19A1* $G^{80}A$ polymorphism and chemotherapy induced leukopenia. Lung cancer patients having *SLCO1B3* $A^{1683\text{-}5676}G$ had a high incidence of wild-type genotype in grade 0 compared to grades 1-4 (88.2% *vs.* 85.4%). However, none of the *SLC19A1* ($G^{80}A$), *SLCO1B1* ($A^{388}G$, $T^{521}C$), and *SLCO1B3* ($A^{1683\text{-}5676}G$) were not associated with the risk of developing leukopenia (Table 5.78 (a)).

Table 5.78 (a): Leukopenia-haematological toxicity (grades 0 vs, 1-4; grades 0-1 vs 2-4) associated with *SLC* polymorphisms

Leukopenia												
Genotype rs1051266 SLC19A1 (G80A)	**Grade of toxicity 0, n (%) N=349**	**Grade of toxicity 1-4, n (%) N=55**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**	**Genotype rs1051266 SLC19A1 (G80A)**	**Grade of toxicity 0-1, n (%) N=387**	**Grade of toxicity 2-4, n (%) N=17**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
GG	107 (30.6)	15 (27.2)	1.00 (Reference)	-	1.00 (Reference)		GG	120 (31.0)	2 (11.7)	1.00 (Reference)		1.00 (Reference)	
GA	168 (48.1)	29 (52.7)	1.23 (0.63-2.40)	0.54	1.06 (0.46-2.42)	0.87	GA	187 (48.3)	10 (58.8)	3.20 (0.69-14.89)	0.13	0.80 (0.12-5.22)	0.8
AA	74 (21.2)	11 (20)	1.06 (0.46-2.43)	0.8	1.09 (0.39-3.04)	0.85	AA	80 (20.6)	5 (29.4)	3.75 (0.71-19.8)	0.11	4.17 (0.74-23.48)	0.10
GA+AA	242 (69.3)	40 (72.7)	1.17 (0.62-2.22)	0.61	1.07 (0.50-2.29)	0.85	GA+AA	267 (68.9)	15 (88.2)	3.37 (0.75-14.97)	0.11	1.95 (0.40-9.43)	0.40
Genotype rs2306283 SLCO1B1 (A388G)	**Grade of toxicity 0, n (%) N=349**	**Grade of toxicity 1-4, n (%) N=55**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**	**Genotype rs2306283 SLCO1B1 (A388G)**	**Grade of toxicity 0-1, n (%) N=387**	**Grade of toxicity 2-4, n (%) N=17**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
AA	83 (23.7)	14 (25.4)	1.00 (Reference)	-	1.00 (Reference)		AA	92 (23.7)	5 (29.4)	1.00 (Reference)		1.00 (Reference)	
AG	173 (49.5)	26 (47.2)	0.89 (0.44-1.79)	0.74	0.90 (0.38-2.12)	0.81	AG	190 (49.0)	9 (52.9)	0.87 (0.28-2.67)	0.81	1.04 (0.23-4.75)	0.9
GG	93 (26.6)	15 (27.2)	0.95 (0.43-2.09)	0.91	0.69 (0.25-1.90)	0.47	GG	105 (27.1)	3 (17.6)	0.52 (0.12-2.26)	0.38	0.35 (0.04-2.59)	0.31
AG+GG	266 (76.2)	41 (74.5)	0.91 (0.47-1.75)	0.78	0.84 (0.37-1.90)	0.68	AG+GG	295 (76.2)	12 (70.5)	0.74 (0.25-2.18)	0.59	0.76 (0.19-3.06)	0.70
Genotype rs4149056 SLCO1B1 (T521C)	**Grade of toxicity 0, n (%) N=349**	**Grade of toxicity 1-4, n (%) N=55**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**	**Genotype rs4149056 SLCO1B1 (T521C)**	**Grade of toxicity 0-1, n (%) N=387**	**Grade of toxicity 2-4, n (%) N=17**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
TT	323 (92.5)	53 (96.3)	1.00 (Reference)	-	1.00 (Reference)		TT	359 (92.7)	17 (100)	1.00 (Reference)	-	1.00 (Reference)	-
TC	26 (7.4)	2 (3.6)	0.46 (0.10-2.03)	0.31	0.62 (0.12-3.03)	0.56	TC	28 (7.23)	0	0.36 (0.02-6.14)	-	-	-
Genotype rs11045585 SLCO1B3 (1683-5676A>G)	**Grade of toxicity 0, n (%) N=349**	**Grade of toxicity 1-4, n (%) N=55**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**	**Genotype rs11045585 SLCO1B3 (1683-5676A>G)**	**Grade of toxicity 0-1, n (%) N=387**	**Grade of toxicity 2-4, n (%) N=17**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
AA	308 (88.2)	47 (85.4)	1.00 (Reference)		1.00 (Reference)		AA	341 (88.1)	14 (82.3)	1.00 (Reference)	-	1.00 (Reference)	-
AG	40 (11.4)	8 (14.5)	1.31 (0.57-2.97)	0.51	1.18 (0.43-3.20)	0.74	AG	45 (11.6)	3 (17.6)	1.62 (0.44-5.87)	0.45	0.55 (0.06-4.79)	0.59
GG	1 (0.2)	0	-	-	-	-	GG	1 (0.25)	0	-	-	-	-
AG+GG	41	8 (14.5)	1.27 (0.56-2.89)	0.55	1.13 (0.41-3.04)	0.80	AG+GG	46 (11.8)	3 (17.6)	1.58 (0.43-5.73)	0.53	0.06 (4.63)	0.57

OR[1] Crude odds ratio, 95% CI: 95% confidence interval, **AOR[2]**Adjusted Odds ratio data were calculated by unconditional logistic regression and adjusted for age, gender, performance status, and regimen. A p-value < 0.05 was considered statistically significant

A total of 406 patients' toxicity data was available in the case of anemia (Table 5.78 (b). 25.1% of individuals were classified in grade 0 toxicity, 66.9% of patients were categorized in low/intermediate toxicity (grade 1-2), and 7.38% were classified in severe toxicity (grade 3-4). As observed for *SLCO1B1* $T^{521}C$ polymorphism, 7.14% of individuals harbored single copy mutant allele (*TC*). Table 5.78 (b) shows that subjects with the heterozygous genotype (*TC*) had a reduced risk of developing anemia (OR=0.44, 95% CI=0.20-0.96; p=**0.04**) when compared to the patients with a wild type genotype (*TT*).

For *SLC19A1* $G^{80}A$ polymorphism, on comparing individuals with severe toxicity (grades 3-4) *vs.* no/low-grade (grade 0-2) toxicity, we observed that 26.6% of patients in grade 3-4 anemia harboring heterozygous (*GA*) genotype showed a reduced risk of anemia (OR=0.35, 95% CI=0.14-1.74; p=**0.02**) as compared to the wild type genotype (*GG*). Also, we did not find any association between the *SLCO1B3* polymorphisms and other hematological toxicities such as anemia and other AEs due to chemotherapy.

Table 5.78 (b): Anaemia-Haematological toxicity (grades 0 vs, 1-4; grades 0-1 vs 2-4, and grades 0-2 *vs.* 3-4) associated with *SLC* polymorphisms

Anaemia													
Genotype rs1051266 SLC19A1 (G80A)	**Grade of toxicity 0, n(%) N=102**	**Grade of toxicity 1-4, n(%) N=304**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs2306283 SLCO1B1 (A388G)**	**Grade of toxicity 0-1 n(%) N=246**	**Grade of toxicity 2-4 n(%)160**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GG	26(25.4)	97(31.9)	1.00 (Reference)		1.00 (Reference)		AA	59(23.9)	37(23.1)	1.00 (Reference)		1.00 (Reference)	
GA	50(49.0)	148(48.6)	0.79(0.46-1.35)	0.39	0.93(0.44-1.95)	0.85	AG	126(51.2)	74(46.2)	0.93(0.56-1.54)	0.79	0.86(0.45-1.63)	0.65
AA	26(25.4)	59(19.6)	0.60(0.32-1.14)	0.12	0.55(0.24-1.25)	0.15	GG	61(24.7)	49(30.6)	1.28(0.73-2.23)	0.38	0.95(0.46-1.92)	0.88
GA+AA	76(74.5)	207(68.0)	0.73(0.44-1.21)	0.22	0.80(0.41-1.56)	0.51	AG+GG	187(76.0)	123(76.8)	1.04(0.65-1.67)	0.84	0.90(0.49-1.64)	0.74
Genotype rs2306283 SLCO1B1 (A388G)	**Grade of toxicity 0, n(%) N=102**	**Grade of toxicity 1-4, n(%) N=304**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs11045585 SLCO1B3 (1683-5676A>G)**	**Grade of toxicity 0-1 n(%) N=246**	**Grade of toxicity 2-4 n(%)160**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	25(24.5)	71(23.3)	1.00 (Reference)		1.00 (Reference)		AA	217(88.2)	140(87.5)	1.00 (Reference)		1.00 (Reference)	
AG	50(49.0)	150(49.3)	1.05(0.60-1.84)	0.84	0.79(0.35-1.79)	0.58	AG	28(11.3)	20(12.5)	1.10(0.60-2.04)	0.74	1.14(0.53-2.46)	0.72
GG	27(26.4)	83(27.3)	1.08(0.57-2.03)	0.80	0.73(0.29-1.83)	0.50	GG	1(0.40)	0	-	-	-	-
AG+GG	77(75.4)	233(76.6)	1.06(0.63-1.79)	0.81	0.78(0.36-1.69)	0.53	AG+GG	29(11.7)	20(12.5)	1.06(0.58-1.96)	0.82	1.07(0.50-2.27)	0.86
Genotype rs4149056 SLCO1B1 (T521C)	**Grade of toxicity 0, n(%) N=102**	**Grade of toxicity 1-4, n(%) N=304**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs4149056 SLCO1B1 (T521C)**	**Grade of toxicity 0-1 n(%) N=246**	**Grade of toxicity 2-4 n(%)160**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
TT	90(88.2)	287(94.4)	1.00 (Reference)		1.00 (Reference)		TT	226(91.8)	151(94.3)	1.00 (Reference)		1.00 (Reference)	
TC	12(11.7)	17(5.5)	0.44(0.20-0.96)	**0.04**	0.54(0.17-1.67)	0.29	TC	20(8.13)	9(5.62)	0.67(0.29-1.51)	0.34	0.64(0.22-1.84)	0.41
Genotype rs11045585 SLCO1B3 (1683-5676A>G)	**Grade of toxicity 0, n(%) N=102**	**Grade of toxicity 1-4, n(%) N=304**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs1051266 SLC19A1 (G80A)**	**Grade of toxicity 0-2 n(%), N=376**	**Grade of toxicity 3-4 n(%),N=30**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	91(89.2)	266(87.5)	1.00 (Reference)		1.00 (Reference)		GG	110(29.2)	13(43.3)	1.00 (Reference)		1.00 (Reference)	
AG	11(10.7)	37(12.17)	1.15(0.56-2.34)	0.69	1.26(0.47-3.36)	0.63	GA	190(50.5)	8(26.6)	0.35(0.14-0.88)	**0.02**	0.55(0.17-1.74)	0.30
GG	0	1(0.32)	-	-	-	-	AA	76(20.2)	9(30)	1.0(0.40-2.46)	0.9	1.61(0.52-4.98)	0.40
AG+GG	11(10.7)	38(12.5)	1.18(0.57-2.40)	0.64	1.29(0.48-3.42)	0.60	GA+AA	266(70.7)	17(56.6)	0.54(0.25-1.15)	0.11	0.86(0.33-2.22)	0.76

Genotype rs1051266 SLC19A1 (G80A)	**Grade of toxicity 0-1 n(%) N=246**	**Grade of toxicity 2-4 n(%) N=160**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs2306283 SLCO1B1 (A388G)**	**Grade of toxicity 0-2 n(%), N=376**	**Grade of toxicity 3-4 n(%), N=30**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GG	76(30.8)	47(29.3)	1.00 (Reference)		1.00 (Reference)		AA	87(23.1)	9(30)	1.00 (Reference)		1.00 (Reference)	
GA	119(48.3)	79(49.3)	1.07(0.67-1.70)	0.76	1.66(0.92-2.98)	0.08	AG	188(50)	12(40	0.61(0.25-1.51)	0.29	0.56(0.18-1.69)	0.30
AA	51(20.7)	34(21.2)	1.07(0.61-1.89)	0.79	1.19(0.58-2.45)	0.63	GG	101(26.8)	9(30)	0.86(0.32-2.26)	0.76	0.53(0.15-1.82)	0.31
GA+AA	170(69.1)	113(70.6)	1.07(0.69-1.65)	0.74	1.49(0.86-2.58)	0.14	AG+GG	289(76.8)	21(70)	0.70(0.31-1.58)	0.39	0.56(0.20-1.52)	0.25
Genotype rs4149056 SLCO1B1 (T521C)	**Grade of toxicity 0-2 n(%), N=376**	**Grade of toxicity 3-4 n(%), N=30**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs11045585 SLCO1B3 (1683-5676A>G)**	**Grade of toxicity 0-2 n(%), N=376**	**Grade of toxicity 3-4 n(%), N=30**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
TT	350(93)	27(90)	1.00 (Reference)		1.00 (Reference)		AA	332(88.2)	25(83.3)	1.00 (Reference)		1.00 (Reference)	
TC	26(6.91)	3(10)	1.49(0.42-5.26)	0.53	2.55(0.49-13.17)	0.26	AG	43(11.4)	5(16.6)	1.54(0.56-4.24)	0.39	1.49(0.45-4.96)	0.50
CC	0	0	-	-	-	-	GG	1(0.26)	0	-	-	-	-
							AG+GG	44(11.7)	5(16.6)	1.50(0.54-4.14)	0.42	1.48(0.44-4.89)	0.52

OR[1] Crude odds ratio, 95% CI: 95% confidence interval, **AOR[2]**Adjusted Odds ratio data were calculated by unconditional logistic regression and adjusted for age, gender, performance status, and regimen. A p-value < 0.05 was considered statistically significant

For analysis of absolute neutrophil count (ANC), we compared no grade toxicity (grade 0) *vs.* low/intermediate/severe toxicity (grade 1-4) and intermediate/severe toxicity (grades 2-4) *vs.* no/low-grade (grades 0-1) toxicity as shown in Table 5.78 (c). A total of 367 patients' toxicity data was available for ANC. For *SLC19A1* $G^{80}A$ polymorphism, when no grade toxicity (grade 0) was compared with low/intermediate/severe toxicity (grade 1-4), our results showed that *SLC19A1* ($G^{80}A$), *SLCO1B1* ($T^{521}C$), and *SLCO1B3* ($A^{1683\text{-}5676}G$) polymorphisms were not associated with developing neutropenia toxicity in lung cancer patients undergoing platinum-based doublet chemotherapy.

Table 5.78 (c): Neutropenia-haematological toxicity (grades 0 *vs.* 1-4; grades 0-1 *vs.* 2-4) associated with *SLC* polymorphisms

		Neutropenia				
Genotype rs1051266 SLC19A1 (G80A)	**Grade of toxicity 0, n (%)N=333**	**Grade of toxicity 1-4, n (%) N=34**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
GG	106 (31.8)	8 (23.5)	1.00 (Reference)		1.00 (Reference)	
GA	158 (47.4)	19 (55.8)	1.59 (0.67-3.77)	0.28	0.93 (0.32-2.66)	0.89
AA	69 (20.7)	7 (20.5)	1.34 (0.46-3.87)	0.58	1.42 (0.43-4.69)	0.56
GA+AA	227 (68.1)	26 (76.4)	1.51 (0.66-3.46)	0.32	1.08 (0.42-2.80)	0.86
Genotype rs2306283 SLCO1B1 (A388G)	**Grade of toxicity 0, n (%)N=333**	**Grade of toxicity 1-4, n (%) N=34**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
AA	80 (24.02)	8 (23.5)	1.00 (Reference)		1.00 (Reference)	
AG	163 (48.9)	17 (50)	1.04 (0.43-2.51)	0.9	0.65 (0.23-1.84)	0.42
GG	90 (27.02)	926.4)	1.00 (0.36-2.71)	1	0.39 (0.10-1.47)	0.16
AG+GG	253 (75.9)	26 (76.4)	1.02 (0.44-2.36)	0.94	0.58 (0.22-1.52)	0.27
Genotype rs11045585 SLCO1B3 (1683-5676A>G)	**Grade of toxicity 0, n (%)N=333**	**Grade of toxicity 1-4, n (%) N=34**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
AA	293 (87.9)	29 (85.2)	1.00 (Reference)		1.00 (Reference)	
AG	39 (11.7)	5 (14.7)	1.29 (0.47-3.54)	0.61	0.91 (0.24-3.36)	0.89
GG	1 (0.30)	0	-	-	-	-
AG+GG	40 (12.01)	5 (14.7)	1.26 (0.46-3.45)	0.64	0.87 (0.23-3.21)	0.84
Genotype rs1051266 SLC19A1 (G80A)	**Grade of toxicity 0-1, n (%)N=349**	**Grade of toxicity 2-4, n (%) N=18**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
GG	109 (31.2)	5 (27.7)	1.00 (Reference)		1.00 (Reference)	
GA	169 (48.4)	8 (44.4)	1.03 (0.32-3.23)	0.9	0.79 (0.20-3.17)	0.75
AA	71 (20.3)	5 (27.7)	1.53 (0.42-5.49)	0.51	2.20 (0.53-9.09)	0.27
GA+AA	240 (68.7)	13 (72.2)	1.18 (0.41-3.39)	0.75	1.21 (0.36-4.05)	0.75
Genotype rs2306283 SLCO1B1 (A388G)	**Grade of toxicity 0-1, n (%)N=349**	**Grade of toxicity 2-4, n (%) N=18**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
AA	82 (23.4)	6 (33.3)	1.00 (Reference)		1.00 (Reference)	
AG	171 (48.9)	9 (50)	0.71 (0.24-1.08)	0.54	0.46 (0.14-1.52)	0.20
GG	96 (27.5)	3 (16.6)	0.42 (0.10-1.76)	0.23	0.17 (0.02-1.04)	0.05
AG+GG	267 (76.5)	12 (66.6)	0.61 (0.22-1.68)	0.34	0.35 (0.11-1.07)	0.06

Genotype rs11045585 SLCO1B3 (1683-5676A>G)	Grade of toxicity 0-1, n (%)N=349	Grade of toxicity 2-4, n (%) N=18	OR[1] (95% CI)	P	AOR[2] (95% CI)	P
AA	306 (87.6)	16 (88.8)	1.00 (Reference)		1.00 (Reference)	
AG	42 (12.03)	2 (11.11)	0.91 (0.20-4.10)	0.9	0.88 (0.19-4.02)	0.87
GG	1 (0.28)	0	-	-	-	-
AG+GG	43 (12.3)	2 (11.11)	0.88 (0.19-4.0)	0.87	0.88 (0.18-4.27)	0.87

OR[1] Crude odds ratio, 95% CI: 95% confidence interval, **AOR[2]**Adjusted Odds ratio data were calculated by unconditional logistic regression and adjusted for age, gender, performance status, and regimen. A p-value < 0.05 was considered statistically significant

Chemotherapy-induced thrombocytopenia is a potentially lethal consequence resulting in chemotherapy dosage delays, reductions, or discontinuance. Keeping this complication in view, we have analyzed the impact of SLC variants in causing thrombocytopenia. As shown in table 5.78 (d), we compared low/intermediate/severe toxicity (grades 1-3) *vs.* no grade (grade 0) toxicity. For thrombocytopenia, there were 367 patients with available toxicity data, of which 32.1% (18) patients with *SLCO1B1* $A^{388}G$ polymorphism harboring a single copy of mutant allele (*AG*) were associated with a lower incidence of thrombocytopenia when compared to the patients carrying wild type alleles (*AA*) (OR=0.41, 95% CI=0.20-0.82; p=**0.01**). After adjusting for confounding variables such as age, gender, regimen, and performance status, the reduced risk in heterozygous genotype (*AG*) for causing thrombocytopenia was consistent when compared to wild-type genotype (*AA*) (AOR=0.35, 95% CI=0.14-0.85; p=**0.02**). Variants of *SLC19A1* ($G^{80}A$), *SLCO1B1* ($T^{521}C$), and *SLCO1B3* ($A^{1683\text{-}5676}G$) were not associated with developing thrombocytopenia in lung cancer patients undergoing platinum-based doublet chemotherapy.

Table 5.78 (d): Thrombocytopenia-haematological toxicity (grades 0 *vs.* 1-4; grades 0-1 *vs.* 2-4) associated with *SLC* polymorphisms

			Thrombocytopenia			
Genotype rs1051266 SLC19A1 (G80A)	**Grade of toxicity 0, n (%)N=342**	**Grade of toxicity 1-3, n (%) N=56**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
GG	107 (31.2)	13 (23.21)	1.00 (Reference)	-	1.00 (Reference)	
GA	167 (48.8)	28 (50)	1.38 (0.68-2.78)	0.36	1.21 (0.52-2.82)	0.64
AA	68 (19.8)	15 (26.7)	1.81 (0.81-4.05)	0.14	1.65 (0.62-4.34)	0.30
GA+AA	235 (68.7)	43 (76.7)	1.50 (0.77-2.91)	0.22	1.32 (0.60-2.90)	0.48
Genotype rs2306283 SLCO1B1 (A388G)	**Grade of toxicity 0, n (%)N=342**	**Grade of toxicity 1-3, n (%) N=56**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
AA	77 (22.5)	19 (33.9)	1.00 (Reference)		1.00 (Reference)	
AG	177 (51.7)	18 (32.1)	0.41 (0.20-0.82)	**0.01**	0.35 (0.14-0.85)	**0.02**
GG	88 (25.7)	19 (33.9)	0.87 (0.43-1.77)	0.71	0.75 (0.30-1.81)	0.52
AG+GG	265 (77.4)	37 (66.0)	0.56 (0.30-1.03)	0.06	0.50 (0.23-1.07)	0.07
Genotype rs4149056 SLCO1B1 (T521C)	**Grade of toxicity 0, n (%)N=342**	**Grade of toxicity 1-3, n (%) N=56**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
TT	318 (92.9)	53 (94.6)	1.00 (Reference)	-	1.00 (Reference)	
TC	24 (7.01)	3 (5.35)	0.75 (0.21-2.57)	0.64	0.75 (0.16-3.57)	0.72
Genotype rs11045585 SLCO1B3 (1683-5676A>G)	**Grade of toxicity 0, n (%)N=342**	**Grade of toxicity 1-3, n (%) N=56**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
AA	300 (87.7)	49 (87.5)	1.00 (Reference)		1.00 (Reference)	
AG	41 (11.9)	7 (12.5)	1.04 (0.44-2.46)	0.91	1.07 (0.37-3.03)	0.89
GG	1 (0.29)	0	-	-	-	-
AG+GG	42 (12.2)	7 (12.5)	1.02 (0.43-2.4)	0.96	1.05 (0.37-2.96)	0.92

OR[1] Crude odds ratio, 95% CI: 95% confidence interval, **AOR[2]**Adjusted Odds ratio data were calculated by unconditional logistic regression and adjusted for age, gender, performance status, and regimen. A p-value < 0.05 was considered statistically significant

5.30.2 Association of SLC polymorphism with nephrotoxicity

Renal functions are assessed during chemotherapy as chemotherapeutic drugs are eliminated *via* the kidneys and nephrotoxic. We have evaluated chemotherapy-induced nephrotoxicity by comparing intermediate/severe toxicity (grades 3-5) *vs.* no/low grade (grades 1-2) toxicity, as shown in table 5.79. Data from 366 patients were available to assess the association between the SLC polymorphisms and nephrotoxicity. Lung carcinoma patients with heterozygous(*AG*) genotype for $A^{1683\text{-}5676}G$ polymorphism showed a lower incidence of nephrotoxicity when compared to the patients with wild-type genotype (*AA*) (OR=0.35, 95% CI=0.17-0.72; p=**0.002)**. When confounding characteristics such as age, gender, regimen, and performance status were taken into account, the reduced risk of nephrotoxicity in heterozygous and mutant genotype (*AG+GG*) was consistent when compared to wild-type genotype (*AA*) (AOR=0.38, 95% CI=0.15-0.98; p=**0.04**). However, *SLC19A1* ($G^{80}A$) and *SLCO1B1* ($A^{388}G$, $T^{521}C$) polymorphisms were not associated with developing nephrotoxicity in lung cancer patients undergoing platinum-based doublet chemotherapy.

Table 5.79: Nephrological toxicity (grades 0 vs, 1-4; grades 0-1 vs 2-4) associated with *SLC* polymorphisms

		Nephrotoxicity				
Genotype rs1051266 SLC19A1 (G80A)	**Grade of toxicity 1, n (%)N=47**	**Grade of toxicity 2-5, n (%)N=319**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
GG	15 (31.9)	101 (31.6)	1.00 (Reference)	-	1.00 (Reference)	
GA	26 (55.3)	153 (47.9)	0.87 (0.44-1.73)	0.69	0.45 (0.16-1.30)	0.14
AA	6 (12.7)	65 (20.3)	1.60 (0.59-4.35)	0.34	0.96 (0.25-3.63)	0.9
GA+AA	32 (68.0)	218 (68.3)	1.01 (0.52-1.95)	0.9	0.62 (0.24-1.62)	0.33
Genotype rs2306283 SLCO1B1 (A388G)	**Grade of toxicity 1, n (%)N=47**	**Grade of toxicity 2-5, n (%)N=319**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
AA	15 (31.9)	81 (25.3)	1.00 (Reference)		1.00 (Reference)	
AG	17 (36.1)	157 (49.2)	1.71 (0.81-3.6)	0.15	1.41 (0.48-4.13)	0.52
GG	15 (31.9)	81 (25.3)	-	-	-	-
AG+GG	32 (68.0)	268 (84.0)	1.37 (0.70-2.67)	0.34	1.31 (0.49-3.47)	0.58
Genotype rs4149056 SLCO1B1 (T521C)	**Grade of toxicity 1, n (%)N=47**	**Grade of toxicity 2-5, n (%)N=319**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
TT	43 (91.4)	297 (93.1)	1.00 (Reference)	-	1.00 (Reference)	
TC	4 (8.51)	22 (6.89)	0.79 (0.26-2.42)	0.68	0.53 (0.10-2.71)	0.44
Genotype rs11045585 SLCO1B3 (1683-5676A>G)	**Grade of toxicity 1, n (%)N=47**	**Grade of toxicity 2-5, n (%)N=319**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
AA	40 (85.1)	281 (88.0)	1.00 (Reference)		1.00 (Reference)	
AG	6 (12.7)	38 (11.9)	0.90 (0.35-2.26)	0.82	1.71 (0.36-8.1)	0.49
GG	1 (2.12)	0	-	-	-	-

AG+GG	7 (14.8)	38 (11.9)	0.77 (0.32-1.84)	0.56	1.08 (0.29-4.07)	0.90
Genotype rs1051266 SLC19A1 (G80A)	**Grade of toxicity 1-2, n (%)N=179**	**Grade of toxicity 3-5, n (%)N=187**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
GG	59 (32.9)	57 (30.4)	1.00 (Reference)		1.00 (Reference)	
GA	90 (50.2)	89 (47.5)	1.02 (0.64-1.63)	0.92	1.13 (0.58-2.19)	0.70
AA	30 (16.7)	41 (21.9)	1.41 (0.78-2.56)	0.25	1.29 (0.53-3.14)	0.56
GA+AA	120 (67.0)	130 (69.5)	1.12 (0.72-1.74)	0.61	1.18 (0.64-2.18)	0.59
Genotype rs2306283 SLCO1B1 (A388G)	**Grade of toxicity 1-2, n (%)N=179**	**Grade of toxicity 3-5, n (%)N=187**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
AA	46 (25.6)	50 (26.7)	1.00 (Reference)		1.00 (Reference)	
AG	84 (46.9)	90 (48.12)	0.83 (0.51-1.35)	0.46	1.41 (0.67-2.93)	0.35
GG	49 (27.3)	47 (25.1)	0.74 (0.42-1.30)	0.30	1.34 (0.60-2.97)	0.46
AG+GG	133 (74.3)	137 (73.2)	0.80 (0.51-1.26)	0.34	1.37 (0.70-2.70)	0.35
Genotype rs4149056 SLCO1B1 (T521C)	**Grade of toxicity 1-2, n (%)N=179**	**Grade of toxicity 3-5, n (%)N=187**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
TT	168 (93.8)	172 (91.9)	1.00 (Reference)	-	1.00 (Reference)	-
TC	11 (6.14)	15 (8.02)	1.33 (0.59-2.98)	0.48	1.04 (0.33-3.24)	0.94
Genotype rs11045585 SLCO1B3 (1683-5676A>G)	**Grade of toxicity 1-2, n (%)N=179**	**Grade of toxicity 3-5, n (%)N=187**	**OR[1] (95% CI)**	**P**	**AOR[2] (95% CI)**	**P**
AA	147 (82.1)	174 (93.0)	1.00 (Reference)	-	1.00 (Reference)	-
AG	31 (17.31)	13 (6.95)	0.35 (0.17-0.72)	**0.002**	0.41 (0.16-1.07)	0.06
GG	1 (0.55)	0	-	-	-	-
AG+GG	32 (17.8)	13 (6.95)	0.34 (0.17-0.67)	**0.002**	0.38 (0.15-0.98)	**0.04**

OR[1] Crude odds ratio, 95% CI: 95% confidence interval, **AOR[2]**Adjusted Odds ratio data were calculated by unconditional logistic regression and adjusted for age, gender, performance status, and regimen. A p-value < 0.05 was considered statistically significant

5.30.3 Association of SLC polymorphisms and gastrointestinal toxicity

Additionally, we investigated gastrointestinal (GI) toxicity by comparing patients with low toxicity (grade 1) grade to individuals with intermediate-high toxicity (grade 2). We evaluated diarrhea, anorexia, constipation, and nausea-vomiting (Table 5.80).

Table 5.80: Gastrointestinal toxicity with CTC grades in lung cancer patients

Toxicity	Adverse events	n	Toxicity profiles	
			Grade 1, n (%)	**Grade 2-4, n (%)**
Gastrointestinal toxicity	Diarrhea	133	61 (45.8)	72 (54.1)
	Constipation	101	31 (30.6)	70 (69.3)
	Anorexia	122	8 (6.55)	114 (93.4)
	Nausea-vomiting	116	53 (45.6)	63 (54.3)

Lung cancer patient with the heterozygous genotype (*AG*) for *SLC19A1* $A^{388}G$ polymorphism has a reduced risk of having constipation as compared to the patients harboring wild-type genotype (*AA*) (AOR=0.17, 95% CI=0.03-0.87, p=**0.03**) as shown in Table 5.81. The reduced risk was consistent when we compared the combined heterozygous and mutant (*AG+GG*) genotype with the wild type (*AA*) genotype (AOR=0.22, 95% CI=0.05-0.97; p=**0.04**). For *SLCO1B1* $A^{388}G$, lung cancer patients with heterozygous genotype (AG) revealed a 3.87-fold increased risk of having constipation (AOR=3.87, 95% CI=0.91-16.48; p=**0.04**) as compared to the patients with wild type genotype (*AA*).

In nausea/vomiting, the comparison was made between intermediate/ severe toxicity (grades 2-3) *vs.* low-grade (grade 1) toxicity. Among 116 patients, 54.3% experienced grade 2-3 nausea/vomiting. For *SLCO1B1* $A^{388}G$ polymorphism, a lower incidence of nausea/vomiting was observed in patients with mutant genotype (*GG*) as compared to patients with wild-type genotype (*AA*) (AOR= 0.22; 95%CI=0.05-1.03, p=0.05 (Table 5.81). We did not find any association between the *SLC* polymorphisms, gastrointestinal toxicities such as diarrhea and anorexia, and other AEs due to chemotherapy.

Table 5.81: Gastrointestinal toxicity (grades 1 *vs.* 2-4; grades 1-2 *vs.* 3-4) associated with *SLC* polymorphisms

Diarrhoea

Genotype rs1051266 SLC19A1 (G80A)	Grade of toxicity 1, n(%)N=61	Grade of toxicity 2-4, n(%) N=72	OR[1] (95% CI)	P	AOR[2](95% CI)	P	Genotype rs1051266 SLC19A1 (G80A)	Grade of toxicity 1-2, n(%),N=105	Grade of toxicity 3-4, n(%),N=28	OR[1] (95% CI)	P	AOR[2](95% CI)	P
GG	20(32.7)	17(23.6)	1.00 (Reference)		1.00 (Reference)		GG	30(28.5)	7(25)	1.00 (Reference)		1.00 (Reference)	
GA	25(40.9)	42(58.3)	1.97(0.87-4.46)	0.10	2.20(0.79-6.05)	0.12	GA	50(47.6)	17(60.7)	1.45(0.54-3.92)	0.45	0.97(0.31-3.0)	0.95
AA	16(26.2)	13(18.0)	0.95(0.36-2.53)	0.92	0.90(0.29-2.77)	0.86	AA	25(23.8)	4(14.28)	0.68(0.17-2.61)	0.58	0.25(0.04-1.46)	0.12
GA+AA	41(67.2)	55(76.3)	1.57(0.73-3.38)	0.24	1.59(0.64-3.93)	0.31	GA+AA	75(71.4)	21(75)	1.20(0.46-3.11)	0.70	0.67(0.22-1.98)	0.47
Genotype rs2306283 SLCO1B1 (A388G)	**Grade of toxicity 1, n(%)N=61**	**Grade of toxicity 2-4, n(%) N=72**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs2306283 SLCO1B1 (A388G)**	**Grade of toxicity 1-2, n(%),N=105**	**Grade of toxicity 3-4, n(%),N=28**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	11(18.0)	16(22.2)	1.00 (Reference)		1.00 (Reference)		AA	21(20)	6(21.4)	1.00 (Reference)		1.00 (Reference)	
AG	37(60.6)	39(54.1)	0.72(0.29-1.76)	0.47	0.92(0.32-2.65)	0.88	AG	60(57.1)	16(57.1)	0.93(0.32-2.69)	0.89	0.95(0.27-3.25)	0.94
GG	13(21.3)	17(23.6)	0.89(0.31-2.57)	0.84	0.57(0.14-2.2)	0.43	GG	24(22.8)	6(21.4)	0.87(0.24-3.12)	0.83	0.25(0.04-1.61)	0.14
AG+GG	50(81.9)	56(77.7)	0.77(0.32-1.81)	0.55	0.84(0.30-2.32)	0.74	AG+GG	84(80)	22(78.5)	0.91(0.33-2.54)	0.86	0.84(0.25-2.7)	0.78
Genotype rs4149056 SLCO1B1 (T521C)	**Grade of toxicity 1, n(%)N=61**	**Grade of toxicity 2-4, n(%) N=72**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs4149056 SLCO1B1 (T521C)**	**Grade of toxicity 1-2, n(%),N=105**	**Grade of toxicity 3-4, n(%),N=28**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
TT	56(91.8)	69(95.8)	1.00 (Reference)		1.00 (Reference)		TT	98(93.3)	27(96.4)	1.00 (Reference)	-	1.00 (Reference)	-
TC	5(8.19)	3(4.16)	0.48(0.11-2.12)	0.33	0.75(0.14-4.03)	0.74	TC	7(6.66)	1(3.57)	0.51(0.06-4.39)	0.54	0.68(0.06-6.71)	0.74
Genotype rs11045585 SLCO1B3 (1683-5676A>G)	**Grade of toxicity 1, n(%)N=61**	**Grade of toxicity 2-4, n(%) N=72**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs11045585 SLCO1B3 (1683-5676A>G)**	**Grade of toxicity 1-2, n(%),N=105**	**Grade of toxicity 3-4, n(%),N=28**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
AA	52(85.2)	64(88.8)	1.00 (Reference)		1.00 (Reference)		AA	94(89.5)	22(78.5)	1.00 (Reference)		1.00 (Reference)	
AG	9(14.7)	8(11.11)	0.72(0.26-2.00)	0.53	0.57(0.17-1.94)	0.37	AG	11(10.4)	6(21.4)	2.33(0.77-6.98)	0.13	1.82(0.45-7.25)	0.39

Anorexia

Genotype rs1051266 SLC19A1 (G80A)	Grade of toxicity 1, n(%),N=8	Grade of toxicity 2-3, n(%) N=114	OR[1] (95% CI)	P	AOR[2](95% CI)	P	Genotype rs2306283 SLCO1B1 (A388G)	Grade of toxicity 1, n(%),N=8	Grade of toxicity 2-3, n(%) N=114	OR[1] (95% CI)	P	AOR[2](95% CI)	P
GG	2(25)	37(32.4)	1.00 (Reference)		1.00 (Reference)		AA	2(25)	26(22.8)	1.00 (Reference)		1.00 (Reference)	
GA	3(37.5)	55(48.2)	0.99(0.15-6.22)	0.9	0.43(0.03-5.16)	0.51	AG	4(50)	61(53.5)	1.17(0.20-6.80)	0.85	2.63(0.31-21.97)	0.36
AA	3(37.5)	22(19.2)	0.39(0.06-2.56)	0.33	0.18(0.009-3.51)	0.25	GG	2(25)	27(23.6)	1.03(0.13-7.92)	0.9	1.87(0.16-20.7)	0.60
GA+AA	6(75)	77(67.5)	0.69(0.13-3.6)	0.66	0.41(0.04-4.0)	0.44	AG+GG	6(75)	88(77.1)	1.12(0.21-5.92)	0.86	2.50(0.37-16.71)	0.34
Genotype rs4149056 SLCO1B1 (T521C)	**Grade of toxicity 1, n(%),N=8**	**Grade of toxicity 2-3, n(%) N=114**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs11045585 SLCO1B3 (1683-5676A>G)**	**Grade of toxicity 1, n(%),N=8**	**Grade of toxicity 2-3, n(%) N=114**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**

TT	7(87.5)	110(96.4)	1.00 (Reference)	-	1.00 (Reference)	-	AA	7((87.5)	99(86.8)	1.00 (Reference)		1.00 (Reference)	
TC	1(12.5)	4(3.50)	0.25(0.02-2.59)	0.24	0.27(0.019-3.82)	0.33	AG	1(12.5)	15(13.1)	1.06(0.12-9.23)	0.9	0.61(0.05-6.65)	0.68
Constipation													
Genotype rs1051266 SLC19A1 (G80A)	**Grade of toxicity 1, n(%),N= 31**	**Grade of toxicity 2, n(%) N=70**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs2306283 SLCO1B1 (A388G)**	**Grade of toxicity 1, n(%),N=31**	**Grade of toxicity 2, n(%) N=70**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GG	4(12.9)	25(35.7)	1.00 (Reference)		1.00 (Reference)		AA	8(25.8)	15(20.8)	1.00 (Reference)		1.00 (Reference)	
GA	20(64.5)	27(38.5)	0.21(0.06-0.71)	**0.01**	0.17(0.03-0.87)	**0.03**	AG	16(51.6)	36(51.4)	1.2(0.42-3.39)	0.73	3.87(0.91-16.48)	**0.04**
AA	7(22.5)	18(25.7)	0.41(0.10-1.61)	0.20	0.24(0.03-1.85)	0.17	GG	7(22.5)	19(27.1)	1.44(0.42-4.90)	0.55	2.22(0.51-9.64)	0.28
GA+AA	27(87.0)	45(64.2)	0.26(0.08-0.84)	**0.02**	0.22(0.05-0.97)	**0.04**	AG+GG	23(74.1)	55(78.5)	1.27(0.47-3.42)	0.62	2.87(0.82-10.04)	0.09
Genotype rs4149056 SLCO1B1 (T521C)	**Grade of toxicity 1, n(%),N= 31**	**Grade of toxicity 2, n(%) N=70**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs11045585 SLCO1B3 (1683-5676A>G)**	**Grade of toxicity 1, n(%),N=31**	**Grade of toxicity 2, n(%) N=70**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
TT	29(93.5)	67(95.7)	1.00 (Reference)		1.00 (Reference)		AA	28(90.3)	63(87.5)	1.00 (Reference)		1.00 (Reference)	
TC	2(6.45)	3(4.28)	0.64(0.10-4.09)	0.64	1.15(0.10-12.6)	0.9	AG	3(9.67)	7(10)	1.03(0.24-4.30)	0.96	1.47(0.26-8.26)	0.65
Nausea-vomiting													
Genotype rs1051266 SLC19A1 (G80A)	**Grade of toxicity 1, n(%),N= 53**	**Grade of toxicity 2-3, n(%) N=63**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs2306283 SLCO1B1 (A388G)**	**Grade of toxicity 1, n(%),N=53**	**Grade of toxicity 2-3, n(%) N=63**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
GG	17(32.0)	21(33.3)	1.00 (Reference)	-	1.00 (Reference)	-	AA	14(26.4)	15(23.8)	1.00 (Reference)		1.00 (Reference)	
GA	25(47.1)	30(47.6)	0.97(0.42-2.23)	0.94	0.97(0.37-2.55)	0.95	AG	23(43.3)	37(58.7)	1.50(0.61-3.67)	0.37	1.39(0.49-3.97)	0.53
AA	11(20.7)	12(19.0)	0.88(0.31-2.49)	0.81	0.64(0.19-2.12)	0.47	GG	16(30.1)	11(17.4)	0.64(0.22-1.84)	0.41	0.22(0.05-1.03)	0.05
GA+AA	36(67.9)	42(66.6)	0.94(0.43-2.05)	0.88	0.95(0.38-2.32)	0.91	AG+GG	39(73.5)	48(76.1)	1.14(0.49-2.66)	0.74	0.86(0.32-2.28)	0.77
Genotype rs4149056 SLCO1B1 (T521C)	**Grade of toxicity 1, n(%),N= 53**	**Grade of toxicity 2-3, n(%) N=63**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**	**Genotype rs11045585 SLCO1B3 (1683-5676A>G)**	**Grade of toxicity 1, n(%),N=53**	**Grade of toxicity 2-3, n(%) N=63**	**OR[1] (95% CI)**	**P**	**AOR[2](95% CI)**	**P**
TT	49(92.4)	60(95.2)	1.00 (Reference)		1.00 (Reference)		AA	45(84.9)	55(87.3)	1.00 (Reference)		1.00 (Reference)	
TC	4(7.54)	3(4.76)	0.61(0.13-2.86)	0.53	0.79(0.14-4.46)	0.79	AG	8(15.0)	8(12.6)	0.81(0.28-2.35)	0.70	0.60(0.17-2.10)	0.43

OR[1] Crude odds ratio, 95% CI: 95% confidence interval, **AOR[2]**Adjusted Odds ratio data were calculated by unconditional logistic regression and adjusted for age, gender, performance status, and regimen. A p-value < 0.05 was considered statistically significant

CHAPTER 6

DISCUSSION

6.1 Thymidylate synthase

Thymidylate synthase (TS) is a validated chemotherapeutic target, and its involvement in DNA synthesis and repair has provided the impetus for evaluating this enzyme. Reports suggest that the 5′-UTR and 3′-UTR polymorphisms in the *TS* gene might affect cancer treatment efficiency (Zhou *et al*., 2012; Olaussen *et al*., 2006), although the results have not been consistent (Edler *et al*., 2002). So, it is prerogative and clinically relevant to understand and minimize the inter-individual variation in drug responsiveness, sensitivity, and toxicity that shall play a crucial factor in determining the best successful treatment option for lung cancer patients. Given the impact of these polymorphisms on TS levels, it is crucial to investigate their role in lung cancer, as the extent and duration of TS inhibition with chemotherapy drugs might affect patients' responses.

6.1.1 Prognostic implications of TS gene polymorphisms in patients with lung cancer

In light of the present data so far evaluated, we endeavored to assess the role of *TS* polymorphisms in influencing the survival rates in lung cancer patients towards platinum-based doublet chemotherapy treatment. Our results suggest that the mutant genotype (*3R3R*) of *TSER* polymorphism and wild-type genotype of *1494del6 (+6/+6bp)* polymorphism is associated with reduced survival in the lung cancer patients who underwent platinum-based doublet chemotherapy. Similar results were reported by Danenberg, who reported that *3R/3R* allele is associated with high *TS* gene expression and the mutant (*3R3R*) genotype is associated with poor survival in NSCLC patients (Danenberg, 2004). Our results are in line with Iacopetta *et al.,* who reported inferior overall survival in patients harbouring mutant *(3R3R)* genotype than those individuals with heterozygous *(2R3R)* and wild genotype *(2R2R)* genotype (Iacopetta *et al*., 2001). Similar results have been reported in rectal and colorectal cancer (Marsh *et al*., 2001; Villafranca *et al*., 2001).

Contrary to our results, Arevalo *et al.* reported superior overall survival in mutant genotype (*3R3R*) of *TSER* polymorphism in advanced NSCLC patients of European ethnicity (Arévalo *et al*., 2014). Our results showed significant differences between the subjects harbouring different *TSER* genotypes at 36 months of survival and OS in both recessive and co-dominant models, likewise to the results revealed by Lectome and Ferraz *et al*. (Lecomte *et al*., 2004). Additionally, our results showed no association between tumour stage, primary tumour extension, and lymph

node invasion with *TSER* polymorphic variants. Our study is in line with Hu *et al.,* who assessed 5' and 3'-UTR *TS* polymorphisms in Asian lung cancer patients, and no particular genotype of *TSER* polymorphism appeared to associate with clinicopathological features and overall response rate. (Hu *et al*., 2012).

For *TS 1494del6* polymorphism, our data showed a lack of significant association between this polymorphism and overall survival. Similar findings were reported by a study in the Caucasian population of advanced NSCLC patients (Arévalo *et al*., 2014). On the contrary, Hu *et al*. reported longer overall survival in NSCLC patients with mutant genotype (*-6bp/-6bp*) (Hu *et al*., 2012). As we also found better survival in the mutant genotype of *1494del6* polymorphism, we would like to explain the increasing survival trend from our outcomes. At 36 months, in all the genetic models, a better survival time was noted for mutant genotype *(-6bp/-6bp)* subjects compared to the patients harbouring wild-type genotype *(+6bp/+6bp).* Hence, the *6 bp* deletion allele seems essential in predicting lung cancer patients' survival time. A study by Lima *et al*. revealed that deletion of 6 *bp* is correlated to the decrease in the TS mRNA stability and expression and better survival (Lima *et al*., 2014). Another report by Wang *et al.,* and Biason *et al.,* also reported that patients with 6 bp deletion had better overall survival in the NSCLC and ovarian cancer patients undergoing chemotherapy, respectively (Wang *et al*., 2013; Biason *et al*., 2012). However, we did not find any association between *1494del6* polymorphism in predicting overall survival and response rate in LC patients undergoing platinum-based doublet chemotherapy. This disparity can be explained by the significant clinical differences in both populations.

6.1.2 Mechanistic explanation of the impact of TS polymorphism on survival

TS expression has been well-correlated as a critical prognostic factor in patients having lung cancer and undergoing chemotherapy (Donner *et al*., 2019). Studies in the past have shown that the *3R* allele or *6 bp* insertion (TTAAAG in 3'-UTR) allele significantly increases the mRNA levels in lung cancer tissues as well as other carcinomas (Shintani *et al*., 2003; Marsh *et al*., 2001; Pullarkat *et al*., 2001; Mandola *et al*., 2003; Kawakami & Watanabe, 2003; Mandola *et al*., 2004). Figure 6.1 illustrates the mechanism of the Thymidylate synthase inhibition by chemotherapeutic drugs and its impact on TS polymorphisms Mechanistically, the *TS* gene is self-regulatory at the translational level, with the protein binding to its mRNA and blocking translation (Chu & Allegra,

1996). The strict regulation of TS activity reveals the necessity of maintaining a balance between dUMP and dTMP. TS inhibition results in the depletion of dTTP pools and elevates dUMP, which leads to DNA damage (Aherne *et al*., 1996). Alternatively, increased TS activity can lead to an elevated concentration of dTTP, which may lead to a complex feedback mechanism to reset the dNTP pool. This increase in the dNTP pool increases DNA replication errors, leading to more chances of cancer risk. Thus, the enhanced expression of the *3R* and *6bp* insertion allele may stimulate cell proliferation and prevent apoptosis (Sakoff & Ackland, 2000).

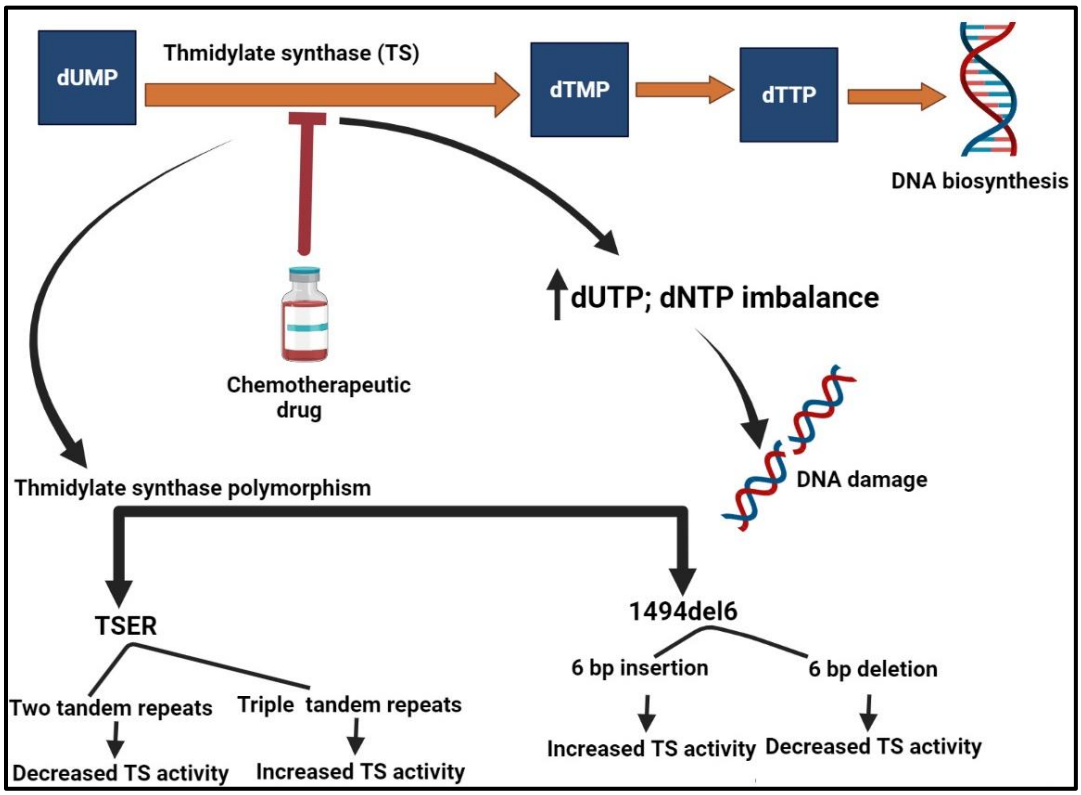


Figure 6.1: Mechanism of the *Thymidylate synthase* inhibition by chemotherapeutic drugs and its impact on *TS* polymorphisms

6.1.3 Impact of combined genotypes in TS polymorphism with OS

Furthermore, only studying *TSER* or *6bp* insertion/deletion polymorphisms was seen to have different results from the different patient groups and cancer types. Therefore, studying the combined genotype of *TSER* and *1494del6* polymorphisms is more worthwhile than studying the individual genotype alone. In our results, patients with (*3R3R*) + (-6/-6) [TSER high/1494del6 low] genotypes showed a decreased OS as compared with (*2R2R*) + (+6/+6) [TSER low/ 1494del6

high]. Contrary to our results, Dotor *et al*., reported that (*3R3R*) + (-6/-6) [TSER high/1494del6 low] haplotype had a higher OS as compared to (*2R2R*) + (6bp/+6p) [TSER low/ 1494del6 high] haplotype (Dotor *et al*., 2006). The contradictory outcomes may be attributed to ethnicity and clinical features of patients, such as the method utilized to measure TS expression and heterogeneity of the tumour histology, which could all play a role.

On evaluating the survival differences between increased TS expression haplotypes (*3R3R*) + *(+6/+6*) with low TS expression haplotypes (*2R2R*) + (*-6/-6*), we observed substantial higher OS in low expression haplotypes [(2R2R) + (-6/-6)]. As expected, low TS expression should show better survival; our results followed Kawakami *et al*., who presented that subjects with low TS expression have the better outcome, OS, and disease-free survival for gastric cancer patients (Kawakami *et al*., 2002). On the contrary, the study by Afzal *et al*. revealed that low TS expression genotypes (*2R2R*) + (-6/-6) had a worse prognosis than other genotypes (Afzal *et al*., 2011). However, our results support two main issues that might be focused on the elucidation of the data. Increased TS levels due to high TS expression genotypes may interfere with the mechanism of action of chemotherapeutic drugs. Also, this may promote cancer aggressiveness (Voeller *et al*., 2004; Liu *et al*., 2002).

6.1.4 Prognostic impact of TS polymorphism based on histology and regimen

We have also evaluated *TS* polymorphisms' role in predicting lung cancer patients' overall survival based on histological subtypes. There was no significant association observed between TS polymorphism and OS. We observed a trend in favor of better survival in ADCC patients with *TSER* polymorphism harbouring wild-type genotype (*2R2R*) as compared to the mutant genotype *(3R3R)*; however, in *1494del6* polymorphism, better survival was observed in mutant genotype *(-6/-6)* as compared to the wild type *(+6/+6)*. The low TS activity in the *TSER* wild type and *1494del6* mutant type could be a reason for better prognosis in these groups (Lecomte *et al*., 2004). Despite a trend in OS, the difference did not reach the threshold of significance, and we did not observe any association between these polymorphisms and OS.

Carboplatin and cisplatin have been considered influential in combination with drugs such as pemetrexed, paclitaxel, and docetaxel in response to treatment. Our results revealed reduced

survival in mutant genotype (*3R3R*) patients undergoing pemetrexed-cisplatin/ carboplatin therapy compared to the wild-type genotype (*2R2R*); however, our data did not achieve a substantial correlation between *TSER* polymorphism and OS. Our results are in line with a study by Shimizu *et al*., who revealed no significant correlation between TS expression and efficacy of pemetrexed-based chemotherapy in lung cancer patients (Shimizu *et al*., 2012). Smit *et al.* revealed no association between low and high TS expression genotypes and clinical outcomes of NSCLC patients treated with pemetrexed (Smit *et al*., 2009). On the contrary, a study has suggested that mutant genotype (*3R3R*) is associated with better survival in NSCLC patients receiving pemetrexed (Arévalo *et al*., 2014). These conflicting results can be explained by the clinical characteristics of patients, such as ethnicity and heterogeneity of the tumour histology.

6.2 Impact of TS polymorphisms in predicting toxicity in LC patients undergoing platinum-based doublet chemotherapy

6.2.1 Association of TS polymorphisms with hematological toxicity

We have also evaluated whether *TS* polymorphisms were associated with increased toxicity among platinum-based chemotherapy-treated lung cancer patients. We found a significant association between genotypes of *TSER* and *1494del6* polymorphism in developing the risk of leukopenia. The cells that were affected the most were WBCs. The lifespan of platelets is about ten days, whereas the lifespan of granulocytes is 6-8h. This could explain disparities in observed hematological toxicities (Seung, 2013). Mechanistically, Thymidylate synthase is a folate-dependent enzyme that catalyzes the reductive dUMP by reducing reduced folate (CH_2THF) to dTMP (Chu & Gollerkeri, 2002). A study by Schreurs *et al.* reported that chemotherapy reduces the level of folic acid after a few months, which leads to folate deficiency (Schreurs *et al*., 1985). Due to this deficiency, the levels of dUMP are elevated, further increasing the dUTP levels. The levels of uracil are found to be elevated in bone marrow cells and leukocyte DNA of folate-deficient individuals. Folate deficiency induces uracil misincorporation leading to DNA damage in hematopoietic cells (Blount *et al*., 1997). This might be a possible reason for the increased risk of leukopenia in chemotherapy-treated cancer patients. We found that subjects with the heterozygous genotype *(2R3R*) of the *TSER* polymorphism had a 2.30-fold increased risk of developing leukopenia toxicity on comparing intermediate/severe toxicity (grade 1-4) to those with

the absence of any toxicity (grade 0). Marginal risk of having leukopenia (1.14-fold) was observed in the mutant genotype of *TSER* polymorphism compared to wild type genotype (*2R2R*) in comparing grade 1-4 toxicity to grade 0 toxicity. Kotnik *et al.* demonstrated that the *3R3R* genotype was correlated with a reduced risk of leukopenia, mucositis, and thrombocytopenia (Kotnik *et al*., 2011).

Similarly, Erculj *et al.* also reported a reduced risk of developing leukopenia in patients harbouring the *3R3R* genotype than in wild-type genotype (*2R2R*) (Erčulj *et al*., 2012). We observed that mutant genotype *(-6/-6bp)* of *1494del6* polymorphism had a 2.57-fold enhanced risk of developing leukopenia on comparing intermediate/severe toxicity (grade 1-4) to those with the absence of any toxicity (grade 0). A study reported that carriers of deletion allele were likely to develop grade 3 or 4 leukopenia. This correlation was not found in previously reported studies in patients with acute lymphoblastic leukemia (Zgheib *et al*., 2014).

We observed a reduced risk of developing anemia in patients harbouring heterozygous genotype (*2R3R*) and mutant type genotype (*3R3R*) by comparing severe toxicity (grade 3-4) with the absence of any/ intermediate toxicity (grade 0-2). A similar study reported substantially less frequent grade≥ 2 among the mutant genotype (*3R3R*) as compared to wild type genotype (*2R2R*) (OR=0.36, p=**0.019**) (Erčulj *et al*., 2012). On the contrary, another study by Ongaro *et al*. reported a substantial risk of anemia in patients with the *3R3R* genotype (Ongaro *et al*., 2009). Abbasian *et al.* reported a significant correlation between grade 3 anemia and the *2R2R* genotype (**p=0.009**) (Abbasian *et al*., 2020). In *1494del6* polymorphism, mutant genotype *(-6/-6bp*) showed an almost 2-fold risk of developing anemia when compared to the wild-type genotype *(+6/+6*) (p=**0.04**). This could be explained by the fact that folate is essential in RBC formation and is also the common target of chemotherapeutic drugs (Robien, 2005). These drugs inhibit folate metabolism, and via this, TS is inhibited, and purine synthesis is hindered. A deficient folic acid impairs the maturation of RBCs and thus results in anemia (Koury & Ponka, 2004). Our results suggest that the *6bp* deletion allele is correlated with the development of anemia.

6.2.2 Association of TS polymorphisms with gastrointestinal toxicity

Gastrointestinal (GI) toxicity is a common side effect of chemotherapeutic drugs in lung cancer patients. The high proliferation index of gastrointestinal mucosa makes it prone to toxicity from chemotherapy (Fiore & Cutsem, 2009). We observed that *TSER* polymorphism was associated with gastrointestinal toxicity. A substantial risk of 5.14-fold constipation was found in heterozygous genotype *(2R3R)* when intermediate grade 2 toxicity was compared to low toxicity (grade 1). Similarly, mutant genotype (*3R3R*) of *TSER* polymorphism showed a 3.21-fold increased risk of constipation compared to wild-type genotype (*2R2R*). It is difficult to assess the incidence rate of chemotherapy-induced constipation among all cancer patients due to a lack of literature. However, specific chemotherapeutic agents like cisplatin and vinca alkaloids like vincristine, vinblastine, and vinorelbine induce accurate chemotherapy-induced constipation in up to 80%–90% of patients (McQuade *et al*., 2016). The above facts suggest that heterozygous *(2R3R)* and mutant genotypes *(3R3R)* of *TSER* polymorphism are associated with the risk of constipation.

Our results also revealed the increased risk of nausea/vomiting in patients harbouring heterozygous genotype *(+6/-6)* of *1494del6* when compared to intermediate/high (grade 2-4) and those with low toxicity (grade 1). Lung cancer patients with combined heterozygous genotype *(+6/-6)* and mutant genotype *(-6/-6)* showed an increased risk of nausea/vomiting with an odd of 2.85 as compared to wild-type genotype *(+6/+6)*. The intensity and pattern of chemotherapy-induced emesis are determined by several factors, including the patient's characteristics and the type of chemotherapy, dose schedule, and regimen used. Cisplatin is the most emetic agent, causing nausea and vomiting in more than 90% of individuals who are not given prophylactic antiemetics at 50 mg/m^2 or above. Carboplatin, methotrexate, doxorubicin, chlormethine, and cyclophosphamide have a significant emetogenic potential (Doherty, 1999). Our results strongly suggest that *TS 1494del6* polymorphism is substantially associated with chemotherapy-induced nausea/vomiting.

6.3 Prognostic implications of ABC gene polymorphisms in patients with lung cancer

6.3.1 Impact of ABCB1 ($C^{1236}T$, $C^{3435}T$, and $G^{2677}A/T$) polymorphisms in predicting overall survival

We have analyzed the role of *ABC* gene polymorphisms in influencing prognosis in lung cancer patients receiving platinum-based doublet chemotherapy. Our results from the North Indian lung carcinoma patients suggest that patients with at least one mutant allele (*CT*) for *ABCB1* $C^{1236}T$ had poor survival compared to the wild-type genotype (*CC*). Our outcomes are in line with Yang *et al*., who revealed that the *T* allele of *ABCB1* $C^{1236}T$ *i*s associated with poor survival in osteosarcoma patients (Yang *et al*., 2013). Similarly, Schaich *et al*. reported that homozygous (*CC*) alleles had superior overall survival than the heterozygous variants (Schaich *et al*., 2009).

The mechanistic explanation is that the *ABCB1* $C^{1236}T$ polymorphism influences the mRNA stability (Frittitta *et al*., 2001), and the allele-specific changes might influence the downstream mRNA splicing, translational control, or regulation in RNA folding. It is also possible that translational activity is reduced (Shen *et al*., 1999). This account for lower ABCB1 expression in patients having wild (*CC)* genotype as compared to the heterozygous *(CT)*, leading to reduced drug efflux and enhanced survival in such patients (Schaich *et al*., 2009). On the contrary, some studies have shown that carriers of the *T* allele of the $C^{1236}T$ are correlated with superior OS in Chinese B cell lymphoma patients, NSCLC, and colorectal cancer (Ni *et al*., 2016; Wu *et al*., 2013; Chen *et al*., 2021). Intriguingly, another study by Han *et al*. did not find any association between *ABCB1* $C^{1236}T$ polymorphism and prognosis in advanced NSCLC patients undergoing cisplatin-based chemotherapy. These discrepancies could be attributed to ethnicity, patient clinical features, and tumour histological heterogeneity, which could play an important role. However, more studies are required to understand how the $C^{1236}T$ polymorphism affects *MDR1* gene expression and protein function.

Our results for $C^{3435}T$ showed no association between $C^{3435}T$ and prognosis in lung cancer patients. Our results concur with a study that assessed the role of $C^{3435}T$ polymorphism in NSCLC patients and did not observe any substantial differences among the genotypes while studying overall survival (Gandara *et al*., 2009). Violas *et al*. also reported a lack of association between

$C^{3435}T$ polymorphism and prognosis in NSCLC patients undergoing platinum-based doublet chemotherapy (Vinolas *et al*., 2011). Also, Johnatty *et al.* found no correlation between the $C^{3435}T$ variant and overall survival in ovarian cancer patients (Johnatty *et al*., 2008). Besides this, our data did not show any association between *ABCB1* $G^{2677}A/T$ polymorphism and overall survival. Our results are in line with Kim *et al.,* who found no association between the overall survival and data analyzed for *ABCB1* $G^{2677}A/T$ (Kim *et al*., 2015).

6.3.2 Impact of ABCC1 ($G^{3173}A$ and $G^{2168}A$) polymorphisms in predicting overall survival

Our results for *ABCC1* $G^{3173}A$ polymorphism revealed that patients having mutant genotypes *(AA)* had a worse prognosis when compared to wild-type *(GG)* genotypes. In several investigations, overexpression of *ABCC1* has been associated with drug resistance in lung carcinoma cells (Triller *et al*., 2006; Yeh *et al*., 2005). Patients with high ABCC1 expression have poorer prognosis and treatment responses (Kadioglu *et al*., 2020). It has been reported that the *ABCC1* mutation may impact chemotherapeutic efficacy in lung carcinoma patients. However, we could not find any supporting article to validate our findings due to the fewer reports on *ABCC1* $G^{3173}A$ polymorphism and its associated role in predicting survival in carcinoma patients. Even though the number of patients with mutant genotypes was low, our findings revealed a substantial link between variant alleles and poor survival in lung cancer patients. The *ABCC1* $G^{2168}A$ polymorphism observed a lack of association in predicting overall survival. Also, no correlation was found between $G^{2168}A$ polymorphism and chemotherapy response. Our results align with Yin *et al*., who reported no significant association between the $G^{2168}A$ polymorphism and chemotherapy response in Chinese lung cancer patients (Yin *et al*., 2011).

6.3.3 Association of ABCC2 $G^{4544}A$ polymorphisms in predicting overall survival

This study reports a significant correlation between poor overall survival and the *A* allele of *ABCC2* $G^{4544}A$ polymorphism. Our results align with Cuffe *et al.,* who depicted the poor survival in NSCLC patients who had *A* allele of $G^{4544}A$ polymorphism (Cuffe *et al*., 2016). Studies have also shown that the expression of *ABCC2* is low in normal lung tissue, while its elevated levels are reported in lung cancer (Sandusky *et al*., 2002). *ABCC2* is expressed at high levels in the proximal renal tubule, the primary location of platinum-based drug clearance, and exports glutathione-

conjugated cisplatin outside the cell (Schaub *et al*., 1999). ABCC2 has been associated with the development of platinum resistance, as increased *ABCC2* mRNA expression is responsible for resistance to cisplatin by lowering intracellular drug accumulation and hence DNA adduct formation (Liedert *et al*., 2003). *In vivo* studies, particularly data on lung cancer, also suggest a strong correlation between ABCC2 expression and platinum resistance (Ushijima *et al*., 2007); however other studies were not as conclusive (Filipits *et al*., 2007).

6.3.4 Association of ABCG2 $G^{4544}A$ polymorphisms in predicting overall survival

Further, our data did not demonstrate any association with the role of the *ABCG2* $C^{421}A$ polymorphism in predicting survival differences in lung cancer patients. Our results align with the meta-analysis study where authors reported no significant correlation between *ABCG2* $C^{421}A$ polymorphism in predicting prognosis or any other clinical outcomes in lung cancer patients undergoing chemotherapy (Fu *et al.*, 2020). Chen *et al.* did not show any substantial correlation between $C^{421}A$ variation and prognosis in Chinese advanced NSCLC patients (Chen *et al.*, 2015).

6.3.5 Correlation of ABC gene polymorphism and prognosis based on histology and regimen

When we stratified our analysis of *ABC* gene polymorphism based on histology, we found that adenocarcinoma patients with homozygous wild alleles (*CC*) for *ABCB1* $C^{1236}T$ and $C^{3435}T$ had better survival as compared to their associated carrier alleles (*CT*). This can be supported by the study by Van *et al*., who found that the expression of *ABCB1* efflux transporter is higher in the adenocarcinoma than in the histology subtype of squamous cell carcinoma (Van Eerden *et al*., 2021). The significantly higher survival in wild-type patients is associated with low ABCB1 mRNA levels and prolonged prognosis (Kadioglu *et al*., 2020). Furthermore, this reduced expression of *ABCB1* mRNA levels leading to reduced efflux transporter activity explains our results with better survival in wild-type genotype (*CC*) of $C^{1236}T$ and $C^{3435}T$ in patients who were administered with a combination of cisplatin/carboplatin and pemetrexed. Contrary to our results, a study by Narumiya *et al.* reported that the wild-type genotype *(CC)* of $C^{3435}T$ is associated with a worse prognosis in patients having adenocarcinoma (Narumiya *et al*., 2011).

Our results revealed that SCLC patients with *ABCB1* $G^{2677}A$ harbouring heterozygous genotype *(GA)* had poor survival than the wild-type genotype *(GG)*. Numerous studies have reported the role of *ABCB1* polymorphism in NSCLC patients; however, very few studies have reported the role of these polymorphisms in SCLC. Astonishingly, increased ABCB1 expression was linked to poorer outcomes in some, though, not all the reports in SCLC patients were treated with chemotherapy (Knez *et al*., 2011). This also explains the poor prognosis in the heterozygous genotype (*GA*) of *ABCB1* $G^{2677}A$ polymorphism administered with cisplatin/carboplatin and irinotecan. Contrary to our results, a previous study reported survival differences in SCLC patients, but their results did not find any significant association between $G^{2677}T/A$ polymorphism and overall survival (Knez *et al*., 2012). The probable reason for the disparate results could be the small population size and fewer scientific contributions of these polymorphisms in small cell lung cancer. None of the previous studies have examined the association between *ABCB1* $G^{2677}A$ polymorphism and SCLC.

Regarding histology, our results for ADCC patients showed poor survival in the mutant genotype *(AA)* of $G^{3173}A$ polymorphism. Studies in the past have shown that MRP1 is abundantly expressed in ADCC specimens, and its expression correlates with the tumour's glandular differentiation (Sugawara *et al*., 1995). A poor prognosis in the NSCLC patients expressing high levels of ABCC1 treated with chemotherapy has also been discussed before (Berger *et al*., 2005; Oshika *et al*., 1998). The *ABCC1* $G^{3173}A$ polymorphism has been evaluated in various diseases and, to a lesser extent, in cancer. This variant's exact impact is inconsistent, making it hard to conclude the meticulous role of $G^{3173}A$ polymorphism in lung cancer.

We also found that ADCC and SCLC patients harbouring at least one mutant allele *(GA)* for *ABCC2* $G^{4544}A$ polymorphism had lower survival than wild-type genotypes *(GG)*. Young *et al*. reported that the MRP2 protein levels were elevated in the NSCLC cell lines compared to the SCLC cell lines; also, ABCC2 overexpression has been well reported to cause platinum resistance (Young *et al*., 2001). Studies have also shown that MRP2-positive individuals had substantially shorter survival than the MRP2-negative subjects (p = **0.007**) (Filipits *et al*., 2007). Furthermore, these high ABCC2 protein levels lead to increased efflux transporter activity of the chemotherapeutic drug, thereby justifying our results with reduced survival in heterozygous

genotype (*GA*) of *ABCC2* $G^{4544}A$ in patients who were administered with a combination of cisplatin/carboplatin and pemetrexed.

The patients who underwent platinum-based docetaxel-containing chemotherapy and had mutant genotype for *ABCB1* $C^{1236}T$ showed better survival than those with wild-type genotypes. Chen *et al.* reported that *ABCB1* $C^{1236}T$ had prognostic significance in patients who received taxane-containing chemotherapy (Chen *et al*., 2021). The impact of genetic mutations on survival could be attributed to polymorphisms that induce alterations in the encoded amino acids, disrupting the normal function of particular drug transporters and reducing P-gp expression. These modifications can cause changes in a drug's pharmacokinetics, as well as lower amounts of the drug being pumped out of the cell, leading to drug accumulation in the cell, which can have a detrimental effect on therapeutic efficacy (Zhou *et al*., 2015; Björn *et al*., 2018). According to Bosch *et al*., the $C^{1236}T$ homozygous mutant had decreased docetaxel clearance (Bosch *et al*., 2006). This could be the probable reason to explain the association of $C^{1236}T$ polymorphism in predicting OS in patients administered with cisplatin/carboplatin and docetaxel.

6.4 Genetic polymorphisms of ABC gene and the toxicity of chemotherapy treatment in lung cancer patients

Many theories have been proposed regarding the association between overexpression of ABC transporters and multidrug resistance. Nevertheless, few theories have been reported regarding the role of these ABC gene polymorphisms in correlation with carcinoma patients' toxicity. Therefore, comprehending and mitigating inter-individual variation in drug receptivity, sensitivity, and toxicity, including its vital role in determining the most effective therapy option for patients with lung cancer, is essential and clinically relevant.

6.4.1 ABC gene polymorphisms as a determinant of hematological toxicity

We have investigated the association of ABC polymorphisms and toxicity in patients with lung cancer undergoing platinum-based chemotherapy. According to our findings, patients with at least one mutant allele *(GA)* in *ABCC1* $G^{3173}A$ had an increased risk of developing anemia and leukopenia. Chemotherapeutic drugs affect folate metabolism, so reducing folate causes aberrant

DNA synthesis that promotes genomic instability, contributing to the notion that a deficiency of folic acid hampers RBC maturation, leading to anemia (Schreurs *et al*., 1985). Additionally, Yin *et al*. showed that the *ABCC1* $G^{3173}A$ ($Arg^{1058}Gln$) mutation had been related to decreased chemotherapeutic drug resistance (Yin *et al*., 2009). Many other factors are responsible for modulating a patient's clinical outcome, such as type of mutation and inter-individual variations.

Also, our data regarding the assessment of survival and other clinical outcomes of lung cancer patients having *ABCC1* $G^{3173}A$ polymorphism has shown higher survival in patients having at least one mutant allele *(GA)* as compared to the wild-type *(GG)* genotype (MST= 8.57 vs. 8.37). In addition, our results demonstrated better response in patients with $G^{3173}A$ polymorphism and undergoing platinum-based chemotherapy. Patients with *ABCC1* $G^{3173}A$ mutation can probably retain more drugs than wild-type *(GG)* patients, because of which a better prognosis and response to chemotherapy have been noted. However, more drug bioavailability to a cell is correlated to adverse events. It is plausible that this is the reason for high toxicity in patients with *ABCC1* $G^{3173}A$ polymorphism. We found a strong correlation between *ABCC1* $G^{3173}A$ mutations and hematological toxicity. The mechanism elucidating alteration in the transport of the cytotoxic chemotherapy impacts erythropoiesis remains unknown. These mutations could potentially affect intracellular doses of the delivered chemotherapeutic agent in cell type-specific methods. Another explanation is that some cells are susceptible to unavoidable variations in a dose of a specific metabolite. Also, it is plausible that the effect on erythropoiesis could be due to a specialized involvement of the damaged gene during erythropoiesis (Theile & Wizgall, 2021).

Additionally, our results showed an increased risk of anemia in patients with heterozygous genotypes for *ABCC2* $G^{4544}A$ polymorphism. Our results support a study by Elens *et al.*, who showed that mutation $G^{4544}A$ in the *ABCC2* gene leads to the impairment of the ATPase activity and is correlated with the increased cellular accumulation of the substrates of ABCC2, which might lead to cellular toxicity (Elens *et al.*, 2011).

6.4.2 Correlation of ABC gene polymorphisms in predicting nephrotoxicity

Our results showed reduced risk of developing nephrotoxicity in patients with *ABCB1* $G^{2677}A$ and *ABCC1* $G^{3173}A$ mutation. The *ABCB1* gene is expressed in the proximal tubule, collecting duct of cells, and thick loop of Henle in the kidney (Ernest & Bello-Reuss, 1998). The kidney is the main

organ in clearing platinum-based chemotherapy drugs (Burger *et al*., 2011). Various drugs' propensity to cause nephrotoxicity are excreted in the urine by active renal secretion and glomerular filtration (Ho & Kim, 2005). Walsh *et al*. have reported overexpression of the *ABCB1* gene in renal carcinoma (Walsh *et al*., 2009).

Furthermore, MRP1 overexpression has also been associated with multidrug resistance in lung, ovarian, breast, kidney, and pancreatic cancer (Poku & Iram, 2022; Barrand *et al*., 1993; Larkin *et al*., 2004; Li *et al*., 2009). Mohammad *et al*. reported that overexpression of drug transporter is responsible for the increased drug efflux in cancer (Mohammad *et al*., 2018). So, keeping these facts in view, our results suggest that *ABCB1* $G^{2677}A$ and *ABCC1* $G^{3173}A$ polymorphism is correlated with the protective effect from nephrotoxicity in cancer patients undergoing platinum-based treatment doublet chemotherapy.

6.4.3 Correlation of ABC gene polymorphism with gastrointestinal toxicity of platinum-based chemotherapy in lung cancer patients

The gastrointestinal system is the first line of protection against drugs and other xenobiotics in the human body. The expression of *ABCB1, ABCC1,* and *ABCG2* have been reported at the apical surface of epithelial cells in the intestine (Leslie *et al*., 2005). Our results showed that people with at least one mutant allele for *ABCB1* $C^{1236}T$ and $C^{3435}T$ polymorphism are correlated with the protective effect of GI toxicity. Nausea/vomiting is caused by cisplatin as an emetic agent and causes such side effects in 90% of patients who are not administered antiemetics (50mg/m^2 or above) (Doherty, 1999). Due to high p-glycoprotein (P-gp) levels in the GI tract and overexpression of MDR1 in the carcinoma cells, the efflux capacity of the various chemotherapeutic drugs such as taxanes and vinca alkaloids is increased (El-Awady *et al*., 2017). Chemotherapeutic drugs are probably eliminated faster, thus not showing nausea or vomiting-like side effects. A report by Qian *et al*. showed lower odds of gastrointestinal toxicity in patients with at least one allele for *ABCB1* $C^{3435}T$ variant in NSCLC patients undergoing platinum-based chemotherapy; however, substantial significance was not achieved in any model (Qian *et al*., 2016).

Our results also demonstrated the protective effect of gastrointestinal toxicity in people harbouring heterozygous genotype for *ABCG2* $C^{421}A$ polymorphism, and Delou *et al*. reported that

the *ABCG2* gene is highly expressed in the GI tract (Delou *et al*., 2009). In the case of *ABCC1* $G^{3173}A$ polymorphism, our outcomes showed that patients having heterozygous genotype *(GA)* and undergoing platinum-based chemotherapy were associated with an increased risk of chemotherapy-induced diarrhea. Understanding the exact incidence of chemotherapy-induced toxicity in lung cancer patients is difficult due to the lack of studies. The severity and degree of chemotherapy-induced toxicity are determined by many parameters, such as the patient's attributes, regimen type, type of chemotherapy, and dose schedule.

6.4.4 Association of ABC gene polymorphism with drug induced-hepatic toxicity

A drug-induced hepatic injury is an adverse event of grave concern because of its severe implications. Hepatic toxicity of platinum is characterized by elevated transaminases, bilirubin, and ALP (Cao *et al*., 2015). Cisplatin causes immediate DNA strand breakage in the basal epithelium, producing reactive oxygen species and causing cellular injury. Oxidative stress is a hallmark of mitochondrial damage and plays a significant role in this toxicity (Hassan *et al*., 2020). Oxidative damage to the mitochondrial cell reduces the efficacy of oxidative phosphorylation and, in the presence of Ca^{2+}, causes a transition in mitochondrial permeability (Custódio *et al*., 2009). Damage to the plasma membrane and protein leakage is the leading causes of high ALT levels in the serum (McGill, 2016). Despite significant research over the previous few decades, the basic mechanisms of chemotherapeutic drug induce damage remains a mystery.

On the other hand, genetic mutations in an individual are crucial in developing hepatic injury (Ulzurrun *et al*., 2014). Our results demonstrated a high risk of hepatotoxicity in lung patients with *ABCB1* $C^{1236}T$ and *ABCC1* $G^{3173}A$ variation. P-gp is expressed in the canalicular membrane of the liver, where it aids biliary drug excretion (Yu *et al*., 2010). Reports have suggested the crucial role of ABCB1 in a specific form of liver toxicity (Ulzurrun *et al*., 2014). Additionally, studies have suggested low levels of MRP1 expression in a healthy human liver (Leslie *et al*., 2005). Reduced expression of MRP1 in the liver probably reduces the drug efflux from the cell, and the concentration of the drug in the cell increases, leading to hepatic toxicity.

6.5 Prognostic implications of SLC gene polymorphisms in patients with lung cancer

6.5.1 Impact of SLC19A1 $G^{80}A$ polymorphisms in predicting overall survival

We have attempted to investigate the significance of *SLC* polymorphisms in affecting prognosis and its correlation with adverse events in lung carcinoma patients undergoing platinum-based chemotherapeutic treatment. The statistical analysis in North Indian lung carcinoma patients points out that variant genotype (*AA*) of *SLC19A1* $G^{80}A$ variation is not correlated in predicting survival. Our results align with Collin *et al.*, who found no correlation of $G^{80}A$ polymorphism in recessive, dominant, or additive models in prostate cancer (Collin *et al*., 2009). A study by Liu *et al*. did not find any significant association between *RFC* $G^{80}A$ polymorphism and prognosis in lung cancer (Liu *et al*., 2008). However, we can explain the increased prognostic trend in lung carcinoma patients with variant genotype (*AA*) of *SLC19A1* $G^{80}A$ polymorphism. The *A* allele of $G^{80}A$ might have a greater influx propensity for the substrates, resulting in improved bioavailability and chemotherapeutic drug intake (Lima *et al*., 2014), thereby leading to better survival outcomes in the individuals with the mutant genotype (*AA*). Contrary to our outcomes, Jabeen *et al.* have reported increased survival in the patients with reference-type genotype (*GG*) in comparison to the mutant genotype (*AA*) of *SLC19A1* $G^{80}A$ polymorphism (Jabeen *et al*., 2015). These incongruent results could be attributed to patients' clinical characteristics and ethnic differences.

6.5.2 Impact of SLCO1B1 gene polymorphisms in predicting overall survival

In patients with *SLCO1B1* $A^{388}G$ polymorphism, our results demonstrated that the patients with variant genotype (*GG*) had the same median survival time as the wild-type genotype *(AA)*. Our results are in concordance with *Lee et al.*, who put forth that *SLCO1B1* $A^{388}G$ is associated with an unaltered transport function (Figure 6.2), so there would be no modification in the drug influx, which could be the likely cause for no difference in median survival time between mutant *(GG)* and wild type genotypes *(AA)* (Lee & Ho, 2017). On the contrary, Sissung *et al.* stated that $A^{388}G$ polymorphism is correlated with the altered SLCO1B1 transport function, leading to modifications in the transmembrane domain structure (Sissung *et al*., 2012). While Liutkevicie *et al.* put forth that the *G* allele of $A^{388}G$ is associated with the increased SLCO1B1 function, proposing increased functional activity of the transporter (Liutkeviciene *et al*., 2018). *SLCO1B1* polymorphism has

been reported by numerous researchers, who have linked the $A^{388}G$ mutation to an increase, decrease, or no effect on the transporter activity (Niemi *et al.*, 2011).

Our results for *SLCO1B1* $T^{521}C$ polymorphism showed reduced survival in patients with variant genotype (*CC*) as compared to both homozygous (*TT*) and heterozygous genotype (*TC*), though the substantial significance between $T^{521}C$ polymorphism and overall survival was not achieved. Studies in the past have shown that the *C* allele of $T^{521}C$ is correlated with decreased activity of SLCO1B1 (Gong & Kim, 2013). Due to this reduced transporter activity, patients harbouring the variant allele (*C*) of $T^{521}C$ polymorphism may have a reduced inflow of drugs, which can explain the lower overall survival in lung carcinoma patients (Figure 6.2). On the contrary, Feng *et al.* have shown better survival in gastric carcinoma patients having mutant *(CC)* and heterozygous *(TC)* genotypes (Feng *et al.*, 2018).

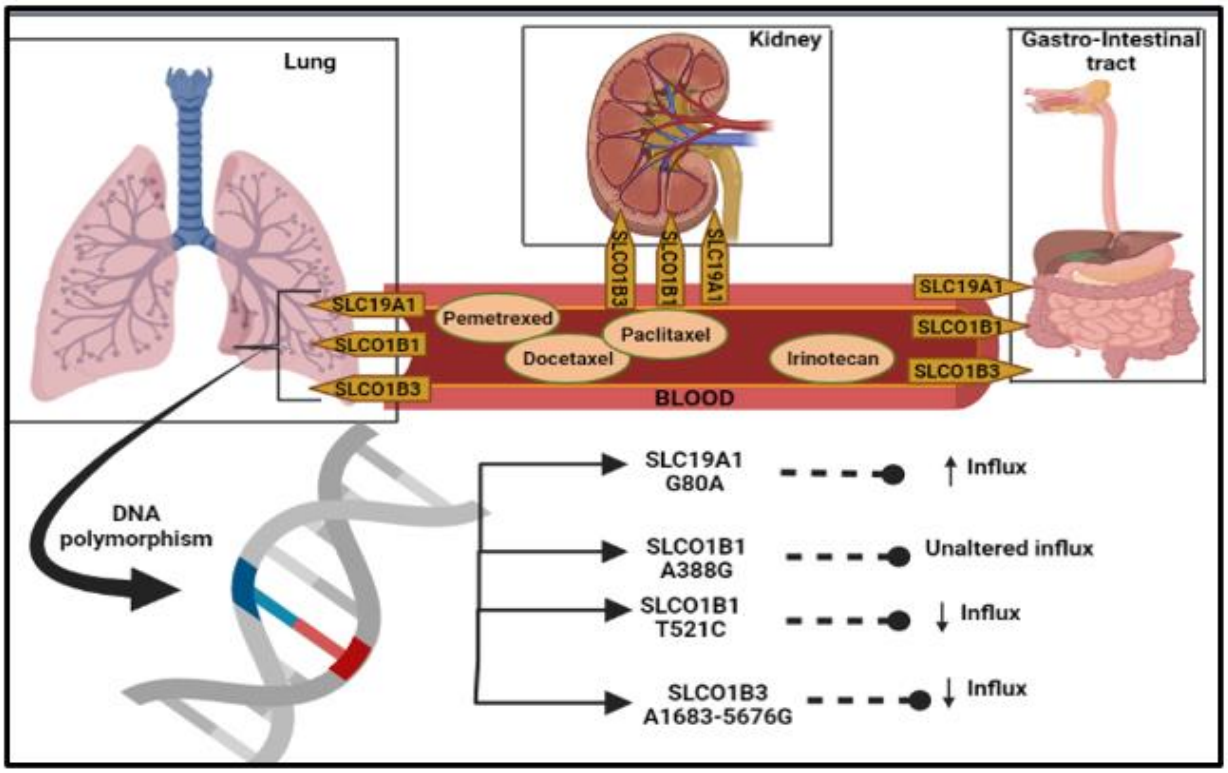


Figure 6.2: Impact of SLC transporter polymorphisms in predicting overall survival

6.5.3 Correlation of SLC polymorphism in predicting survival based on histology

We have also assessed the impact of SLC polymorphism on overall survival based on histology. Our findings demonstrated that ADCC patients with the mutant genotype *(AA)* of the *SLC19A1* $G^{80}A$ polymorphism had a better prognosis (**p=0.04**). Mechanistically, the RFC1 protein is encoded by the main influx transporter *SLC19A1*. It mediates folate uptake in the cell and transports antifolate chemotherapeutic agents. RFC1 is substantially expressed in lung cancer patients (Li *et al*., 2018). It has been reported that in adenocarcinoma cells, RFC1 proteins are overexpressed (Nunez *et al*., 2012).

Furthermore, studies have linked the downregulation of the RFC1 protein to impaired drug transport and developing resistance (Lima *et al*., 2014; Gorlick *et al*., 1997). The overexpression of RFC1 in ADCC patients explains our results with better survival in the mutant genotype *(AA)* of *SLC19A1* $G^{80}A$ polymorphism. Even though *SLC19A1* is the most common antifolate uptake transporter, our outcomes did not show any relationship of $G^{80}A$ polymorphism in association with the survival of the patients undergoing pemetrexed and cisplatin/carboplatin treatment. Our results align with the various studies on NSCLC (Tiseo *et al*., 2012; Smit *et al*., 2009; Goricar *et al*., 2014). Our findings also revealed that SCLC patients with the mutant genotype *(AA)* for *SLC19A1* $G^{80}A$ polymorphism had a better prognosis (p=**0.04**). To the best of our knowledge, none of the previous studies has looked at the role of *SLC19A* $G^{80}A$ polymorphism in SCLC, as most of the research has focused on its impact on NSCLC. Based on our findings, we believe that the *SLC19A1* $G^{80}A$ polymorphism may improve overall survival in SCLC patients.

6.5.4 Correlation of SLC polymorphism in predicting survival based on regimen stratification

As per our results, patients who were given docetaxel along with cisplatin/ carboplatin and were heterozygous carriers *(AG)* for the *SLCO1B3* $(A^{1683\text{-}5676}G)$ polymorphism had a significantly shorter survival time as compared to the wild-type *(AA)* genotype. No supporting studies were found because the pharmacogenetics of *SLCO1B3* is little understood, and previous *in vivo* and *in vitro* studies gave inconsistent results regarding the functional effects of the *SLCO1B3* polymorphisms. Chew *et al*. revealed that the *SLCO1B3* $(A^{1683\text{-}5676}G)$ variant allele is related to altered docetaxel disposition (Chew *et al*., 2012). However, our investigation did not find any patients with mutant genotype *(GG)* for *SLCO1B3* $(A^{1683\text{-}5676}G)$ polymorphism administered

docetaxel and cisplatin/carboplatin therapy, and we could not assess the role of its variant genotype on the survival of lung cancer patients. Chew *et al*. have mentioned that inter-individual variability in docetaxel disposition is influenced by *SLCO1B3* pharmacogenetics (Chew *et al*., 2011). However, more research is needed to investigate the functional characterization of *SLCO1B3*-associated docetaxel transport, which may help researchers better understand the molecular basis for docetaxel disposal disposition.

6.6 Role of SLC polymorphism in predicting toxicity in LC patients undergoing platinum-based doublet chemotherapy

6.6.1 Correlation of SLC polymorphisms with hematological toxicity

SLC polymorphisms and their association with toxicity amongst platinum-based chemotherapeutic-treated lung carcinoma patients have also been evaluated. Our results showed that subjects with at least one mutant allele *(AG)* in *SLCO1B1* $A^{388}G$ had a significantly lower risk of developing thrombocytopenia. Ieiri *et al.* have reported that the presence of the *G* allele at the codon 388 can modulate the activity of SLCO1B1(Ieiri *et al*., 2009). The systemic concentration of the chemotherapeutic drug strongly depends on the activity of SLCO1B1. An increase in the SLCO1B1 activity and subsequent increase in the hepatic clearance of the chemotherapeutic drug may lead to reduced systemic exposure and hence less toxicity (Bins *et al*., 2016). This may explain the reduced odds of having thrombocytopenia in the heterozygous *(AG)* genotype of $A^{388}G$, as the occurrence of chemotherapy-induced thrombocytopenia varies according to the treatment used. Our findings showed a significant association of *SLC19A1* $G^{80}A$ polymorphism with a reduced incidence of anemia. The carrier genotype *(GA)* of $G^{80}A$ polymorphism showed a substantial protective effect from hematological toxicity-anemia in lung cancer patients.

6.6.2 Correlation of SLC polymorphisms with nephrological toxicity

Our results showed a low risk of nephrotoxicity in patients with carrier genotype *(AG)* for $A^{1683-5676}G$ polymorphism as compared to grades 3-5 with grades 1-2. Mechanistically, the kidney is the main organ in eliminating platinum-based chemotherapeutic drugs (Burger *et al.*, 2011). Many drugs, particularly those with a propensity for nephrotoxicity, are eliminated in the urine by active

tubular secretion and glomerular filtration (Ho & Kim, 2005). *In vitro* studies have shown that *OATP1B1* and *OATP1B3* transport cisplatin and carboplatin, and the toxicity of these drugs in human tumour cells was enhanced with increased *OATP1B3* mRNA expression (Lancaster *et al*., 2013). Hilgendorf *et al.* reported low mRNA expression of *SLCO1B3* in the kidney (Hilgendorf *et al*., 2007). Also, it has been reported that the $A^{1683\text{-}5676}G$ variant may lead to the reduced activity of *SLCO1B3,* which might vary the therapeutic efficacy (Yamada *et al*., 2014). These findings explain that *SLCO1B3* has reduced expression in the kidney, leading to reduced drug uptake and thereby showing a reduced risk of causing nephrotoxicity.

6.6.3 Correlation of SLC polymorphisms with gastrointestinal toxicity

Gastrointestinal (GI) toxicity is a typical adverse effect of chemotherapy treatments in lung carcinoma patients. We observed that carrier genotypes of *SLC19A1* $G^{80}A$ and *SLCO1B1* $A^{388}G$ polymorphisms were associated with protective effects from gastrointestinal toxicity. Some studies state that *SLCO1B1* polymorphisms are correlated with the increased risk of gastrointestinal toxicity in acute lymphoblastic leukemia (Treviño *et al*., 2009). In the case of *SLC19A1*, *Lima et al.* found that *G* carriers of $G^{80}A$ polymorphism were linked to chemotherapy-associated gastrointestinal toxicity (Lima *et al*., 2014), whereas various other studies did not find any correlations with the toxicity (Plaza-Plaza *et al*., 2012; Owen *et al*., 2013). Therefore, these conflicting data on *SLC* polymorphism and chemotherapy-related gastrointestinal toxicity must be clarified.

CHAPTER 7

CONCLUSION

Notable findings from the present investigation

- *TSER* polymorphism was associated with poor overall survival in lung cancer patients.
- Males having *TSER* polymorphism and possessing mutant genotype *(3R3R)* showed reduced survival as compared to the males having wild type *(2R2R)* and mutant genotype *(3R3R)*.
- Stage IV lung cancer patients with *TSER* variation and mutant genotype *(3R3R)* showed worse prognosis as compared to the wild-type *(2R2R)* and heterozygous genotypes *(2R3R)*.
- In *TSER* polymorphism, the individuals with good performance status (ECOG-PS-0,1) carrying mutant genotype (*3R3R)* showed reduced survival as compared to combined heterozygous and wild type genotype (*2R2R+2R3R)*.
- The individuals who had limited functional capacity or bad performance status (ECOG-2,3,4) and had combined heterozygous and mutant type genotype (*2R3R+3R3R)* for *TSER* polymorphism showed worse survival as compared to the patients with wild type genotype *(2R2R)*.
- In *1494 ins/del6* polymorphism, lung cancer patients who had limited functional ability or bad performance status (ECOG-2,3,4) and carried at least one mutant allele *(+6/-6)* had better survival as compared to the patients harboring wild-type alleles *(+6/+6)*.
- Combinatorial analysis of *TSER* and *1494del6* polymorphism showed better prognosis in subjects carrying both the wild and heterozygous alleles [(*2R3R)* + (*+6/-6)* and (*2R2R)* + (*+6/+6)]* as compared to patients having the homozygous variant alleles for both the genotypes (*3R3R+ -6/-6 bp*).
- Reduced TS expression (*2R2R + -6/-6*) in lung cancer patients may lead to better survival outcomes than in patients with increased TS expression (*3R3R+ +6/+6bp*).
- Patients harboring the heterozygous *(+6/-6bp)* genotype for 1*494de6* polymorphism were less likely to manifest metastasis than subjects carrying the wild type *(+6/+6)* genotype.
- Regarding analysis of the toxicity profile, the mutant genotype *(-6/-6bp)* for *1494del6* polymorphism exhibited a 6.44-fold high risk of developing leukopenia as compared to the wild-type genotype *(+6/+6bp)*.
- Further, lung cancer patients with mutant genotype (*-6/-6bp)* for *1494del6* polymorphism had a 2-fold risk of developing anemia as compared to patients with wild genotype (*+6/+6bp)* (p=**0.04**).

- Additionally, the combined genotype (*2R3R+3R3R*) for *TSER* polymorphism showed decreased risk of developing anemia as compared to wild type genotype (*2R2R*) (p=**0.03**).
- Lung carcinoma patients with the heterozygous genotype *(2R3R)* for *TSER* polymorphism had a 4.76-fold risk of constipation compared to the subjects having wild-type genotype (*2R2R)* (p=**0.0016**).
- For *1494 ins/del6* polymorphism, an increased risk of nausea/vomiting was observed in patients with mutant genotype (*-6/-6bp)* with an odds of 2.77 as compared to patients with wild-type genotype *(+6/+6bp)* (p=**0.03**).
- In *ABCB1* $C^{1236}T$ polymorphism, lung cancer patients carrying heterozygous genotype *(CT)* showed poor overall survival as compared to the wild (*CC*) genotype (p=**0.04**).
- Further, patients with ADCC and *ABCB1* $C^{1236}T$ polymorphism harboring heterozygous (*CT)* genotype had lower survival as compared to the patients having wild type (*CC)* genotype (p=**0.03**).
- *ABCB1* $C^{1236}T$ polymorphism appears to be a potential prognostic marker for OS of lung cancer patients who underwent cisplatin/carboplatin and docetaxel therapy.
- The lung cancer subjects harboring heterozygous genotype (*CT)* for *ABCB1* $C^{1236}T$ and treated with the pemetrexed and cisplatin/carboplatin had substantially poor survival as compared to the patients with wild type *(CC)* genotype p=**0.02**).
- For *ABCB1* $C^{3435}T$, a drastic decrease in survival and the high hazard ratio was observed in the ADCC patients having heterozygous genotype (*CT*) when compared with the wild type genotype *(CC)* (p=**0.04**).
- Patients treated with the pemetrexed and cisplatin/carboplatin and having combined heterozygous and mutant type genotype (*CT+TT)* for *ABCB1* $C^{3435}T$ had poor median survival time as compared with the wild type genotype (*CC*) (p=**0.02**).
- SCLC patients having *ABCB1* $G^{2677}A$ polymorphism and carrying one copy of mutant allele (*GA)* showed poor survival as compared with the patients having both copies wild type alleles (*GG)* (p=**0.004**).
- Irinotecan therapy is associated with poor prognosis for lung cancer patients with *ABCB1* $G^{2677}A$ polymorphism as heterozygous genotype *(GA)* showed worse survival as compared to the wild type genotype *(GG)* (p=**0.007**).

- Stage III lung cancer patients with heterozygous genotype for *ABCB1* $G^{2677}A$ polymorphism had 3-times reduced survival time as compared to the reference genotype *(GG)*.
- In *ABCB1* $C^{3435}T$ polymorphism, individuals harboring heterozygous (*CT*) and mutant genotype *(TT)* are at potential risk of manifesting metastasis as compared to wild-type genotype *(CC)*.
- Mutant genotype *(AA)* of *ABCC1* $G^{3173}A$ polymorphism conferred poor prognosis in lung cancer patients (p=**0.01**).
- Further, in ADCC patients, polymorphism of *ABCC1* $G^{3173}A$ appears to be a potential prognostic marker as mutant genotype *(AA)* was associated with poor survival outcomes (p=**0.001).**
- Lung cancer subjects with heterozygous genotypes for *ABCC2* $G^{4544}A$ were associated with poor survival.
- Pemetrexed therapy is associated with poor prognosis for lung cancer patients carrying heterozygous genotypes for *ABCC2* $G^{4544}A$ polymorphism.
- Non-smokers with heterozygous genotype *(GA)* for *ABCC2* $G^{4544}A$ had poor survival and high death ratio compared to the wild-type genotype.
- Regarding analyzing the toxicity profile, lung cancer patients with at least one mutant allele *(GA)* in *ABCC1* $G^{3173}A$ had an increased risk of developing anemia and leukopenia.
- We observed that lung cancer patients with the *GA* (heterozygous) genotype for *ABCC2* $G^{4544}A$ polymorphism had a 5.63-fold risk of developing anemia (p=**0.03**).
- *ABCB1* $G^{2677}A$ and *ABCC1* $G^{3173}A$ polymorphism is correlated with the nephrotoxicity protective effect in cancer patients undergoing platinum-based doublet chemotherapy treatment.
- Lung cancer patients with one mutant allele for *ABCB1* $C^{3435}T$ polymorphism were correlated with the protective effect of GI toxicity, *i.e.*, constipation.
- Similarly, patients with mutant alleles for *ABCB1* $C^{1236}T$ polymorphism were correlated with a reduced risk of having nausea or vomiting (p=**0.02**).
- Our results for *ABCC1* $G^{3173}A$ showed that patients having heterozygous genotype *(GA)* undergoing platinum-based chemotherapy were associated with an increased risk of chemotherapy-induced diarrhea.

- A high risk of hepatotoxicity was observed in lung patients with heterozygous genotypes for *ABCB1 $C^{1236}T$* and *ABCC1 $G^{3173}A$* variation.
- Genetic variants of *$G^{2677}T$* polymorphism were correlated with the protective effect of hepatic toxicity in lung cancer patients who underwent platinum-based doublet chemotherapy.
- The *SLC19A1 $G^{80}A$* appears to be a potential prognostic factor for overall survival in ADCC and SCLC patients (p=**0.04).**
- The individuals who had limited functional capacity or bad performance status (ECOG-2,3,4) and had mutant genotype (*AA)* for *SLC19A1 $G^{80}A$* polymorphism showed better survival as compared to the patients with combined wild type and heterozygous genotype *(GG+GA)*.
- Patients with *SLCO1B1 $A^{388}G$* polymorphism were associated with no difference in median survival time between mutant *(GG)* and wild-type genotypes *(AA)* (p=**0.04)**. Therefore, *SLCO1B1* polymorphism has been correlated with unaltered transporter activity.
- Stage III lung cancer patients with heterozygous genotype *(AG)* of *SLCO1B1 $A^{388}G$* variation showed 2-times better prognosis as compared to the wild-type *(AA)* genotype.
- On the contrary, stage IV lung cancer patients with heterozygous *(AG)* and mutant alleles *(GG)* of *SLCO1B1 $A^{388}G$* variation showed poor overall survival as compared to the wild-type *(AA)* genotype.
- Smokers with mutant genotype *(AA)* for *SLCO1B1 $A^{388}G$* had poor survival and high death ratio compared to wild-type genotypes (p=**0.03**).
- Docetaxel therapy is associated with poor prognosis for lung cancer patients with *SLCO1B3 $A^{1683\text{-}5676}G$* polymorphism as heterozygous genotype *(AG)* showed worse survival as compared to the wild type genotype *(AA)* (p=**0.02**).
- Lung cancer subjects with the heterozygous genotype for *SLC19A1 $G^{80}A$* and *SLCO1B1 $T^{521}C$* polymorphism had a reduced risk of developing anemia.
- Patients with *SLCO1B1 $A^{388}G$* polymorphism harboring a single copy of mutant allele (*AG*) were associated with a lower incidence of thrombocytopenia.
- Lung carcinoma patients with heterozygous (*AG*) genotype for *$A^{1683\text{-}5676}G$* polymorphism showed a lower incidence of nephrotoxicity when compared to the patients with wild-type genotype (*AA*).

In conclusion, this is the very first Indian study focusing on membrane transporters and chemotherapeutic target genes with large sample size and their connection with clinicopathological variables. The stratified analysis was carried out to estimate risk for subgroups based on parameters such as tumor stage, lymph node invasion, primary tumor extension, metastasis, and response rate. This is the first study to evaluate overall survival in North Indian lung cancer patients receiving platinum-based doublet chemotherapy. The current investigation is the first study in the North Indian region to analyze the SNPs for overall survival and clinical outcomes. The link between *ABC*, *SLC*, and *TS* SNPs and toxicities associated with chemotherapy is being assessed for the first time in this investigation. These SNPs could also be exploited as biomarkers, assisting in the formulation of individualized chemotherapy regimens depending on genotype and determining the relationship between these polymorphisms and clinical outcomes for the development of personalized therapeutics.

Future prospects of the study

Membrane transporters play a crucial role in translocating chemotherapeutic drugs across the cancer cell membrane, thus influencing chemotherapy efficiency and toxicity. Genetic polymorphisms in the transporters genes and drug target can influence the chemotherapeutic response in patients with lung cancer. These polymorphisms in the *ABC*, *SLC*, and *TS* genes are worth paying more attention to, for they may serve as a platform for predicting overall survival in lung cancer patients. It would be possible to individualize treatment for patients with lung cancer and reduce the resistance and toxicity conferred by chemotherapy by better understanding prognostic markers. Advanced research in the future should be considered to assess the role of these genetic variants in transporter genes and folate metabolism pathways to determine the role of particular polymorphism in pharmacological activity, pharmacokinetics, and toxicity of chemotherapeutic drugs. Such findings could contribute to pharmacogenetically improved and personalized drug dosing, accounting for the chemotherapeutic drug's pharmacokinetic disparities between individuals.

Strengths and weaknesses of the current study

Strengths:

1) The current investigation has been carried out in large sample size compared to the other studies, which can increase the reliability of the results.
2) All patients were administered platinum-based doublet chemotherapy; everyone was treated at the PGIMER hospital only.
3) The patient recruitment and other clinical parameters were collected independently, irrespective of the associated polymorphism.

Limitations:

1) Our study only includes advanced stage III and IV lung cancer patients, as carcinoma is often detected at a late stage. This could be because of community alertness or its similarity to other diseases like tuberculosis.
2) Many patients who receive chemotherapy at advanced stages III and IV of cancer cannot achieve the treatment's endpoint, weakening the research's scope on chemotherapy response.
3) Most people who visited the PGIMER clinic for the treatment were from rural areas in Northern India. As it was difficult for them to travel from their region, many patients in remote areas did not regularly visit the hospital.
4) Due to the shortage of clinical data, we could not conduct progression-free survival (PFS) analysis on the present study group.
5) Our study had a limited sample size of SCLC patients.
6) In our study, few patients had grade 3-4 toxicity, while most had intermediate or low-grade toxicity (grade 1-2). Some study participants experienced significant adverse effects that prompted delaying or terminating the treatment. Hence, it is imperative to conduct multiple center studies with many cases.

CHAPTER 8

BIBLIOGRAPHY

- Abbasian, M. H., Ansarinejad, N., Abbasi, B., Iravani, M., Ramim, T., Hamedi, F., & Ardekani, A. M. (2020). The Role of Dihydropyrimidine Dehydrogenase and Thymidylate Synthase Polymorphisms in Fluoropyrimidine-Based Cancer Chemotherapy in an Iranian Population. Avicenna Journal of Medical Biotechnology, 12(3), 157.
- Abbasian, M. H., Ansarinejad, N., Ramim, T., Fardad, F., & Abbasi, B. (2022). SLC19A1 and Thymidylate Synthase Polymorphisms do not Predict Survival Outcome in Non-Small-Cell Lung Cancer Treated with Pemetrexed-Cisplatin Chemotherapy Regimen. Middle East Journal of Cancer.
- Abdel-Haleem, A. M., El-Zeiry, M. I., Mahran, L. G., Abou-Aisha, K., Rady, M. H., Rohde, J., Spahn-Langguth, H. (2011). Expression of RFC/SLC19A1 is associated with tumor type in bladder cancer patients. PloS One, 6(7), e21820.
- Acquaviva, J., Wong, R., & Charest, A. (2009). The multifaceted roles of the receptor tyrosine kinase ROS in development and cancer. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer, 1795(1), 37-52.
- Adams, J. M., & Cory, S. (2018). The BCL-2 arbiters of apoptosis and their growing role as cancer targets. Cell Death & Differentiation, 25(1), 27-36.
- Adjei, A. A., Mandrekar, S. J., Dy, G. K., Molina, J. R., Adjei, A. A., Gandara, D. R., & Zinner, R. G. (2010). Phase II trial of pemetrexed plus bevacizumab for second-line therapy of patients with advanced non–small-cell lung cancer: NCCTG and SWOG study N0426. Journal of clinical oncology, 28(4), 614.
- Adjei, A. A., Salavaggione, O. E., Mandrekar, S. J., Dy, G. K., Ziegler, K. L. A., Endo, C., Adjei, A. A. (2010). Correlation between polymorphisms of the reduced folate carrier gene (SLC19A1) and survival after pemetrexed-based therapy in non-small cell lung cancer: a North Central Cancer Treatment Group-based exploratory study. Journal of Thoracic Oncology, 5(9), 1346–1353.
- Afzal, S., Gusella, M., Jensen, S. A., Vainer, B., Vogel, U., Andersen, J. T., Adleff, V. (2011). The association of polymorphisms in 5-fluorouracil metabolism genes with outcome in adjuvant treatment of colorectal cancer. Pharmacogenomics, 12(9), 1257–1267.
- Aherne, G. W., Hardcastle, A., Raynaud, F., & Jackman, A. L. (1996). Immunoreactive dUMP and TTP pools as an index of thymidylate synthase inhibition; effect of tomudex (ZD1694) and a nonpolyglutamated quinazoline antifolate (CB30900) in L1210 mouse leukaemia cells. Biochemical Pharmacology, 51(10), 1293–1301.
- Aisner, D. L., Nguyen, T. T., Paskulin, D. D., Le, A. T., Haney, J., Schulte, N., & Varella-Garcia, M. (2014). ROS1 and ALK Fusions in Colorectal Cancer, with Evidence of Intratumoral Heterogeneity for Molecular DriversROS1 and ALK Fusions in CRC. Molecular Cancer Research, 12(1), 111-118.
- Akasaka, K., Kaburagi, T., Yasuda, S., Ohmori, K., Abe, K., Sagara, H., Imai, Y. (2010). Impact of functional ABCG2 polymorphisms on the adverse effects of gefitinib in Japanese patients with non–small-cell lung cancer. Cancer Chemotherapy and Pharmacology, 66(4), 691–698.
- Akopyan, G., & Bonavida, B. (2006). Understanding tobacco smoke carcinogen NNK and lung

tumorigenesis. International Journal of Oncology, 29(4), 745–752.

- Al-Abdulla, R., Perez-Silva, L., Abete, L., Romero, M. R., Briz, O., & Marin, J. J. G. (2019). Unraveling 'The Cancer Genome Atlas' information on the role of SLC transporters in anticancer drug uptake. Expert Review Of Clinical Pharmacology, 12(4), 329–341.
- Alam, K., Crowe, A., Wang, X., Zhang, P., Ding, K., Li, L., & Yue, W. (2018). Regulation of organic anion transporting polypeptides (OATP) 1B1-and OATP1B3-mediated transport: an updated review in the context of OATP-mediated drug-drug interactions. International Journal of Molecular Sciences, 19(3), 855.
- Alrefai, W. A., & Gill, R. K. (2007). Bile acid transporters: structure, function, regulation and pathophysiological implications. Pharmaceutical Research, 24(10), 1803–1823.
- Ankathil, R. (2010). Tobacco, genetic susceptibility and lung cancer. Tobacco Use Insights, 3, TUI-S2819.
- Arévalo, E., Castañón, E., López, I., Salgado, J., Collado, V., Santisteban, M., … Patiño-García, A. (2014). Thymidylate synthase polymorphisms in genomic DNA as clinical outcome predictors in a European population of advanced non-small cell lung cancer patients receiving pemetrexed. Journal of Translational Medicine, 12(1), 1–9.
- Aronica, E., Gorter, J. A., Redeker, S., Van Vliet, E. A., Ramkema, M., Scheffer, G. L., Baayen, J. C. (2005). Localization of breast cancer resistance protein (BCRP) in microvessel endothelium of human control and epileptic brain. Epilepsia, 46(6), 849–857.
- Austin Doyle, L., & Ross, D. D. (2003). Multidrug resistance mediated by the breast cancer resistance protein BCRP (ABCG2). Oncogene, 22(47), 7340–7358.
- Bade, B. C., & Cruz, C. S. Dela. (2020). Lung cancer 2020: epidemiology, etiology, and prevention. Clinics in Chest Medicine, 41(1), 1–24.
- Bailey-Dell, K. J., Hassel, B., Doyle, L. A., & Ross, D. D. (2001). Promoter characterization and genomic organization of the human breast cancer resistance protein (ATP-binding cassette transporter G2) gene. Biochimica et Biophysica Acta (BBA)-Gene Structure and Expression, 1520(3), 234–241.
- Bakos, É., & Homolya, L. (2007). Portrait of multifaceted transporter, the multidrug resistance-associated protein 1 (MRP1/ABCC1). Pflügers Archiv-European Journal of Physiology, 453(5), 621–641.
- Balça-Silva, J., Matias, D., Do Carmo, A., Dubois, L. G., Gonçalves, A. C., Girão, H., … Sarmento-Ribeiro, A. B. (2017). Glioblastoma entities express subtle differences in molecular composition and response to treatment. Oncology Reports, 38(3), 1341–1352.
- Ballestero, M. R., Monte, M. J., Briz, O., Jimenez, F., Gonzalez-San Martin, F., & Marin, J. J. G. (2006). Expression of transporters potentially involved in the targeting of cytostatic bile acid derivatives to colon cancer and polyps. Biochemical Pharmacology, 72(6), 729–738.
- Bandariavičiūtė, R., Ražanauskas, D., Daniūnaitė, K., Lazutka, J. R., Jankevičius, F., & Jarmalaitė, S. (2015). Frequent down-regulation of ABC transporter genes in prostate cancer. BMC Cancer, 15.
- Barrand, M. A., Rhodes, T., Center, M. S., & Twentyman, P. R. (1993). Chemosensitisation and

drug accumulation effects of cyclosporin A, PSC-833 and verapamil in human MDR large cell lung cancer cells expressing a 190k membrane protein distinct from P-glycoprotein. European Journal of Cancer, 29(3), 408–415.

- Bartlett, J. M., & White, A. (2003). Extraction of DNA from whole blood. PCR protocols, 29-31.
- Baskar, R., Lee, K. A., Yeo, R., & Yeoh, K. W. (2012). Cancer and radiation therapy: current advances and future directions. International journal of medical sciences, 9(3), 193.
- Battista, T., Fiorillo, A., Chiarini, V., Genovese, I., Ilari, A., & Colotti, G. (2020). Roles of Sorcin in drug resistance in cancer: one protein, many mechanisms, for a novel potential anticancer drug target. Cancers, 12(4), 887.
- Baxevanos, P., & Mountzios, G. (2018). Novel chemotherapy regimens for advanced lung cancer: have we reached a plateau? Annals of Translational Medicine, 6(8).
- Begg, C. B., & Zhang, Z. F. (1994). Statistical analysis of molecular epidemiology studies employing case-series. Cancer Epidemiology and Prevention Biomarkers, 3(2), 173-175.
- Behera, D., & Balamugesh, T. (2012). Lung cancer in India. Indian J Chest Dis Allied Science 2004; 46: 269, 81.
- Beis, K. (2015). Structural basis for the mechanism of ABC transporters. Biochemical Society Transactions, 43(5), 889–893.
- Benderra, Z., Faussat, A.-M., Sayada, L., Perrot, J.-Y., Chaoui, D., Marie, J.-P., & Legrand, O. (2004). Breast cancer resistance protein and P-glycoprotein in 149 adult acute myeloid leukemias. Clinical Cancer Research, 10(23), 7896–7902.
- Bensberg, M., Rundquist, O., Selimović, A., Lagerwall, C., Benson, M., Gustafsson, M., ... & Nestor, C. E. (2021). TET2 as a tumor suppressor and therapeutic target in T-cell acute lymphoblastic leukemia. Proceedings of the National Academy of Sciences, 118(34), e2110758118.
- Berger, W., Setinek, U., Hollaus, P., Zidek, T., Steiner, E., Elbling, L., … Micksche, M. (2005). Multidrug resistance markers P-glycoprotein, multidrug resistance protein 1, and lung resistance protein in non-small cell lung cancer: prognostic implications. Journal of Cancer Research and Clinical Oncology, 131(6), 355–363.
- Bhangal, G., Halford, S., Wang, J., Roylance, R., Shah, R., & Waxman, J. (2000). Expression of the multidrug resistance gene in human prostate cancer. Urologic Oncology: Seminars and Original Investigations, 5(3), 118–121. Elsevier.
- Bian, Y., Song, C., Cheng, K., Dong, M., Wang, F., Huang, J., Zou, H. (2014). An enzyme assisted RP-RPLC approach for in-depth analysis of human liver phosphoproteome. Journal of Proteomics, 96, 253–262.
- Biason, P., Visentin, M., Talamini, R., Stopar, A., Giorda, G., Lucia, E., Toffoli, G. (2012). Polymorphic thymidylate synthase gene impacts on overall survival of patients with epithelial ovarian cancer after platinum-based chemotherapy. Pharmacogenomics, 13(14), 1609–1619.
- Bins, S., Lenting, A., El Bouazzaoui, S., van Doorn, L., Oomen-de Hoop, E., Eskens, F. A. L. M., Mathijssen, R. H. J. (2016). Polymorphisms in SLCO1B1 and UGT1A1 are associated with

sorafenib-induced toxicity. Pharmacogenomics, 17(14), 1483–1490.

- Birch, A. H., Arcand, S. L., Oros, K. K., Rahimi, K., Watters, A. K., Provencher, D., & Tonin, P. N. (2011). Chromosome 3 anomalies investigated by genome wide SNP analysis of benign, low malignant potential and low grade ovarian serous tumours. PloS one, 6(12), e28250.
- Björn, N., Jakobsen Falk, I., Vergote, I., & Green, H. (2018). ABCB 1 Variation Affects Myelosuppression, Progression-free Survival and Overall Survival in Paclitaxel/Carboplatin-treated Ovarian Cancer Patients. Basic & Clinical Pharmacology & Toxicology, 123(3), 277–287.
- Blount, B. C., Mack, M. M., Wehr, C. M., MacGregor, J. T., Hiatt, R. A., Wang, G., … Ames, B. N. (1997). Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proceedings of the National Academy of Sciences, 94(7), 3290–3295.
- Borst, P., Evers, R., Kool, M., & Wijnholds, J. (2000). A family of drug transporters: the multidrug resistance-associated proteins. Journal of the National Cancer Institute, 92(16), 1295–1302.
- Borst, P., Zelcer, N., & Van De Wetering, K. (2006). MRP2 and 3 in health and disease. Cancer Letters, 234(1), 51–61.
- Bosch, T. M., Huitema, A. D. R., Doodeman, V. D., Jansen, R., Witteveen, E., Smit, W. M., … Beijnen, J. H. (2006). Pharmacogenetic screening of CYP3A and ABCB1 in relation to population pharmacokinetics of docetaxel. Clinical Cancer Research, 12(19), 5786–5793.
- Brahmer, J., Reckamp, K. L., Baas, P., Crinò, L., Eberhardt, W. E. E., Poddubskaya, E., … Holgado, E. (2015). Nivolumab versus docetaxel in advanced squamous-cell non–small-cell lung cancer. New England Journal of Medicine, 373(2), 123–135.
- Bray, F., & Soerjomataram, I. (2015). The changing global burden of cancer: transitions in human development and implications for cancer prevention and control. Cancer: Disease Control Priorities, 3, 23–44.
- Brinkmann, U., & Eichelbaum, M. (2001). Polymorphisms in the ABC drug transporter gene MDR1. The Pharmacogenomics Journal, 1(1), 59–64.
- Brown, P. (2016). The cobas® EGFR mutation test v2 assay. Future Oncology, 12(4), 451-452.
- Bruckmueller, H., & Cascorbi, I. (2021). ABCB1, ABCG2, ABCC1, ABCC2, and ABCC3 drug transporter polymorphisms and their impact on drug bioavailability: what is our current understanding? Expert Opinion on Drug Metabolism & Toxicology, 17(4), 369–396.
- Bugde, P., Biswas, R., Merien, F., Lu, J., Liu, D.-X., Chen, M., … Li, Y. (2017). The therapeutic potential of targeting ABC transporters to combat multi-drug resistance. Expert Opinion on Therapeutic Targets, 21(5), 511–530.
- Bukhtoyarov, O. V, & Samarin, D. M. (2015). Pathogenesis of cancer: Cancer reparative trap. Journal of Cancer Therapy, 6(05), 399.
- Burbee, D. G., Forgacs, E., Zöchbauer-Müller, S., Shivakumar, L., Fong, K., Gao, B., ... & Minna, J. D. (2001). Epigenetic inactivation of RASSF1A in lung and breast cancers and malignant phenotype suppression. Journal of the National Cancer Institute, 93(9), 691-699.

- Burger, H., Loos, W. J., Eechoute, K., Verweij, J., Mathijssen, R. H. J., & Wiemer, E. A. C. (2011). Drug transporters of platinum-based anticancer agents and their clinical significance. Drug Resistance Updates, 14(1), 22–34.
- Buxhofer-Ausch, V., Secky, L., Wlcek, K., Svoboda, M., Kounnis, V., Briasoulis, E., … Thalhammer, T. (2013). Tumor-specific expression of organic anion-transporting polypeptides: transporters as novel targets for cancer therapy. Journal of Drug Delivery, 2013.
- Cabanero, M., Sangha, R., Sheffield, B. S., Sukhai, M., Pakkal, M., Kamel-Reid, S., … Butts, C. (2017). Management of EGFR-mutated non-small-cell lung cancer: practical implications from a clinical and pathology perspective. Current Oncology, 24(2), 111–119.
- Cao, S., Wang, C., Ma, H., Yin, R., Zhu, M., Shen, W., … Hu, Z. (2015). Genome-wide association study on platinum-induced hepatotoxicity in non-small cell lung cancer patients. Scientific Reports, 5(1), 1–8.
- Caronia, D., Patino-Garcia, A., Peréz-Martínez, A., Pita, G., Moreno, L. T., Zalacain-Díez, M., Benítez, J. (2011). Effect of ABCB1 and ABCC3 polymorphisms on osteosarcoma survival after chemotherapy: a pharmacogenetic study. PLoS One, 6(10), e26091.
- Carter, B. W., Glisson, B. S., Truong, M. T., & Erasmus, J. J. (2014). Small cell lung carcinoma: staging, imaging, and treatment considerations. Radiographics, 34(6), 1707–1721.
- Cha, P.-C., Mushiroda, T., Zembutsu, H., Harada, H., Shinoda, N., Kawamoto, S., … Sasaki, K. (2009). Single nucleotide polymorphism in ABCG2 is associated with irinotecan-induced severe myelosuppression. Journal of Human Genetics, 54(10), 572–580.
- Chamizo, C., Zazo, S., Dómine, M., Cristóbal, I., García-Foncillas, J., Rojo, F., & Madoz-Gúrpide, J. (2015). Thymidylate synthase expression as a predictive biomarker of pemetrexed sensitivity in advanced non-small cell lung cancer. BMC Pulmonary Medicine, 15(1), 1–7.
- Chang, A. B., Lin, R., Studley, W. K., Tran, C. V, & Saier Milton H, J. (2004). Phylogeny as a guide to structure and function of membrane transport proteins. Molecular Membrane Biology, 21(3), 171–181.
- Chaudhry, R., & Bordoni, B. (2022). Anatomy , Thorax , Lungs. 1–5.
- Chen, J.-Y., Chen, H.-J., & Chen, P.-F. (2021). Association of expression and genotypes of thymidylate synthase in non-small cell lung cancer patients with different clinicopathological characteristics. Pteridines, 32(1), 39–47.
- Chen, Q., Lin, W., Yang, J., Lin, M., Lin, X., Huang, J., … Chen, Y. (2020). Predictive value of two polymorphisms in ABCB1, rs1045642 and rs1128503, in prognosis following taxane-containing chemotherapy: A meta-analysis.
- Chen, Q., Lin, W., Yang, J., Lin, M., Lin, X., Weng, Y., & Chen, Y. (2021). Prognostic Value of Two Polymorphisms, rs1045642 and rs1128503, in ABCB1 Following Taxane-based Chemotherapy: A Meta-Analysis. Asian Pacific Journal of Cancer Prevention: APJCP, 22(1), 3.
- Chen, S., Huo, X., Lin, Y., Ban, H., Lin, Y., Li, W., … Xu, X. (2010). Association of MDR1 and ERCC1 polymorphisms with response and toxicity to cisplatin-based chemotherapy in non-small-cell lung cancer patients. International Journal of Hygiene and Environmental Health, 213(2), 140–145.

- Chen, X., Chen, D., Yang, S., Ma, R., Pan, Y., Li, X., & Ma, S. (2015). Impact of ABCG2 polymorphisms on the clinical outcome of TKIs therapy in Chinese advanced non-small-cell lung cancer patients. Cancer Cell International, 15(1), 1–7.
- Chen, X., Wu, S., Tang, L., Ma, L., Wang, F., Feng, H., ... & Han, Z. (2019). Mesenchymal stem cells overexpressing heme oxygenase-1 ameliorate lipopolysaccharide-induced acute lung injury in rats. Journal of Cellular Physiology, 234(5), 7301-7319.
- Chen, Y., Zhou, H., Yang, S., & Su, D. (2021). Increased ABCC2 expression predicts cisplatin resistance in non-small cell lung cancer. Cell Biochemistry and Function, 39(2), 277–286.
- Chew, S. C., Sandanaraj, E., Singh, O., Chen, X., Tan, E. H., Lim, W. T., … Chowbay, B. (2012). Influence of SLCO1B3 haplotype-tag SNPs on docetaxel disposition in Chinese nasopharyngeal cancer patients. British Journal of Clinical Pharmacology, 73(4), 606–618.
- Chew, S.-C., Singh, O., Chen, X., Ramasamy, R. D., Kulkarni, T., Lee, E. J. D., … Chowbay, B. (2011). The effects of CYP3A4, CYP3A5, ABCB1, ABCC2, ABCG2 and SLCO1B3 single nucleotide polymorphisms on the pharmacokinetics and pharmacodynamics of docetaxel in nasopharyngeal carcinoma patients. Cancer Chemotherapy and Pharmacology, 67(6), 1471–1478.
- Choi, J. R., Kim, J.-O., Kang, D. R., Shin, J.-Y., Zhang, X. H., Oh, J. E., … Kang, J.-H. (2015). Genetic variations of drug transporters can influence on drug response in patients treated with docetaxel chemotherapy. Cancer Research and Treatment: Official Journal of Korean Cancer Association, 47(3), 509–517.
- Choudhuri, S, & Klaassen, C. D. (2018). Molecular Biology of ABC Transporters.
- Choudhuri, Supratim, & Klaassen, C. D. (2006). Structure, function, expression, genomic organization, and single nucleotide polymorphisms of human ABCB1 (MDR1), ABCC (MRP), and ABCG2 (BCRP) efflux transporters. International Journal of Toxicology, 25(4), 231–259.
- Christie, E. L., Pattnaik, S., Beach, J., Copeland, A., Rashoo, N., Fereday, S., … Lamb, G. (2019). Multiple ABCB1 transcriptional fusions in drug resistant high-grade serous ovarian and breast cancer. Nature Communications, 10(1), 1–10.
- Chu, E., & Allegra, C. J. (1996). The role of thymidylate synthase as an RNA binding protein. Bioessays, 18(3), 191–198.
- Chu, E., & Gollerkeri, A. (2002). Resistance to inhibitor compounds of thymidylate synthase.
- Chu, E., & Sartorelli, A. C. (2018). Cancer chemotherapy. Lange's Basic and Clinical Pharmacology, 948–976.
- Chu, P. G., & Weiss, L. M. (2002). Expression of cytokeratin 5/6 in epithelial neoplasms: an immunohistochemical study of 509 cases. Modern Pathology, 15(1), 6–10.
- Ciribilli, Y., Singh, P., Inga, A., & Borlak, J. (2016). c-Myc targeted regulators of cell metabolism in a transgenic mouse model of papillary lung adenocarcinoma. Oncotarget, 7(40), 65514.
- Cole, Susan P C. (2014). Multidrug resistance protein 1 (MRP1, ABCC1), a "multitasking" ATP-binding cassette (ABC) transporter. Journal of Biological Chemistry, 289(45), 30880–30888.
- Coleman, M. D. (2020). Human drug metabolism. John Wiley & Sons.

- Coleman, W. B., & Tsongalis, G. J. (2017). Cancer epidemiology: incidence and etiology of human neoplasms. In The molecular basis of human cancer (pp. 1–24). Springer.
- Collin, S. M., Metcalfe, C., Zuccolo, L., Lewis, S. J., Chen, L., Cox, A., … Smith, G. D. (2009). Association of Folate-Pathway Gene Polymorphisms with the Risk of Prostate Cancer: a Population-Based Nested Case-Control Study, Systematic Review, and Meta-analysisFolate-Pathway Gene Polymorphisms and Prostate Cancer. Cancer Epidemiology, Biomarkers & Prevention, 18(9), 2528–2539.
- Conrad, S., Kauffmann, H.-M., Ito, K., Leslie, E. M., Deeley, R. G., Schrenk, D., & Cole, S. P. C. (2002). A naturally occurring mutation in MRP1 results in a selective decrease in organic anion transport and in increased doxorubicin resistance. Pharmacogenetics and Genomics, 12(4), 321–330.
- Conseil, G., Arama-Chayoth, M., Tsfadia, Y., & Cole, S. P. C. (2019). Structure-guided probing of the leukotriene C4 binding site in human multidrug resistance protein 1 (MRP1; ABCC1). The FASEB Journal, 33(10), 10692–10704.
- Cordova-Delgado, M., Pinto, M. P., Retamal, I. N., Muñoz-Medel, M., Bravo, M. L., Fernández, M. F., ... & Garrido, M. (2019). High proportion of potential candidates for immunotherapy in a chilean cohort of gastric cancer patients: Results of the FORCE1 study. Cancers, 11(9), 1275.
- Corrigan, A., Walker, J. L., Wickramasinghe, S., Hernandez, M. A., Newhouse, S. J., Folarin, A. A., … Marinaki, A. M. (2014). Pharmacogenetics of pemetrexed combination therapy in lung cancer: pathway analysis reveals novel toxicity associations. The Pharmacogenomics Journal, 14(5), 411–417.
- Corsini, A., & Bortolini, M. (2013). Drug-induced liver injury: The role of drug metabolism and transport. The Journal of Clinical Pharmacology, 53(5), 463–474.
- Cortejoso, L., García, M. I., García-Alfonso, P., González-Haba, E., Escolar, F., Sanjurjo, M., & López-Fernández, L. A. (2013). Differential toxicity biomarkers for irinotecan-and oxaliplatin-containing chemotherapy in colorectal cancer. Cancer Chemotherapy and Pharmacology, 71(6), 1463–1472.
- Cosaert, J., & Quoix, E. (2002). Platinum drugs in the treatment of non-small-cell lung cancer. British Journal of Cancer, 87(8), 825–833.
- Costi, M. P., Ferrari, S., Venturelli, A., Calo, S., Tondi, D., & Barlocco, D. (2005). Thymidylate synthase structure, function and implication in drug discovery. Current Medicinal Chemistry, 12(19), 2241–2258.
- Cuffe, S., Azad, A. K., Qiu, X., Qiu, X., Brhane, Y., Kuang, Q., … Cheng, D. (2016). ABCC2 polymorphisms and survival in the Princess Margaret cohort study and the NCIC clinical trials group BR. 24 trial of platinum-treated advanced stage non-small cell lung cancer patients. Cancer Epidemiology, 41, 50–56.
- Cui, Y., König, J., Nies, A. T., Pfannschmidt, M., Hergt, M., Franke, W. W., … Keppler, D. (2003). Detection of the human organic anion transporters SLC21A6 (OATP2) and SLC21A8 (OATP8) in liver and hepatocellular carcinoma. Laboratory Investigation, 83(4), 527–538.

- Cully, M., You, H., Levine, A. J., & Mak, T. W. (2006). Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis. Nature Reviews Cancer, 6(3), 184-192.
- Cusatis, G., Gregorc, V., Li, J., Spreafico, A., Ingersoll, R. G., Verweij, J., … Sparreboom, A. (2006). Pharmacogenetics of ABCG2 and adverse reactions to gefitinib. Journal of the National Cancer Institute, 98(23), 1739–1742.
- Custódio, J. B. A., Cardoso, C. M. P., Santos, M. S., Almeida, L. M., Vicente, J. A. F., & Fernandes, M. A. S. (2009). Cisplatin impairs rat liver mitochondrial functions by inducing changes on membrane ion permeability: prevention by thiol group protecting agents. Toxicology, 259(1–2), 18–24.
- Cuya, S. M., Bjornsti, M.-A., & van Waardenburg, R. C. A. M. (2017). DNA topoisomerase-targeting chemotherapeutics: what's new? Cancer Chemotherapy and Pharmacology, 80(1), 1–14.
- Czabotar, P. E., Lessene, G., Strasser, A., & Adams, J. M. (2014). Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. Nature reviews Molecular cell biology, 15(1), 49-63.
- Daly AK, Aithal GP, Leathart JB, et al. Genetic susceptibility to diclofenac-induced hepatotoxicity: contribution of UGT2B7, CYP2C8, and ABCC2 genotypes. Gastroenterology. 2007;132(1):272-281.
- Danenberg, P. V. (2004). Pharmacogenomics of thymidylate synthase in cancer treatment. Frontiers in Bioscience-Landmark, 9(4), 2484–2494.
- Darshan, M. S., Loftus, M. S., Thadani-Mulero, M., Levy, B. P., Escuin, D., Zhou, X. K., … Tagawa, S. T. (2011). Taxane-Induced Blockade to Nuclear Accumulation of the Androgen Receptor Predicts Clinical Responses in Metastatic Prostate CancerMT-Dependent AR Transport Underlines Taxane Efficacy in PC. Cancer Research, 71(18), 6019–6029.
- Dasari, S., & Tchounwou, P. B. (2014). Cisplatin in cancer therapy: molecular mechanisms of action. European Journal of Pharmacology, 740, 364–378.
- Davies, K. D., Le, A. T., Theodoro, M. F., Skokan, M. C., Aisner, D. L., Berge, E. M., & Doebele, R. C. (2012). Identifying and targeting ROS1 gene fusions in non–small cell lung cancer. Clinical Cancer Research, 18(17), 4570-4579.
- de Faria, G. P., de Oliveira, J. A., de Oliveira, J. G. P., Romano, S. de O., Neto, V. M., & Maia, R. C. (2008). Differences in the expression pattern of P-glycoprotein and MRP1 in low-grade and high-grade gliomas. Cancer Investigation, 26(9), 883–889.
- de Man, F. M., Goey, A. K. L., van Schaik, R. H. N., Mathijssen, R. H. J., & Bins, S. (2018). Individualization of irinotecan treatment: a review of pharmacokinetics, pharmacodynamics, and pharmacogenetics. Clinical Pharmacokinetics, 57(10), 1229–1254.
- Dean, M., Hamon, Y., & Chimini, G. (2001). The human ATP-binding cassette (ABC) transporter superfamily. Journal of Lipid Research, 42(7), 1007–1017.
- Deaton, A. M., & Bird, A. (2011). CpG islands and the regulation of transcription. Genes & development, 25(10), 1010-1022.

- Delou, J. M. A., Lopes, A. G., & Capella, M. A. M. (2009). Unveiling the role of multidrug resistance proteins in hypertension. Hypertension, 54(2), 210–216.
- Deschamps, P., Réty, S., Bareille, J., & Leulliot, N. (2017). Crystal structure of the active form of native human thymidylate synthase in the absence of bound substrates. Acta Crystallographica Section F: Structural Biology Communications, 73(6), 336–341.
- Desjardins, P., & Conklin, D. (2010). NanoDrop microvolume quantitation of nucleic acids. JoVE (Journal of Visualized Experiments), (45), e2565.
- Desnoyer, A., Broutin, S., Delahousse, J., Maritaz, C., Blondel, L., Mir, O., … Paci, A. (2020). Pharmacokinetic/pharmacodynamic relationship of therapeutic monoclonal antibodies used in oncology: part 2, immune checkpoint inhibitor antibodies. European Journal of Cancer, 128, 119–128.
- Di Fiore, F., & Van Cutsem, E. (2009). Acute and long-term gastrointestinal consequences of chemotherapy. Best Practice & Research Clinical Gastroenterology, 23(1), 113–124.
- Dogu, G. G., Kargi, A., Turgut, S., Ayada, C., Taskoylu, B. Y., Demiray, G., … Ergin, A. (2012). MDR1 single nucleotide polymorphism C3435T in Turkish patients with non-small-cell lung cancer. Gene, 506(2), 404–407.
- Doherty, K. M. (1999). Closing the gap in prophylactic antiemetic therapy: patient factors in calculating the emetogenic potential of chemotherapy. Clinical Journal of Oncology Nursing, 3(3), 113–119.
- Dong, H., Bao, D., Guo, X., Hu, J., Li, X., Wan, S., & Xing, J. (2015). Effect of thymidylate synthase gene polymorphism on the response to chemotherapy and clinical outcome of non-small cell lung cancer patients. Tumor Biology, 36(9), 7151–7157.
- Donner, D. B., Nakakura, E. K., Venook, A. P., Lenz, H.-J., Zhang, W., Hwang, J., … Antonia, R. J. (2019). High thymidylate synthase gene expression predicts poor outcome after resection of hepatocellular carcinoma. PloS One, 14(7), e0219469.
- Dotor, E., Cuatrecases, M., Martínez-Iniesta, M., Navarro, M., Vilardell, F., Guinó, E., … Serrano, T. (2006). Tumor thymidylate synthase 1494del6 genotype as a prognostic factor in colorectal cancer patients receiving fluorouracil-based adjuvant treatment. Journal of Clinical Oncology, 24(10), 1603–1611.
- Downward, J. (2003). Targeting RAS signalling pathways in cancer therapy. Nature reviews cancer, 3(1), 11-22.
- Edler, D., Glimelius, B., Hallström, M., Jakobsen, A., Johnston, P. G., Magnusson, I., … Blomgren, H. (2002). Thymidylate synthase expression in colorectal cancer: a prognostic and predictive marker of benefit from adjuvant fluorouracil-based chemotherapy. Journal of Clinical Oncology, 20(7), 1721–1728.
- Eisenhauer, E., Therasse, P., Bogaerts, J., Schwartz, L. H., Sargent, D., Ford, R., ...& Rubinstein, L. (2009). New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). European Journal of Cancer, 45(2), 228-247.
- El-Awady, R., Saleh, E., Hashim, A., Soliman, N., Dallah, A., Elrasheed, A., & Elakraa, G. (2017). The role of eukaryotic and prokaryotic ABC transporter family in failure of

chemotherapy. Frontiers in Pharmacology, 7, 535.

- Elens, L., Tyteca, D., Panin, N., Courtoy, P., Lison, D., Demoulin, J.-B., & Haufroid, V. (2011). Functional defect caused by the 4544G> A SNP in ABCC2: potential impact for drug cellular disposition. Pharmacogenetics and Genomics, 21(12), 884–893.
- Elens, L., Yombi, J.-C., Lison, D., Wallemacq, P., Vandercam, B., & Haufroid, V. (2009). Association between ABCC2 polymorphism and lopinavir accumulation in peripheral blood mononuclear cells of HIV-infected patients. Pharmacogenomics, 10(10), 1589–1597.
- Erčulj, N., Kotnik, B. F., Debeljak, M., Jazbec, J., & Dolžan, V. (2012). Influence of folate pathway polymorphisms on high-dose methotrexate-related toxicity and survival in childhood acute lymphoblastic leukemia. Leukemia & Lymphoma, 53(6), 1096–1104.
- Ernest, S., & Bello-Reuss, E. (1998). P-glycoprotein functions and substrates: possible roles of MDR1 gene in the kidney. Kidney International. Supplement, 65, S11-7.
- Evangeli, L., Ioannis, S., Valentinos, K., Antigony, M., Elli, I., Eleftheria, H., … Evangelos, B. (2017). SLCO1B3 screening in colorectal cancer patients using High-Resolution Melting Analysis method and immunohistochemistry. Tumor Biology, 39(3), 1010428317691176.
- Faganel Kotnik, B., Grabnar, I., Bohanec Grabar, P., Dolžan, V., & Jazbec, J. (2011). Association of genetic polymorphism in the folate metabolic pathway with methotrexate pharmacokinetics and toxicity in childhood acute lymphoblastic leukaemia and malignant lymphoma. European Journal of Clinical Pharmacology, 67(10), 993–1006.
- Feng, W., Liu, X., Zhao, X., Huang, M., Guo, W., Yin, J., … Zhu, X. (2018). Influence of SLCO1B1 in gastric cancer patients treated with EOF chemotherapy. Oncology Letters, 16(4), 4489–4497.
- Fernandes, S. G., Dsouza, R., Pandya, G., Kirtonia, A., Tergaonkar, V., Lee, S. Y., ... &Khattar, E. (2020). Role of telomeres and Telomeric proteins in human malignancies and their therapeutic potential. Cancers, 12(7), 1901.
- Filipits, M., Haddad, V., Schmid, K., Huynh, A., Dunant, A., André, F., … Soria, J.-C. (2007). Multidrug resistance proteins do not predict benefit of adjuvant chemotherapy in patients with completely resected non–small cell lung cancer: International Adjuvant Lung Cancer Trial Biologic Program. Clinical Cancer Research, 13(13), 3892–3898.
- Friedlaender, A., Drilon, A., Weiss, G. J., Banna, G. L., & Addeo, A. (2020). KRAS as a druggable target in NSCLC: Rising like a phoenix after decades of development failures. Cancer treatment reviews, 85, 101978.
- Frittitta, L., Ercolino, T., Bozzali, M., Argiolas, A., Graci, S., Santagati, M. G., … Tassi, V. (2001). A cluster of three single nucleotide polymorphisms in the 3′-untranslated region of human glycoprotein PC-1 gene stabilizes PC-1 mRNA and is associated with increased PC-1 protein content and insulin resistance–related abnormalities. Diabetes, 50(8), 1952–1955.
- Fu, L., Wang, R., Yin, L., Shang, X., Zhang, R., & Zhang, P. (2020). A meta-analysis of ABCG2 gene polymorphism and non-small cell lung cancer outcomes. Genetics and Molecular Biology, 42.
- Fujita, K., Kubota, Y., Ishida, H., & Sasaki, Y. (2015). Irinotecan, a key chemotherapeutic drug

for metastatic colorectal cancer. World Journal of Gastroenterology, 21(43), 12234.

- Funai, K., Yokose, T., Ishii, G., Araki, K., Yoshida, J., Nishimura, M., … Ochiai, A. (2003). Clinicopathologic characteristics of peripheral squamous cell carcinoma of the lung. The American Journal of Surgical Pathology, 27(7), 978–984.
- Fung, K. L., & Gottesman, M. M. (2009). A synonymous polymorphism in a common MDR1 (ABCB1) haplotype shapes protein function. Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics, 1794(5), 860–871.
- Galbiatti, A. L. S., Castro, R., Caldas, H. C., Padovani, J. A., Pavarino, É. C., & Goloni-Bertollo, E. M. (2013). Alterations in the expression pattern of MTHFR, DHFR, TYMS, and SLC19A1 genes after treatment of laryngeal cancer cells with high and low doses of methotrexate. Tumor Biology, 34(6), 3765–3771.
- Ganapathy, V., Smith, S. B., & Prasad, P. D. (2004). SLC19: the folate/thiamine transporter family. Pflügers Archiv, 447(5), 641–646.
- Gandara, D. R., Kawaguchi, T., Crowley, J., Moon, J., Furuse, K., Kawahara, M., … Williamson, S. K. (2009). Japanese-US common-arm analysis of paclitaxel plus carboplatin in advanced non–small-cell lung cancer: a model for assessing population-related pharmacogenomics. Journal of Clinical Oncology, 27(21), 3540.
- Ganoci, L., Trkulja, V., Živković, M., Božina, T., Šagud, M., Lovrić, M., & Božina, N. (2021). ABCB1, ABCG2 and CYP2D6 polymorphism effects on disposition and response to long-acting risperidone. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 104, 110042.
- Gao, Y., Cui, J., Xi, H., Cai, A., Shen, W., Li, J., … Chen, L. (2016). Association of thymidylate synthase expression and clinical outcomes of gastric cancer patients treated with fluoropyrimidine-based chemotherapy: a meta-analysis. OncoTargets and Therapy, 9, 1339.
- Gardner, A., de Mingo Pulido, Á., &Ruffell, B. (2020). Dendritic cells and their role in immunotherapy. Frontiers in Immunology, 11, 924.
- Gastman, B., Agarwal, P. K., Berger, A., Boland, G., Broderick, S., Butterfield, L. H., … Fong, Y. (2020). Defining best practices for tissue procurement in immuno-oncology clinical trials: consensus statement from the Society for Immunotherapy of Cancer Surgery Committee. Journal for Immunotherapy of Cancer, 8(2).
- Gavhane, Y. N., & Yadav, A. V. (2012). Loss of orally administered drugs in GI tract. Saudi Pharmaceutical Journal, 20(4), 331–344.
- Ge, Y., Haska, C. L., LaFiura, K., Devidas, M., Linda, S. B., Liu, M., … Matherly, L. H. (2007). Prognostic role of the reduced folate carrier, the major membrane transporter for methotrexate, in childhood acute lymphoblastic leukemia: a report from the Children's Oncology Group. Clinical Cancer Research, 13(2), 451–457.
- George, A. M., & Jones, P. M. (2012). Perspectives on the structure–function of ABC transporters: the switch and constant contact models. Progress in Biophysics and Molecular Biology, 109(3), 95–107.
- Ghosh, S., Winter, J. M., Patel, K., & Kern, S. E. (2011). Reexamining a proposal: thymidylate synthase 5'-untranslated region as a regulator of translation efficiency. Cancer Biology &

Therapy, 12(8), 750–755.

- Giacomini, C. P., Sun, S., Varma, S., Shain, A. H., Giacomini, M. M., Balagtas, J., ... & Pollack, J. R. (2013). Breakpoint analysis of transcriptional and genomic profiles uncovers novel gene fusions spanning multiple human cancer types. PLoS genetics, 9(4), e1003464.
- Girard, L., Zöchbauer-Müller, S., Virmani, A. K., Gazdar, A. F., & Minna, J. D. (2000). Genome-wide allelotyping of lung cancer identifies new regions of allelic loss, differences between small cell lung cancer and non-small cell lung cancer, and loci clustering. Cancer research, 60(17), 4894-4906.
- Glavinas, H., Krajcsi, P., Cserepes, J., & Sarkadi, B. (2004). The role of ABC transporters in drug resistance, metabolism and toxicity. Current Drug Delivery, 1(1), 27–42.
- Gomez, H. L., Santillana, S. L., Vallejos, C. S., Velarde, R., Sanchez, J., Wang, X., … Niyikiza, C. (2006). A phase II trial of pemetrexed in advanced breast cancer: clinical response and association with molecular target expression. Clinical Cancer Research, 12(3), 832–838.
- Gong, B., Li, D., Kusko, R., Novoradovskaya, N., Zhang, Y., Wang, S., ... & Jones, W. (2021). Cross-oncopanel study reveals high sensitivity and accuracy with overall analytical performance depending on genomic regions. Genome biology, 22(1), 1-23.
- Gong, I. Y., & Kim, R. B. (2013). Impact of genetic variation in OATP transporters to drug disposition and response. Drug Metabolism and Pharmacokinetics, 28(1), 4–18.
- Gonzalez, N., Moody, T. W., Igarashi, H., Ito, T., & Jensen, R. T. (2008). Bombesin-related peptides and their receptors: recent advances in their role in physiology and disease states. Current opinion in endocrinology, diabetes, and obesity, 15(1), 58.
- Goricar, K., Kovac, V., & Dolzan, V. (2014). Polymorphisms in folate pathway and pemetrexed treatment outcome in patients with malignant pleural mesothelioma. Radiology and Oncology, 48(2), 163–172.
- Gorlick, R. (2000). Methotrexate, its mechanism of action in malignant disease and mechanisms of resistance by malignant cells. In Methotrexate (pp. 9–29). Springer.
- Gorlick, R., Goker, E., Trippett, T., Steinherz, P., Elisseyeff, Y., Mazumdar, M., … Bertino, J. R. (1997). Defective transport is a common mechanism of acquired methotrexate resistance in acute lymphocytic leukemia and is associated with decreased reduced folate carrier expression. Blood, The Journal of the American Society of Hematology, 89(3), 1013–1018.
- Grandori, C., Cowley, S. M., James, L. P., & Eisenman, R. N. (2000). The Myc/Max/Mad network and the transcriptional control of cell behavior. Annual review of cell and developmental biology, 16(1), 653-699.
- Grawenda, A. M., &O'neill, E. (2015). Clinical utility of RASSF1A methylation in human malignancies. British journal of cancer, 113(3), 372-381.
- Gromnicova, R., Romero, I., & Male, D. (2012). Transcriptional control of the multi-drug transporter ABCB1 by transcription factor Sp3 in different human tissues. PLoS One, 7(10), e48189.
- Groopman, E. E., Rasouly, H. M., & Gharavi, A. G. (2018). Genomic medicine for kidney disease. Nature Reviews Nephrology, 14(2), 83-104.

- Gruetz, M., Sticht, H., Glaeser, H., Fromm, M. F., & König, J. (2016). Analysis of amino acid residues in the predicted transmembrane pore influencing transport kinetics of the hepatic drug transporter organic anion transporting polypeptide 1B1 (OATP1B1). Biochimica et Biophysica Acta (BBA)-Biomembranes, 1858(11), 2894–2902.
- Gui, C., & Hagenbuch, B. (2009). Role of transmembrane domain 10 for the function of organic anion transporting polypeptide 1B1. Protein Science, 18(11), 2298–2306.
- Guminski, A. D., Balleine, R. L., Chiew, Y.-E., Webster, L. R., Tapner, M., Farrell, G. C., … Defazio, A. (2006). MRP2 (ABCC2) and cisplatin sensitivity in hepatocytes and human ovarian carcinoma. Gynecologic Oncology, 100(2), 239–246.
- Haga, S., Hinoshita, E., Ikezaki, K., Fukui, M., Scheffer, G. L., Uchiumi, T., & Kuwano, M. (2001). Involvement of the multidrug resistance protein 3 in drug sensitivity and its expression in human glioma. Japanese Journal of Cancer Research, 92(2), 211–219.
- Hagenbuch, B., & Meier, P. J. (2003). The superfamily of organic anion transporting polypeptides. Biochimica et Biophysica Acta (BBA)-Biomembranes, 1609(1), 1–18.
- Hagenbuch, B., & Meier, P. J. (2004). Organic anion transporting polypeptides of the OATP/SLC21 family: phylogenetic classification as OATP/SLCO superfamily, new nomenclature and molecular/functional properties. Pflügers Archiv, 447(5), 653–665.
- Hamada, S., Satoh, K., Hirota, M., Kanno, A., Umino, J., Ito, H., … Shimosegawa, T. (2012). The homeobox gene MSX2 determines chemosensitivity of pancreatic cancer cells via the regulation of transporter gene ABCG2. Journal of Cellular Physiology, 227(2), 729–738.
- Hammad, H., Sarkar, M., Gupta, N., Ardalan, B., & Subbarayan, P. R. (2012). The presence of three repeats in the 5'UTR region of thymidylate synthase (TS) is associated with increased TS mRNA expression in cultured human cancer cell lines in vitro. Oncology Reports, 27(1), 246–249.
- Han, F., Wang, H., Zhang, G., Han, H., Song, B., Li, L., ... & Liang, Z. (2015). Texture feature analysis for computer-aided diagnosis on pulmonary nodules. Journal of digital imaging, 28(1), 99-115.
- Han, J. M., Choi, K. H., Lee, H. H., & Gwak, H. S. (2022). Association between SLCO1B1 polymorphism and methotrexate-induced hepatotoxicity: a systematic review and meta-analysis. Anti-Cancer Drugs, 33(1), 75–79.
- Han, J., Lim, H., Yoo, Y., Shin, E. S., Park, Y. H., Lee, S. Y., … Lee, J. S. (2007). Associations of ABCB1, ABCC2, and ABCG2 polymorphisms with irinotecan-pharmacokinetics and clinical outcome in patients with advanced non-small cell lung cancer. Cancer, 110(1), 138–147.
- Han, J.-Y., Lim, H.-S., Park, Y. H., Lee, S. Y., & Lee, J. S. (2009). Integrated pharmacogenetic prediction of irinotecan pharmacokinetics and toxicity in patients with advanced non-small cell lung cancer. Lung Cancer, 63(1), 115–120.
- Han, J.-Y., Lim, H.-S., Shin, E. S., Yoo, Y.-K., Park, Y. H., Lee, J.-E., … Lee, J. S. (2008). Influence of the organic anion-transporting polypeptide 1B1 (OATP1B1) polymorphisms on irinotecan-pharmacokinetics and clinical outcome of patients with advanced non-small cell lung cancer. Lung Cancer, 59(1), 69–75.

- Hanahan, D., & Weinberg, R. A. (2000). The hallmarks of cancer. Cell, 100(1), 57–70. https://doi.org/10.1016/s0092-8674(00)81683-9
- Hanna, N., Shepherd, F. A., Fossella, F. V, Pereira, J. R., De Marinis, F., Von Pawel, J., … Muller, T. (2004). Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. Journal of Clinical Oncology, 22(9), 1589–1597.
- Hanssen, K. M., Haber, M., & Fletcher, J. I. (2021). Targeting multidrug resistance-associated protein 1 (MRP1)-expressing cancers: Beyond pharmacological inhibition. Drug Resistance Updates, 100795.
- Hassan, H. M., Al-Wahaibi, L. H., Elmorsy, M. A., & Mahran, Y. F. (2020). Suppression of cisplatin-induced hepatic injury in rats through alarmin high-mobility group box-1 pathway by Ganoderma lucidum: theoretical and experimental study. Drug Design, Development and Therapy, 14, 2335.
- He, S.-M., Li, R., R Kanwar, J., & Zhou, S.-F. (2011). Structural and functional properties of human multidrug resistance protein 1 (MRP1/ABCC1). Current Medicinal Chemistry, 18(3), 439–481.
- Hediger, M. A., Clémençon, B., Burrier, R. E., & Bruford, E. A. (2013). The ABCs of membrane transporters in health and disease (SLC series): introduction. Molecular Aspects of Medicine, 34(2–3), 95–107.
- Hediger, M. A., Romero, M. F., Peng, J.-B., Rolfs, A., Takanaga, H., & Bruford, E. A. (2004). The ABCs of solute carriers: physiological, pathological and therapeutic implications of human membrane transport proteins. Pflügers Archiv, 447(5), 465–468.
- Heeke, A. L., Pishvaian, M. J., Lynce, F., Xiu, J., Brody, J. R., Chen, W. J., ... & Isaacs, C. (2018). Prevalence of homologous recombination–related gene mutations across multiple cancer types. JCO precision oncology, 2, 1-13.
- Hiley, C. T., Le Quesne, J., Santis, G., Sharpe, R., de Castro, D. G., Middleton, G., & Swanton, C. (2016). Challenges in molecular testing in non-small-cell lung cancer patients with advanced disease. The Lancet, 388(10048), 1002-1011.
- Hilgendorf, C., Ahlin, G., Seithel, A., Artursson, P., Ungell, A.-L., & Karlsson, J. (2007). Expression of thirty-six drug transporter genes in human intestine, liver, kidney, and organotypic cell lines. Drug Metabolism and Disposition, 35(8), 1333–1340.
- Hirsch, F. R., Scagliotti, G. V., Langer, C. J., Varella-Garcia, M., & Franklin, W. A. (2003). Epidermal growth factor family of receptors in preneoplasia and lung cancer: perspectives for targeted therapies. Lung cancer, 41, 29-42.
- Hitzl, M., Drescher, S., van der Kuip, H., Schäffeler, E., Fischer, J., Schwab, M., … Fromm, M. F. (2001). The C3435T mutation in the human MDR1 gene is associated with altered efflux of the P-glycoprotein substrate rhodamine 123 from CD56+ natural killer cells. Pharmacogenetics and Genomics, 11(4), 293–298.
- Ho, R. H., & Kim, R. B. (2005). Transporters and drug therapy: implications for drug disposition and disease. Clinical Pharmacology & Therapeutics, 78(3), 260–277.

- Hoffmeyer, S., Burk, O., Von Richter, O., Arnold, H. P., Brockmöller, J., Johne, A., … Eichelbaum, M. (2000). Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. Proceedings of the National Academy of Sciences, 97(7), 3473–3478.
- Hong, M. (2017). Biochemical studies on the structure–function relationship of major drug transporters in the ATP-binding cassette family and solute carrier family. Advanced Drug Delivery Reviews, 116, 3–20.
- Hong, M., Li, S., Zhou, F., Thomas, P. E., & You, G. (2010). Putative transmembrane domain 12 of the human organic anion transporter hOAT1 determines transporter stability and maturation efficiency. Journal of Pharmacology and Experimental Therapeutics, 332(2), 650–658.
- Hori, M., Saito, E., Katanoda, K., & Tsugane, S. (2020). Estimation of lifetime cumulative mortality risk of lung cancer by smoking status in Japan. Japanese Journal of Clinical Oncology, 50(10), 1218–1224.
- Hou, Q., Li, S., Li, L., Li, Y., Sun, X., & Tian, H. (2015). Association between SLCO1B1 gene T521C polymorphism and statin-related myopathy risk: a meta-analysis of case–control studies. Medicine, 94(37).
- Hou, Z., & Matherly, L. H. (2014). Biology of the major facilitative folate transporters SLC19A1 and SLC46A1. Current Topics in Membranes, 73, 175–204.
- Housman, G., Byler, S., Heerboth, S., Lapinska, K., Longacre, M., Snyder, N., & Sarkar, S. (2014). Drug resistance in cancer: an overview. Cancers, 6(3), 1769–1792.
- https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcaev3.pdf
- https://www.genecards.org/
- Hu, Q., Li, X., Su, C., Chen, X., Gao, G., Zhang, J., … Zhou, C. (2012). Correlation between thymidylate synthase gene polymorphisms and efficacy of pemetrexed in advanced non-small cell lung cancer. Experimental and Therapeutic Medicine, 4(6), 1010–1016.
- Huang, L., Zhang, T., Xie, C., Liao, X., Yu, Q., Feng, J., … Chen, J. (2013). SLCO1B1 and SLC19A1 gene variants and irinotecan-induced rapid response and survival: a prospective multicenter pharmacogenetics study of metastatic colorectal cancer. PLoS One, 8(10), e77223.
- Huber, R. D. (2005). Identification and functional characterization of human organic anion transporting polypeptides. ETH Zurich.
- Husgafvel-Pursiainen, K., Boffetta, P., Kannio, A., Nyberg, F., Pershagen, G., Mukeria, A., ... & Benhamou, S. (2000). p53 mutations and exposure to environmental tobacco smoke in a multicenter study on lung cancer. Cancer Research, 60(11), 2906-2911.
- Huss, R. (2015). Biomarkers. In Translational regenerative medicine (pp. 235-241). Academic Press.
- Iacopetta, B., Grieu, F., Joseph, D., & Elsaleh, H. (2001). A polymorphism in the enhancer region of the thymidylate synthase promoter influences the survival of colorectal cancer patients treated with 5-fluorouracil. British Journal of Cancer, 85(6), 827–830.
- Ieiri, I., Higuchi, S., & Sugiyama, Y. (2009). Genetic polymorphisms of uptake (OATP1B1,

1B3) and efflux (MRP2, BCRP) transporters: implications for inter-individual differences in the pharmacokinetics and pharmacodynamics of statins and other clinically relevant drugs. Expert Opinion on Drug Metabolism & Toxicology, 5(7), 703–729.

- Imai, Y., Nakane, M., Kage, K., Tsukahara, S., Ishikawa, E., Tsuruo, T., … Sugimoto, Y. (2002). C421A polymorphism in the human breast cancer resistance protein gene is associated with low expression of Q141K protein and low-level drug resistance. Molecular Cancer Therapeutics, 1(8), 611–616.
- Innocenti, F., Kroetz, D. L., Schuetz, E., Dolan, M. E., Ramírez, J., Relling, M., … Ratain, M. J. (2009). Comprehensive pharmacogenetic analysis of irinotecan neutropenia and pharmacokinetics. Journal of Clinical Oncology, 27(16), 2604.
- Ishida, Y., Kawakami, K., Tanaka, Y., Kanehira, E., Omura, K., & Watanabe, G. (2002). Association of thymidylate synthase gene polymorphism with its mRNA and protein expression and with prognosis in gastric cancer. Anticancer Research, 22(5), 2805–2809.
- Isla, D., Sarries, C., Rosell, R., Alonso, G., Domine, M., Taron, M., … Nunez, L. (2004). Single nucleotide polymorphisms and outcome in docetaxel–cisplatin-treated advanced non-small-cell lung cancer. Annals of Oncology, 15(8), 1194–1203.
- Izzedine, H., Hulot, J.-S., Villard, E., Goyenvalle, C., Dominguez, S., Ghosn, J., … Deray, and G. (2006). Association between ABCC2 gene haplotypes and tenofovir-induced proximal tubulopathy. The Journal of Infectious Diseases, 194(11), 1481–1491.
- Jabeen, S., Holmboe, L., Alnæs, G. I. G., Andersen, A. M., Hall, K. S., & Kristensen, V. N. (2015). Impact of genetic variants of RFC1, DHFR and MTHFR in osteosarcoma patients treated with high-dose methotrexate. The Pharmacogenomics Journal, 15(5), 385–390.
- Jada, S. R., Lim, R., Wong, C. I., Shu, X., Lee, S. C., Zhou, Q., … Chowbay, B. (2007). Role of UGT1A1* 6, UGT1A1* 28 and ABCG2 c. 421C> A polymorphisms in irinotecan-induced neutropenia in Asian cancer patients. Cancer Science, 98(9), 1461–1467.
- Jafri, M. A., Ansari, S. A., Alqahtani, M. H., & Shay, J. W. (2016). Roles of telomeres and telomerase in cancer, and advances in telomerase-targeted therapies. Genome medicine, 8(1), 1-18.
- Jain, R., Agarwal, S., Mandava, N. K., Sheng, Y., & Mitra, A. K. (2008). Interaction of dipeptide prodrugs of saquinavir with multidrug resistance protein-2 (MRP-2): evasion of MRP-2 mediated efflux. International Journal of Pharmaceutics, 362(1–2), 44–51.
- Jiang, W. G., Sanders, A. J., Katoh, M., Ungefroren, H., Gieseler, F., Prince, M., … Dhawan, P. (2015). Tissue invasion and metastasis: Molecular, biological and clinical perspectives. Seminars in Cancer Biology, 35, S244–S275. Elsevier.
- Jin, G., Kim, M. J., Jeon, H. S., Choi, J. E., Kim, D. S., Lee, E. B., ... & Park, J. Y. (2010). PTEN mutations and relationship to EGFR, ERBB2, KRAS, and TP53 mutations in non-small cell lung cancers. Lung cancer, 69(3), 279-283.
- Jiye, Y. I. N., & Jianting, Z. (2011). Multidrug resistance-associated protein 1 (MRP1/ABCC1) polymorphism: from discovery to clinical application. Zhong Nan Da Xue Xue Bao. Yi Xue Ban= Journal of Central South University. Medical Sciences, 36(10), 927.

- Johansson, B. G. (1972). Agarose gel electrophoresis. Scandinavian Journal of Clinical and Laboratory Investigation, 29(sup124), 7-19.
- Johnatty, S. E., Beesley, J., Paul, J., Fereday, S., Spurdle, A. B., Webb, P. M., … Group, A. S. (2008). ABCB1 (MDR 1) polymorphisms and progression-free survival among women with ovarian cancer following paclitaxel/carboplatin chemotherapy. Clinical Cancer Research, 14(17), 5594–5601.
- Jung, D., Hagenbuch, B., Gresh, L., Pontoglio, M., Meier, P. J., & Kullak-Ublick, G. A. (2001). Characterization of the human OATP-C (SLC21A6) gene promoter and regulation of liver-specific OATP genes by hepatocyte nuclear factor 1α. Journal of Biological Chemistry, 276(40), 37206–37214.
- Kabiraj, A., Jaiswal, R., Singh, A., Gupta, J., Singh, A., &Samadi, F. M. (2018). Immunohistochemical evaluation of tumor angiogenesis and the role of mast cells in oral squamous cell carcinoma. Journal of cancer research and therapeutics, 14(3), 495.
- Kacew, A. J., Harris, E. J., Lorch, J. H., Haddad, R. I., Chau, N. G., Rabinowits, G., ... & Hanna, G. J. (2019). Chromosome 3q arm gain linked to immunotherapy response in advanced cutaneous squamous cell carcinoma. European Journal of Cancer, 113, 1-9.
- Kadioglu, O., Saeed, M. E. M., Munder, M., Spuller, A., Greten, H. J., & Efferth, T. (2020). Effect of ABC transporter expression and mutational status on survival rates of cancer patients. Biomedicine & Pharmacotherapy, 131, 110718.
- Kalliokoski, A., Neuvonen, M., Neuvonen, P. J., & Niemi, M. (2008). The effect of SLCO1B1 polymorphism on repaglinide pharmacokinetics persists over a wide dose range. British Journal of Clinical Pharmacology, 66(6), 818–825.
- Kamiyama, Y., Matsubara, T., Yoshinari, K., Nagata, K., Kamimura, H., & Yamazoe, Y. (2007). Role of human hepatocyte nuclear factor 4α in the expression of drug-metabolizing enzymes and transporters in human hepatocytes assessed by use of small interfering RNA. Drug Metabolism and Pharmacokinetics, 22(4), 287–298.
- Kaneda, S., Nalbantoglu, J., Takeishi, K., Shimizu, K., Gotoh, O., Seno, T., & Ayusawa, D. (1990). Structural and functional analysis of the human thymidylate synthase gene. Journal of Biological Chemistry, 265(33), 20277–20284.
- Kantharidis, P., El-Osta, S., De Silva, M., Lee, G., Hu, X. F., & Zalcberg, J. (2000). Regulation of MDR1 gene expression: emerging concepts. Drug Resistance Updates, 3(2), 99–108.
- Kapała, J., Mnich, S., & Karpińska, M. (2017). Radon in human environment and Carcinoma–part 1. Polish Journal of Applied Sciences, 3(1), 26-31.
- Karbownik, A., Sobańska, K., Płotek, W., Grabowski, T., Klupczynska, A., Plewa, S., … Szałek, E. (2020). The influence of the coadministration of the p-glycoprotein modulator elacridar on the pharmacokinetics of lapatinib and its distribution in the brain and cerebrospinal fluid. Investigational New Drugs, 38(3), 574–583.
- Kashima, J., Kitadai, R., & Okuma, Y. (2019). Molecular and morphological profiling of lung cancer: a foundation for “next-generation” pathologists and oncologists. Cancers, 11(5), 599.
- Kast, H. R., Goodwin, B., Tarr, P. T., Jones, S. A., Anisfeld, A. M., Stoltz, C. M., … Edwards,

P. A. (2002). Regulation of multidrug resistance-associated protein 2 (ABCC2) by the nuclear receptors pregnane X receptor, farnesoid X-activated receptor, and constitutive androstane receptor. Journal of Biological Chemistry, 277(4), 2908–2915.

- Kaur, G., Nijhawan, R., Gupta, N., Singh, N., & Rajwanshi, A. (2017). Pleural fluid cytology samples in cases of suspected lung cancer: An experience from a tertiary care centre. Diagnostic cytopathology, 45(3), 195-201.
- Kawakami, K., & Watanabe, G. (2003). Identification and functional analysis of single nucleotide polymorphism in the tandem repeat sequence of thymidylate synthase gene. Cancer Research, 63(18), 6004–6007.
- Kawakami, K., Ishida, Y., Danenberg, K. D., Omura, K., Watanabe, G., & Danenberg, P. V. (2002). Functional polymorphism of the thymidylate synthase gene in colorectal cancer accompanied by frequent loss of heterozygosity. Japanese Journal of Cancer Research, 93(11), 1221–1229.
- Kciuk, M., Marciniak, B., & Kontek, R. (2020). Irinotecan—still an important player in cancer chemotherapy: a comprehensive overview. International Journal of Molecular Sciences, 21(14), 4919.
- Keppler, D. (2014). The roles of MRP2, MRP3, OATP1B1, and OATP1B3 in conjugated hyperbilirubinemia. Drug Metabolism and Disposition, 42(4), 561–565.
- Kim, A.-S., Ko, H.-J., Kwon, J.-H., & Lee, J.-M. (2018). Exposure to secondhand smoke and risk of cancer in never smokers: a meta-analysis of epidemiologic studies. International Journal of Environmental Research and Public Health, 15(9), 1981.
- Kim, H., Im, S., Keam, B., Ham, H. S., Lee, K. H., Kim, T. Y., … Han, W. (2015). ABCB 1 polymorphism as prognostic factor in breast cancer patients treated with docetaxel and doxorubicin neoadjuvant chemotherapy. Cancer Science, 106(1), 86–93.
- Kim, I., Kim, H., Kim, D. C., Eom, H., Kong, S., Shin, H., … Lee, G. (2008). 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 Science, 99(12), 2496–2501.
- Kim, Y., & Chen, J. (2018). Molecular structure of human P-glycoprotein in the ATP-bound, outward-facing conformation. Science, 359(6378), 915–919.
- Kitamura, H., Yazawa, T., Okudela, K., Shimoyamada, H., & Sato, H. (2008). Molecular and genetic pathogenesis of lung cancer: differences between small-cell and non-small-cell carcinomas. The Open Pathology Journal, 2(1).
- Kiyotani, K., Mushiroda, T., Kubo, M., Zembutsu, H., Sugiyama, Y., & Nakamura, Y. (2008). Association of genetic polymorphisms in SLCO1B3 and ABCC2 with docetaxel-induced leukopenia. Cancer Science, 99(5), 967–972.
- Klaassen, C. D., & Aleksunes, L. M. (2010). Xenobiotic, bile acid, and cholesterol transporters: function and regulation. Pharmacological Reviews, 62(1), 1–96.
- Knez, L., Košnik, M., Ovčariček, T., Sadikov, A., Sodja, E., Kern, I., & Cufer, T. (2012).

Predictive value of ABCB1 polymorphisms G2677T/A, C3435T, and their haplotype in small cell lung cancer patients treated with chemotherapy. Journal of Cancer Research and Clinical Oncology, 138(9), 1551–1560.

- Knez, Lea, Sodja, E., Kern, I., Košnik, M., & Cufer, T. (2011). Predictive value of multidrug resistance proteins, topoisomerases II and ERCC1 in small cell lung cancer: a systematic review. Lung Cancer, 72(3), 271–279.
- Knight, T., Luedtke, D., Edwards, H., Taub, J. W., & Ge, Y. (2019). A delicate balance–The BCL-2 family and its role in apoptosis, oncogenesis, and cancer therapeutics. Biochemical pharmacology, 162, 250-261.
- Kobayashi, D., Ieiri, I., Hirota, T., Takane, H., Maegawa, S., Kigawa, J., … Terakawa, N. (2005). Functional assessment of ABCG2 (BCRP) gene polymorphisms to protein expression in human placenta. Drug Metabolism and Disposition, 33(1), 94–101.
- Kobayashi, H., Sato, K., Niioka, T., Miura, H., Ito, H., & Miura, M. (2015). Relationship among gefitinib exposure, polymorphisms of its metabolizing enzymes and transporters, and side effects in Japanese patients with non–small-cell lung cancer. Clinical Lung Cancer, 16(4), 274–281.
- Kondo, T., Yamada, K., Noda, K., Nakayama, H., & Kameda, Y. (2002). Radiologic–prognostic correlation in patients with small pulmonary adenocarcinomas. Lung Cancer, 36(1), 49–57.
- König, J. (2011). Uptake transporters of the human OATP family. Drug Transporters, 1–28.
- König, J., Cui, Y., Nies, A. T., & Keppler, D. (2000). Localization and genomic organization of a new hepatocellular organic anion transporting polypeptide. Journal of Biological Chemistry, 275(30), 23161–23168.
- König, J., Nies, A. T., Cui, Y., Leier, I., & Keppler, D. (1999). Conjugate export pumps of the multidrug resistance protein (MRP) family: localization, substrate specificity, and MRP2-mediated drug resistance. Biochimica et Biophysica Acta (BBA)-Biomembranes, 1461(2), 377–394.
- Koury, M. J., & Ponka, P. (2004). New insights into erythropoiesis: the roles. Annu. Rev. Nutr, 24, 105–131.
- Kris, M. G., Johnson, B. E., Berry, L. D., Kwiatkowski, D. J., Iafrate, A. J., Wistuba, I. I., ... & Bunn, P. A. (2014). Using multiplexed assays of oncogenic drivers in lung cancers to select targeted drugs. Jama, 311(19), 1998-2006.
- Kuchler, K. (2011). The ABC of ABCs: multidrug resistance and genetic diseases. The FEBS Journal, Vol. 278, p. 3189. Wiley Online Library.
- Kunická, T., & Souček, P. (2014). Importance of ABCC1 for cancer therapy and prognosis. Drug Metabolism Reviews, 46(3), 325–342.
- Lababede, O., & Meziane, M. A. (2018). The eighth edition of TNM staging of lung cancer: Reference chart and diagrams. The Oncologist, 23(7), 844–848.
- Lamba, J. K., Fridley, B. L., Ghosh, T. M., Yu, Q., Mehta, G., & Gupta, P. (2014). Genetic variation in platinating agent and taxane pathway genes as predictors of outcome and toxicity in advanced non-small-cell lung cancer. Pharmacogenomics, 15(12), 1565–1574.
- Lancaster, C. S., Sprowl, J. A., Walker, A. L., Hu, S., Gibson, A. A., & Sparreboom, A. (2013).

Modulation of OATP1B-Type Transporter Function Alters Cellular Uptake and Disposition of Platinum ChemotherapeuticsOATP1B-Type Transporters Modulate Platinum Drug Uptake. Molecular Cancer Therapeutics, 12(8), 1537–1544.

- Lara Jr, P. N., Natale, R., Crowley, J., Lenz, H. J., Redman, M. W., Carleton, J. E., … Dakhil, S. R. (2009). Phase III trial of irinotecan/cisplatin compared with etoposide/cisplatin in extensive-stage small-cell lung cancer: clinical and pharmacogenomic results from SWOG S0124. Journal of Clinical Oncology, 27(15), 2530.
- Larbcharoensub, N., Sornmayura, P., Sirachainan, E., Wilasrusmee, C., Wanmoung, H., & Janvilisri, T. (2011). Prognostic value of ABCG2 in moderately and poorly differentiated intrahepatic cholangiocarcinoma. Histopathology, 59(2), 235–246.
- Larkin, A., O'Driscoll, L., Kennedy, S., Purcell, R., Moran, E., Crown, J., … Clynes, M. (2004). Investigation of MRP-1 protein and MDR-1 P-glycoprotein expression in invasive breast cancer: A prognostic study. International Journal of Cancer, 112(2), 286–294.
- Lecomte, T., Ferraz, J.-M., Zinzindohoué, F., Loriot, M.-A., Tregouet, D.-A., Landi, B., … Beaune, P. (2004). Thymidylate synthase gene polymorphism predicts toxicity in colorectal cancer patients receiving 5-fluorouracil-based chemotherapy. Clinical Cancer Research, 10(17), 5880–5888.
- Lee, H. H., & Ho, R. H. (2017). Interindividual and interethnic variability in drug disposition: polymorphisms in organic anion transporting polypeptide 1B1 (OATP1B1; SLCO1B1). British Journal of Clinical Pharmacology, 83(6), 1176–1184.
- Lee, S. H., Kim, H. J., Lee, J. S., Lee, I.-S., & Kang, B. Y. (2007). Inhibition of topoisomerase I activity and efflux drug transporters' expression by xanthohumol from hops. Archives of Pharmacal Research, 30(11), 1435–1439.
- Lee, W., Ha, J., & Sugiyama, Y. (2020). Post-translational regulation of the major drug transporters in the families of organic anion transporters and organic anion–transporting polypeptides. Journal of Biological Chemistry, 295(50), 17349–17364.
- Lee, W.-K., & Frank, T. (2021). Teaching an old dog new tricks: reactivated developmental signaling pathways regulate ABCB1 and chemoresistance in cancer. Cancer Drug Resistance, 4(2), 424–452.
- Leslie, E. M., Deeley, R. G., & Cole, S. P. C. (2005). Multidrug resistance proteins: role of P-glycoprotein, MRP1, MRP2, and BCRP (ABCG2) in tissue defense. Toxicology and Applied Pharmacology, 204(3), 216–237.
- Lettnin, A. P., Wagner, E. F., Carrett-Dias, M., dos Santos Machado, K., Werhli, A., Cañedo, A. D., … de Souza Votto, A. P. (2019). Silencing the OCT4-PG1 pseudogene reduces OCT-4 protein levels and changes characteristics of the multidrug resistance phenotype in chronic myeloid leukemia. Molecular Biology Reports, 46(2), 1873–1884.
- Levy, A. S., Sather, H. N., Steinherz, P. G., Sowers, R., La, M., Moscow, J. A., … Gorlick, R. (2003). Reduced folate carrier and dihydrofolate reductase expression in acute lymphocytic leukemia may predict outcome: a Children's Cancer Group Study. Journal of Pediatric Hematology/Oncology, 25(9), 688–695.

- Li, C., Yuan, B., Yu, X., Ju, L., Ma, Y., Guo, D., & Zhang, K. (2020). SLC19A1 May Serve as a Potential Biomarker for Diagnosis and Prognosis in Osteosarcoma. Clinical Laboratory, 66(11).
- Li, J., Li, Z.-N., Du, Y.-J., Li, X.-Q., Bao, Q.-L., & Chen, P. (2009). Expression of MRP1, BCRP, LRP, and ERCC1 in advanced non–small-cell lung cancer: correlation with response to chemotherapy and survival. Clinical Lung Cancer, 10(6), 414–421.
- Li, N., Hong, W., Huang, H., Lu, H., Lin, G., & Hong, M. (2013). Correction: Identification of Amino Acids Essential for Estrone-3-Sulfate Transport within Transmembrane Domain 2 of Organic Anion Transporting Polypeptide 1B1. Plos One, 8(5).
- Li, Q., & Shu, Y. (2014). Role of solute carriers in response to anticancer drugs. Molecular and Cellular Therapies, 2(1), 1–14.
- Li, X., Wei, S., & Chen, J. (2014). Critical appraisal of pemetrexed in the treatment of NSCLC and metastatic pulmonary nodules. OncoTargets and Therapy, 7, 937.
- Li, Y., Gan, S., Ren, L., Yuan, L., Liu, J., Wang, W., … Zhang, F. (2018). Multifaceted regulation and functions of replication factor C family in human cancers. American Journal of Cancer Research, 8(8), 1343.
- Liang, Y., Li, S., & Chen, L. (2015). The physiological role of drug transporters. Protein & Cell, 6(5), 334–350.
- LiCrawford, R. R., Potukuchi, P. K., Schuetz, E. G., & Schuetz, J. D. (2018). Beyond competitive inhibition: regulation of ABC transporters by kinases and protein-protein interactions as potential mechanisms of drug-drug interactions. Drug Metabolism and Disposition, 46(5), 567–580.
- Liedert, B., Materna, V., Schadendorf, D., Thomale, J., & Lage, H. (2003). Overexpression of cMOAT (MRP2/ABCC2) is associated with decreased formation of platinum-DNA adducts and decreased G2-arrest in melanoma cells resistant to cisplatin. Journal of Investigative Dermatology, 121(1), 172–176.
- Lima, A., Bernardes, M., Azevedo, R., Monteiro, J., Sousa, H., Medeiros, R., & Seabra, V. (2014). SLC19A1, SLC46A1 and SLCO1B1 polymorphisms as predictors of methotrexate-related toxicity in Portuguese rheumatoid arthritis patients. Toxicological Sciences, 142(1), 196–209.
- Lima, A., Bernardes, M., Sousa, H., Azevedo, R., Costa, L., Ventura, F., ... & Medeiros, R. (2014). SLC19A1 80G allele as a biomarker of methotrexate-related gastrointestinal toxicity in Portuguese rheumatoid arthritis patients. Pharmacogenomics, 15(6), 807-820.
- Lima, A., Seabra, V., Martins, S., Coelho, A., Araújo, A., & Medeiros, R. (2014). Thymidylate synthase polymorphisms are associated to therapeutic outcome of advanced non-small cell lung cancer patients treated with platinum-based chemotherapy. Molecular Biology Reports, 41(5), 3349–3357.
- Lin, L., Yee, S. W., Kim, R. B., & Giacomini, K. M. (2015). SLC transporters as therapeutic targets: emerging opportunities. Nature Reviews Drug Discovery, 14(8), 543–560.
- Liu, H., Jin, G., Wang, H., Wu, W., Liu, Y., Qian, J., … Hu, Z. (2008). Association of

polymorphisms in one-carbon metabolizing genes and lung cancer risk: a case-control study in Chinese population. Lung Cancer, 61(1), 21–29.

- Liu, J., Long, J., Zhang, S., Fang, X., & Luo, Y. (2013). Polymorphic variants of SLCO1B1 in neonatal hyperbilirubinemia in China. Italian Journal of Pediatrics, 39(1), 1-5.
- Liu, J., Schmitz, J. C., Lin, X., Tai, N., Yan, W., Farrell, M., … Chu, E. (2002). Thymidylate synthase as a translational regulator of cellular gene expression. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1587(2–3), 174–182.
- Liu, X. (2019). ABC family transporters. Drug Transporters in Drug Disposition, Effects and Toxicity, 13–100.
- Liu, X. Y., Witt, T. L., & Matherly, L. H. (2003). Restoration of high-level transport activity by human reduced folate carrier/ThTr1 thiamine transporter chimaeras: role of the transmembrane domain 6/7 linker region in reduced folate carrier function. Biochemical Journal, 369(1), 31–37.
- Liutkeviciene, R., Vilkeviciute, A., Slavinskaite, A., Petrauskaite, A., Tatarunas, V., & Kriauciuniene, L. (2018). Evaluation of serum SLCO1B1 levels and genetic variants of SLCO1B1 rs4149056 and rs2306283 in patients with early and exudative age-related macular degeneration. Gene, 676, 139–145.
- Lockhart, A. C., Harris, E., LaFleur, B. J., Merchant, N. B., Washington, M. K., Resnick, M. B., … Lee, W. (2008). Organic anion transporting polypeptide 1B3 (OATP1B3) is overexpressed in colorectal tumors and is a predictor of clinical outcome. Clinical and Experimental Gastroenterology, 1, 1.
- López-Marqués, R. L., Poulsen, L. R., Bailly, A., Geisler, M., Pomorski, T. G., & Palmgren, M. G. (2015). Structure and mechanism of ATP-dependent phospholipid transporters. Biochimica et Biophysica Acta (BBA)-General Subjects, 1850(3), 461–475.
- Macalou, S., Robey, R. W., Jabor Gozzi, G., Shukla, S., Grosjean, I., Hegedus, T., … Di Pietro, A. (2016). The linker region of breast cancer resistance protein ABCG2 is critical for coupling of ATP-dependent drug transport. Cellular and Molecular Life Sciences, 73(9), 1927–1937.
- Maeno, K., Nakajima, A., Conseil, G., Rothnie, A., Deeley, R. G., & Cole, S. P. C. (2009). Molecular basis for reduced estrone sulfate transport and altered modulator sensitivity of transmembrane helix (TM) 6 and TM17 mutants of multidrug resistance protein 1 (ABCC1). Drug Metabolism and Disposition, 37(7), 1411–1420.
- Mahimkar, M. B., Samant, T. A., Kannan, S., Tulsulkar, J., Pai, P. S., & Anantharaman, D.(2012). Polymorphisms in GSTM1 and XPD genes predict clinical outcome in advanced oral cancer patients treated with postoperative radiotherapy. Molecular carcinogenesis, 51(S1), E94-103.
- Malhotra, J., Malvezzi, M., Negri, E., La Vecchia, C. and Boffetta, P. (2016). Risk factors for lung cancer worldwide. European Respiratory Journal ERJ–00359.
- Maliepaard, M., Scheffer, G. L., Faneyte, I. F., van Gastelen, M. A., Pijnenborg, A. C. L. M., Schinkel, A. H., … Schellens, J. H. M. (2001). Subcellular localization and distribution of the breast cancer resistance protein transporter in normal human tissues. Cancer Research, 61(8), 3458–3464.

- Malik, P. S., & Raina, V. (2015). Lung cancer: prevalent trends & emerging concepts. The Indian journal of medical research, 141(1), 5.
- Malson, J. L., Sims, K., Murty, R., & Pickworth, W. B. (2001). Comparison of the nicotine content of tobacco used in bidis and conventional cigarettes. Tobacco Control, 10(2), 181–183.
- Mandery, K., Sticht, H., Bujok, K., Schmidt, I., Fahrmayr, C., Balk, B., … Glaeser, H. (2011). Functional and structural relevance of conserved positively charged lysine residues in organic anion transporting polypeptide 1B3. Molecular Pharmacology, 80(3), 400–406.
- Mandola, M. V, Stoehlmacher, J., Muller-Weeks, S., Cesarone, G., Yu, M. C., Lenz, H.-J., & Ladner, R. D. (2003). A novel single nucleotide polymorphism within the 5′ tandem repeat polymorphism of the thymidylate synthase gene abolishes USF-1 binding and alters transcriptional activity. Cancer Research, 63(11), 2898–2904.
- Mandola, M. V, Stoehlmacher, J., Zhang, W., Groshen, S., Mimi, C. Y., Iqbal, S., … Ladner, R. D. (2004). A 6 bp polymorphism in the thymidylate synthase gene causes message instability and is associated with decreased intratumoral TS mRNA levels. Pharmacogenetics and Genomics, 14(5), 319–327.
- Manohar, C. F., Bray, J. A., Salwen, H. R., Madafiglio, J., Cheng, A., Flemming, C., … Cohn, S. L. (2004). MYCN-mediated regulation of the MRP1 promoter in human neuroblastoma. Oncogene, 23(3), 753–762.
- Mao, Q., & Unadkat, J. D. (2015). Role of the breast cancer resistance protein (BCRP/ABCG2) in drug transport—an update. The AAPS Journal, 17(1), 65–82.
- Marchetti, A., Ardizzoni, A., Papotti, M., Crinò, L., Rossi, G., Gridelli, C., ... & Pinto, C. (2013). Recommendations for the analysis of ALK gene rearrangements in non–small-cell lung Cancer: a consensus of the Italian Association of Medical Oncology and the Italian Society of Pathology and Cytopathology. Journal of Thoracic Oncology, 8(3), 352-358.
- Marcuello, E., Altés, A., del Rio, E., César, A., Menoyo, A., & Baiget, M. (2004). Single nucleotide polymorphism in the 5′ tandem repeat sequences of thymidylate synthase gene predicts for response to fluorouracil-based chemotherapy in advanced colorectal cancer patients. International Journal of Cancer, 112(5), 733–737.
- Marsh, S., & Hoskins, J. M. (2010). Irinotecan pharmacogenomics. Pharmacogenomics, 11(7), 1003–1010.
- Marsh, S., McKay, J. A., Cassidy, J., & McLeod, H. L. (2001). Polymorphism in the thymidylate synthase promoter enhancer region in colorectal cancer. International Journal of Oncology, 19(2), 383–386.
- Martin, P., Shiau, C. J., Pasic, M., Tsao, M., Kamel-Reid, S., Lin, S., ... & Leighl, N. B. (2016). Clinical impact of mutation fraction in epidermal growth factor receptor mutation positive NSCLC patients. British Journal of Cancer, 114(6), 616-622.
- Martinho, N., Santos, T. C. B., Florindo, H. F., & Silva, L. C. (2019). Cisplatin-membrane interactions and their influence on platinum complexes activity and toxicity. Frontiers in Physiology, 9, 1898.
- Masereeuw, R., & Russel, F. G. M. (2012). Regulatory pathways for ATP-binding cassette

transport proteins in kidney proximal tubules. The AAPS Journal, 14(4), 883–894.

- Massion, P. P., & Carbone, D. P. (2003). The molecular basis of lung cancer: molecular abnormalities and therapeutic implications. Respiratory research, 4(1), 1-15.
- Matherly, L. H., & Hou, Z. (2008). Structure and function of the reduced folate carrier: a paradigm of a major facilitator superfamily mammalian nutrient transporter. Vitamins & Hormones, 79, 145–184.
- Matherly, L. H., Hou, Z., & Deng, Y. (2007). Human reduced folate carrier: translation of basic biology to cancer etiology and therapy. Cancer and Metastasis Reviews, 26(1), 111–128.
- Matherly, L. H., Wilson, M. R., & Hou, Z. (2014). The major facilitative folate transporters solute carrier 19A1 and solute carrier 46A1: biology and role in antifolate chemotherapy of cancer. Drug Metabolism and Disposition, 42(4), 632–649.
- Mathur, P., Sathishkumar, K., Chaturvedi, M., Das, P., Sudarshan, K. L., Santhappan, S., … Roselind, F. S. (2020). Cancer statistics, 2020: report from national cancer registry programme, India. JCO Global Oncology, 6, 1063–1075.
- Matias, D., Balça-Silva, J., da Graça, G. C., Wanjiru, C. M., Macharia, L. W., Nascimento, C. P., … Dos Santos, M. F. (2018). Microglia/astrocytes–glioblastoma crosstalk: Crucial molecular mechanisms and microenvironmental factors. Frontiers in Cellular Neuroscience, 12, 235.
- McCarthy, W. J., Meza, R., Jeon, J., & Moolgavkar, S. H. (2012). Chapter 6: Lung cancer in never smokers: epidemiology and risk prediction models. Risk Analysis: An International Journal, 32, S69-S84.
- McGill, M. R. (2016). The past and present of serum aminotransferases and the future of liver injury biomarkers. EXCLI Journal, 15, 817.
- McQuade, R. M., Stojanovska, V., Abalo, R., Bornstein, J. C., & Nurgali, K. (2016). Chemotherapy-induced constipation and diarrhea: pathophysiology, current and emerging treatments. Frontiers in Pharmacology, 7, 414.
- Meier-Abt, F., Mokrab, Y., & Mizuguchi, K. (2006). Organic anion transporting polypeptides of the OATP/SLCO superfamily: identification of new members in nonmammalian species, comparative modeling and a potential transport mode. The Journal of Membrane Biology, 208(3), 213–227.
- Meijer, O. C., Karssen, A. M., & De Kloet, E. R. (2003). Cell-and tIssue-specific effects of corticosteroids in relation to glucocorticoid resistance: examples from the brain. The Journal of Endocrinology, 178(1), 13–18.
- Meyer zu Schwabedissen, H. E., Böttcher, K., Chaudhry, A., Kroemer, H. K., Schuetz, E. G., & Kim, R. B. (2010). Liver X receptor α and farnesoid X receptor are major transcriptional regulators of OATP1B1. Hepatology, 52(5), 1797–1807.
- Michalska, K., Balcerczak, E., Pietrzak, J., & Żebrowska-Nawrocka, M. (2021). Effects of the SLCO1B1 A388G single nucleotide polymorphism on the development, clinical parameters and survival of multiple myeloma cases in a Polish population.
- Mishra, D. K., Chen, Z., Wu, Y., Sarkissyan, M., Koeffler, H. P., & Vadgama, J. V. (2010). Global Methylation Pattern of Genes in Androgen-Sensitive and Androgen-Independent Prostate

Cancer CellsPromoter Methylation Array in Prostate Cancer. Molecular Cancer Therapeutics, 9(1), 33–45.

- Mishra, G. A., Pimple, S. A., & Shastri, S. S. (2012). An overview of the tobacco problem in India. Indian Journal of Medical and Paediatric Oncology, 33(03), 139-145.
- Mitsudomi, T., Hamajima, N., Ogawa, M., & Takahashi, T. (2000). Prognostic significance of p53 alterations in patients with non-small cell lung cancer: a meta-analysis. Clinical Cancer Research, 6(10), 4055-4063.
- Mitsuuchi, Y., & Testa, J. R. (2002). Cytogenetics and molecular genetics of lung cancer. American journal of medical genetics, 115(3), 183-188.
- Mo, W., & Zhang, J.-T. (2012). Human ABCG2: structure, function, and its role in multidrug resistance. International Journal of Biochemistry and Molecular Biology, 3(1), 1.
- Mochida, Y., Taguchi, K., Taniguchi, S., Tsuneyoshi, M., Kuwano, H., Tsuzuki, T., … Wada, M. (2003). The role of P-glycoprotein in intestinal tumorigenesis: disruption of mdr1a suppresses polyp formation in Apc Min/+ mice. Carcinogenesis, 24(7), 1219–1224.
- Mohammad, I. S., He, W., & Yin, L. (2018). Understanding of human ATP binding cassette superfamily and novel multidrug resistance modulators to overcome MDR. Biomedicine & Pharmacotherapy, 100, 335–348.
- Mohelnikova-Duchonova, B., Brynychova, V., Oliverius, M., Honsova, E., Kala, Z., Muckova, K., & Soucek, P. (2013). Differences in transcript levels of ABC transporters between pancreatic adenocarcinoma and nonneoplastic tissues. Pancreas, 42(4), 707–716.
- Moini, J., Avgeropoulos, N. G., & Badolato, C. (2022). Global Epidemiology of Cancer: Diagnosis and Treatment. John Wiley & Sons.
- Moitra, K., Lou, H., & Dean, M. (2011). Multidrug efflux pumps and cancer stem cells: insights into multidrug resistance and therapeutic development. Clinical Pharmacology & Therapeutics, 89(4), 491–502.
- Mollaoglu, G., Guthrie, M. R., Böhm, S., Brägelmann, J., Can, I., Ballieu, P. M., ... & Oliver, T. G. (2017). MYC drives progression of small cell lung cancer to a variant neuroendocrine subtype with vulnerability to aurora kinase inhibition. Cancer cell, 31(2), 270-285.
- Monosik, R., Stredanský, M., & Sturdik, E. (2012). Biosensors-classification, characterization and new trends. Acta chimica slovaca, 5(1), 109.
- Moro, H., Hattori, N., Nakamura, Y., Kimura, K., Imai, T., Maeda, M., … Ushijima, T. (2020). Epigenetic priming sensitizes gastric cancer cells to irinotecan and cisplatin by restoring multiple pathways. Gastric Cancer, 23(1), 105–115.
- Müller, P. J., Dally, H., Klappenecker, C. N., Edler, L., Jäger, B., Gerst, M., … Drings, P. (2009). Polymorphisms in ABCG2, ABCC3 and CNT1 genes and their possible impact on chemotherapy outcome of lung cancer patients. International Journal of Cancer, 124(7), 1669–1674.
- Muredda, M., Nunoya, K.-I., Burtch-Wright, R. A., Kurz, E. U., Cole, S. P. C., & Deeley, R. G. (2003). Cloning and characterization of the murine and rat mrp1 promoter regions. Molecular Pharmacology, 64(5), 1259–1269.
- Muriithi, W., Macharia, L. W., Heming, C. P., Echevarria, J. L., Nyachieo, A., Niemeyer Filho,

P., & Neto, V. M. (2020). ABC transporters and the hallmarks of cancer: roles in cancer aggressiveness beyond multidrug resistance. Cancer Biology & Medicine, 17(2), 253.

- Murray, M., & Zhou, F. (2017). Trafficking and other regulatory mechanisms for organic anion transporting polypeptides and organic anion transporters that modulate cellular drug and xenobiotic influx and that are dysregulated in disease. British Journal of Pharmacology, 174(13), 1908–1924.
- Mustafa, M., Azizi, A. J., IIIzam, E., Nazirah, A., Sharifa, S., & Abbas, S. (2016). Lung cancer: risk factors, management, and prognosis. IOSR Journal of Dental and Medical Sciences, 15(10), 94-101.
- Muto, M., Onogawa, T., Suzuki, T., Ishida, T., Rikiyama, T., Katayose, Y., … Unno, M. (2007). Human liver-specific organic anion transporter-2 is a potent prognostic factor for human breast carcinoma. Cancer Science, 98(10), 1570–1576.
- Muz, B., de la Puente, P., Azab, F., &Azab, A. K. (2015). The role of hypoxia in cancer progression, angiogenesis, metastasis, and resistance to therapy. Hypoxia, 3, 83.
- Nagy A, Szalai R, Magyari L, Bene J, Toth K,Melegh B. Extreme differences in SLCO1B3 functional polymorphisms in Roma and Hungarian populations.EnvironToxicolPharmacol. 39(3),1246-1251 (2015).
- Nakanishi, T. (2007). Drug transporters as targets for cancer chemotherapy. Cancer Genomics & Proteomics, 4(3), 241–254.
- Namisaki, T., Schaeffeler, E., Fukui, H., Yoshiji, H., Nakajima, Y., Fritz, P., … Nies, A. T. (2014). Differential expression of drug uptake and efflux transporters in Japanese patients with hepatocellular carcinoma. Drug Metabolism and Disposition, 42(12), 2033–2040.
- Narumiya, K., Metzger, R., Bollschweiler, E., Alakus, H., Brabender, J., Drebber, U., … Warnecke-Eberz, U. (2011). Impact of ABCB1 C3435T polymorphism on lymph node regression in multimodality treatment of locally advanced esophageal cancer. Pharmacogenomics, 12(2), 205–214.
- Nedeljković, M., Tanić, N., Prvanović, M., Milovanović, Z., & Tanić, N. (2021). Friend or foe: ABCG2, ABCC1 and ABCB1 expression in triple-negative breast cancer. Breast Cancer, 28(3), 727–736.
- New cancer cases in India by 2020 (2015). (http://icmr.nic.in/icmrsql/archive/2016/7.pdf)
- Ni, W., Ji, J., Dai, Z., Papp, A., Johnson, A. J., Ahn, S., … Li, X. (2010). Flavopiridol pharmacogenetics: clinical and functional evidence for the role of SLCO1B1/OATP1B1 in flavopiridol disposition. PLoS One, 5(11), e13792.
- Ni, Y., Yin, G., Xiao, Z., Fan, L., Wang, L., Wu, Y., … Li, J. (2016). MDR1 polymorphisms have an impact on the prognosis of Chinese diffuse large B cell lymphoma patients. Tumor Biology, 37(1), 1237–1244.
- Nicholson, S. A., Beasley, M. B., Brambilla, E., Hasleton, P. S., Colby, T. V, Sheppard, M. N., … Travis, W. D. (2002). Small cell lung carcinoma (SCLC): a clinicopathologic study of 100 cases with surgical specimens. The American Journal of Surgical Pathology, 26(9), 1184–1197.
- Niemi, M. (2007). Role of OATP transporters in the disposition of drugs.

- Niemi, M., Pasanen, M. K., & Neuvonen, P. J. (2011). Organic anion transporting polypeptide 1B1: a genetically polymorphic transporter of major importance for hepatic drug uptake. Pharmacological Reviews, 63(1), 157–181.
- Nies, A. T., & Keppler, D. (2007). The apical conjugate efflux pump ABCC2 (MRP2). Pflügers Archiv-European Journal of Physiology, 453(5), 643–659.
- Nishimura, M., & Naito, S. (2005). Tissue-specific mRNA expression profiles of human ATP-binding cassette and solute carrier transporter superfamilies. Drug Metabolism and Pharmacokinetics, 20(6), 452–477.
- Nooter, K., Bosman, F. T., Burger, H., Van Wingerden, K. E., Flens, M. J., Scheper, R. J., … Stoter, G. (1996). Expression of the multidrug resistance-associated protein (MRP) gene in primary non-small-cell lung cancer. Annals of Oncology, 7(1), 75–81.
- Noronha, V., Pinninti, R., Patil, V. M., Joshi, A., & Prabhash, K. (2016). Lung cancer in the Indian subcontinent. South Asian journal of cancer, 5(03), 095-103.
- Nunez, M. I., Behrens, C., Woods, D. M., Lin, H., Suraokar, M., Kadara, H., … Franklin, W. (2012). High expression of folate receptor alpha in lung cancer correlates with adenocarcinoma histology and mutation. Journal of Thoracic Oncology, 7(5), 833–840.
- Nyquist, M. D., Prasad, B., & Mostaghel, E. A. (2017). Harnessing solute carrier transporters for precision oncology. Molecules, 22(4), 539.
- Obata, H., Yahata, T., Quan, J., Sekine, M., & Tanaka, K. (2006). Association between single nucleotide polymorphisms of drug resistance-associated genes and response to chemotherapy in advanced ovarian cancer. Anticancer Research, 26(3B), 2227–2232.
- Oizumi, H., Kato, H., Endoh, M., Inoue, T., Watarai, H., & Sadahiro, M. (2014). Techniques to define segmental anatomy during segmentectomy. Annals of Cardiothoracic Surgery, 3(2), 170.
- Ojima, I., Lichtenthal, B., Lee, S., Wang, C., & Wang, X. (2016). Taxane anticancer agents: a patent perspective. Expert Opinion on Therapeutic Patents, 26(1), 1–20.
- Oladipupo, O. A., Dutta, D., & Chong, N. S. (2019). Analysis of chemical constituents in mainstream bidi smoke. BMC Chemistry, 13(1), 1–11.
- Olaussen, K. A., Dunant, A., Fouret, P., Brambilla, E., André, F., Haddad, V., … Popper, H. H. (2006). DNA repair by ERCC1 in non–small-cell lung cancer and cisplatin-based adjuvant chemotherapy. New England Journal of Medicine, 355(10), 983–991.
- Ongaro, A., De Mattei, M., Della Porta, M. G., Rigolin, G., Ambrosio, C., Di Raimondo, F., … Catozzi, L. (2009). Gene polymorphisms in folate metabolizing enzymes in adult acute lymphoblastic leukemia: effects on methotrexate-related toxicity and survival. Haematologica, 94(10), 1391.
- Onoi, K., Chihara, Y., Uchino, J., Shimamoto, T., Morimoto, Y., Iwasaku, M., … Takayama, K. (2020). Immune checkpoint inhibitors for lung cancer treatment: a review. Journal of Clinical Medicine, 9(5), 1362.
- Ooyama, A., Okayama, Y., Takechi, T., Sugimoto, Y., Oka, T., & Fukushima, M. (2007). Genome-wide screening of loci associated with drug resistance to 5-fluorouracil-based drugs. Cancer Science, 98(4), 577–583.

- Orlando, B. J., & Liao, M. (2020). ABCG2 transports anticancer drugs via a closed-to-open switch. Nature Communications, 11(1), 1–11.
- Oshika, Y., Nakamura, M., Tokunaga, T., Fukushima, Y., Abe, Y., Ozeki, Y., … Ueyama, Y. (1998). Multidrug resistance-associated protein and mutant p53 protein expression in non-small cell lung cancer. Modern Pathology: An Official Journal of the United States and Canadian Academy of Pathology, Inc, 11(11), 1059–1063.
- Owen, S. A., Hider, S. L., Martin, P., Bruce, I. N., Barton, A., & Thomson, W. (2013). Genetic polymorphisms in key methotrexate pathway genes are associated with response to treatment in rheumatoid arthritis patients. The Pharmacogenomics Journal, 13(3), 227–234.
- Paik, P. K., Arcila, M. E., Fara, M., Sima, C. S., Miller, V. A., Kris, M. G., ... & Riely, G. J. (2011). Clinical characteristics of patients with lung adenocarcinomas harboring BRAF mutations. Journal of clinical oncology, 29(15), 2046.
- Pan, J., Han, J., Wu, J., Sheng, L., Huang, H., & Yu, Q. (2008). MDR1 single nucleotide polymorphisms predict response to vinorelbine-based chemotherapy in patients with non-small cell lung cancer. Respiration, 75(4), 380–385.
- Panczyk M, Balcerczak E, Piaskowski S, et al. ABCB1 gene polymorphisms and haplotype analysis in colorectal cancer. Int. J. Colorectal Dis. 2009;24(8):895-905.
- Pang, K. S., Rodrigues, A. D., & Peter, R. M. (2010). Enzyme-and Transporter-Based Drug-Drug Interactions. New York: Springer, 2010, 301–307.
- Panov, S. Z. (2005). Molecular biology of the lung cancer. Radiology and Oncology, 39(3).
- Patel, P. R., & De Jesus, O. (2022). CT Scan. In StatPearls [Internet]. StatPearls Publishing.
- Patriquin, L., Merrick, D. T., Hill, D., Holcomb, R. G., Lemieux, M. E., Bennett, G., ... & Bauer II, T. (2015). Early detection of lung cancer with meso tetra (4-carboxyphenyl) porphyrin-labeled sputum. Journal of Thoracic Oncology, 10(9), 1311-1318.
- Patwa, A., & Shah, A. (2015). Anatomy and physiology of respiratory system relevant to anaesthesia. Indian Journal of Anaesthesia, 59(9), 533.
- Pavelić, J., Pavelić, L., Karadža, J., Križanac, Š., Unešić, J., Spaventi, Š., & Pavelić, K. (2002). Insulin-like growth factor family and combined antisense approach in therapy of lung carcinoma. Molecular Medicine, 8(3), 149-157.
- Peng, Y., Zhong, H., Xu, Z., Tu, H., Li, X., & Peng, L. (2021). Pulmonary Lobe Segmentation in CT Images Based on Lung Anatomy Knowledge. Mathematical Problems in Engineering, 2021. https://doi.org/10.1155/2021/5588629
- Pérez-Ramírez, C., Cañadas-Garre, M., Alnatsha, A., Villar, E., Delgado, J. R., Faus-Dáder, M. J., & ÿngel Calleja-Hernández, M. (2016). Pharmacogenetic predictors of toxicity to platinum based chemotherapy in non-small cell lung cancer patients. Pharmacological Research, 111, 877–884.
- Pérez-Ramírez, C., Cañadas-Garre, M., Alnatsha, A., Villar, E., Valdivia-Bautista, J., Faus-Dáder, M. J., & Calleja-Hernández, M. Á. (2019). Pharmacogenetics of platinum-based chemotherapy: impact of DNA repair and folate metabolism gene polymorphisms on prognosis of non-small cell lung cancer patients. The Pharmacogenomics Journal, 19(2), 164–177.

- Petrus, P., Bialesova, L., Checa, A., Kerr, A., Naz, S., Bäckdahl, J., … Hedén, P. (2018). Adipocyte expression of SLC19A1 links DNA hypermethylation to adipose tissue inflammation and insulin resistance. The Journal of Clinical Endocrinology & Metabolism, 103(2), 710–721.
- Pfeifer, G. P., & Besaratinia, A. (2009). Mutational spectra of human cancer. Human genetics, 125(5), 493-506.
- Pienta, K. J. (2001). Preclinical mechanisms of action of docetaxel and docetaxel combinations in prostate cancer. Seminars in Oncology, 28, 3–7. Elsevier.
- Pillot, G. A., Read, W. L., Hennenfent, K. L., Marsh, S., Gao, F., Viswanathan, A., … Govindan, R. (2006). A phase II study of irinotecan and carboplatin in advanced non-small cell lung cancer with pharmacogenomic analysis. Journal of Thoracic Oncology, 1(9), 972–978.
- Plaza-Plaza, J. C., Aguilera, M., Cañadas-Garre, M., Chemello, C., González-Utrilla, A., Faus Dader, M. J., & Calleja, M. A. (2012). Pharmacogenetic polymorphisms contributing to toxicity induced by methotrexate in the southern Spanish population with rheumatoid arthritis. Omics: A Journal of Integrative Biology, 16(11), 589–595.
- Poku, V. O., & Iram, S. H. (2022). A critical review on modulators of Multidrug Resistance Protein 1 in cancer cells. PeerJ, 10, e12594.
- Polgar, O., Robey, R. W., Morisaki, K., Dean, M., Michejda, C., Sauna, Z. E., … Bates, S. E. (2004). Mutational analysis of ABCG2: role of the GXXXG motif. Biochemistry, 43(29), 9448–9456.
- Popat, S., Matakidou, A., & Houlston, R. S. (2004). Thymidylate synthase expression and prognosis in colorectal cancer: a systematic review and meta-analysis. Journal of Clinical Oncology, 22(3), 529–536.
- Pozzi, C., Ferrari, S., Luciani, R., Tassone, G., Costi, M. P., & Mangani, S. (2019). Structural comparison of enterococcus faecalis and human thymidylate synthase complexes with the substrate dUMP and its analogue FdUMP provides hints about enzyme conformational variabilities. Molecules, 24(7), 1257.
- Prabhakar, B., Shende, P., & Augustine, S. (2018). Current trends and emerging diagnostic techniques for lung cancer. Biomedicine & Pharmacotherapy, 106, 1586-1599.
- Pressler, H., Sissung, T. M., Venzon, D., Price, D. K., & Figg, W. D. (2011). Expression of OATP family members in hormone-related cancers: potential markers of progression. PloS One, 6(5), e20372.
- Pu, Z., Zhang, X., Chen, Q., Yuan, X., & Xie, H. (2015). Establishment of an expression platform of OATP1B1 388GG and 521CC genetic polymorphism and the therapeutic effect of tamoxifen in MCF-7 cells. Oncology Reports, 33(5), 2420–2428.
- Pullarkat, S. T., Stoehlmacher, J., Ghaderi, V., Xiong, Y. P., Ingles, S. A., Sherrod, A., … Lenz, H. J. (2001). Thymidylate synthase gene polymorphism determines response and toxicity of 5-FU chemotherapy. The Pharmacogenomics Journal, 1(1), 65–70.
- Pylkkanen, L., Wolff, H., Stjernvall, T., Tuominen, P., Sioris, T., Karjalainen, A., ... & Husgafvel-Pursiainen, K. (2002). Reduced Fhit protein expression and loss of heterozygosity at

FHIT gene in tumours from smoking and asbestos-exposed lung cancer patients. International journal of oncology, 20(2), 285-290.

- Qian, C., Zheng, Y., Wang, Y., Chen, J., Liu, J., Zhou, H., … Liu, Z. (2016). Associations of genetic polymorphisms of the transporters organic cation transporter 2 (OCT2), multidrug and toxin extrusion 1 (MATE1), and ATP-binding cassette subfamily C member 2 (ABCC2) with platinum-based chemotherapy response and toxicity in non-sma. Cancer Communications, 35(1), 1–13.
- Qian, Y., Chai, S., Liang, Z., Wang, Y., Zhou, Y., Xu, X., ... & Wang, K. (2014). KIF5B-RET fusion kinase promotes cell growth by multilevel activation of STAT3 in lung cancer. Molecular cancer, 13(1), 1-11
- Qiao, R., Wu, W., Lu, D., & Han, B. (2016). Influence of single nucleotide polymorphisms in ABCB1, ABCG2 and ABCC2 on clinical outcomes to paclitaxel-platinum chemotherapy in patients with non-small-cell lung cancer. Int J Clin Exp Med, 9(1), 298–307.
- Rady, P. L., Szucs, S., Matalon, R. K., Grady, J., Hudnall, S. D., Kellner, L. H., & Nitowsky, H. (2001). Genetic polymorphism (G80A) of reduced folate carrier gene in ethnic populations. Molecular Genetics and Metabolism, 73(3), 285–286.
- Rajgopal, A., Sierra, E. E., Zhao, R., & Goldman, I. D. (2001). Expression of the reduced folate carrier SLC19A1 in IEC-6 cells results in two distinct transport activities. American Journal of Physiology-Cell Physiology, 281(5), C1579–C1586.
- Ramesh, R., Saeki, T., Templeton, N. S., Ji, L., Stephens, L. C., Ito, I., ... & Roth, J. A. (2001). Successful treatment of primary and disseminated human lung cancers by systemic delivery of tumor suppressor genes using an improved liposome vector. Molecular Therapy, 3(3), 337-350.
- Rami-Porta, R., Crowley, J. J., & Goldstraw, P. (2009). Review the revised TNM staging system for lung cancer. Ann Thorac Cardiovasc Surg, 15(1), 5.
- Ramsey, L. B., Johnson, S. G., Caudle, K. E., Haidar, C. E., Voora, D., Wilke, R. A., … Roden, D. M. (2014). The clinical pharmacogenetics implementation consortium guideline for SLCO1B1 and simvastatin-induced myopathy: 2014 update. Clinical Pharmacology & Therapeutics, 96(4), 423–428.
- Ray, S., Das, S., & Suar, M. (2017). Molecular mechanism of drug resistance. In Drug Resistance in Bacteria, Fungi, Malaria, and Cancer (pp. 47–110). Springer.
- Reddy, K. S. and Gupta, P. C. (2004). Report on Tobacco Control in India (New Delhi, India) New Delhi, India: Ministry of Health and Family Welfare.
- Rees, D. C., Johnson, E., & Lewinson, O. (2009). ABC transporters: the power to change. Nature Reviews Molecular Cell Biology, 10(3), 218–227.
- Righi, L., Papotti, M. G., Ceppi, P., Billè, A., Bacillo, E., Molinaro, L., … Selvaggi, G. (2010). Thymidylate synthase but not excision repair cross-complementation group 1 tumor expression predicts outcome in patients with malignant pleural mesothelioma treated with pemetrexed-based chemotherapy. Journal of Clinical Oncology, 28(9), 1534–1539.

- Rikova, K., Guo, A., Zeng, Q., Possemato, A., Yu, J., Haack, H., ... & Comb, M. J. (2007). Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. Cell, 131(6), 1190-1203.
- Rizzo, R., Spaggiari, F., Indelli, M., Lelli, G., Baricordi, O. R., Rimessi, P., & Ferlini, A. (2010). Association of CYP1B1 with hypersensitivity induced by taxane therapy in breast cancer patients. Breast Cancer Research and Treatment, 124(2), 593–598.
- Robert, C. (2020). A decade of immune-checkpoint inhibitors in cancer therapy. Nature Communications, 11(1), 1–3.
- Robey, R. W., Pluchino, K. M., Hall, M. D., Fojo, A. T., Bates, S. E., & Gottesman, M. M. (2018). Revisiting the role of ABC transporters in multidrug-resistant cancer. Nature Reviews Cancer, 18(7), 452–464.
- Robien, K. (2005). Folate during antifolate chemotherapy: what we know… and do not know. Nutrition in Clinical Practice, 20(4), 411–422.
- Rodenhuis, S., Boerrigter, L., Top, B., Slebos, R. J., Mooi, W. J., van't Veer, L., & van Zandwijk, N. (1997). Mutational activation of the K-ras oncogene and the effect of chemotherapy in advanced adenocarcinoma of the lung: a prospective study. Journal of clinical oncology, 15(1), 285-291.
- Rose, M. G., Farrell, M. P., & Schmitz, J. C. (2002). Thymidylate synthase: a critical target for cancer chemotherapy. Clinical Colorectal Cancer, 1(4), 220–229.
- Rota, M., Bosetti, C., Boccia, S., Boffetta, P., & La Vecchia, C. (2014). Occupational exposures to polycyclic aromatic hydrocarbons and respiratory and urinary tract cancers: an updated systematic review and a meta-analysis to 2014. Archives of toxicology, 88(8), 1479-1490.
- Rubin, B. P., Skarin, A. T., Pisick, E., Rizk, M., & Salgia, R. (2001). Use of cytokeratins 7 and 20 in determining the origin of metastatic carcinoma of unknown primary, with special emphasis on lung cancer. European Journal of Cancer Prevention, 77–82.
- Rudas, M., Filipits, M., Taucher, S., Stranzl, T., Steger, G. G., Jakesz, R., … Pohl, G. (2003). Expression of MRP1, LRP and Pgp in breast carcinoma patients treated with preoperative chemotherapy. Breast Cancer Research and Treatment, 81(2), 149–157.
- Rustemoglu A, Gumus-Akay G, Karakus N, et al. Association analysis of three ABCB1 (MDR1) gene variants (C1236T, G2677A/T and C3435T) and their genotype/haplotype combinations with the familial Mediterranean fever. Xenobiotica. 2014;44(10):933-940.
- Said, H. M., & Nexo, E. (2012). Mechanisms and regulation of intestinal absorption of water-soluble vitamins: cellular and molecular aspects. In Physiology of the Gastrointestinal Tract (pp. 1711–1756). Elsevier.
- Sakoff, J. A., & Ackland, S. P. (2000). Thymidylate synthase inhibition induces S-phase arrest, biphasic mitochondrial alterations and caspase-dependent apoptosis in leukaemia cells. Cancer Chemotherapy and Pharmacology, 46(6), 477–487.
- Salgia, R. (2011). Prognostic significance of angiogenesis and angiogenic growth factors in nonsmall cell lung cancer. Cancer, 117(17), 3889-3899.
- Salvador-Martín, S., García-González, X., García, M. I., Blanco, C., García-Alfonso, P., Robles,

L., … Martínez, V. (2018). Clinical utility of ABCB1 genotyping for preventing toxicity in treatment with irinotecan. Pharmacological Research, 136, 133–139.

- Sancho, V., Di Florio, A., W Moody, T., & T Jensen, R. (2011). Bombesin receptor-mediated imaging and cytotoxicity: review and current status. Current drug delivery, 8(1), 79-134.
- Sandoval, P., & Hagenbuch, B. (2021). Drug Transport—Uptake.
- Sandusky, G. E., Mintze, K. S., Pratt, S. E., & Dantzig, A. H. (2002). Expression of multidrug resistance-associated protein 2 (MRP2) in normal human tissues and carcinomas using tissue microarrays. Histopathology, 41(1), 65–74.
- Sarankó, H., Tordai, H., Telbisz, Á., Özvegy-Laczka, C., Erdős, G., Sarkadi, B., & Hegedűs, T. (2013). Effects of the gout-causing Q141K polymorphism and a CFTR ΔF508 mimicking mutation on the processing and stability of the ABCG2 protein. Biochemical and Biophysical Research Communications, 437(1), 140–145.
- Sato, M., Shay, J. W., & Minna, J. D. (2020). Immortalized normal human lung epithelial cell models for studying lung cancer biology. Respiratory Investigation, 58(5), 344-354.
- Scagliotti, G. V., Selvaggi, G., Novello, S., & Hirsch, F. R. (2004). The biology of epidermal growth factor receptor in lung cancer. Clinical Cancer Research, 10(12), 4227s-4232s.
- Schaich, M., Kestel, L., Pfirrmann, M., Robel, K., Illmer, T., Kramer, M., … Krex, D. (2009). A MDR1 (ABCB1) gene single nucleotide polymorphism predicts outcome of temozolomide treatment in glioblastoma patients. Annals of Oncology, 20(1), 175–181.
- Schaller, L., & Lauschke, V. M. (2019). The genetic landscape of the human solute carrier (SLC) transporter superfamily. Human Genetics, 138(11), 1359–1377.
- Schaub, T. P., Kartenbeck, Jür., KÖNIG, J., Spring, H., DÖRsam, J., Staehler, G., … Keppler, D. (1999). Expression of the MRP2 gene-encoded conjugate export pump in human kidney proximal tubules and in renal cell carcinoma. Journal of the American Society of Nephrology, 10(6), 1159–1169.
- Schinkel, A. H., & Jonker, J. W. (2012). Mammalian drug efflux transporters of the ATP binding cassette (ABC) family: an overview. Advanced Drug Delivery Reviews, 64, 138–153.
- Schramm, F., Schaefer, L., & Wygrecka, M. (2022). EGFR Signaling in Lung Fibrosis. Cells, 11(6), 986.
- Schreurs, W. H., Odink, J., Egger, R. J., Wedel, M., & Bruning, P. F. (1985). The influence of radiotherapy and chemotherapy on the vitamin status of cancer patients. International Journal for Vitamin and Nutrition Research. Internationale Zeitschrift Fur Vitamin-Und Ernahrungsforschung. Journal International de Vitaminologie et de Nutrition, 55(4), 425–432.
- Schulte, R. R., & Ho, R. H. (2019). Organic anion transporting polypeptides: emerging roles in cancer pharmacology. Molecular Pharmacology, 95(5), 490–506.
- Schumann, T., König, J., Henke, C., Willmes, D. M., Bornstein, S. R., Jordan, J., … Birkenfeld, A. L. (2020). Solute carrier transporters as potential targets for the treatment of metabolic disease. Pharmacological Reviews, 72(1), 343–379.
- Scotto, K. W. (2003). Transcriptional regulation of ABC drug transporters. Oncogene, 22(47), 7496–7511.

- Seborova, K., Vaclavikova, R., Soucek, P., Elsnerova, K., Bartakova, A., Cernaj, P., … Dvorak, P. (2019). Association of ABC gene profiles with time to progression and resistance in ovarian cancer revealed by bioinformatics analyses. Cancer Medicine, 8(2), 606–616.
- Sekido, Y., Fong, K. M., & Minna, J. D. (2003). Molecular genetics of lung cancer. Annual review of medicine, 54(1), 73-87.
- Sekido, Y., Fong, K., & Minna, J. (2001). Cancer-principles and practice of oncology. sl Vol. 1.
- Sekine, I., Yamamoto, N., Nishio, K., & Saijo, N. (2008). Emerging ethnic differences in lung cancer therapy. British Journal of Cancer, 99(11), 1757–1762.
- Seung, A. H. (2013). Adverse effects of chemotherapy and targeted agents. Applied Therapeutics: The Clinical Use of Drugs. 10rd Ed. Wolters Kluwer: Lippincott Williams & Wilkins, 2109–2142.
- Shah, F., Cancer, G., Vajaria, B., Diagnostics, Q., Patel, K., Cancer, T. G., … Cancer, G. (2011). A Review on Salivary Genomics and Proteomics Biomarkers in Oral Cancer Franky D . Shah , Rasheedunnisa Begum ,. (June 2018). https://doi.org/10.1007/s12291-011-0149-8
- Shah, S. A., Jameeullah, U. F., Jamsheed, M., & Saifullah, A. A. (2021). Amplification of ABCG2 Transporter Gene Exon 8 and 14 of Diabetic Patients. J Clin Med Res, 3(3), 1–15.
- Sharma, P., & Sharma, S. (2022). In silico screening and analysis of single-nucleotide polymorphic variants of the ABCC2 gene affecting Dubin–Johnson syndrome. Arab Journal of Gastroenterology.
- Sharma, P., Singh, N., & Sharma, S. (2021). ATP binding cassette transporters and cancer: revisiting their controversial role. Pharmacogenomics, 22(18), 1211–1235.
- Sharma, P., Singh, N., & Sharma, S. (2022). Polymorphism in Thymidylate Synthase Gene Predicts Survival and Toxicity in North Indian Lung Cancer Patients Undergoing Platinum-Based Doublet Chemotherapy. Oncology.
- Shen, B., Dong, P., Li, D., & Gao, S. (2011). Expression and function of ABCG2 in head and neck squamous cell carcinoma and cell lines. Experimental and Therapeutic Medicine, 2(6), 1151–1157.
- Shen, L. X., Basilion, J. P., & Stanton Jr, V. P. (1999). Single-nucleotide polymorphisms can cause different structural folds of mRNA. Proceedings of the National Academy of Sciences, 96(14), 7871–7876.
- Sherr, C. J. (2000). The Pezcoller lecture: cancer cell cycles revisited. Cancer research, 60(14), 3689-3695.
- Shi, L., Wang, L., Hou, J., Zhu, B., Min, Z., Zhang, M., ... & Wang, X. (2015). Targeting roles of inflammatory microenvironment in lung cancer and metastasis. Cancer and Metastasis Reviews, 34(2), 319-331.
- Shi, Q., Zhang, Z., Neumann, A. S., Li, G., Spitz, M. R., & Wei, Q. (2005). Case–control analysis of thymidylate synthase polymorphisms and risk of lung cancer. Carcinogenesis, 26(3), 649-656.
- Shibuya, M. (2011). Vascular endothelial growth factor (VEGF) and its receptor (VEGFR) signaling in angiogenesis: a crucial target for anti-and pro-angiogenic therapies. Genes & cancer, 2(12), 1097-1105.

- Shimizu, T., Nakanishi, Y., Nakagawa, Y., Tsujino, I., Takahashi, N., Nemoto, N., & Hashimoto, S. (2012). Association between expression of thymidylate synthase, dihydrofolate reductase, and glycinamide ribonucleotide formyltransferase and efficacy of pemetrexed in advanced non-small cell lung cancer. Anticancer Research, 32(10), 4589–4596.
- Shintani, Y., Ohta, M., Hirabayashi, H., Tanaka, H., Iuchi, K., Nakagawa, K., … Matsuda, H. (2003). New prognostic indicator for non-small-cell lung cancer, quantitation of thymidylate synthase by real-time reverse transcription polymerase chain reaction. International Journal of Cancer, 104(6), 790–795.
- Shitara, Y., Maeda, K., Ikejiri, K., Yoshida, K., Horie, T., & Sugiyama, Y. (2013). Clinical significance of organic anion transporting polypeptides (OATPs) in drug disposition: their roles in hepatic clearance and intestinal absorption. Biopharmaceutics & Drug Disposition, 34(1), 45–78.
- Siegel, R. L., Miller, K. D., Fuchs, H. E., & Jemal, A. (2021). Cancer Statistics, 2021. CA: A Cancer Journal for Clinicians, 71(1), 7–33. https://doi.org/10.3322/caac.21654
- Silvers, A. L. (2002). Gene expression analysis of human non-small cell lung cancer subtypes, adenocarcinoma and squamous cell carcinoma, and mouse lung epithelial cell lines: Implications in lung tumorigenesis. Medical College of Ohio.
- Singh, N., & Agrawal, S. (2021). Lung Cancer in India. Journal of Thoracic Oncology, 16(8), 1250–1266. https://doi.org/10.1016/j.jtho.2021.02.004
- Sissung, T. M., Troutman, S. M., Campbell, T. J., Pressler, H. M., Sung, H., Bates, S. E., & Figg, W. D. (2012). Transporter polymorphisms affect normal physiology, diseases, and pharmacotherapy. Discovery Medicine, 13(68), 19.
- Sithanandam, G., Smith, G. T., Masuda, A., Takahashi, T., Anderson, L. M., & Fornwald, L. W. (2003). Cell cycle activation in lung adenocarcinoma cells by the ErbB3/phosphatidylinositol 3-kinase/Akt pathway. Carcinogenesis, 24(10), 1581-1592.
- Słomka, M., Sobalska-Kwapis, M., Korycka-Machała, M., Bartosz, G., Dziadek, J., & Strapagiel, D. (2015). Genetic variation of the ABC transporter gene ABCC1 (Multidrug resistance protein 1–MRP1) in the Polish population. BMC Genetics, 16(1), 1–16.
- Smit, E. F., Burgers, S. A., Biesma, B., Smit, H. J. M., Eppinga, P., Dingemans, A.-M. C., … Van Zandwijk, N. (2009). Randomized phase II and pharmacogenetic study of pemetrexed compared with pemetrexed plus carboplatin in pretreated patients with advanced non–small-cell lung cancer. Journal of Clinical Oncology, 27(12), 2038–2045.
- Smith, E. E., Conseil, G., & Cole, S. P. C. (2021). Conserved amino acids in the region connecting membrane spanning domain 1 to nucleotide binding domain 1 are essential for expression of the MRP1 (ABCC1) transporter. PloS One, 16(2), e0246727.
- Socinski, M. A., Jotte, R. M., Cappuzzo, F., Orlandi, F., Stroyakovskiy, D., Nogami, N., … Barlesi, F. (2018). Atezolizumab for first-line treatment of metastatic nonsquamous NSCLC. New England Journal of Medicine, 378(24), 2288–2301.

- Soda, M., Takada, S., Takeuchi, K., Choi, Y. L., Enomoto, M., Ueno, T., ... & Mano, H. (2008). A mouse model for EML4-ALK-positive lung cancer. Proceedings of the National Academy of Sciences, 105(50), 19893-19897.
- Sohn, J. W., Lee, S. Y., Lee, S. J., Kim, E. J., Cha, S. I., Kim, C. H., … Park, J. Y. (2006). MDR1 polymorphisms predict the response to etoposide–cisplatin combination chemotherapy in small cell lung cancer. Japanese Journal of Clinical Oncology, 36(3), 137–141.
- Sone, K., Oguri, T., Uemura, T., Takeuchi, A., Fukuda, S., Takakuwa, O., … Ohkubo, H. (2019). Genetic variation in the ATP binding cassette transporter ABCC10 is associated with neutropenia for docetaxel in Japanese lung cancer patients cohort. BMC Cancer, 19(1), 1–9.
- Song, G., Chung, J.-E., Yee, J., Lee, K.-E., Park, K., & Gwak, H.-S. (2021). Effects of SLCO1B1 and SLCO1B3 Genetic Polymorphisms on Valsartan Pharmacokinetics in Healthy Korean Volunteers. Journal of Personalized Medicine, 11(9), 862.
- Song, S., Tian, B., Zhang, M., Gao, X., Jie, L., Liu, P., & Li, J. (2021). Diagnostic and prognostic value of thymidylate synthase expression in breast cancer. Clinical and Experimental Pharmacology and Physiology, 48(2), 279–287.
- Song, W., Li, D., Tao, L., Luo, Q., & Chen, L. (2020). Solute carrier transporters: the metabolic gatekeepers of immune cells. Acta Pharmaceutica Sinica B, 10(1), 61–78.
- Søreide, K. (2009). Receiver-operating characteristic curve analysis in diagnostic, prognostic and predictive biomarker research. Journal of clinical pathology, 62(1), 1-5.
- Sprowl, J. A., Gregorc, V., Lazzari, C., Mathijssen, R. H., Loos, W. J., & Sparreboom, A. (2012). Associations between ABCC2 polymorphisms and cisplatin disposition and efficacy. Clinical Pharmacology & Therapeutics, 91(6), 1022–1026.
- Srinivas, N., Rachakonda, S., & Kumar, R. (2020). Telomeres and telomere length: a general overview. Cancers, 12(3), 558.
- Steenland, K., Mannetje, A., Boffetta, P., Stayner, L., Attfield, M., Chen, J., ... & Checkoway, H. (2001). Pooled exposure–response analyses and risk assessment for lung cancer in 10 cohorts of silica-exposed workers: an IARC multicentre study. Cancer Causes & Control, 12(9), 773-784.
- Stöckel, B., König, J., Nies, A. T., Cui, Y., Brom, M., & Keppler, D. (2000). Characterization of the 5′-flanking region of the human multidrug resistance protein 2 (MRP2) gene and its regulation in comparison withthe multidrug resistance protein 3 (MRP3) gene. European Journal of Biochemistry, 267(5), 1347–1358.
- Stolarczyk, E. I., Reiling, C. J., Pickin, K. A., Coppage, R., Knecht, M. R., & Paumi, C. M. (2012). Casein kinase 2α regulates multidrug resistance-associated protein 1 function via phosphorylation of Thr249. Molecular Pharmacology, 82(3), 488–499.
- Strimbu, K., & Tavel, J. A. (2010). What are biomarkers?. Current Opinion in HIV and AIDS, 5(6), 463.
- Sugawara, I., Yamada, H., Nakamura, H., Sumizawa, T., Akiyama, S., & Masunaga, A. (1996). Preferential expression of the multidrug-resistance-associated protein (MRP) in adenocarcinoma of the lung. Lung Cancer, 2(14), 400–401.

- Sugawara, I., Yamada, H., Nakamura, H., Sumizawa, T., Akiyama, S., Masunaga, A., & Itoyama, S. (1995). Preferential expression of the multidurg-resistance-associated protein (MRP) in adenocarcinoma of the lung. International Journal of Cancer, 64(5), 322–325.
- Sukowati, C. H. C., Rosso, N., Pascut, D., Anfuso, B., Torre, G., Francalanci, P., … Tiribelli, C. (2012). Gene and functional up-regulation of the BCRP/ABCG2 transporter in hepatocellular carcinoma. BMC Gastroenterology, 12(1), 1–8.
- Sun, R., Ying, Y., Tang, Z., Liu, T., Shi, F., Li, H., … Lai, R. (2020). The emerging role of the SLCO1B3 protein in cancer resistance. Protein and Peptide Letters, 27(1), 17–29.
- Sun, S., Cai, J., Yang, Q., Zhu, Y., Zhao, S., & Wang, Z. (2016). Prognostic value and implication for chemotherapy treatment of ABCB1 in epithelial ovarian cancer: a meta-analysis. PloS One, 11(11), e0166058.
- Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians, 71(3), 209–249.
- Svoboda, M., Riha, J., Wlcek, K., Jaeger, W., & Thalhammer, T. (2011). Organic anion transporting polypeptides (OATPs): regulation of expression and function. Current Drug Metabolism, 12(2), 139–153.
- Svoboda, M., Wlcek, K., Taferner, B., Hering, S., Stieger, B., Tong, D., … Jäger, W. (2011). Expression of organic anion-transporting polypeptides 1B1 and 1B3 in ovarian cancer cells: relevance for paclitaxel transport. Biomedicine & Pharmacotherapy, 65(6), 417–426.
- Szczyrek, M., Mlak, R., Krawczyk, P., Wojas-Krawczyk, K., Powrózek, T., Szudy-Szczyrek, A., … Milanowski, J. (2017). Polymorphisms of genes encoding multidrug resistance proteins as a predictive factor for second-line docetaxel therapy in advanced non-small cell lung cancer. Pathology & Oncology Research, 23(3), 607–614.
- Takahashi, M. (2022). RET receptor signaling: Function in development, metabolic disease, and cancer. Proceedings of the Japan Academy, Series B, 98(3), 112-125.
- Tamura, A., Wakabayashi, K., Onishi, Y., Takeda, M., Ikegami, Y., Sawada, S., … Ishikawa, T. (2007). Re-evaluation and functional classification of non-synonymous single nucleotide polymorphisms of the human ATP-binding cassette transporter ABCG2. Cancer Science, 98(2), 231–239.
- Tamura, T., Kurishima, K., Nakazawa, K., Kagohashi, K., Ishikawa, H., Satoh, H., & Hizawa, N. (2015). Specific organ metastases and survival in metastatic non-small-cell lung cancer. Molecular and clinical oncology, 3(1), 217-221.
- Tanaka, F., Wada, H., Fukui, Y., & Fukushima, M. (2011). Thymidylate synthase (TS) gene expression in primary lung cancer patients: a large-scale study in Japanese population. Annals of Oncology, 22(8), 1791–1797.
- Tang, D., Kang, R., Berghe, T. V., Vandenabeele, P., & Kroemer, G. (2019). The molecular machinery of regulated cell death. Cell research, 29(5), 347-364.
- Tang, L., Zhang, C., He, H., Pan, Z., Fan, D., He, Y., … Li, Y. (2018). Associations between

ABCG2 gene polymorphisms and gefitinib toxicity in non-small cell lung cancer: a meta-analysis. OncoTargets and Therapy, 11, 665.

- Tang, Y., Hou, J., Li, G., Song, Z., Li, X., Yang, C., … Xu, Y. (2014). ABCG2 regulates the pattern of self-renewing divisions in cisplatin-resistant non-small cell lung cancer cell lines. Oncology Reports, 32(5), 2168–2174.
- Taxak, N., & Bharatam, P. V. (2014). Drug metabolism. Resonance, 19(3), 259–282.
- Taylor, N. M. I., Manolaridis, I., Jackson, S. M., Kowal, J., Stahlberg, H., & Locher, K. P. (2017). Structure of the human multidrug transporter ABCG2. Nature, 546(7659), 504–509.
- Thakkinstian, A., McElduff, P., D'Este, C., Duffy, D., & Attia, J. (2005). A method for meta-analysis of molecular association studies. Statistics in Medicine, 24(9), 1291-1306.
- Thandra, K. C., Barsouk, A., Saginala, K., Aluru, J. S., & Barsouk, A. (2021). Epidemiology of lung cancer. Contemporary Oncology/Współczesna Onkologia, 25(1), 45-52.
- Thawani, R., McLane, M., Beig, N., Ghose, S., Prasanna, P., Velcheti, V., & Madabhushi, A. (2018). Radiomics and radiogenomics in lung cancer: a review for the clinician. Lung cancer, 115, 34-41.
- Theile, D., & Wizgall, P. (2021). Acquired ABC-transporter overexpression in cancer cells: transcriptional induction or Darwinian selection? Naunyn-Schmiedeberg's Archives of Pharmacology, 394(8), 1621–1632.
- Thomas, C., & Tampé, R. (2020). Structural and mechanistic principles of ABC transporters. Annual Review of Biochemistry, 89, 605–636.
- Thomas, C., & Thomas, L. (2009). Renal failure—measuring the glomerular filtration rate. Deutsches Ärzteblatt International, 106(51-52), 849.
- Thomson, M. M. S., Hines, R. N., Schuetz, E. G., & Meibohm, B. (2016). Expression patterns of organic anion transporting polypeptides 1B1 and 1B3 protein in human pediatric liver. Drug Metabolism and Disposition, 44(7), 999–1004.
- Three-Year Report of Population Based Cancer Registries 2012-2014. (March, 2016).
- Tiseo, M., Giovannetti, E., Tibaldi, C., Camerini, A., Di Costanzo, F., Barbieri, F., … Smit, E. F. (2012). Pharmacogenetic study of patients with advanced non-small cell lung cancer (NSCLC) treated with second-line pemetrexed or pemetrexed–carboplatin. Lung Cancer, 78(1), 92–99.
- To, K. K. W., Robey, R. W., Knutsen, T., Zhan, Z., Ried, T., & Bates, S. E. (2009). Escape from hsa-miR-519c enables drug-resistant cells to maintain high expression of ABCG2. Molecular Cancer Therapeutics, 8(10), 2959–2968.
- Travis, W. D., Brambilla, E., Noguchi, M., Nicholson, A. G., Geisinger, K., Yatabe, Y., … Franklin, W. (2013). Diagnosis of lung cancer in small biopsies and cytology: implications of the 2011 International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification. Archives of Pathology and Laboratory Medicine, 137(5), 668–684.
- Treviño, L. R., Shimasaki, N., Yang, W., Panetta, J. C., Cheng, C., Pei, D., … Pui, C.-H. (2009). Germline genetic variation in an organic anion transporter polypeptide associated with methotrexate pharmacokinetics and clinical effects. Journal of Clinical Oncology, 27(35), 5972.

- Triller, N., Korošec, P., Kern, I., Košnik, M., & Debeljak, A. (2006). Multidrug resistance in small cell lung cancer: expression of P-glycoprotein, multidrug resistance protein 1 and lung resistance protein in chemo-naive patients and in relapsed disease. Lung Cancer, 54(2), 235–240.
- Tulsyan, S., Mittal, R. D., & Mittal, B. (2016). The effect of ABCB1 polymorphisms on the outcome of breast cancer treatment. Pharmacogenomics and Personalized Medicine, 9, 47.
- Ujiie, H., Tomida, M., Akiyama, H., Nakajima, Y., Okada, D., Yoshino, N., ... & Tanzawa, H. (2012). Serum hepatocyte growth factor and interleukin-6 are effective prognostic markers for non-small cell lung cancer. Anticancer research, 32(8), 3251-3258.
- Ulrich, C. M., Bigler, J., Velicer, C. M., Greene, E. A., Farin, F. M., & Potter, J. D. (2000). Searching expressed sequence tag databases: discovery and confirmation of a common polymorphism in the thymidylate synthase gene. Cancer Epidemiology Biomarkers & Prevention, 9(12), 1381–1385.
- Ulrich, C. M., Rankin, C., Toriola, A. T., Makar, K. W., Altug-Teber, Ö., Benedetti, J. K., … Lenz, H. (2014). Polymorphisms in folate-metabolizing enzymes and response to 5-fluorouracil among patients with stage II or III rectal cancer (INT-0144; SWOG 9304). Cancer, 120(21), 3329–3337.
- Ulzurrun, E., Stephens, C., Ruiz-Cabello, F., Robles-Diaz, M., Saenz-López, P., Hallal, H., … Lucena, M. I. (2014). Selected ABCB1, ABCB4 and ABCC2 polymorphisms do not enhance the risk of drug-induced hepatotoxicity in a Spanish cohort. PLoS One, 9(4), e94675.
- Ushijima, R., Takayama, K., Izumi, M., Harada, T., Horiuchi, Y., Uchino, J., … Nakanishi, Y. (2007). Immunohistochemical expression of MRP2 and clinical resistance to platinum-based chemotherapy in small cell lung cancer. Anticancer Research, 27(6C), 4351–4358.
- Vaclavikova, R., Nordgard, S. H., Alnaes, G. I. G., Hubackova, M., Kubala, E., Kodet, R., … Kristensen, V. N. (2008). Single nucleotide polymorphisms in the multidrug resistance gene 1 (ABCB1): effects on its expression and clinicopathological characteristics in breast cancer patients. Pharmacogenetics and Genomics, 18(3), 263–273.
- Valles, I., Pajares, M. J., Segura, V., Guruceaga, E., Gomez-Roman, J., Blanco, D., ... & Pio, R. (2012). Identification of novel deregulated RNA metabolism-related genes in non-small cell lung cancer.
- Van der Deen, M., De Vries, E. G. E., Timens, W., Scheper, R. J., Timmer-Bosscha, H., & Postma, D. S. (2005). ATP-binding cassette (ABC) transporters in normal and pathological lung. Respiratory Research, 6(1), 1–16.
- Van Eerden, R. A. G., van Doorn, L., Femke, M., Heersche, N., Doukas, M., van den Bosch, T. P. P., … Ibrahim, E. (2021). Tissue Type Differences in ABCB1 Expression and Paclitaxel Tissue Pharmacokinetics in Patients With Esophageal Cancer. Frontiers in Pharmacology, 12.
- Vavricka, S. R., Jung, D., Fried, M., Grützner, U., Meier, P. J., & Kullak-Ublick, G. A. (2004). The human organic anion transporting polypeptide 8 (SLCO1B3) gene is transcriptionally repressed by hepatocyte nuclear factor 3β in hepatocellular carcinoma. Journal of Hepatology, 40(2), 212–218.

- Villafranca, E., Okruzhnov, Y., Dominguez, M. A., García-Foncillas, J., Azinovic, I., Martínez, E., … Salgado, E. (2001). Polymorphisms of the repeated sequences in the enhancer region of the thymidylate synthase gene promoter may predict downstaging after preoperative chemoradiation in rectal cancer. Journal of Clinical Oncology, 19(6), 1779–1786.
- Vinolas, N., Provencio, M., Reguart, N., Cardenal, F., Alberola, V., Sanchez-Torres, J. M., … Moreno, I. (2011). Single nucleotide polymorphisms in MDR1 gen correlates with outcome in advanced non-small-cell lung cancer patients treated with cisplatin plus vinorelbine. Lung Cancer, 71(2), 191–198.
- Virmani, A. K., Rathi, A., Sathyanarayana, U. G., Padar, A., Huang, C. X., Cunnigham, H. T., ... & Gazdar, A. F. (2001). Aberrant methylation of the adenomatous polyposis coli (APC) gene promoter 1A in breast and lung carcinomas. Clinical Cancer Research, 7(7), 1998-2004.
- Vodenkova, S., Buchler, T., Cervena, K., Veskrnova, V., Vodicka, P., & Vymetalkova, V. (2020). 5-fluorouracil and other fluoropyrimidines in colorectal cancer: Past, present and future. Pharmacology & Therapeutics, 206, 107447.
- Voeller, D., Rahman, L., & Zajac-Kaye, M. (2004). Elevated levels of thymidylate synthase linked to neoplastic transformation of mammalian cells. Cell Cycle, 3(8), 1003–1005.
- Walia, H. K., Sharma, P., Singh, N., & Sharma, S. (2022). Immunotherapy in Small Cell Lung Cancer Treatment: a Promising Headway for Future Perspective. Current Treatment Options in Oncology, 1-27.
- Walsh, N., Kennedy, S., Larkin, A.-M., Tryfonopoulos, D., Eustace, A. J., Mahgoub, T., … Ballot, J. (2010). Membrane transport proteins in human melanoma: associations with tumour aggressiveness and metastasis. British Journal of Cancer, 102(7), 1157–1162.
- Walsh, N., Larkin, A., Kennedy, S., Connolly, L., Ballot, J., Ooi, W., … O'Driscoll, L. (2009). Expression of multidrug resistance markers ABCB1 (MDR-1/P-gp) and ABCC1 (MRP-1) in renal cell carcinoma. BMC Urology, 9(1), 1–7.
- Wang, J.-Q., Yang, Y., Cai, C.-Y., Teng, Q.-X., Cui, Q., Lin, J., … Chen, Z.-S. (2021). Multidrusistance proteins (MRPs): Structure, function and the overcoming of cancer multidrug resistance. Drug Resistance Updates, 54, 100743.
- Wang, L., Wang, R., Pan, Y., Sun, Y., Zhang, J., & Chen, H. (2014). The pemetrexed-containing treatments in the non-small cell lung cancer, is-/low thymidylate synthase expression better than+/high thymidylate synthase expression: a meta-analysis. BMC Cancer, 14(1), 1–9.
- Wang, X., Wang, Y., Wang, Y., Cheng, J., Wang, Y., & Ha, M. (2013). Association of thymidylate synthase gene 3'-untranslated region polymorphism with sensitivity of non-small cell lung cancer to pemetrexed treatment: TS gene polymorphism and pemetrexed sensitivity in NSCLC. Journal of Biomedical Science, 20(1), 1–8.
- Wee, P., & Wang, Z. (2017). Epidermal growth factor receptor cell proliferation signaling pathways. Cancers, 9(5), 52.
- Wei, H., Lu, X., Shang, L., Xu, G., Hu, J., Che, D., … Yu, Y. (2011). Polymorphisms of ERCC1 C118T/C8092A and MDR1 C3435T predict outcome of platinum-based chemotherapies in advanced non-small cell lung cancer: a meta-analysis. Archives of Medical Research, 42(5),

412–420.

- Weiss. (2010, April 5). An Introduction to Lung Cancer. Retrieved June 22, 2022, from http://cancergrace.org/lung/2010/04/05/an-introduction-to-lung-cancer/
- Weissfeld, J. L., Diergaarde, B., Nukui, T., Buch, S., Pennathur, A., Socinski, M. A., … Romkes, M. (2014). Inherited variation in the ATP-binding cassette transporter ABCB1 and survival after chemotherapy for stage III–IV lung cancer. Journal of Thoracic Oncology, 9(9), 1264–1271.
- Wen, X., Joy, M. S., & Aleksunes, L. M. (2017). In vitro transport activity and trafficking of MRP2/ABCC2 polymorphic variants. Pharmaceutical Research, 34(8), 1637–1647.
- Wenskus, J. K., Vincent, D., Hempel, M., &Reinshagen, K. (2021). Hirschsprung Disease–Clinical Relevance of RET Mutations. ZeitschriftfürGeburtshilfe und Neonatologie, 225(01), 80-82.
- Whetstine, J. R., Flatley, R. M., & Matherly, L. H. (2002). The human reduced folate carrier gene is ubiquitously and differentially expressed in normal human tissues: identification of seven non-coding exons and characterization of a novel promoter. Biochemical Journal, 367(3), 629–640.
- Whetstine, J. R., Gifford, A. J., Witt, T., Liu, X. Y., Flatley, R. M., Norris, M., … Matherly, L. H. (2001). Single nucleotide polymorphisms in the human reduced folate carrier: characterization of a high-frequency G/A variant at position 80 and transport properties of the His27 and Arg27 carriers. Clinical Cancer Research, 7(11), 3416–3422.
- Wilkens, S. (2015). Structure and mechanism of ABC transporters. F1000prime Reports, 7.
- Wilkes, G. M. (2018). Targeted therapy: attacking cancer with molecular and immunological targeted agents. Asia-Pacific Journal of Oncology Nursing, 5(2), 137–155.
- Williamson, G., Aeberli, I., Miguet, L., Zhang, Z., Sanchez, M.-B., Crespy, V., … Glavinas, H. (2007). Interaction of positional isomers of quercetin glucuronides with the transporter ABCC2 (cMOAT, MRP2). Drug Metabolism and Disposition, 35(8), 1262–1268.
- Wilson, P. M., Danenberg, P. V, Johnston, P. G., Lenz, H.-J., & Ladner, R. D. (2014). Standing the test of time: targeting thymidylate biosynthesis in cancer therapy. Nature Reviews Clinical Oncology, 11(5), 282–298.
- Wistuba, I. I., Behrens, C., Virmani, A. K., Mele, G., Milchgrub, S., Girard, L., ... & Minna, J. D. (2000). High resolution chromosome 3p allelotyping of human lung cancer and preneoplastic/preinvasive bronchial epithelium reveals multiple, discontinuous sites of 3p allele loss and three regions of frequent breakpoints. Cancer Research, 60(7), 1949-1960.
- Wojnowski, L., Kulle, B., Schirmer, M., Schlüter, G., Schmidt, A., Rosenberger, A., … Suk, E.-K. (2005). NAD (P) H oxidase and multidrug resistance protein genetic polymorphisms are associated with doxorubicin-induced cardiotoxicity. Circulation, 112(24), 3754–3762.
- World Health Organization. (2019). International agency for research on cancer.
- Wright, J. L., Kwon, E. M., Ostrander, E. A., Montgomery, R. B., Lin, D. W., Vessella, R., … Mostaghel, E. A. (2011). Expression of SLCO transport genes in castration-resistant prostate cancer and impact of genetic variation in SLCO1B3 and SLCO2B1 on prostate cancer outcomes. Cancer Epidemiology, Biomarkers & Prevention, 20(4), 619–627.

- Wright, S. R., Boag, A. H., Valdimarsson, G., Hipfner, D. R., Campling, B. G., Cole, S. P., & Deeley, R. G. (1998). Immunohistochemical detection of multidrug resistance protein in human lung cancer and normal lung. Clinical Cancer Research: An Official Journal of the American Association for Cancer Research, 4(9), 2279–2289.
- Wu, H., Kang, H., Liu, Y., Xiao, Q., Zhang, Y., Sun, M., … Yao, W. (2013). Association of ABCB1 genetic polymorphisms with susceptibility to colorectal cancer and therapeutic prognosis. Pharmacogenomics, 14(8), 897–911.
- Wu, K., House, L., Liu, W., & Cho, W. C. S. (2012). Personalized targeted therapy for lung cancer. International Journal of Molecular Sciences, 13(9), 11471–11496.
- Wu, L., Zhang, W., Jia, S., Zhao, X., Zhou, D., Xu, A., … Zheng, S. (2018). Mutation analysis of the ABCC2 gene in Chinese patients with Dubin-Johnson syndrome. Experimental and Therapeutic Medicine, 16(5), 4201–4206.
- Wu, S., & Fu, L. (2018). Tyrosine kinase inhibitors enhanced the efficacy of conventional chemotherapeutic agent in multidrug resistant cancer cells. Molecular Cancer, 17(1), 1–13.
- Xiang, X., Jada, S. R., Li, H. H., Fan, L., San Tham, L., Wong, C. I., … Goh, B. C. (2006). Pharmacogenetics of SLCO1B1 gene and the impact of* 1b and* 15 haplotypes on irinotecan disposition in Asian cancer patients. Pharmacogenetics and Genomics, 16(9), 683–691.
- Xie, F., Ding, X., & Zhang, Q.-Y. (2016). An update on the role of intestinal cytochrome P450 enzymes in drug disposition. Acta Pharmaceutica Sinica B, 6(5), 374–383.
- Xu, S., Yang, J., Xu, S., Zhu, Y., Zhang, C., Liu, L., ... & Xing, X. (2018). Lymphatic vessel density as a prognostic indicator in Asian NSCLC patients: a meta-analysis. BMC pulmonary medicine, 18(1), 1-8.
- Xu, Y., Wan, B., Chen, X., Zhan, P., Zhao, Y., Zhang, T., … Hochwald, S. N. (2019). The association of PD-L1 expression with the efficacy of anti-PD-1/PD-L1 immunotherapy and survival of non-small cell lung cancer patients: a meta-analysis of randomized controlled trials. Translational Lung Cancer Research, 8(4), 413.
- Yamada, A., Maeda, K., Ishiguro, N., Tsuda, Y., Igarashi, T., Ebner, T., … Sugiyama, Y. (2011). The impact of pharmacogenetics of metabolic enzymes and transporters on the pharmacokinetics of telmisartan in healthy volunteers. Pharmacogenetics and Genomics, 21(9), 523–530.
- Yamada, A., Maeda, K., Kiyotani, K., Mushiroda, T., Nakamura, Y., & Sugiyama, Y. (2014). Kinetic interpretation of the importance of OATP1B3 and MRP2 in docetaxel-induced hematopoietic toxicity. CPT: Pharmacometrics & Systems Pharmacology, 3(7), 1–10.
- Yan, P. W., Huang, X. E., Yan, F., Xu, L., & Jiang, Y. (2011). Influence of MDR1 gene codon 3435 polymorphisms on outcome of platinum-based chemotherapy for advanced non small cell lung cancer. Asian Pac J Cancer Prev, 12(9), 2291–2294.
- Yang, J., Wang, Z.-G., Cai, H.-Q., Li, Y.-C., & Xu, Y.-L. (2013). Effect of variation of ABCB1 and ABCC3 genotypes on the survival of bone tumor cases after chemotherapy. Asian Pacific Journal of Cancer Prevention, 14(8), 4595–4598.
- Yee, S. W., Gong, L., Badagnani, I., Giacomini, K. M., Klein, T. E., & Altman, R. B. (2010). SLC19A1 pharmacogenomics summary. Pharmacogenetics and Genomics, 20(11), 708.

- Yeh, J.-J., Hsu, N.-Y., Hsu, W.-H., Tsai, C.-H., Lin, C.-C., & Liang, J.-A. (2005). Comparison of chemotherapy response with P-glycoprotein, multidrug resistance-related protein-1, and lung resistance-related protein expression in untreated small cell lung cancer. Lung, 183(3), 177–183.
- Yi, T., Cho, S.-G., Yi, Z., Pang, X., Rodriguez, M., Wang, Y., … Liu, M. (2008). Thymoquinone inhibits tumor angiogenesis and tumor growth through suppressing AKT and extracellular signal-regulated kinase signaling pathways. Molecular Cancer Therapeutics, 7(7), 1789–1796.
- Yim DJ, Kim OJ, An HJ, Kang H, Ahn DH, HwangSG, KimNK. Polymorphisms of thymidylate synthase gene 5′-and 3′-untranslated region and risk of gastric cancer in Koreans. Anticancer Res.2010; 30(6): 2325-2330.
- Yin JY, Huang Q, Yang Y, et al. Characterization and analyses of multidrug resistance-associated protein 1 (MRP1/ABCC1) polymorphisms in Chinese population. Pharmacogenet. Genomics. 2009; 19(3):206.
- Yin, B., Li, Y., Lu, P., Wang, H., Pei, K., Liang, J., … Xiao, J. (2017). Advances in pharmacogenomic studies of docetaxel-a. Int J Clin Exp Med, 10(5), 7450–7456.
- Yin, J., Han, L., Huang, Q., Xu, X., Zhou, H., & Liu, Z. (2011). ABCC1 polymorphism Arg723Gln (2168G> A) is associated with lung cancer susceptibility in a Chinese population. Clinical and Experimental Pharmacology and Physiology, 38(9), 632–637.
- Yin, J.-Y., Huang, Q., Yang, Y., Zhang, J.-T., Zhong, M.-Z., Zhou, H.-H., & Liu, Z.-Q. (2009). Characterization and analyses of multidrug resistance-associated protein 1 (MRP1/ABCC1) polymorphisms in Chinese population. Pharmacogenetics and Genomics, 19(3), 206.
- Yin, J.-Y., Huang, Q., Zhao, Y.-C., Zhou, H.-H., & Liu, Z.-Q. (2012). Meta-analysis on pharmacogenetics of platinum-based chemotherapy in non small cell lung cancer (NSCLC) patients. PloS One, 7(6), e38150.
- Yip, P. Y., Yu, B., Cooper, W. A., Selinger, C. I., Ng, C. C., Kennedy, C. W., ... & O'Toole, S. A. (2013). Patterns of DNA mutations and ALK rearrangement in resected node negative lung adenocarcinoma. Journal of Thoracic oncology, 8(4), 408-414.
- Young, L. C., Campling, B. G., Cole, S. P. C., Deeley, R. G., & Gerlach, J. H. (2001). Multidrug resistance proteins MRP3, MRP1, and MRP2 in lung cancer: correlation of protein levels with drug response and messenger RNA levels. Clinical Cancer Research, 7(6), 1798–1804.
- Yu, M., Zhang, W., Qin, L., Tian, L., & Zhou, C. (2010). Enhancement of P-glycoprotein expression by hepatocyte transplantation in carbon tetrachloride-induced rat liver. The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology, 293(7), 1167–1174.
- Yue, M., Yang, J., Jin, M., Steiert, B., Xiang, Y., Zhang, H., … Gui, C. (2019). Gly45 and Phe555 in transmembrane domains 1 and 10 are critical for the activation of organic anion transporting polypeptide 1B3 by epigallocatechin gallate. Journal of Agricultural and Food Chemistry, 67(32), 9079–9087.
- Zair, Z. M., & Singer, D. R. J. (2016a). Efflux transporter variants as predictors of drug toxicity in lung cancer patients: systematic review and meta-analysis. Pharmacogenomics, 17(9), 1089–1112.

- Zair, Z. M., & Singer, D. R. J. (2016b). Influx transporter variants as predictors of cancer chemotherapy-induced toxicity: systematic review and meta-analysis. Pharmacogenomics, 17(10), 1189–1205.
- Zappa, C., & Mousa, S. A. (2016). Non-small cell lung cancer: current treatment and future advances. Translational Lung Cancer Research, 5(3), 288.
- Zawadzka, I., Jeleń, A., Pietrzak, J., Żebrowska-Nawrocka, M., Michalska, K., Szmajda-Krygier, D., … Balcerczak, E. (2020). The impact of ABCB1 gene polymorphism and its expression on non-small-cell lung cancer development, progression and therapy–preliminary report. Scientific Reports, 10(1), 1–10.
- Zgheib, N. K., Akra-Ismail, M., Aridi, C., Mahfouz, R., Abboud, M. R., Solh, H., & Muwakkit, S. A. (2014). Genetic polymorphisms in candidate genes predict increased toxicity with methotrexate therapy in Lebanese children with acute lymphoblastic leukemia. Pharmacogenetics and Genomics, 24(8), 387–396.
- Zhang, C., Xu, C., Gao, X., & Yao, Q. (2022). Platinum-based drugs for cancer therapy and anti-tumor strategies. Theranostics, 12(5), 2115.
- Zhang, W., Shannon, W. D., Duncan, J., Scheffer, G. L., Scheper, R. J., & McLeod, H. L. (2006). Expression of drug pathway proteins is independent of tumour type. The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland, 209(2), 213–219.
- Zhang, X., Pu, Z., Ge, J., Shen, J., Yuan, X., & Xie, H. (2015). Association of CYP2D6* 10, OATP1B1 A388G, and OATP1B1 T521C polymorphisms and overall survival of breast cancer patients after tamoxifen therapy. Medical Science Monitor: International Medical Journal of Experimental and Clinical Research, 21, 563.
- Zhao, L. N., Björklund, M., Caldez, M. J., Zheng, J., & Kaldis, P. (2021). Therapeutic targeting of the mitochondrial one-carbon pathway: perspectives, pitfalls, and potential. Oncogene, 40(13), 2339–2354.
- Zhao, R., Gao, F., Wang, Y., Diaz, G. A., Gelb, B. D., & Goldman, I. D. (2001). Impact of the reduced folate carrier on the accumulation of active thiamin metabolites in murine leukemia cells. Journal of Biological Chemistry, 276(2), 1114–1118.
- Zhao, R., Matherly, L. H., & Goldman, I. D. (2009). Membrane transporters and folate homeostasis: intestinal absorption and transport into systemic compartments and tissues. Expert Reviews in Molecular Medicine, 11.
- Zheng C, Yang H, Wang Q, et al. Association analysis of five SNP variants with gout in the Minnan population in China. Turk. J. Med. Sci .2016;46(2):361-367.
- Zhou, F., He, X., Liu, H., Zhu, Y., Jin, T., Chen, C., … Chen, Z. (2012). Functional polymorphisms of circadian positive feedback regulation genes and clinical outcome of Chinese patients with resected colorectal cancer. Cancer, 118(4), 937–946.
- Zhou, S.-F. (2008). Structure, function and regulation of P-glycoprotein and its clinical relevance in drug disposition. Xenobiotica, 38(7–8), 802–832.
- Zhou, Z., Chen, Q., Zuo, D., Wang, H., Hua, Y., & Cai, Z. (2015). ABCB1 (rs1128503) polymorphism and response to chemotherapy in patients with malignant tumors-evidences from

a meta-analysis. International Journal of Clinical and Experimental Medicine, 8(1), 265.

- Zhu, V. W., Upadhyay, D., Schrock, A. B., Gowen, K., Ali, S. M., & Ou, S. H. I. (2016). TPD52L1-ROS1, a new ROS1 fusion variant in lung adenosquamous cell carcinoma identified by comprehensive genomic profiling. Lung cancer, 97, 48-50.
- Zochbauer-Muller, S., Gazdar, A. F., & Minna, J. D. (2002). Molecular pathogenesis of lung cancer. Annual review of physiology, 64, 681.
- Zöchbauer-Müller, S., Wistuba, I. I., Minna, J. D., & Gazdar, A. F. (2000). Fragile histidine triad (FHIT) gene abnormalities in lung cancer. Clinical lung cancer, 2(2), 141-145.

CHAPTER 9

APPENDIX

Appendix-I ETHICS COMMITTEE REPORT

Institutional Ethics Committee
Postgraduate Institute of Medical Education and Research, Chandigarh

Prof. K.N. Pathak
Chairman

Phone No. 0172-2755266

Dr. Samir Malhotra
Convener

No. PGI/IEC/2018/ 001310 Dated: - 08-09-2018

To: Dr. Navneet Singh, Dept. of Pulmonary Medicine, PGIMER, Chandigarh.
From: IEC Office
Date: 29.08.2018
IEC #: IEC-04/2018-884
Subject: Genetic polymorphism in phase I/II and ABC transporter gens in lung cancer pateint undergoing platinum based chemotherapy and its association with toxcity and clinical outcome.

==========

The Institutional Ethics Committee of PGIMER, Chandigarh reviewed and discussed the above mentioned Project in its meeting, which was held on 14/15.05.2018 and sent the observations for your clarification, after receiving your reply the same is APPROVED by Institutional Ethics committee. The following documents were reviewed:-

(a) Study Protocol (including amendments, if any)
(b) Patient Information Sheet and Informed Consent Form (including updates, if any)
(c) Principal Investigator's current CV.
(d) IEC form

The following members of the IEC were present at the meeting held on 29.08.2018 at 03:00 PM in the Board Room of Kairon Block, PGIMER, Chandigarh:-

Sr. No	Name of the member	Designation	Affiliation to PGI
1.	Prof. K. N. Pathak	Chairman	No
2.	Justice V. K. Jhanji	Member (Legal)	No
3.	Dr. Virender Kumar	Member (Lay Person)	No
4.	Dr. O. P. Katare	Member (Basic Scientist)	No
5.	Dr. Sukhbir Kaur,	Member (Basic Science)	No
6.	Dr. Anil Monga	Member (Social Scientist)	No
7.	Dr. Pallab Ray	Member (Basic Science)	Yes
8.	Dr. Ashish Bhalla	Member (Clinician)	Yes
9.	Dr. Neelam Aggarwal	Member (Clinician)	Yes
10.	Dr. Ritambra Nadda	Member (Basic Sciences)	Yes
11.	Dr. Kanya Mukhopadhyay	Member (Clinician)	Yes
12.	Dr. Manish Modi	Member (Clinician)	Yes
13.	Dr. Nusrat Shafiq	Member (Pharmacologist)	Yes
14.	Dr. P.V.M. Lakshmi	Member	Yes
15.	Dr. Samir Malhotra	Convener	Yes

The Institutional Ethics Committee expects to be informed about the progress of the study, please use the Annual Progress Report Form available on the PGIMER website. Please note that it is the responsibility of the PI to inform the IEC/DSMC regarding any SAE occurring in the course of the study, or any changes in the protocol and patient information sheet /informed consent form, if applicable. You are also requested to provide a copy of the final report in the Study Completion Form available on the PGIMER website. Please be advised that your study can be periodically monitored by the IEC.

Yours sincerely,

(Samir Malhotra)
Convener
Instt. Ethics Committee, PGIMER, Chandigarh.

INSTITUTIONAL ETHICS COMMITTEE (IEC)

Department of Biotechnology

THAPAR INSTITUTE
OF ENGINEERING & TECHNOLOGY
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Review Letter number IEC/TIET/DBT/09/03/18

Dated: 27.09.2018

The Institutional ethics committee of Thapar Institute of Engineering & Technology, Patiala, in its Sixth (06) meeting held on 27.09.2018, in the office of Head, Department of Biotechnology, reviewed and discussed the project submitted by Dr. Siddharth Sharma as PI from Department of Biotechnology.

TIET/DBT/IEC/09/03/18: Genetic polymorphism in Phase I/II and ABC transporter genes in Lung Cancer Patient undergoing Platinum based chemotherapy and its association with toxicity and clinical outcomes.

The members reviewed the following documents and raised queries which were satisfactorily answered by Dr. Siddharth Sharma.

a) Proposal & Protocol

b) Available Safety Information

c) Cell line/ Genetic material storage and discard procedure

It is understood that the study would be conducted under the PI's direction strictly as per the submitted protocol. In case of any deviation from the approved methods, the study documents will have to be resubmitted to ethics committee for re-approval.

Members	**Signature**
Dr. Manoj Baranwal (Member)	
Dr. Siddharth Sharma (Member)	
Dr. Vikas Handa (Member)	
Dr. Sanjai Saxena (Member Secretary)	

The present approval is for 01 year only; the investigators are advised to take a re-approval after one year. 06 Monthly compliance report is a must for seeking re-approval.

Signature
Prof. Moushumi Ghosh
Chairman, Institutional Ethics Committee

HEAD
Department of Biotechnology
Thapar Institute of Engineering & Technology
Patiala - 147004, Punjab

POSTGRADUATE INSTITUTE OF MEDICAL EDUCATION & RESEARCH, CHANDIGARH
MEDICAL EDUCATION AND RESEARCH CELL
PHONE: 0172-2755568

No: 79/179-Edu-18 / 4913 Dated: 05/12/2018

To

Dr. Navneet Singh
Additional Professor
Department of Pulmonary Medicine
PGIMER, Chandigarh

Subject: Collaborative Research project titled **"Genetic Polymorphism in Phase I/II and ABC Transporter Genes in Lung Cancer patient undergoing platinum based chemotherapy and its association with toxicity and clinical outcomes"**

Please refer to your letter no. 2765 dated 02/11/2018 on the subject cited above.

Approval of the ICRC Committee is herewith conveyed for the collaborative research project titled "Genetic Polymorphism in Phase I/II and ABC Transporter Genes in Lung Cancer patients undergoing platinum based chemotherapy and its association with toxicity and clinical outcomes"

5/12

Sub-Dean (Research)
Medical Education & Research Cell
PGIMER, Chandigarh

Appendix-II PATIENT CONSENT FORM

Patient consent form

Name of the participant: _____

Name of the Principal (Co-) Investigators: _____

Name of the Institution: _____

Name and address of the sponsoring (funding) agency(ies): _____

_____

Documentation of the informed consent

I, … … … … … … … … … … . have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in the project "**Genetic Polymorphisms in Phase I/II and ABC Transporter Genes in Lung Cancer patient undergoing platinum based chemotherapy and its association with toxicity and clinical outcomes**"

(1) I have read and understood consent form and the information provided to me.

(2) I have had the consent document explained to me.

(3) I have been explained about the nature of the study.

(4) My rights and responsibilities have been explained to me by the investigator.

(5) I have the right to not be a subject in the above study and am not willing to give sample

(6) I have informed the investigator of all the treatments I am taking or have taken in the past … … . months including any *desi* (alternative) treatments.

(7) Patient data and confidentiality have been explained to me.

(8) I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Government agencies, and ethics committee. I understand that they may inspect my original records.

(9) My identity will be kept confidential if my data are publicly presented.

(10) I have had my questions answered to my satisfaction.

(11) I have decided to be in the research study.

I am aware, that if I have any questions during this study, I should contact the concerned authorities. By signing this consent from, I attest that the information given in this document. I will be given a copy of this consent document.

For adult participants

Name and signature / thumb impression of the participant (or legal representative if participant incompetent):

_____ (Name) _____ (Signature)

Date: _____ Time: _____

Name and signature of impartial witness (required for illiterate patients):

_____ (Name) _____ (Signature)

Date:_____ Time: _____

Address and contact number of the impartial witness: _____

_____

Name and signature of the Investigator or his representative obtaining consent:

_____ (Name) _____ (Signature)

_____ (Date)

Investigator Certificate

I certify that all the elements including the nature of the research study and possible risks of the above study as described in this consent document have been fully explained to the subject. In my judgment, the participant possesses the legal capacity to give informed consent to participate in this research and is voluntarily and knowingly giving informed consent to participate,

Signature of the Investigator: _____ Dated:_____

Name of the Investigator: _____

Appendix-III PATIENT INFORMATION SHEET

PATIENT INFORMATION SHEET

Department of Biotechnology, Thapar University &
Dept. of Pulmonary Medicine, PGIMER, Chandigarh

Investigator: Siddharth Sharma, Ph.D.
Co-Investigator: Dr. Navneet Singh, M.D, D.M

Name of Patient _____

Address (Rural/Urban) _____

C.R Number _____

Date of Sampling _____

Type of Cancer _____

Stage of Cancer _____

Sex _____

Age _____

Education (Years) _____

Occupation _____

Marital Status _____

Smoking Status

1. Smoker or Non-Smoker _____

2. Type of Smoking

a) Cigarette b) Beedi c) Hookah d) Cigarette+Beedi e) Beedi+Hookah f) Any other form of smoke

3. No. of Cigarettes/ Beedis smoked

a) Per day_____

b) Per Week _____

c) Amount of tobacco added to hookah _____

4. a) Age at which started Smoking _____

b) How many years have you smoked _____

5. Time taken to finish cigarette (in min) _____

6. Brand of smoke preferred _____

7. Do you chew tobacco/betelnut

a) Yes b) No

If yes amount chewed _____

8. Any familial case of Smoking related cancer in your family: a) Yes b) No

9. Are you only one to smoke in your family

Alcohol Consumption

1. Do you consume alcohol
   a) Yes b) No
2. Intake of Alcohol
   a) Daily b) 3-5 times a week c) 1-2 times a week d) once a week e) once a month
3. How much alcohol do you consume in ml_____
4. What type of alcohol do you consume _____

Documentation of the informed consent

I, … … … … … … … … … …. have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in the project "**Genetic Polymorphisms in Phase I/II and ABC Transporter Genes in Lung Cancer patient undergoing platinum based chemotherapy and its association with toxicity and clinical outcomes**"

(1) I have read and understood consent form and the information provided to me.

(2) I have had the consent document explained to me.

(3) I have been explained about the nature of the study.

(4) My rights and responsibilities have been explained to me by the investigator.

(5) I have the right to not be a subject in the above study and am not willing to give sample

(6) I have informed the investigator of all the treatments I am taking or have taken in the past … … . months including any *desi* (alternative) treatments.

(7) Patient data and confidentiality have been explained to me.

(8) I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Government agencies, and ethics committee. I understand that they may inspect my original records.

(9) My identity will be kept confidential if my data are publicly presented.

(10) I have had my questions answered to my satisfaction.

(11) I have decided to be in the research study.

I am aware, that if I have any questions during this study, I should contact the concerned authorities. By signing this consent from, I attest that the information given in this document. I will be given a copy of this consent document.

Appendix-IV

- 10% SDS: Dissolved 1g of SDS in 10ml of deionised water.
- 0.5M EDTA: Dissolved 9.306g of disodium salt of EDTA in 20ml of deionised water, and then adjusted the pH to 8.0 by 1 M sodium hydroxide. Sterilized the solution by autoclaving.
- 10 mg/ml Proteinase K: Dissolved 10mg Proteinase K in 1ml of double distilled water. Sterilized the solution by autoclaving.
- 100mM Tris-Cl (pH 8.0): Dissolved 0.32g of Tris-Cl in 10 ml of deionised water, then adjusted the pH to 8.0 by 1M sodium hydroxide. Sterilized the solution by autoclaving.
- 5M Sodium chloride (NaCl): Dissolved 5.85g of sodium chloride in 20ml of deionised water. Sterilized the solution by autoclaving.
- 10mg/ml Proteinase K: Dissolved 10mg Proteinase K in 1ml of double distilled water. Sterilized the solution by autoclaving.
- 1mg/ml BSA: Dissolved 100mg of BSA in 100ml of deionised sterile water and kept at 4 ˚C overnight.
- Ethidium Bromide (10mg/ml): Dissolved 1g of ethidium bromide in 100ml of water. Mixed the solution properly.
- 5X TBE buffer: Dissolved 54g of Tris base and 27.5g of boric acid in 980ml of double distilled water and then added 20ml of 0.5 EDTA. Sterilized the solution by autoclaving.
- Magnesium chloride ($MgCl_2$) (100mM): Dissolved 0.41gms of MgCl2 in 20ml of deionised water and sterilized by autoclaving.
- Triton X- 100 (10%): Took 100 µl of TritonX-100 and mixed with 900 µl of deionised water and mixed properly**.**
- Sucrose (1M): Dissolved 3.41 g of sucrose in 10 ml of deionised water and sterilized by autoclaving.
- TE buffer (pH 8.0): Added 1ml of 100mM Tris-Cl (pH 8.0) and 200 µl of 0.5M EDTA solution to 8.8 ml of deioinsed water. Sterilized the solution.

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RESEARCH ARTICLE

WILEY

Impact of *ABCB1*, *ABCC1*, *ABCC2*, and *ABCG2* variants in predicting prognosis and clinical outcomes of north Indian lung cancer patients undergoing platinum-based doublet chemotherapy

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Abstract

Background: ABC transporters are membrane proteins expressed in the lungs and are crucial for efflux of various chemotherapeutic agents. Polymorphisms of ABC transporters have a certain impact on the transporter activity because their expression levels may influence the extent and longevity of chemotherapeutic drug outflow, affecting patient outcomes. The present study aimed to assess the impact of *ABCB1*, *ABCC1/2*, and *ABCG2* gene variants in predicting prognosis and clinical outcomes in lung carcinoma patients.

Methods: In total, 502 lung cancer patients undergoing platinum-based chemotherapy were recruited in this prospective study. Genotyping of *ABCB1* ($C^{1236}T$, $C^{3435}T$, and $G^{2677}T/A$), *ABCC1* ($G^{3173}A$ and $G^{2168}A$), *ABCC2* ($G^{4544}A$), and *ABCG2* ($C^{421}A$) polymorphisms in Northern Indian lung carcinoma patients were evaluated using polymerase chain reaction-restriction fragment length polymorphism analysis.

Results: Poor survival outcomes were noted in patients carrying a heterozygous genotype (*CT*) for the *ABCB1* $C^{1236}T$ polymorphism compared to the wild-type genotype (*CC*) ($p = 0.04$). The mutant genotype (*AA*) for *ABCC1* $G^{3173}A$ exhibited a lower median survival time compared to the reference genotype (*GG*) ($p = 0.009$). Lower survival was observed in individuals carrying a heterozygous genotype (*GA*) for *ABCC2* $G^{4544}A$ polymorphism compared to the wild-type genotype (*GG*) ($p = 0.017$). Small cell lung cancer patients with the *ABCB1* $G^{2677}A$ polymorphism having a heterozygous genotype (*GA*) showed poor survival compared to the wild-type genotype (*GG*) ($p = 0.03$). For *ABCC1* $G^{3173}A$, adenocarcinoma patients having a mutant genotype (*AA*) had reduced survival compared to the wild-type (*GG*) genotype ($p = 0.03$). For *ABCB1* $C^{3435}T$, individuals carrying a heterozygous (*CT*) ($p = 0.018$) and mutant (*TT*) genotype ($p = 0.007$) had poor survival compared to the wild-type (*CC*) genotype in patients treated with pemetrexed and cisplatin. The patients administered cisplatin and irinotecan and having mutant alleles (*AA*) for the *ABCB1* $G^{2677}A$ polymorphism showed a lower survival compared to the individuals carrying wild-type alleles (*GG*) ($p = 0.009$).

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Conclusions: Our findings suggest that *ABCB1* $C^{1236}T$, *ABCB1* $C^{3435}T$, *ABCB1* $G^{2677}A$, *ABCC1* $G^{3173}A$, and *ABCC2* $G^{4544}A$ are involved in predicting prognosis. Genotyping of the *ABC* polymorphism is essential for predicting prognosis in lung carcinoma patients.

KEYWORDS
ABC transporter, lung cancer, platinum-based chemotherapy, polymorphism, survival

1 | INTRODUCTION

Lung cancer is the primary cause of mortality and morbidity across the globe and has a five-year survival rate of only 15%, with a median survival time of less than 10 months.[1] Even though smoking is a well-known significant risk for lung cancer, only approximately 20% of those who smoke throughout their lifetimes get the disease.[2] Lung cancer diagnosed in less developed countries accounts for more than half of all cases, in accordance with the International Agency for Research on Cancer (IARC).[3] Patients with lung cancer are either treated with surgery, chemotherapy or radiation. Patients with localized non-small cell lung cancer (NSCLC) are treated predominantly with surgery, whereas patients with non-localized NSCLC are treated chiefly with radiochemotherapy. The most common treatment for small cell lung cancer (SCLC) is chemotherapy, combined with or without further radiotherapy.

NSCLC accounts for around 85% of cases and is usually detected late, and chemotherapy remains the standard treatment. Platinum-based doublet chemotherapy is the usual first-line treatment for such patients. However, with apprximately 19–30% of people responding, the results are poor, whereas the average response time is only 3.6 months.[4] This shows that insights into both inherent and acquired mechanisms of platinum resistance are essential, with genetic variability accounting for the observed inter-patient disparities in response and clinical manifestations. One of the most well-known mechanisms of platinum resistance is the enhanced outflow of chemotherapeutic drugs by subfamilies of the adenosine triphosphate-binding cassette (ABC) transporters. The ABC transporters comprise a vast family of proteins that use ATP hydrolysis energy to translocate many endogenous and exogenous substances across the cell membrane.[5] There are 48 members of the ATP transporter family in humans, which are classified into seven subfamilies. Among these, ABCB1 (multidrug resistance-1/MDR1), ABCC1 (multidrug resistance-associated protein-1/MRP1), ABCC2(multidrug resistance-associated protein-2/MRP2), and ABCG2 (breast cancer resistance protein/BCRP) are well reported for their associated role in multidrug resistance.

ABCB1 is expressed in the kidney, lung, liver, and small intestine, and its gene is found on chromosome 7q21.6.[6] In a normal lung, expression of *ABCB1* is reported on the apical surface of bronchi, bronchiolar epithelium, and at the plasma membrane of the alveolar macrophages, where it plays a vital role in removing the exogenous compounds from a lung lumen.[7] In the case of lung cancer, the expression of *ABCB1* is initially low, although this can change after the chemotherapy because of acquired drug resistance.[8] Studies have shown that ABCB1 confers resistance to chemotherapeutic drugs, such as cisplatin and etoposide, and genetic polymorphisms can impact substrate specificity.[9] More than 50 variants have been investigated in *ABCB1*, but polymorphisms $C^{1236}T$ (exon 12), $C^{3435}T$ (exon 34), and $G^{2677}T/A$ (exon 21) have received significant attention.[10] Li et al.[11] reported that overexpression of ABCB1 has been associated with resistance to various chemotherapeutic drugs in cancer patients. This modulation in expression might also impact the prognosis in such patients.

ABCC1 is expressed in almost every human body tissue, with more significant amounts in the lung, kidneys, testis, and muscles. *ABCC1* is found in bronchial epithelium and alveolar macrophages.[12] Polymorphisms of the *MRP1* gene or regulatory genes may influence the function of *ABCC1* in the lung. Studies have demonstrated that a single mutation in the *ABCC1* gene can lead to the loss of transport of specific ABCC1 substrates, implying that even slight variations in the *ABCC1* gene could result in MRP1 protein functional abnormalities.[13] Our study assessed the role of $G^{3173}A$ and $G^{2168}A$ polymorphism in the *ABCC1* gene. Kadioglu et al.[14] reported that higher expression of *ABCC1* is associated with poor prognosis and worse clinical outcomes. Reports have also discussed the role of *ABCC2* $G^{4544}A$ polymorphism in correlation with impaired ATPase activity and it is high in hepatic cells. There are increasing numbers of studies stating that this variant may influence the cellular disposition of the various chemotherapeutic drugs.[15] To date, only few studies have reported the role of *ABCC2* $G^{4544}A$ in predicting prognosis in lung cancer patients.

ABCG2 (BCRP) is the half-transporter located on chromosome 4q22 and plays a role in toxin resistance and resistance to various chemotherapeutic treatments. Yanase et al.[16] reported that the polymorphic variant $C^{421}A$ results in glutamine substituting a lysine at position 141 and is linked to differences in protein activity and drug sensitivity in cell lines. Gardner et al.[17] reported that the wild-type genotype of *ABCG2* $C^{421}A$ encodes for a functional protein and has a higher chance of survival.

In the present study, we have assessed the role of *ABCB1*, *ABCC1*, *ABCC2*, and *ABCG2* gene polymorphisms in predicting the overall survival and various clinicopathological outcomes in North Indian lung cancer patients undergoing platinum-based doublet chemotherapy. The findings may aid in a better understanding of the molecular mechanisms causing abnormal ABC expression, anticancer drug transport,

and therapy efficacy and determine whether these transporters may be used as diagnostic and prognostic molecular markers. To the best of our knowledge, no research examining the role of these genetic polymorphisms in lung carcinoma patients from North India has been conducted.

2 | MATERIALS AND METHODS

2.1 | Study design

The current prospective study included lung carcinoma patients who approached our lung cancer clinic (i.e. Post-Graduate Institute of Medical Education and Research, PGIMER, Chandigarh, India). The blood samples were collected from 502 patients with lung cancer at PGIMER, Chandigarh, India. For 3 years (January 2019 to January 2022), newly diagnosed lung carcinoma patients with confirmed SCLC or NSCLC were enrolled in the study. The Ethics Committee of PGIMER, Chandigarh, India, authorized the study.

2.2 | Participants

Patients with lung cancer who met the following inclusion criteria were eligible to participate: (i) lung carcinoma diagnosis confirmed by cytology or histology; (ii) stage IIIA/B to stage IV patients (not scheduled for the surgery); (iii) untreated with respect to undergoing definitive platinum-based chemotherapy (cisplatin/carboplatin along with pemetrexed, docetaxel, gemcitabine, paclitaxel, or irinotecan); (iv) a performance status (i.e. PS) of 0–2 according to the Eastern Cooperative Oncology Group (ECOG); (v) according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria, at least one bi-dimensionally quantifiable lesion; and (vi) informed written consent acquired. The exclusion criteria comprised: (i) non-bronchogenic malignancies; (ii) saarcomas; (iii) pleural mesothelioma; (iv) distant metastasis tumors to the lungs or pleura; and (v) initial-stage (resectable) lung carcinoma.

Kidney function tests, a hepatic panel, a complete blood count (CBC), a chest radiograph, and a contrast-enhanced computed tomography scan of the thorax (also in the upper abdomen) were all performed on the patients at the start of the study. Before each treatment cycle, CBC, renal function, and liver tests were performed 3–4 weeks after the previous chemotherapy round. A CBC was performed 10 days after the initial chemotherapy treatment and any subsequent rounds if necessary. The patients' demographic characteristics (age and performance status), tumor stage, cancer histology, and smoking habits were also recorded. The clinical parameters and other necessary data were collected from PGIMER, Chandigarh, India. The Strengthening the Reporting of Observational studies in Epidemiology (STROBE) and STrengthening the REporting of Genetic Association Studies (STREGA) criterion was used to report the study.[18,19]

2.3 | Chemotherapy regimen

A 1-h infusion of irinotecan (100 mg/m^2), docetaxel (75 mg/m^2), pemetrexed (500 mg/m^2) or paclitaxel (175 mg/m^2) was given, preceded by a 3-hour intravenous administration of cisplatin (70 mg/m^2). As is usual protocol at PGIMER, four cycles of chemotherapy treatment were given before a tumor response assessment. The tumor response was examined before completing the four cycles and, if there was any unfavorable adverse reaction or clinical-radiological signs of cancer progression, chemotherapy was paused if necessary. Subjects who responded to chemotherapy effectively were given two further treatment cycles (i.e. a maximum of six cycles). The RECIST criteria were used to assess tumor response. During chemotherapy, patients were followed up every 3 weeks, and then every 3 months for span of three years. A telephone follow-up approach was utilized to follow-up patients. Overall survival was assessed from the chemotherapy to fatality or the last follow-up date.

2.4 | Genotyping of ABC variants

The genomic DNA was extracted using the phenol-chloroform separation procedure from 3–5 ml of blood. The isolated genomic DNA was stored at −20°C until use. Each single nucleotide polymorphism (SNP) was genotyped using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) or allele-specific PCR (AS-PCR) method in the Department of Biotechnology of Thapar Institute of Engineering and Technology, Patiala, India. PCR-RFLP for the four SNPs of *ABCB1* gene$C^{1236}T$ (rs1128503), $C^{3435}T$ (rs1045642), $G^{2677}A$ (rs2032582), and $G^{2677}T$ (rs2032582) was performed as previously reported.[20,21] For the *ABCC1* polymorphism, PCR-RFLP of the two polymorphisms $G^{2168}A$ (rs4148356) and $G^{3173}A$ (rs41410450) was carried out as previously described.[22] For *ABCC2* $G^{4544}A$ (rs8187710) polymorphism, the genotyping was carried out as previously reported.[23] *ABCG2* variant, $C^{421}A$ (rs2231142) was genotyped by AS-PCR. The genotyping method and primers used are described in the previous study by Zheng et al.[24]

The PCR (25 μl) reaction contained 1 × PCR buffer, 0.5 μM forward primer, 0.5 μM reverse primers, 200 μM dNTPs, 100 μg/ml bovine serum albumin, 1 U of Taq polymerase (DyNAzyme II; Thermo Scientific, Waltham, MA, USA) and 200 ng of DNA to amplify the required fragment. As indicated previously, the amplified product was digested with appropriate restriction enzymes. The digested product was separated into a polyacrylamide gel or agarose gel and subjected to staining by ethidium bromide to determine the restriction patterns. The analysis of all the above-stated polymorphisms was performed by one group (i.e. Principal Investigator [SS] and Co-Principal investigator [NS]) and genotyping by PCR- RFLP or AS-PCR was performed by the first investigator (PS). There was no involvement of any other group when performing the present study. The genotypes were assigned in smaller batches by analyzing the restriction pattern of the specific enzymes to associated polymorphisms. Two individuals verified the

results of genotyping to ensure no errors. Additionally, 20% of the samples were chosen at random once more for genotyping to ensure absolute validity.

2.5 | Statistical analysis

The current research focused on the Northern Indian population, including information on gender, age, and smoking habits. We used a chi-squared goodness-of-fit test to evaluate whether cases were in line with the Hardy–Weinberg equilibrium (i.e. $p^2 + q^2 + 2pq = 1$; where p represents the frequency of wild-type and q depicts the frequency of mutant type alleles). PCR-RFLP or AS-PCR methods were utilized to infer the genotypes of the lung cancer cases. The adjusted odds ratio (AOR) and 95% confidence interval (CI) were computed by logistic regression analysis after adjustment with different confounding parameters using MedCalc, version 14.8.1 (Medcalc software, Ostend, Belgium). Descriptive analysis is represented as the mean ± SD, or number/percentages. Overall survival (OS) for all patients was computed from the first day of chemotherapy treatment to the demise or the last follow-up date. The univariate Kaplan–Meier approach calculated the median OS time and log-rank p value. Multivariate Cox regression analysis was used for adjusting different covariates to determine the polymorphism's independent influence on the overall survival. $p < 0.05$ was considered statisticaly significant in all analyses. To address the issue of missing data, frequent visits to PGIMER, Chandigarh, were carried out and the available data was collected. The current investigated North Indian population did not require a method to evaluate relatedness because the individuals were not closely related.

3 | RESULTS

3.1 | Patients and treatment outcomes

The demographic features of the case group, such as age, smoking status, gender, packyears, tumor size, lymph node invasion (N), metastases (M), tumor staging, and various clinical parameters, are summarized in Table 1. In the present study, 502 lung cancer cases were enrolled, and genotyping was carried out on each sample. Polymorphism analysis of *ABCB1* ($C^{1236}T$, $C^{3435}T$, $G^{2677}A$, $G^{2677}T$), *ABCC1* ($G^{2168}A$, $G^{3173}A$), *ABCC2* ($G^{4544}A$), and *ABCG2* ($C^{421}A$) gene was performed. The mean age of the patients was 60 ± 9.67 years (range 30–86 years). In the studied patients, males outnumbered females by 401 (79.8%) to 101 (20.1%). Smokers accounted for 78.8% of the group, being more significant than non-smokers (21.1%). Smokers had an average of 22.81 ± 23.52 pack-years. When stratified based on TNM staging, 32.2% and 58.1% of patients were diagnosed with stage III and IV lung tumor. Patients with T1 and T2 tumor sizes had low frequencies of 6.17% and 8.7%, respectively, whereas those with T3 and T4 tumor sizes had high frequencies of 18.32% and 60.1%, respectively. At the time of diagnosis, 54.5% of patients had already presented metastasis, whereas 42.8% had no metastatic involvement. N0 incidence was found in 13.7% subjects for lymph node invasion, whereas N1, N2, N3, and N4 had an incidence of 7.7%, 43.8%, 31.8%, and 0.19%, respectively. Histologically, 41.4% of patients were diagnosed with (ADCC), 41.8% had squamous cell carcinoma (SQCC), and 15.3% were detected with SCLC. In addition, the cases were distinguished according to ECOG and Karnofsky's status (KPS); 88 participants had KPS scores below 60, 256 patients had KPS scores of 70–80, and 138 subjects had KPS scores of 90–100. As per the ECOG score, 43.8% (220) of patients had ECOG scores 0–1, 37.2% (187) had ECOG 2, and 15.1% (76) had ECOG scores of 3–4. Patients in the case group were given cisplatin/carboplatin-based therapy, with 113 receiving pemetrexed, 45 receiving irinotecan, 86 receiving paclitaxel, 46 receiving docetaxel, 31 receiving gemcitabine, 15 receiving gefitinib, 17 receiving etoposide, and 17 receiving other regimens.

3.2 | Overall survival analysis for *ABCB1*, *ABCC1*, *ABCC2*, and *ABCG2* genes

The correlation of the ABC polymorphisms with survival in 483 lung cancer samples was investigated. At the time of analysis, 84.4% of patients were dead, whereas 15.5% of patients were alive after 3 years. *ABC* SNPs association with lung cancer survival was investigated using univariate and multivariate analysis after adjusting for demographic parameters such as smoking status, age, histology, tumor stage, regimen, and ECOG (Table 2). In the present analysis, we employed four potential models (dominant, recessive, co-dominant, and additive) to determine the relationship between ABC polymorphism and OS in lung carcinoma patients. The best-fit model presented the results shown in Table 2. For the *ABCB1* $C^{1236}T$ polymorphism, in the co-dominant model, our data revealed a lower median survival time in patients having a heterozygous (*CT*) genotype compared to wild-type (*CC*) genotype (median survival time [MST] = 7.7 versus 8.57, HR = 1.13, log-rank $p = 0.27$). After adjusting with the different covariates such as age, gender, stage, smoking status, histology, regimen, and performance status, the results showed poor survival outcomes in the heterozygous (*CT*) genotype compared to the wild-type (*CC*) genotype (HR = 1.34, $p = 0.04$) (Figure 1A). Our results reveal no associations between different genotypes of *ABCB1* $C^{3435}T$, $G^{2677}A$, and $G^{2677}T$ polymorphisms and prognosis in lung cancer patients.

For the *ABCC1* $G^{3173}A$ variant, in the co-dominant model, patients with both mutant alleles (*AA*) exhibited a significantly lower MST than patients with the wild-type genotype (*GG*) (MST 1.63 versus 8.37, HR = 4.15, 95% CI = 1.40–12.2, log-rank $p = 0.009$) (Figure 1B). Patients having a mutant genotype (*AA*) had a high odds (HR = 2.45, 95% CI = 1.19–5.04, $p = 0.01$) after adjusting for various covariates in the Cox regression model (Table 2). Our results reveal no associations between different genotypes of *ABCC1* $G^{2168}A$ polymorphism and overall survival in lung cancer patients.

Survival time was substantially lower in patients with one copy of a mutant allele (*GA*) for the *ABCC2* $G^{4544}A$ polymorphism than in patients with the wild-type genotype (*GG*) (MST 4.37 versus 8.4,

TABLE 1 Patient characteristics

Variable	Total ($N = 502$)	Cases, *n* (%)	Cases, *n* (%)	Total (*N*)	Cases, *n* (%)
Gender	502		**Lymph Node**	502	
Male		401 (79.8)	N0		69 (13.7)
Female		101 (20.1)	N1		39 (7.7)
Age (years)	502	60 ± 9.67	N2		220 (43.8)
		30–86	N3		160 (31.8)
Smoking status	502		N4		1 (0.19)
Smokers		396 (78.8)	Unknown		13 (2.58)
Non-smokers		106 (21.1)	**Performance status**	502	
Pack years	502		KPS (below 60)		88 (17.5)
Mean ± SD		22.81 ± 23.52	KPS (70–80)		256 (50.9)
Overall survival	483		KPS (90–100)		138 (27.4)
Dead		408 (84.4)	Unknown		20 (3.98)
Alive		75 (15.5)	ECOG (0–1)		220 (43.8)
TNM staging	502		ECOG (2)		187 (37.2)
I		5 (0.9)	ECOG (3–4)		76 (15.1)
II		19 (3.78)	Unknown		19 (3.7)
III		162 (32.2)	**Histological types**	495	207 (41.8)
IV		292 (58.1)	SQCC		205 (41.4)
Unclassified		24 (4.7)	ADCC		76 (15.3)
			SCLC		7 (1.41)
			Others		
Tumor Size	502		**Chemotherapy regimen**	370	
Tx		19 (3.78)	Pemetrexed cis/carboplatin		113 (30.5)
T1		31 (6.17)	Irinotecan cis/carboplatin		45 (12.1)
T2		44 (8.7)	Docetaxel cis/carboplatin		46 (12.4)
T3		92 (18.32)	Paclitaxel cis/carboplatin		86 (23.2)
T4		302 (60.1)	Gemcitabine cis/carboplatin		31 (8.37)
Unknown		14 (2.78)	Gefitinib		15 (4.05)
Metastasis	502		Etoposide		17 (4.59)
M0		215 (42.8)	Others		17 (4.59)
M1		274 (54.5)	**Chemotherapy response**	260	
Unknown		13 (2.58)	CR/PR		103 (39.6)
			SD/PD		157 (60.3)

HR = 2.16, 95% CI = 1.14–4.09, log-rank $p = 0.017$). After adjusting with multiple covariates, a hazard ratio of 1.88 was noted in the heterozygous group (*GA*) compared to the patients in the wild-type group (*GG*) (HR = 1.88, 95% CI = 0.96–3.70, $p = 0.04$) (Figure 1C). Our data did not reveal any association between different genotypes of *ABCG2* $C^{421}A$ polymorphism and OS in lung carcinoma patients.

3.3 | Relationship of ABC polymorphism with the OS in lung cancer histological subtypes

Furthermore, we have determined the importance of the ABC polymorphism in predicting survival in distinct subgroups stratified by histology, as shown in Table 3 and the Supporting information (-Table S1). For the *ABCB1* $C^{1236}T$ polymorphism, patients with ADCC harboring a heterozygous (*CT*) genotype had a lower survival compared to the patients having a wild-type (*CC*) genotype (MST = 8.8 versus 7.57 months, log-rank $p = 0.32$). After multivariate analysis, the hazard ratio substantially increased in the patients with the *ABCB1* $C^{1236}T$ polymorphism having a heterozygous genotype (*CT*) compared to the wild-type (*CC*) genotype (HR = 1.69, 95% CI = 1.04–2.74, $p = 0.03$) (Figure 2A). For *ABCB1* $C^{3435}T$, in the co-dominant model, a drastic decrease in survival was observed in the ADCC patients having a heterozygous genotype (*CT*) compared to the wild-type genotype (*CC*) (6.8 months versus 13.07 months, HR = 1.92, 95% CI = 1.01–3.64, $p = 0.04$) (Figure 2B). ADCC patients with the *ABCB1* $G^{2677}A$

TABLE 2 Univariate and multivariate analysis for *ABC* variants

						Univariate analysis			Multivariate analysis	
Genotype rs1128503 ABCB1 (C1236T)	**Cases, *n* (%), *N* = 483**	**Dead, *n* (%), *N* = 408**	**Alive *n* (%), *N* = 75**	**$\chi 2$**	***p* value**	**MST (months)**	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
CC	268 (55.4)	226 (55.3)	42 (56)			8.57	–	1.0 (reference)	–	–
CT	144 (29.8)	126 (30.8)	18 (24)	2.70	0.25	7.7	0.27	1.13 (0.90–1.41)	1.34 (1.00–1.80)	**0.04**
TT	71 (14.6)	56 (13.7)	15 (20)			7.6	0.51	0.90 (0.68–1.21)	0.98 (0.66–1.45)	0.92
Dominant model										
CC	268 (55.4)	226 (55.3)	42 (56)	0.009	0.92	8.6	–	1.0 (reference)	–	–
CT + TT	215 (44.5)	182 (44.6)	33 (44)			7.67	0.61	1.05 (0.86–1.27)	1.18 (0.91–1.53)	0.21
Recessive model										
CC + CT	412 (85.3)	352 (86.2)	60 (80)	1.98	0.15	8.37	–	1.0 (reference)	–	–
TT	71 (14.6)	56 (13.7)	15 (20)			7.6	0.35	0.88 (0.67–1.15	0.88 (0.61–1.29)	0.53
Additive model										
CC + TT	339 (70.1)	282 (69.1)	57 (76)	1.43	0.23	8.4	–	1.0 (reference)	–	–
CT	144 (29.8)	126 (30.8)	18 (24)			7.7	0.19	1.15 (0.92–1.43)	1.32 (0.99–1.75)	0.05
						Univariate analysis			**Multivariate analysis**	
Genotype rs1045642 ABCB1 (C3435T)	**Cases, *n* (%), *N* = 483**	**Dead *n* (%), *N* = 408**	**Alive, *n* (%), *N* = 75**	**$\chi 2$**	***p* value**	**MST (months)**	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
CC	93 (19.2)	76 (18.6)	17 (22.6)			8.4	–	1.0 (reference)	–	–
CT	199 (41.2)	171 (41.9)	28 (37.3)	0.86	0.64	7.57	0.20	1.18 (0.91–1.54)	1.32 (0.92–1.92)	0.12
TT	191 (39.5)	161 (39.4)	30 (40)			8.73	0.31	0.87 (0.66–1.14)	1.14 (0.79–1.65)	0.46
Dominant model										
CC	93 (19.2)	76 (18.6)	17 (22.6)	0.66	0.41	8.4	–	1.0 (reference)	–	–
CT + TT	390 (80.7)	332 (81.3)	58 (77.3)			8.26	0.21	1.16 (0.91–1.47)	1.21 (0.87–1.69)	0.25
Recessive model										
CC + CT	292 (60.4)	247 (60.5)	45 (60)	0.007	0.93	7.8	–	1.0 (reference)	–	–
TT	191 (39.5)	161 (39.4)	30 (40)			8.73	0.9	0.99 (0.81–1.21)	0.97 (0.74–1.27)	0.85

TABLE 2 (Continued)

Genotype rs1045642 ABCB1 (C3435T)	Cases, *n* (%), *N* = 483	Dead *n* (%), *N* = 408	Alive, *n* (%), *N* = 75	χ2	*p* value	Univariate analysis			Multivariate analysis	
						MST (months)	Log-rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
Additive model										
CC + TT	284 (58.7)	237 (58.0)	47 (62.6)	0.54	0.45	8.6	–	1.0 (reference)	–	–
CT	199 (41.2)	171 (41.9)	28 (37.3)			7.57	0.29	1.11 (0.91–1.35)	1.17 (0.89–1.53)	0.24
Genotype rs2032582 ABCB1 (G2677A)	**Cases, *n* (%), *N* = 483**	**Dead, *n* (%), *N* = 408**	**Alive, *n* (%), *N* = 75**	**χ2**	***p* value**	**Univariate analysis**			**Multivariate analysis**	
						MST (months)	**Log-ranl *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
GG	431 (89.2)	363 (88.9)	68 (90.6)	0.18	0.66	8.6	–	1.0 (reference)	–	–
GA	52 (10.7)	45 (11.0)	7 (9.3)			5.37	0.12	1.31 (0.93–1.84)	1.20 (0.81–1.78)	0.35
AA	0 (0)	0 (0)	0 (0)			–	–	–	–	–
Genotype rs2032582 ABCB1 (G2677T)	**Cases, *n* (%), *N* = 483**	**Dead, *n* (%), *N* = 408**	**Alive, *n* (%), *N* = 75**	**χ2**	***p* value**	**Univariate analysis**			**Multivariate analysis**	
						MST (months)	**Log-ranl *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
GT	250 (51.7)	211 (51.7)	39 (52)	0.002	0.96	7.5	–	1.0 (reference)	–	–
TT	233 (52.3)	197 (48.2)	36 (48)			8.8	0.52	0.93 (0.77–1.14)	0.97 (0.75–1.27)	0.8
Genotype rs41410450 ABCC1 (G3173A)	**Cases, *n* (%), *N* = 483**	**Dead, *n* (%), *N* = 408**	**Alive, *n* (%), *N* = 75**	**χ2**	***p* value**	**Univariate analysis**			**Multivariate analysis**	
						MST (months)	**Log-ranl *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
GG	373 (77.2)	312 (76.4)	61 (81.3)			8.37	–	1.0 (reference)	–	–
GA	102 (21.2)	88 (21.5)	14 (18.6)	1.97	0.38	8.57	0.81	0.97 (0.76–1.23)	1.14 (0.83–1.56)	0.41
AA	8 (1.6)	8 (1.9)	0 (0)			1.63	**0.009**	4.15 (1.40–12.2)	2.45 (1.19–5.04)	**0.01**
Dominant model										
GG	373 (77.2)	312 (76.4)	61 (81.3)	0.85	0.35	8.37	–	1.0 (reference)	–	–
GA + AA	110 (22.7)	96 (23.5)	14 (18.6)			8.26	0.83	1.02 (0.81–1.29)	1.16 (0.85–1.58)	0.34
Recessive model										
GG + GA	475 (98.3)	400 (98.0)	75 (100)	1.49	0.22	8.43	–	1.0 (reference)	–	–

(Continues)

TABLE 2 (Continued)

Genotype rs41410450 ABCC1 (G3173A)	Cases, *n* (%), *N* = 483	Dead, *n* (%), *N* = 408	Alive, *n* (%), *N* = 75	χ2	*p* value	Univariate analysis			Multivariate analysis	
						MST (months)	Log-ranl *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
AA	8 (1.6)	8 (1.9)	0 (0)			1.63	**0.007**	4.51 (1.50–13.55)	2.53 (1.24–5.17)	**0.01**

Genotype rs4148356 ABCC1 (G2168A)	Cases, *n* (%), *N* = 483	Dead, *n* (%), *N* = 408	Alive, *n* (%), *N* = 75	χ2	*p* value	Univariate analysis			Multivariate analysis	
						MST (months)	Log-ranl *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
GG	467 (96.6)	395 (96.8)	72 (96)	0.13	0.71	8.40	–	1.0 (reference)	–	–
GA	16 (3.3)	13 (3.1)	3 (4)			6.3	0.81	1.07 (0.60–1.89)	1.51 (0.78–2.90)	0.21

Genotype rs8187710 ABCC2 (G4544A)	Cases, *n* (%), *N* = 483	Dead, *n* (%), *N* = 408	Alive, *n* (%), *N* = 75	χ2	*p* value	Univariate analysis			Multivariate analysis	
						MST (months)	Log-ranl *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	p
GG	466 (96.4)	391 (95.8)	75 (100)	3.2	0.07	8.4	–	1.0 (reference)	–	–
GA	17 (3.5)	17 (4.1)	0 (0)			4.37	**0.017**	2.16 (1.14–4.09)	1.88 (0.96–3.70)	**0.04**

Genotype rs2231142 ABCG2 (C421A)	Cases, *n* (%), *N* = 483	Dead, *n* (%), *N* = 408	Alive, *n* (%), *N* = 75	χ2	*p* value	Univariate analysis			Multivariate analysis	
						MST (months)	Log-ranl *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
CC	419 (86.7)	353 (86.5)	66 (88)	0.12	0.72	8.37	–	1.0 (reference)	–	–
CA	64 (13.2)	55 (13.4)	9 (12)			7.67	0.87	1.02 (0.76–1.36)	0.94 (0.64–1.38)	0.77

[a]Unadjusted Hazards ratio for Kaplan meier analysis,
[b]hazards ratio after adjustment for age, sex, smoking, stage, regimen, histology, KPS, ECOG.

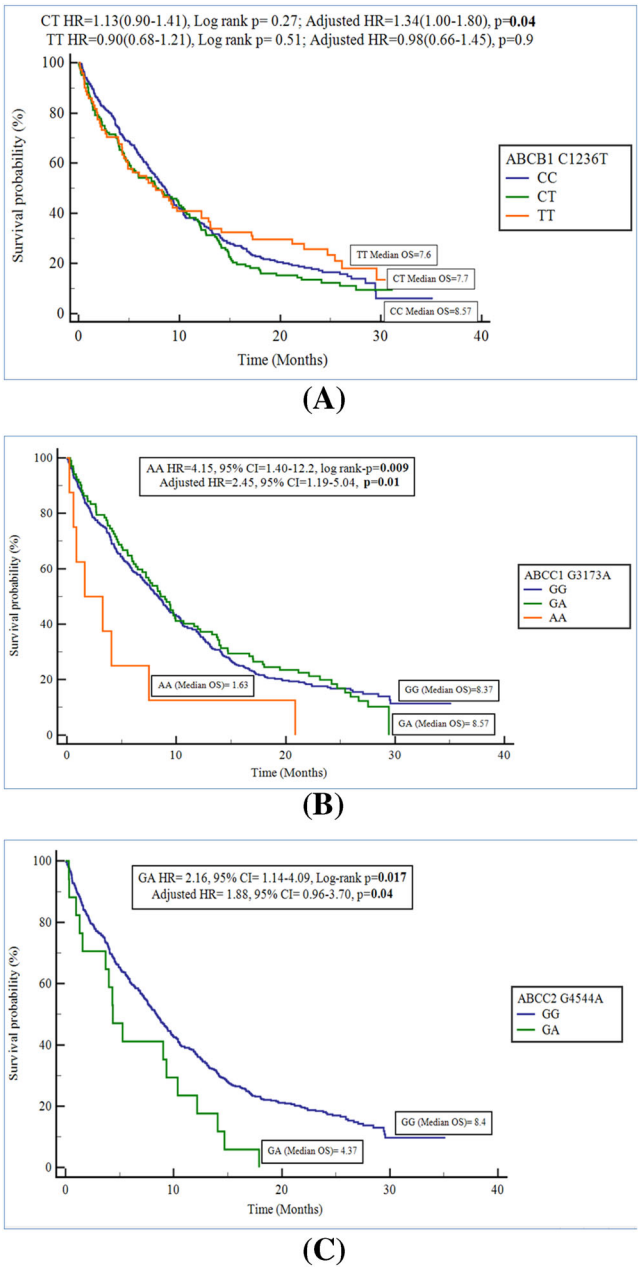


FIGURE 1 (A) Kaplan–Meier curves illustrating the association between overall survival in different genotypes of *ABCB1 C1236T* polymorphism in lung cancer patients. CC represents wild-type genotype, CT represents heterozygous genotype, and TT represents homozygous for mutant genotype. (B) Kaplan–Meier curves illustrating the association between overall survival in different genotypes of *ABCC1 G3173A* polymorphism in lung cancer patients. GG represents wild-type genotype, GA represents heterozygous genotype, and AA represents homozygous for mutant genotype). (C) Kaplan–Meier curves illustrating the association between overall survival in different genotypes of *ABCC2 G4544A* polymorphism in lung cancer patients. GG represents wild-type genotype, GA represents heterozygous genotype

and $G^{2677}T$ polymorphism did not show any association in predicting the survival of lung cancer patients. For the *ABCC1* $G^{3173}A$ polymorphism, our results revealed that ADCC patients having a variant genotype (*AA*) had a reduced OS compared to the patients harboring a reference-type genotype (*GG*) (MST = 3.27 versus 8.5, HR = 4.44, 95% CI = 1.10–17.08, $p = 0.03$) (Figure 2C). Even though there were fewer patients harboring the mutant genotype (*AA*), after multivariate Cox regression, our findings still revealed an increase in the hazard ratio in patients with mutant genotypes (*AA*) (HR = 13.7, 95% CI = 2.70–70.12, $p = 0.001$) (Table 3). None of the ADCC patients revealed any prognostic significance for the *ABCC1* ($G^{2168}A$), *ABCC2* ($G^{4544}A$), and *ABCG2* ($C^{421}A$) polymorphisms (see Supporting information, Table S1).

Our data for SQCC patients revealed no association between different genotypes of *ABCB1* ($C^{1236}T$, $C^{3435}T$, $G^{2677}A$, $G^{2677}T$), *ABCC1* ($G^{3173}A$, $G^{2168}A$), *ABCC2* ($G^{4544}A$), and *ABCG2* ($C^{421}A$) polymorphisms and prognosis (see Supporting information, Table S1).

SCLC patients having $G^{2677}A$ polymorphism and carrying one copy of mutant allele (*GA*) showed poor survival compared to patients having both copies of the wild-type alleles (*GG*) (MST = 4.53 versus 10.4, HR = 3.72, 95% CI = 1.08–12.83, $p = 0.03$). After adjusting with various covariates, the heterozygous genotype (*GA*) for $G^{2677}A$ showed a hazard ratio of 5.54 compared to the wild-type (*GG*) genotype (HR = 5.54, 95% CI = 1.69–18.06, $p = 0.0045$). None of the other *ABCB1* polymorphisms ($C^{1236}T$, $C^{3435}T$, and $G^{2677}T$) showed any association in the prognosis of SCLC patients (see Supporting information, Table S1). However, SCLC patients having the *ABCC2* $G^{4544}A$ polymorphism and harboring a heterozygous genotype (*GA*) showed a poor survival time compared to subjects having the reference genotype (*AA*) (MST = 4.0 versus 9.93, HR = 15.3, $p = 0.03$). Additionally, no association in survival was found in SCLC patients having the *ABCC1* ($G^{3173}A$, $G^{2168}A$) and *ABCG2* ($C^{421}A$) polymorphisms.

3.4 | Prognosis of lung carcinoma patients based on various chemotherapy regimens

Patients with lung carcinoma were given platinum-based chemotherapy and pemetrexed, paclitaxel, docetaxel, and irinotecan. As a result, we chose to investigate the modifying effects of the ABC polymorphism and its relationship with chemotherapy and overall patient survival. The patients administered cisplatin/carboplatin and docetaxel and having both copies of the mutant allele (*TT*) for the *ABCB1* $C^{1236}T$ polymorphism showed a substantially higher OS compared to the reference type genotype (*CC*) (MST 9.7 versus 8.7, HR = 0.30, $p = 0.04$) (Figure 3A and Table 4). Even in the recessive model, survival remained significantly high for mutant genotype (*TT*) compared to combined wild-type and heterozygous genotype (*CC*+*CT*). No association in survival was found in patients administered cisplatin/carboplatin and docetaxel having *ABCB1* ($C^{3435}T$, $G^{2677}A$, and $G^{2677}T$), *ABCC1* ($G^{3173}A$ and $G^{2168}A$), and *ABCG2* ($C^{421}A$) polymorphisms (see Supporting information, Table S2).

Similarly, no correlation was found in cisplatin/carboplatin and paclitaxel regimen in either of the the *ABCB1* ($C^{1236}T$, $C^{3435}T$, $G^{2677}A$, and $G^{2677}T$), *ABCC1* ($G^{3173}A$ and $G^{2168}A$), *ABCC2* ($G^{4544}A$) and *ABCG2* ($C^{421}A$) polymorphisms (see Supporting information, Table S2).

TABLE 3 Association of *ABCB1* SNPs on overall survival according to histology

ADCC										
						Univariate analysis			Multivariate analysis	
Genotype rs1128503 ABCB1 (C1236T)	**Cases, *n* (%), *N* = 191**	**Dead, *n* (%), *N* = 152**	**Alive, *n* (%), *N* = 39**	**χ2**	***p* value**	**MST (months)**	**Log-ranl *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
CC	104 (54.4)	82 (53.9)	22 (56.4)			8.8	–	1.0 (reference)	–	–
CT	54 (28.2)	45 (29.6)	9 (23.0)	0.79	0.67	7.57	0.32	1.20 (0.82–1.76)	1.68 (1.03–2.73)	**0.03**
TT	33 (17.2)	25 (16.4)	8 (20.5)			9.77	0.79	0.94 (0.60–1.46)	1.05 (0.58–1.92)	0.85
Dominant model										
CC	104 (54.4)	82 (53.9)	22 (56.4)	0.07	0.78	8.8	–	1.0 (reference)	–	–
CT + TT	87 (45.5)	70 (46.0)	17 (43.5)			8.57	0.60	1.08 (0.79–1.50)	1.35 (0.88–2.05)	0.16
Recessive model										
CC + CT	158 (82.7)	83.5 (127)	31 (79.4)	0.11	0.73	8.23	–	1.0 (reference)	–	–
TT	33 (17.2)	25 (16.4)	8 (20.5)			9.77	0.53	0.87 (0.58–1.32)	0.86 (0.49–1.52)	0.61
						Univariate analysis			**Multivariate analysis**	
Genotype rs1045642 ABCB1 (C3435T)	**Cases, *n* (%), *N* = 191**	**Dead, *n* (%), *N* = 152**	**Alive, *n* (%), *N* = 39**	**χ2**	***p* value**	**MST (months)**	**Log-ranl *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
CC	45 (23.5)	33 (21.7)	12 (30.7)	1.67	0.43	13.07	–	1.0 (reference)	–	–
CT	69 (36.1)	55 (36.1)	14 (35.8)			6.8	0.18	1.33 (0.87–2.03)	1.92 (1.01–3.64)	**0.04**
TT	77 (40.3)	64 (42.1)	13 (33.3)			9.2	0.10	1.40 (0.93–2.12)	1.52 (0.83–2.80)	0.17
Dominant model										
CC	45 (23.5)	33 (21.7)	12 (30.7)	1.40	0.23	13.07	–	1.0 (reference)	–	–
CT + TT	146 (76.4)	119 (78.2)	27 (69.2)			7.97	0.11	1.33 (0.93–1.91)	1.72 (0.99–3.01)	0.05
Recessive model										
CC + CT	114 (59.6)	88 (57.8)	26 (66.6)	0.98	0.32	7.8	–	1.0 (reference)	–	–
TT	77 (40.3)	64 (42.1)	13 (33.3)			9.2	0.53	0.90 (0.64–1.25)	0.94 (0.61–1.45)	0.8

Genotype rs41410450 ABCC1 (G3173A)	Cases, *n* (%), *N* = 191	Dead, *n* (%), *N* = 152	Alive, *n* (%), *N* = 39	χ2	*p* value	Univariate analysis			Multivariate analysis	
						MST (months)	Log-ranl *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
GG	153 (80.1)	121 (79.6)	32 (82.0)	1.31	0.51	8.5	–	1.0 (reference)	–	–
GA	33 (17.2)	26 (17.1)	7 (17.9)			9.7	0.65	0.90 (0.60–1.37)	1.46 (0.83–2.58)	0.18
AA	5 (2.6)	5 (3.2)	0 (0)			3.27	**0.03**	4.44 (1.10–17.8)	13.7 (2.7–70.12)	**0.001**
GA + AA	38 (19.8)	31 (20.3)	7 (17.9)			8.97	0.94	1.01 (0.68–1.50)	1.61 (0.93–2.77)	0.08

Genotype rs8187710 ABCC2 (G4544A)	Cases, *n* (%), *N* = 191	Dead, *n* (%), *N* = 152	Alive, *n* (%), *N* = 39	χ2	*p* value	Univariate analysis			Multivariate analysis	
						MST (months)	Log-ranl *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
GG	184 (96.3)	145 (95.3)	39 (100)	1.85	0.17	8.8	–	1.0 (reference)	–	–
GA	7 (3.66)	7 (4.6)	0 (0)			4.37	**0.04**	3.03 (1.02–8.97)	1.64 (0.56–4.77)	0.35

SCLC

Genotype rs2032582 ABCB1 (G2677A)	Cases, *n* (%), *N* = 75	Dead, *n* (%), *N* = 71	Alive, *n* (%), *N* = 4	χ2	*p* value	Univariate analysis			Multivariate analysis	
						MST (months)	Log-ranl *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
GG	69 (92)	65 (91.5)	4 (100)	0.36	0.54	10.4	–	1.0 (reference)	–	–
GA	6 (8)	6 (8.4)	0 (0)			4.53	**0.03**	3.72 (1.08–12.83)	5.54 (1.69–18.06)	**0.0045**

Genotype rs8187710 ABCC2 (G4544A)	Cases, *n* (%), *N* = 75	Dead, *n* (%), *N* = 71	Alive, *n* (%), *N* = 4	χ2	*p* value	Univariate analysis			Multivariate analysis	
						MST (months)	Log-ranl *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
GG	71 (94.6)	67 (94.3)	4 (100)	0.23	0.62	9.93	–	1.0 (reference)	–	–
GA	4 (5.3)	4 (5.6)	0 (0)			4.0	0.20	2.92 (0.5–15.1)	15.3 (1.29–182.0)	**0.03**

[a]Unadjusted Hazards ratio for Kaplan meier analysis,
[b]hazards ratio after adjustment for age, sex, smoking, stage, regimen, histology, KPS, ECOG.

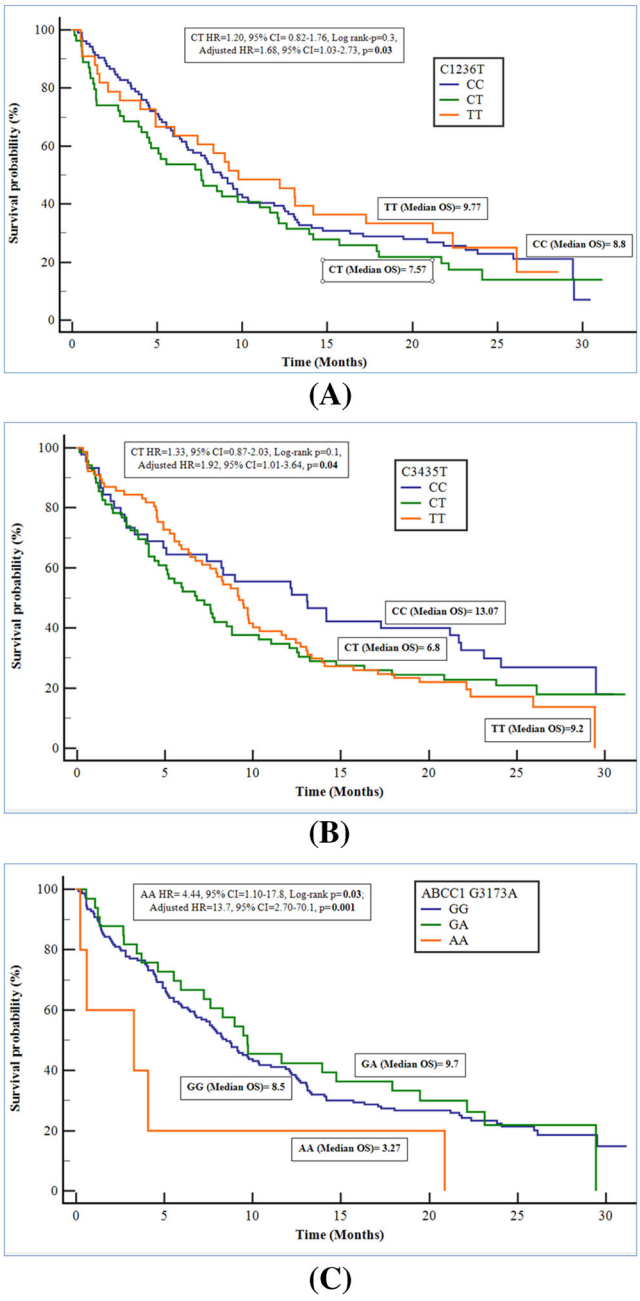


FIGURE 2 (A) Kaplan–Meier curves illustrating the association between overall survival in different genotypes of *ABCB1 C1236T* polymorphism in ADCC patients. CC represents wild-type genotype, CT represents heterozygous genotype, and TT represents homozygous for mutant genotype. (B) Kaplan–Meier curves illustrating the association between overall survival in different genotypes of *ABCB1 C3435T* polymorphism in ADCC patients. CC represents wild-type genotype, CT represents heterozygous genotype, and TT represents homozygous for mutant genotype. (C) Kaplan–Meier curves illustrating the association between overall survival in different genotypes of *ABCC1 G3173A* polymorphism in ADCC patients. GG represents wild-type genotype, GA represents heterozygous genotype, and AA represents homozygous for mutant genotype

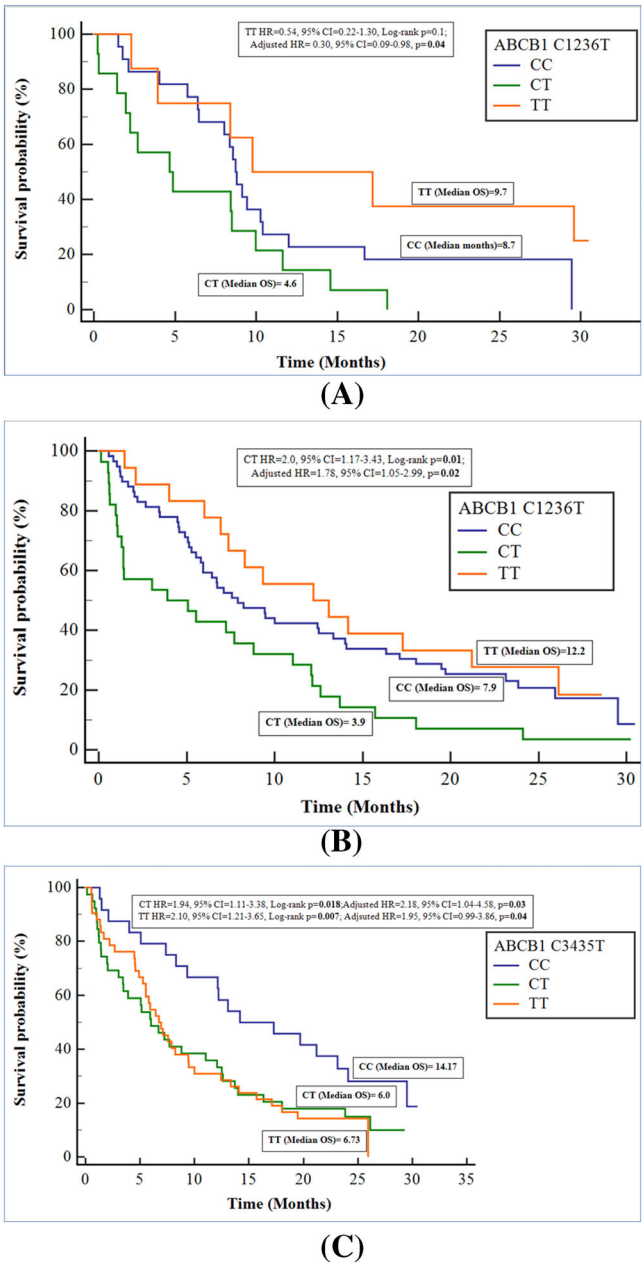


FIGURE 3 (A) Kaplan–Meier curves illustrating the association between overall survival in different genotypes of *ABCB1 C1236T* polymorphism in patients administered with cisplatin/carboplatin and docetaxel. CC represents wild-type genotype, CT represents heterozygous genotype, and TT represents homozygous for mutant genotype. (B) Kaplan–Meier curves illustrating the association between overall survival in different genotypes of *ABCB1 C1236T* polymorphism in patients administered with cisplatin/carboplatin and pemetrexed. CC represents wild-type genotype, CT represents heterozygous genotype, and TT represents homozygous for mutant genotype

Lung cancer patients having a heterozygous genotype (*CT*) for *ABCB1* $C^{1236}T$ and treated with the pemetrexed and cisplatin/carboplatin had substantially poor survival compared to patients with the wild-type (CC) genotype (MST = 3.9 versus 7.9 months, HR = 2.0, 95% CI = 1.17–3.43, $p = 0.01$) (Table 4). After adjusting with different factors such as stage, smoking, age, performance status, and histology, the hazard ratio was 1.78 (HR = 1.78, 95% CI = 1.05–

TABLE 4 Association of *ABC* SNPs on overall survival according to regimen

Cisplatin/carboplatin+docetaxel										
						Univariate analysis			Multivariate analysis	
Genotype rs1128503 ABCB1 (C1236T)	Cases, *n* (%), *N* = 44	Dead, *n* (%), *N* = 39	Alive, *n* (%), *N* = 5	$\chi 2$	*p* value	MST (months)	Log-rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
CC	22 (50.0)	19 (48.7)	3 (60.0)			8.7	–	1.0 (reference)	–	–
CT	14 (31.8)	14 (35.8)	0 (0)	3.38	0.18	4.6	0.08	1.82 (0.86–3.88)	1.88 (0.76–4.67)	0.16
TT	8 (18.1)	6 (15.3)	2 (40.0)			9.7	0.17	0.54 (0.22–1.30)	0.30 (0.09–0.98)	**0.04**
Dominant model										
CC	22 (50)	19 (48.7)	3 (60.0)	0.22	0.63	8.73	–	1.0 (reference)	–	–
CT + TT	22 (50)	20 (51.2)	2 (40.0)			8.4	0.78	1.09 (0.57–2.07)	0.84 (0.40–1.78)	0.66
Recessive model										
CC + CT	36 (81.8)	33 (84.6)	3 (60.0)	1.76	0.18	8.43	–	1.0 (reference)	–	–
TT	8 (18.1)	6 (15.3)	2 (40.0)			9.77	0.06	0.49 (0.23–1.04)	0.34 (0.12–0.97)	**0.04**
Cisplatin/carboplatin+pemetrexed										
						Univariate analysis			Multivariate analysis	
Genotype rs1128503 ABCB1 (C1236T)	Cases, *n* (%), *N* = 105	Dead, *n* (%), *N* = 89	Alive, *n* (%), *N* = 16	$\chi 2$	*p* value	MST (months)	Log-rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
CC	59 (56.1)	48 (53.9)	11 (68.7)			7.9	–	1.0 (reference)	–	–
CT	28 (26.6)	27 (30.3)	1 (6.2)	4.16	0.12	3.9	**0.01**	2.0 (1.17–3.43)	1.78 (1.05–2.99)	**0.02**
TT	18 (17.1)	14 (15.7)	4 (25)			12.2	0.46	0.80 (0.45–1.42)	0.83 (0.41–1.64)	0.59
Dominant model										
CC	59 (56.1)	48 (53.9)	11 (68.7)	1.19	0.27	7.9	–	1.0 (reference)	–	–
CT + TT	46 (43.8)	41 (46.0)	5 (31.2)			7.37	0.24	1.28 (0.84–1.96)	1.25 (0.79–1.96)	0.32
Recessive model										
CC + CT	87 (82.8)	75 (84.2)	12 (75)	0.81	0.36	7.1	–	1.0 (reference)	–	–
TT	18 (17.1)	14 (15.7)	4 (25)			12.2	0.17	0.70 (0.421.16)	0.69 (0.36–1.32)	0.27

Genotype rs1045642 ABCB1 (C3435T)	Cases, *n* (%), *N* = 105	Dead, *n* (%), *N* = 89	Alive, *n* (%), *N* = 16	χ2	*p* value	Univariate analysis			Multivariate analysis	
						MST (months)	Log-rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
CC	24 (22.8)	18 (20.2)	6 (37.5)			14.17	–	1.0 (reference)	–	–
CT	39 (37.1)	34 (38.2)	5 (31.2)	2.30	0.31	6.0	**0.018**	1.94 (1.11–3.38)	2.18 (1.04–4.58)	**0.03**
TT	42 (40)	37 (41.5)	5 (31.2)			6.73	**0.007**	2.10 (1.21–3.65)	1.95 (0.99–3.86)	**0.04**
Dominant model										
CC	24 (22.8)	18 (20.2)	6 (37.5)	2.27	0.13	14.17	–	1.0 (reference)	–	–
CT + TT	81 (77.1)	71 (79.7)	10 (62.5)			6.7	**0.007**	1.86 (1.18–2.93)	2.04 (1.10–3.77)	**0.02**
Recessive model										
CC + CT	63 (60)	52 (58.4)	11 (68.7)	0.59	0.43	9.33	–	1.0 (reference)	–	–
TT	42 (40)	37 (41.5)	5 (31.2)			6.73	0.14	1.39 (0.89–2.18)	1.07 (0.68–1.69)	0.76

Genotype rs8187710 ABCC2 (G4544A)	Cases, *n* (%), *N* = 105	Dead, *n* (%), *N* = 89	Alive, *n* (%), *N* = 16	χ2	*p* value	Univariate analysis			Multivariate analysis	
						MST (months)	Log-rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
GG	101 (96.1)	85 (95.5)	16 (100)	0.74	0.38	7.9	–	1.0 (reference)	–	–
GA	4 (3.8)	4 (4.4)	0 (0)			1.3	**0.03**	5.96 (1.15–30.8)	3.11 (1.06–9.11)	**0.03**

Cisplatin/carboplatin+Irinotecan

Genotype rs2032582 ABCB1 (G2677A)	Cases, *n* (%), *N* = 43	Dead, *n* (%), *N* = 40	Alive, *n* (%), *N* = 3	χ2	*p* value	Univariate analysis			Multivariate analysis	
						MST (months)	Log-rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
GG	38 (88.3)	35 (87.5)	3 (100)	0.41	0.51	10.57	–	1.0 (reference)	–	–
GA	5 (11.6)	5 (12.5)	0 (0)			4.53	**0.009**	7.99 (1.67–38.09)	5.0 (1.55–16.65)	**0.0071**

[a]Unadjusted hazards ratio for Kaplan–Meier analysis,
[b]Hazards ratio after adjustment for age, sex, smoking, stage, regimen, histology, KPS, and ECOG.

2.99, $p = 0.02$) (Figure 3B). In the same line, patients treated with the pemetrexed and cisplatin/carboplatin having both a heterozygous (*CT*) (MST = 6.0 vs 14.17, HR = 1.94, 95% CI = 1.11–3.38, log-rank $p = 0.018$) and mutant (*TT*) genotype (MST = 6.73 vs 14.17, HR = 2.10, 95% CI = 1.21–3.65, log-rank $p = 0.007$) for *ABCB1* $C^{3435}T$ had an approximately 2.5 times poorer OS compared to the reference type genotype (*CC*). After adjusting with multiple covariates, the risk of death remained approximately two times higher in both heterozygous (*CT*) (HR = 2.18, 95% CI = 1.04–4.58, $p = 0.03$) and mutant (*TT*) genotypes (HR = 1.95, 95% CI = 0.99–3.86, $p = 0.04$) (Figure 3C). For the *ABCC2* $G^{4544}A$ polymorphism, patients with one copy of a mutant allele (*GA*) and undergoing cis/carboplatin and pemetrexed revealed a reduced survival compared to the reference genotype (*GG*) subjects (MST = 1.3 versus 7.9, HR = 5.96, log-rank $p = 0.03$). After adjusting with the confounding factors, the risk of death remained high compared to the wild-type genotype (*GG*) (HR = 3.11, 95% CI = 1.06–9.11, $p = 0.03$) (Table 4). Our data did not show any association between different genotypes of *ABCB1 G2677A/T*, *ABCC1* ($G^{3173}A$ and $G^{2168}A$), *ABCG2* $C^{421}A$ polymorphisms and OS in patients undergoing cisplatin/carboplatin and pemetrexed chemotherapy (see Supporting information, Table S2).

Patients administered cisplatin/carboplatin and irinotecan and having one copy of a mutant allele (*GA*) for the *ABCB1* $G^{2677}A$ polymorphism showed a substantially lower survival than the reference genotype (*GG*) (MST = 4.53 versus 10.57, HR = 7.99, log-rank $p = 0.009$). Upon multivariate analysis, the risk of death of the former genotype was 5.0 times higher compared to the wild-type (*GG*) genotype (HR = 5.0, 95% CI = 1.55–16.65, $p = 0.007$), as shown in Table 4. Furthermore, the patients administered irinotecan and cisplatin/carboplatin did not exhibit any significant association between the OS and *ABCB1* ($C^{1236}T$, $C^{3435}T$, and $G^{2677}T$), *ABCC1* ($G^{3173}A$, $G^{2168}A$) and *ABCG2* ($C^{421}A$), as shown in the Supporting information (Table S2).

3.5 | Correlation of ABC polymorphism with OS based on gender stratification

In lung carcinoma patients, 85.05% of males and 82.1% of females were dead. Our results demonstrated no association between overall survival in males and different genotypes of *ABCB1* ($C^{1236}T$, $C^{3435}T$, $G^{2677}A$, $G^{2677}T$), *ABCC1* ($G^{2168}A$, $G^{3173}A$), *ABCC2* ($G^{4544}A$), and *ABCG2* ($C^{421}A$) gene polymorphisms, as illustrated in the Supporting information (Table S3). By contrast, in the *ABCB1* $C^{3435}T$ polymorphism, in the co-dominant model, lower survival was observed in females harboring a mutant type (*TT*) genotype compared to the wild-type (*CC*) genotype (MST = 14.17 versus 8.23, HR = 1.60, log-rank $p = 0.12$) (Table 5). After the Cox regression model, the hazard ratio increased three times in females carrying homozygous mutant alleles (*TT*) in (HR = 3.17, 95% CI = 1.10–9.11, $p = 0.03$). Even in the dominant model, the hazard ratio remained high in the combined heterozygous and mutant genotype (*CT*+*TT*) compared to the reference genotype (*CC*) (HR = 2.52, $p = 0.02$) (Table 5). Furthermore, none of the other polymorphisms in *ABCB1* ($C^{1236}T$, $G^{2677}A$, $G^{2677}T$), *ABCC1* ($G^{2168}A$, $G^{3173}A$), *ABCC2* ($G^{4544}A$), and *ABCG2* ($C^{421}A$) were associated with predicting the prognosis in the females having lung cancer (see Supporting information, Table S3).

3.6 | Correlation of clinicopathological features and ABC polymorphic variants

The individuals were classified by cancer stage, tumor extension, lymph node invasion, and metastasis to assess the relationship between ABC polymorphism and clinical-pathological parameters (see Supporting information, Table S4). Patients with distant metastasis accounted for 56.03% of lung cancer patients, whereas 43.9% had no metastasis. When a comparison was made between the patients with M1 and M0 in *ABCB1* $C^{3435}T$, a two times increased risk of M1 lung carcinoma was observed in the heterozygous (*TC*) and mutant (*TT*) genotype compared to in patients with the wild-type homozygous genotype (*CC*) (AOR = 2.27, $p = 0.01$). Using the dominant model, after adjusting with covariates such as smoking, gender, age, and regimen, an increased risk of developing metastases was observed in the combined heterozygous and mutant (*CT*+*TT*) genotype compared to patients with the wild-type homozygous genotype (*CC*) (AOR = 2.20, 95% CI = 1.18–4.07, $p = 0.01$). There was a no significant association between tumor extension, tumor stage, and lymph node involvement with any *ABCB1*, *ABCC1*, *ABCC2*, and *ABCG2* polymorphic variants.

3.7 | ABC polymorphisms and their response to chemotherapy

The correlation between ABC polymorphisms and the treatment outcomes of response rate was examined using univariate logistic regression. The Supporting information (Table S5) highlights the correlation between ABC polymorphisms and the chemotherapeutic response. Patients with stable and progressing diseases were classified as poor responders, whereas those with partial or complete responses were classified as good responders. There was no statistical correlation between the ABC polymorphism and the platinum-based chemotherapy treatment response. As a result, we conclude that none of the ABC polymorphisms significantly predicts the chemotherapeutic response and clinical benefit.

4 | DISCUSSION

The importance of efflux transporters in ensuring drug absorption, dispersion, metabolism, and elimination in the human tissues/organs and regulating physiological homeostasis is no longer a point of debate. As the functional roles of several ABC transporters are revealed and their significance in cancer biology is highlighted, it has been established that these proteins are associated with various hallmarks and promote oncogenic abilities. ABC transporters are actively involved in

TABLE 5 Association of ABC SNPs on overall survival in females

	Female									
						Univariate analysis			Multivariate analysis	
Genotype rs1045642 ABCB1 (C3435T)	**Cases, *n* (%), *N* = 95**	**Dead, *n* (%), *N* = 78**	**Alive, *n* (%), *N* = 17**	**χ2**	***p* value**	**MST (months)**	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
CC	21 (22.1)	17 (21.7)	4 (23.5)			14.17	–	1.0 (reference)	–	–
CT	41 (43.1)	33 (42.3)	8 (47.05)	0.26	0.87	8.5	0.49	1.22 (0.68–2.17)	2.06 (0.76–5.55)	0.15
TT	33 (34.7)	28 (35.8)	5 (29.4)			8.23	0.12	1.60 (0.87–2.94)	3.17 (1.10–9.11)	**0.03**
Dominant model										
CC	21 (22.1)	17 (21.7)	4 (23.5)	0.02	0.87	14.17	–	1.0 (reference)	–	–
CT + TT	74 (77.8)	61 (78.2)	13 (76.4)			8.36	0.28	1.31 (0.79–2.18)	2.52 (1.13–5.62)	**0.02**
Recessive model										
CC + CT	62 (65.2)	50 (64.1)	12 (70.5)	0.25	0.61	9.03	–	1.0 (reference)	–	–
TT	33 (34.7)	28 (35.8)	5 (29.4)			8.23	0.25	1.32 (0.81–2.16)	1.75 (0.89–3.41)	0.09
						Univariate analysis			**Multivariate analysis**	
Genotype rs41410450 ABCC1 (G3173A)	**Cases, *n* (%), *N* = 95**	**Dead, *n* (%), *N* = 78**	**Alive, *n* (%), *N* = 17**	**χ2**	***p* value**	**MST (months)**	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
GG	68 (71.5)	54 (69.2)	14 (82.3)			8.91	–	1.0 (reference)	–	–
GA	23 (24.2)	20 (25.6)	3 (17.6)	1.57	0.45	9.7	0.64	1.13 (0.66–1.92)	1.06 (0.45–2.49)	0.8
AA	4 (4.2)	4 (5.1)	0 (0)			8.91	0.05	4.62 (0.96–22.2)	400.83 (17.9–8,956)	**0.0002**
GA + AA	27 (28.4)	24 (30.76)	3 (17.6)			9.46	0.37	0.79 (0.47–1.31)	1.30 (0.58–2.93)	0.51
						Univariate analysis			**Multivariate analysis**	
Genotype rs4148356 ABCC1 (G2168A)	**Cases, *n* (%), *N* = 95**	**Dead, *n* (%), *N* = 78**	**Alive, *n* (%), *N* = 17**	**χ2**	***p* value**	**MST (months)**	**Log-rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
GG	92 (96.8)	75 (96.1)	17 (100)	0.66	0.41	9.03	–	1.0 (reference)	–	–
GA	3 (3.1)	3 (3.8)	0 (0)			1.33	**0.04**	7.49 (1.04–53.8)	1.63 (0.26–9.99)	0.59

[a]Unadjusted hazards ratio for Kaplan–Meier analysis,

[b]Hazards ratio after adjustment for age, sex, smoking, stage, regimen, histology, KPS, and ECOG.

transporting chemotherapeutic agents through biological membranes in many organs, and gene mutations influence drug efflux. Polymorphisms in ABC transporters account for genetic disparities and contribute to multidrug resistance. The critical mechanism implicated in imparting multidrug resistance is the increased expression of ABC transporters, which can accelerate the efflux of chemotherapeutic drugs from cancerous cells, reducing the intracellular drug concentration and altering the treatment efficacy and prognosis in lung tumor patients. Knowing that these polymorphisms have a particular impact on the ABC transporter activity, it is critical to investigate their role in lung carcinoma because their expression levels may influence the extent and longevity of chemotherapeutic drug outflow, affecting patient outcomes.

We have analyzed the role of ABC polymorphisms in influencing prognosis in lung cancer patients receiving platinum-based doublet chemotherapy. The results from the North Indian lung carcinoma patients suggest that patients with mutant alleles (*TT*) for *ABCB1* $C^{1236}T$ had a lower survival than the wild-type genotype (*CC*). According to Yang et al.,[25] the *T* allele of *ABCB1* $C^{1236}T$ *is* associated with poor survival in osteosarcoma patients. Similarly, Schaich et al.[26] reported that homozygous (*CC*) alleles had superior overall survival than the heterozygous variants. The *ABCB1* $C^{1236}T$ polymorphism may influence mRNA stability,[27] and the allele-specific changes might influence the downstream mRNA splicing, translational control or regulation in RNA folding. It is also possible that translational activity will be reduced.[28] This accounts for the lower ABCB1 expression in patients having a *CC* genotype compared to the heterozygous genotype and also the reduced drug efflux and enhanced survival in such patients.[26]

By contrast, some studies have shown that Chinese B cell lymphoma patients with the *T* allele of the *ABCB1* $C^{1236}T$ polymorphism had an increased OS.[29] These discrepancies could be attributed to ethnicity, patient clinical features, and tumor histological heterogeneity, which could play an important role. However, many more studies are required to understand how the *ABCB1* $C^{1236}T$ polymorphism affects *MDR1* gene expression and its protein function. The effect of *ABCB1* $C^{3435}T$ polymorphisms on clinical outcomes has been demonstrated in several studies, although the results were inconclusive.[30,31] Our results for *ABCB1* $C^{3435}T$ showed better survival outcomes in patients with mutant genotype (*TT*); however, substantial differences were not achieved, and no association was found between *ABCB1* $C^{3435}T$ and prognosis. Our results concur with those of Johnatty et al.,[32] who found no correlation between the *ABCB1* $C^{3435}T$ variant and overall survival in ovarian cancer patients. However, many contrary reports state a strong correlation of the mutant allele (*TT*) of *ABCB1* $C^{3435}T$ to predict a better OS.[33] The trend in our survival analysis can be explained mechanistically by a few studies that state that the *T* allele at *ABCB1* $C^{3435}T$ attributes to the lower functional activity of the protein, which enhances the bioavailability of the chemotherapeutic drugs and better survival.[30] Other than this, our data did not show any association between *ABCB1* $G^{2677}A/T$ polymorphism and overall survival. Our results are in line with Kim et al.,[34] who found no association in the overall survival and data analyzed for *ABCB1* $G^{2677}A/T$.

When we stratified our analysis of *ABCB1* polymorphism based on histology, we found that adenocarcinoma patients who have homozygous wild-type alleles (*CC*) for *ABCB1* $C^{1236}T$ and $C^{3435}T$ had better survival compared to their associated carrier alleles (*CT*). This can be supported by the study by Eerden et al.,[35] who found that the expression of *ABCB1* efflux transporter is higher in the adenocarcinoma than in the other histology subtypes of squamous cell carcinoma. The significantly higher survival in wild-type patients is associated with the low ABCB1 mRNA levels and prolonged prognosis.[14] Furthermore, this reduced expression of *ABCB1* mRNA levels leading to reduced efflux transporter activity explains our results regarding better survival for the wild-type genotype (*CC*) of *ABCB1* $C^{1236}T$ and $C^{3435}T$ in patients who were administered a combination of cisplatin/carboplatin and pemetrexed. By contrast to our results, a study by Narumiya et al.,[36] reported that the wild-type genotype (*CC*) of *ABCB1* $C^{3435}T$ is associated with a worse prognosis in patients having adenocarcinoma. Our results revealed that SCLC patients with *ABCB1* $G^{2677}A$ harboring heterozygous genotype (*GA*) had a poor survival compared to the wild-type genotype (*GG*). Numerous studies have discussed the role of the *ABCB1* polymorphism in NSCLC patients; however, few studies have reported the role of these polymorphisms in SCLC. It is not unexpected that increased ABCB1 expression was linked to poorer outcomes in some, but not all, of the reports in SCLC patients treated with chemotherapy.[37] This also explains the poor prognosis of heterozygous genotype (*GA*) *of ABCB1* $G^{2677}A$ administered with cisplatin/carboplatin and irinotecan.

By contrast to our results, a previous study reported survival differences in SCLC patients, but somehow their results did not find any significant association between *ABCB1* $G^{2677}T/A$ polymorphism and overall survival.[38] The probable reason for the disparate results could be the small population size and fewer contributions of these polymorphisms in small cell lung cancer. None of the previous studies have examined the association between the *ABCB1* $G^{2677}A$ polymorphism and SCLC.

The patients administered with platinum-based docetaxel-containing chemotherapy with a mutant genotype for *ABCB1*$C^{1236}T$ showed a better survival than those with a wild-type genotype. Chen et al.[39] reported that *ABCB1* $C^{1236}T$ had prognostic significance for OS in patients who received taxane-containing chemotherapy. The impact of genetic mutations on survival could be attributed to polymorphisms that induce alterations in the encoded amino acids, disrupting the normal function of particular drug transporters and reducing P-gp expression. These modifications can cause changes in the pharmacokinetics of a drug, as well as the lower amounts of the drug being pumped out of the cell, leading to drug accumulation in the cell, which can have a detrimental effect on therapeutic efficacy.[40,41] According to Bosch et al.,[42] the *ABCB1* $C^{1236}T$ homozygous mutant had decreased docetaxel clearance. This could be the probable reason explaining the association of *ABCB1* $C^{1236}T$ polymorphism in predicting OS in patients administered with cisplatin/carboplatin and docetaxel.

ABCC1 $G^{3173}A$ is another missense mutation found on the membrane-spanning domain 3.[43] Our results for the *ABCC1* $G^{3173}A$

polymorphism revealed that patients having mutant genotypes (*AA*) had worse prognoses compared to wild-type (*GG*) genotypes. In several investigations, overexpression of *ABCC1* has been associated with drug resistance in lung carcinoma cells.[44,45] Patients with high ABCC1 expression have a poorer prognosis and poorer treatment responses.[14] It has been reported that the *ABCC1* mutation may impact chemotherapeutic efficacy in lung carcinoma patients. However, we could not find any supporting article in the literature to validate our findings as a result of the fewer reports on the *ABCC1* $G^{3173}A$ polymorphism and its associated role in predicting survival in carcinoma patients. Even though the number of patients with mutant genotypes was low, our findings revealed a substantial link between variant alleles and poor survival in lung cancer patients. Lack of association was observed between the *ABCC1* $G^{2168}A$ polymorphism in predicting overall survival. Also, no correlation was found between the *ABCC1* $G^{2168}A$ polymorphism and chemotherapy response. Our results align with Yin et al.,[46] who reported no significant association between the *ABCC1* $G^{2168}A$ polymorphism and chemotherapy response in Chinese lung cancer patients.

The present study reports a significant correlation between poor overall survival and the A allele of the *ABCC2* $G^{4544}A$ polymorphism. Our results align with Cuffe et al.,[15] who depicted the poor survival in NSCLC patients in *A* allele for *ABCC2* $G^{4544}A$ polymorphism. Studies have also shown that the expression of *ABCC2* is low in normal lung tissue, whereas its elevated levels are reported in lung cancer.[47] *ABCC2* is expressed at high levels in the proximal renal tubule, the primary location of platinum-based drug clearance, exporting glutathione-conjugated cisplatin outside the cell.[48] ABCC2 has been associated with the development of platinum resistance because increased *ABCC2* mRNA expression is responsible for resistance to cisplatin by lowering intracellular drug accumulation and hence formation of DNA adducts.[49] *In vivo* studies, particularly data in lung cancer, also suggest a correlation between ABCC2 expression and platinum resistance,[50] although other studies were not as conclusive.[51]

Our data did not demonstrate any association with the role of the *ABCG2* $C^{421}A$ polymorphism in predicting survival differences in lung cancer patients; however, we did see survival trends. Our results align with Steinbach et al.,[52] who found no difference in overall survival between the groups, when they used the median BCRP expression as a cut-off for low and high expression. We observed increased survival in patients with wild-type genotype for *ABCG2* $C^{421}A$ polymorphism compared to the heterozygous genotype. Similar survival trends were shown by Gardner et al.,[17] who stated that the wild-type ABCG2 encodes for a complete functional protein and has a higher chance of survival. The probable reason for the contradictory results could be the massive difference in population size, inter-individual variation, and disparity in therapeutic responses in lung cancer patients.

It is important to note that the present study has certain limitations because carcinoma is discovered late, and our study only included patients with stage III/IV lung cancer. This could be because of a lack of community awareness or resemblance to various diseases, mainly tuberculosis. Many patients receiving chemotherapy at an advanced stage of carcinoma could not meet the treatment's outcome, limiting the scope of our investigation on the chemotherapy response. Most of those seeking treatment at PGIMER come from rural regions in North India. Many patients in remote areas did not attend the hospital frequently because it was challenging for them to travel to the hospital. We could not perform a progression-free survival analysis in the present study because of a lack of clinical information. On the other hand, our study has various advantages, including recruiting a large number of lung cancer patients. Second, patients receiving platinum-based doublet chemotherapy were recruited and treated at the same hospital. Third, patients were enrolled regardless of the ABC polymorphism information, and clinical data were collected separately.

5 | CONCLUSIONS

In the present study, we assessed the role of *ABCB1*, *ABCC1*, *ABCC2*, and ABCG2 polymorphisms in predicting the prognosis of Northern India lung cancer patients. We used genotyping analyses to examine whether the ABC transporter variants affect patient clinical outcomes. Our results revealed that ABC variants play a vital role in regulating ABC gene expression, altering the activity of the efflux transporter, and affecting the overall survival in lung carcinoma patients. This is the first study to examine the effect of ABC polymorphism in lung carcinoma patients from Northern India. Our findings suggest that genotyping for the ABC variations could be a valuable tool for predicting whether patients with lung cancer will benefit most from platinum-based chemotherapy. However, many studies predict contradictory findings regarding transporter activity. Further research into the influence of the ABC variants on transporter activity is needed in the future to determine the role of a particular variation in the pharmacokinetics and pharmacological actions of chemotherapeutic drugs. Such understanding could improve personalized drug doses, correlating with chemotherapeutic drug pharmacokinetic differences between patients.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

ETHICAL STATEMENT

The current study followed the Declaration of Helsinki and Good Clinical Practice guidelines. The Approval No. for the approved study is IEC-04/2018–884. All patients provided their written informed consent.

DATA AVAILABILITY STATEMENT

All of the data generated from this research is presented in the present study. Any further queries can be directed to the corresponding author.

REFERENCES

1. Molina JR, Yang P, Cassivi SD, et al. Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship. In: *Mayo Clin. Proc.* Vol.83(5. Elsevier; 2008:584-594. doi:10.1016/S0025-6196(11)60735-0
2. Walia HK, Singh N, Sharma S. MTHFR polymorphism as a predictive biomarker for gastrointestinal and hematological toxicity in north Indian adenocarcinoma patients. *J Chemother*. 2021;1-15(5):326-340. doi:10.1080/1120009X.2021.1997008
3. Bray F. Transitions in human development and the global cancer burden. World cancer report 34. 2014.
4. Ardizzoni A, Boni L, Tiseo M, et al. Cisplatin-versus carboplatin-based chemotherapy in first-line treatment of advanced non–small-cell lung cancer: an individual patient data meta-analysis. *J Natl Cancer Inst*. 2007;99(11):847-857. doi:10.1093/jnci/djk196
5. Dean M, Hamon Y, Chimini G. The human ATP-binding cassette (ABC) transporter superfamily. *J Lipid Res*. 2001;42(7):1007-1017. doi:10.1016/S0022-2275(20)31588-1
6. Marzolini C, Paus E, Buclin T, Kim RB. Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. *Clin Pharmacol Ther*. 2004;75(1):13-33. doi:10.1016/j.clpt.2003.09.012
7. Scheffer GL, Pijnenborg AC, Smit EF, et al. Multidrug resistance related molecules in human and murine lung. *J Clin Pathol*. 2002;55(5):332-339. doi:10.1136/jcp.55.5.332
8. Campa D, Müller P, Edler L, et al. A comprehensive study of polymorphisms in ABCB1, ABCC2 and ABCG2 and lung cancer chemotherapy response and prognosis. *Int J Cancer*. 2012;131(12):2920-2928. doi:10.1002/ijc.27567
9. Liu L, Fan L, Peng X, Hu D, Zhou H. MDR1 C2005T polymorphism changes substrate specificity. *Cancer Chemother Pharmacol*. 2010;66(3):617-623. doi:10.1007/s00280-010-1308-y
10. Han JY, Lim HS, Yoo YK, et al. Associations of ABCB1, ABCC2, and ABCG2 polymorphisms withirinotecan pharmacokinetics and clinical outcome in patients with advanced non small cell lung cancer. *Cancer*. 2007;110(1):138-147. doi:10.1002/cncr.22760
11. Li YH, Wang YH, Li Y, Yang L. *MDR1* gene polymorphisms and clinical relevance. *Acta Genetica Sinica*. 2006;33(2):93-104. doi:10.1016/S0379-4172(06)60027-9
12. Leslie EM, Deeley RG, Cole SPC. Multidrug resistance proteins in toxicology: role of P-glycoprotein, MRP1, MRP2 and BCRP (ABCG2) in tissue defense. *Toxicol Appl Pharmacol*. 2005;204(3):216-237. doi:10.1016/j.taap.2004.10.012
13. van der Deen M, de Vries EGE, Timens W, Scheper RJ, Timmer-Bosscha H, Postma DS. ATP-binding cassette (ABC) transporters in normal and pathological lung. *Respir Res*. 2005;6(1):1, 59-6. doi:10.1186/1465-9921-6-59
14. Kadioglu O, Saeed ME, Munder M, Spuller A, Greten HJ, Efferth T. Effect of ABC transporter expression and mutational status on survival rates of cancer patients. *Biomed Pharmacother*. 2020;131:110718. doi:10.1016/j.biopha.2020.110718
15. Cuffe S, Azad AK, Qiu X, et al. ABCC2 polymorphisms and survival in the Princess Margaret cohort study and the NCIC clinical trials group BR. 24 trial of platinum-treated advanced stage non-small cell lung cancer patients. *Cancer Epidemiol*. 2016;41:50-56. doi:10.1016/j.canep.2015.12.012
16. Yanase K, Tsukahara S, Mitsuhashi J, Sugimoto Y. Functional SNPs of the breast cancer resistance protein-therapeutic effects and inhibitor development. *Cancer Lett*. 2006;234(1):73-80. doi:10.1016/j.canlet.2005.04.039
17. 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
18. Little J, Higgins JP, Ioannidis JP, et al. STrengthening the REporting of genetic association studies (STREGA)—an extension of the STROBE statement. *Genetic Epidemiol: Off Publ Intl Genetic Epidemiol Soc*. 2009;33(7):581-598. doi:10.1002/gepi.20410
19. Cuschieri S. The STROBE guidelines. *Saudi J Anaesth*. 2019;13(Suppl 1):S31-S34. doi:10.4103/sja.SJA_543_18
20. Rustemoglu A, Gumus-Akay G, Karakus N, Yigit S, Sahin S, Tasliyurt T. Association analysis of three ABCB1 (MDR1) gene variants (C1236T, G2677A/T and C3435T) and their genotype/haplotype combinations with the familial Mediterranean fever. *Xenobiotica*. 2014;44(10):933-940. doi:10.3109/00498254.2014.915071
21. Panczyk M, Balcerczak E, Piaskowski S, Jamroziak K, Pasz-Walczak G, Mirowski M. ABCB1 gene polymorphisms and haplotype analysis in colorectal cancer. *Int J Colorectal Dis*. 2009;24(8):895-905. doi:10.1007/s00384-009-0724-0
22. Yin JY, Huang Q, Yang Y, et al. Characterization and analyses of multidrug resistance-associated protein 1 (MRP1/ABCC1) polymorphisms in Chinese population. *Pharmacogenet Genomics*. 2009;19(3):206-216. doi:10.1097/FPC.0b013e328323f680
23. Daly AK, Aithal GP, Leathart JB, Swainsbury RA, Dang TS, Day CP. Genetic susceptibility to diclofenac-induced hepatotoxicity: contribution of UGT2B7, CYP2C8, and ABCC2 genotypes. *Gastroenterology*. 2007;132(1):272-281. doi:10.1053/j.gastro.2006.11.023
24. Zheng C, Yang H, Wang Q, Rao H, Diao Y. Association analysis of five SNP variants with gout in the Minnan population in China. *Turk J Med Sci*. 2016;46(2):361-367. doi:10.3906/sag-1409-58
25. Yang J, Wang ZG, Cai HQ, Li YC, Xu YL. Effect of variation of ABCB1 and ABCC3 genotypes on the survival of bone tumor cases after chemotherapy. *Asian Pac J Cancer Prev*. 2013;14(8):4595-4598. doi:10.7314/APJCP.2013.14.8.4595
26. Schaich M, Kestel L, Pfirrmann M, et al. A MDR1 (ABCB1) gene single nucleotide polymorphism predicts outcome of temozolomide treatment in glioblastoma patients. *Ann Oncol*. 2009;20(1):175-181. doi:10.1093/annonc/mdn548
27. Frittitta L, Ercolino T, Bozzali M, et al. A cluster of three single nucleotide polymorphisms in the 3′-untranslated region of human glycoprotein PC-1 gene stabilizes PC-1 mRNA and is associated with increased PC-1 protein content and insulin resistance–related abnormalities. *Diabetes*. 2001;50(8):1952-1955. doi:10.2337/diabetes.50.8.1952
28. Shen LX, Basilion JP, Stanton VP. Single-nucleotide polymorphisms can cause different structural folds of mRNA. *Proc Natl Acad Sci USA*. 1999;96(14):7871-7876. doi:10.1073/pnas.96.14.7871
29. Ni Y, Yin G, Xiao Z, et al. MDR1 polymorphisms have an impact on the prognosis of Chinese diffuse large B cell lymphoma patients. *Tumor Biol*. 2016;37(1):1237-1244. doi:10.1007/s13277-015-3930-0
30. Chang H, Rha SY, Jeung HC, et al. Association of the ABCB1 gene polymorphisms 2677G> T/A and 3435C> T with clinical outcomes of paclitaxel monotherapy in metastatic breast cancer patients. *Ann Oncol*. 2009;20(2):272-277. doi:10.1093/annonc/mdn624
31. Pan JH, Han JX, Wu JM, Huang HN, Yu QZ, Sheng LJ. MDR1 single nucleotide polymorphism G2677T/A and haplotype are correlated with response to docetaxel-cisplatin chemotherapy in patients with non-small-cell lung cancer. *Respiration*. 2009;78(1):49-55. doi:10.1159/000158454
32. Johnatty SE, Beesley J, Paul J, et al. ABCB1 (MDR 1) polymorphisms and progression-free survival among women with ovarian cancer following paclitaxel/carboplatin chemotherapy. *Clin Cancer Res*. 2008;14(17):5594-5601. doi:10.1158/1078-0432.CCR-08-0606
33. Hoffmeyer S, Burk O, von Richter O, et al. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc Natl Acad Sci USA*. 2000;97(7):3473-3478. doi:10.1073/pnas.97.7.3473
34. Kim HJ, Im SA, Keam B, et al. ABCB 1 polymorphism as prognostic factor in breast cancer patients treated with docetaxel and

doxorubicin neoadjuvant chemotherapy. *Cancer Sci*. 2015;106(1):86-93. doi:10.1111/cas.12560
35. Van Eerden RA, van Doorn L, de Man FM, et al. Tissue type differences in ABCB1 expression and paclitaxel tissue pharmacokinetics in patients with esophageal cancer. *Front Pharmacol*. 2021;12:759146. doi:10.3389/fphar.2021.759146
36. Narumiya K, Metzger R, Bollschweiler E, et al. Impact of ABCB1 C3435T polymorphism on lymph node regression in multimodality treatment of locally advanced esophageal cancer. *Pharmacogenomics*. 2011;12(2):205-214. doi:10.2217/pgs.10.174
37. Knez L, Sodja E, Kern I, Košnik M, Cufer T. Predictive value of multidrug resistance proteins, topoisomerases II and ERCC1 in small cell lung cancer: a systematic review. *Lung Cancer*. 2011;72(3):271-279. doi:10.1016/j.lungcan.2011.02.014
38. Knez L, Košnik M, Ovčariček T, et al. Predictive value of ABCB1 polymorphisms G2677T/a, C3435T, and their haplotype in small cell lung cancer patients treated with chemotherapy. *J Cancer Res Clin Oncol*. 2012;138(9):1551-1560. doi:10.1007/s00432-012-1231-1
39. Chen Q, Lin W, Yang J, Lin M, Weng Y, Chen Y. Prognostic value of two polymorphisms, rs1045642 and rs1128503, in ABCB1 following Taxane-based chemotherapy: a meta-analysis. *Asian Pac J Cancer Prev*. 2021;22(1):3-10. doi:10.31557/APJCP.2021.22.1.3
40. Zhou Z, Chen Q, Zuo D, Wang H, Hua Y, Cai Z. ABCB1 (rs1128503) polymorphism and response to chemotherapy in patients with malignant tumors-evidences from a meta-analysis. *Int J Clin Exp Med*. 2015;8(1):265-272.
41. Bjorn N, Jakobsen Falk I, Vergote I, Green H. ABCB1 variation affects myelosuppression, progression-free survival and overall survival in paclitaxel/carboplatin-treated ovarian cancer patients. *Basic Clin Pharmacol Toxicol*. 2018;123:277-287.
42. Bosch TM, Huitema AD, Doodeman VD, et al. Pharmacogenetic screening of CYP3A and ABCB1 in relation to population pharmacokinetics of docetaxel. *Clin Cancer Res*. 2006;12(19):5786-5793. doi:10.1158/1078-0432.CCR-05-2649
43. Ito S, Ieiri I, Tanabe M, Suzuki A, Higuchi S, Otsubo K. Polymorphism of the ABC transporter genes, MDR1, MRP1 and MRP2/cMOAT, in healthy Japanese subjects. *Pharmacogenet Genomics*. 2001;11(2):175-184. doi:10.1097/00008571-200103000-00008
44. Triller N, Korošec P, Kern I, Košnik M, Debeljak A. Multidrug resistance in small cell lung cancer: expression of P-glycoprotein, multidrug resistance protein 1 and lung resistance protein in chemo-naive patients and in relapsed disease. *Lung Cancer*. 2006;54(2):235-240. doi:10.1016/j.lungcan.2006.06.019
45. Yeh JJ, Hsu NY, Hsu WH, Tsai CH, Lin CC, Liang JA. Comparison of chemotherapy response with P-glycoprotein, multidrug resistance-related protein-1, and lung resistance-related protein expression in untreated small cell lung cancer. *Lung*. 2005;183(3):177-183. doi:10.1007/s00408-004-2532-1
46. Yin JY, Han LF, Huang Q, Xu XJ, Zhou HH, Liu ZQ. ABCC1 polymorphism Arg723Gln (2168G> a) is associated with lung cancer susceptibility in a Chinese population. *Clin Exp Pharmacol Physiol*. 2011;38(9):632-637. doi:10.1111/j.1440-1681.2011.05571.x
47. Sandusky GE, Mintze KS, Pratt SE, Dantzig AH. Expression of multidrug resistance associated protein 2 (MRP2) in normal human tissues and carcinomas using tissue microarrays. *Histopathology*. 2002;41(1):65-74. doi:10.1046/j.1365-2559.2002.01403.x
48. Schaub TP, Kartenbeck J, König J, et al. Expression of the MRP2 gene-encoded conjugate export pump in human kidney proximal tubules and in renal cell carcinoma. *J Am Soc Nephrol*. 1999;10(6):1159-1169. doi:10.1681/ASN.V1061159
49. Liedert B, Materna V, Schadendorf D, Thomale J, Lage H. Overexpression of cMOAT (MRP2/ABCC2) is associated with decreased formation of platinum-DNA adducts and decreased G2-arrest in melanoma cells resistant to cisplatin. *J Invest Dermatol*. 2003;121(1):172-176. doi:10.1046/j.1523-1747.2003.12313.x
50. Ushijima R, Takayama K, Izumi M, et al. Immunohistochemical expression of MRP2 and clinical resistance to platinum-based chemotherapy in small cell lung cancer. *Anticancer Res*. 2007;27(6C):4351-4358.
51. Filipits M, Haddad V, Schmid K, et al. Multidrug resistance proteins do not predict benefit of adjuvant chemotherapy in patients with completely resected non–small cell lung cancer: international adjuvant lung cancer trial biologic program. *Clin Cancer Res*. 2007;13(13):3892-3898. doi:10.1158/1078-0432.CCR-06-2446
52. Steinbach D, Sell W, Voigt A, Hermann J, Zintl F, Sauerbrey A. BCRP gene expression is associated with a poor response to remission induction therapy in childhood acute myeloid leukemia. *Leukemia*. 2002;16(8):1443-1447. doi:10.1038/sj.leu.2402541

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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RESEARCH ARTICLE

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Genetic variations in *ABC* transporter genes as a predictive biomarker for toxicity in North Indian lung cancer patients undergoing platinum-based doublet chemotherapy

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Abstract

ATP-binding cassette (ABC) transporters are expressed in various human tissues and play a vital role in the efflux of various chemotherapeutic drugs. The current study has assessed genetic variants of *ABCB1*, *ABCC1*, *ABCC2*, and *ABCG2* genes in 407 lung cancer patients undergoing platinum-based doublet chemotherapy. The association of *ABCB1* ($C^{1236}T$, $C^{3435}T$, and $G^{2677}T/A$), *ABCC1* ($G^{3173}A$ and $G^{2168}A$),*ABCC2* ($G^{4544}A$), and *ABCG2* ($C^{421}A$) polymorphisms with chemotherapy-induced adverse events were assessed, and statistical analysis was conducted. Our data showed that patients harboring heterozygous (*GA*) genotype for *ABCC1* $G^{3173}A$ had an increased risk of developing leukopenia (odds ratio [OR] = 1.88, $p = 0.04$) and anemia (adjusted odds ratio [AOR] = 2.70, $p = 0.03$). For *ABCC2* $G^{4544}A$ polymorphism, patients harboring one copy of the mutant (*GA*) allele showed an increased risk of developing anemia (OR = 4.24, $p = 0.03$). After adjusting with various confounding factors, the heterozygous (*GA*) genotype showed a 5.63-fold increased risk of developing anemia (AOR = 5.63, $p = 0.03$). The *ABCB1* $G^{2677}A$ (OR = 0.37, $p = 0.008$) and *ABCC1* $G^{3173}A$ (OR = 0.54, $p = 0.04$) polymorphism showed a lower incidence of developing nephrotoxicity. In *ABCG2* $C^{421}A$ polymorphism, patients harboring heterozygous (*CA*) genotype had a lower incidence of having diarrhea (OR = 0.25, $p = 0.04$). An increased risk of having diarrhea was observed in the heterozygous genotype (*GA*) for *ABCC1* $G^{3173}A$ polymorphism (AOR = 2.78, $p = 0.04$). An increased risk of liver injury was found in the patients carrying heterozygous genotype of the *ABCC1* $G^{3173}A$ (OR = 2.06, $p = 0.02$) and *ABCB1* $C^{1236}T$ (OR = 1.85, $p = 0.01$). This study demonstrates the role of polymorphic variations in *ABCB1*, *ABCC1*, *ABCC2*, and *ABCG2* in predicting hematological, nephrotoxicity, gastrointestinal, and hepatotoxicity.

KEYWORDS
ABC transporter, lung cancer, polymorphism, risk, toxicity

1 | INTRODUCTION

Lung carcinoma is a malignant tumor reported in both males and females and is also one of the significant causes of death globally.[1] Small cell lung cancer (SCLC) and non-SCLC (NSCLC) are the two main types of lung malignancy, where NSCLC accounts for the majority of all cases of lung cancer.[2] As early diagnosis is challenging, most individuals are detected with late-stage cancer when surgery is impossible.[3] Platinum-based drugs, like cisplatin, oxaliplatin, and carboplatin, have been suggested as first-line

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treatment for patients with malignancy, in combination with other cytotoxic agents.[4] These drugs have an antitumor effect as platinum-based drugs interfere with DNA repair, suppressing and gradually killing cancerous cells.[5] Regrettably, platinum can impede the growth of healthy cells as well.

Even though platinum-based chemotherapeutic drugs are efficacious in cancer treatment, platinum-induced toxicity is a significant consequence of such therapies. Platinum-based drugs cause adverse effects such as hepatotoxicity, nephrotoxicity, gastrointestinal (GI) toxicity, and hepatotoxicity, hindering the treatment's efficacy. Adverse reactions might lead to dose reductions, treatment delays, chemotherapy termination, and life-threatening problems. Additionally, there is heterogeneity among patients regarding platinum-induced toxicities.[6] So, personalized therapy aims to identify people who are more likely to get benefits from anticancerous chemotherapy or experience fewer adverse events (AEs). There are mounting shreds of evidence to support that genetic polymorphisms play a role in causing disparities in platinum-related toxicity.[7] So, it is crucial to assess the correlation between genetic polymorphisms and cisplatin/carboplatin-induced drug toxicity to receive maximum benefit from personalized therapy.

The physiological role of efflux transporter genes in the translocation of the chemotherapeutic agents by a biological membrane into the other organs is well stated, and mutations in such transporters can significantly affect the outflow of the drugs. Single nucleotide polymorphisms (SNPs) in the ATP-binding cassette (ABC) transporter genes alter the functions or expressions of the ABC proteins'. The aberrant expression of these transporter genes might affect interindividual drug tissue concentration and its therapeutic effects. The impact of *ABCB1* ($C^{1236}T$, $C^{3435}T$, and $G^{2677}T/A$), *ABCC1* ($G^{3173}A$ and $G^{2168}A$), *ABCC2* ($G^{4544}A$), and *ABCG2* ($C^{421}A$) gene polymorphisms in predicting toxicity has been studied in some tumors.[8–10]

A study by Han et al. reported an association between *ABCB1* $G^{2677}T/A$ polymorphism and severe grade neutropenia in NSCLC patients of the Korean population who underwent a combination of platinum and irinotecan regimen. Another *ABCB1* $C^{3435}T$ polymorphism has been strongly correlated with grade 3 diarrhea.[11,12] However, Pan et al. reported no association between $C^{3435}T$ and $G^{2677}T/A$ polymorphism and AEs in NSCLC patients who underwent cisplatin-based chemotherapy in NSCLC patients.[13] Another study reported no association between *ABCB1* $C^{3435}T$ and $G^{2677}T/A$ polymorphism and the risk of GI, hematological, nephrological, or hepatic toxicity in lung cancer patients who underwent platinum-based chemotherapy.[14] A recent study by Rierra et al. showed an association between *ABCB1* $C^{1236}T$ polymorphism and grade 3–4 GI toxicity in patients undergoing irinotecan-based chemotherapy.[15] Another study by Choi et al. revealed that the variant genotype of *ABCB1* $C^{3435}T$ was correlated with an increased risk of anemia and neutropenia.[16]

Mutation $G^{4544}A$ in the *ABCC2* gene leads to the impairment of the ATPase activity and is correlated with increased cellular accumulation of the substrates of ABCC2, which might lead to cellular toxicity.[17] However, the reports for *ABCC2* gene polymorphism concerning lung cancer are very trivial, as the *ABCC2* gene has been extensively studied in the context of Dubin Johnson Syndrome. Sprowl et al. reported no substantial correlation between $G^{4544}A$ polymorphism in predicting nephrotoxicity in patients who underwent cisplatin therapy.[18]

ABCG2 $C^{421}A$ variation is responsible for the reduced activity of ABCG2. Genetic variation of *ABCG2* may play a role in the alteration of transporter activity for the chemotherapeutic drug and enhance the systemic circulation of the drugs, which leads to myelosuppression.[19] A report has depicted this variation's role in the elevated risk of diarrhea in NSCLC patients.[20] These efficient evidence have suggested the crucial role of ABC gene polymorphisms in predicting toxicity in lung cancer patients undergoing platinum-based chemotherapy. Hence, uncovering genetic variations associated with ABC transporter genes is essential in personalizing treatment to increase therapeutic response while reducing drug toxicity. So, the present study aims to find the correlation of ABC polymorphisms, that is, *ABCB1* ($C^{123}6T$, $C^{3435}T$, *and* $G^{2677}T/A$), *ABCC1* ($G^{3173}A$ *and* $G^{2168}A$), *ABCC2* ($G^{4544}A$), *and ABCG2* ($C^{421}A$) in modulating the AEs in lung cancer patients undergoing platinum-based doublet chemotherapy. To the best of our knowledge, the impact of the *ABC* gene polymorphisms in predicting toxicity has not been studied in North Indian lung cancer patients undergoing platinum-based chemotherapy.

2 | METHODS

2.1 | Sample collection and study design

The current study included lung cancer patients who visited the author's clinic, that is, the Post Graduate Institute of Medical Education and Research (PGIMER). For 3 years, the study recruited newly diagnosed individuals with histologically or cytologically proven NSCLC or SCLC. The institute's ethics board approved the research study (Approval No. IEC-04/2018-884). Patients with lung carcinoma who met all of the following criteria were eligible to participate: (i) Lung cancer diagnosis confirmed by cytology or histology (ii) Stage IIIB–stage IV or stage IIIA (not scheduled for surgery). (iii) Untreated and intending platinum-based chemotherapy treatment (platinum agent [Cisplatin or Carboplatin] in combination with docetaxel, paclitaxel, gemcitabine, pemetrexed, or irinotecan). (iv) A performance status (PS) of range 0–2 from the Eastern Cooperative Oncology Group (ECOG). (v) At least one bi-dimensionally quantifiable lesion based on the Response Evaluation Criteria in Solid Tumors (RECIST) criteria. (vi) Adequate organ function, which can be defined as absolute neutrophil count greater than 1500/μl, platelet count greater than 100,000/μl, and creatinine, liver enzyme, and alanine aminotransferase (ALT) level less than two times the upper limits of normal. (vii) Informed written consent was taken. Among the exclusion criteria were (i) Non-Bronchogenic tumors. (ii) Primary pleural tumors –mesothelioma (iii) Sarcomas (iv) Metastatic tumors in lungs or pleural (v) Early-stage (resectable) lung cancer.

Patients underwent renal function tests, a complete blood count (CBC), a contrast-enhanced computed tomography scan of the thorax (that also includes the upper abdomen), liver function tests (LFT), and a chest radiograph at the commencement of this investigation.

The participants' demographic details (gender, age, and performance status), stage of the disease, tumor histology, and smoking status were documented. A CBC was performed 10 days after the first chemotherapy session, and any additional cycles were required. Before each treatment cycle and 3–4 weeks after the last chemotherapy session, CBC, renal function, and liver test was conducted. The STROPS checklist was used to report this paper.[21]

2.2 | Chemotherapy toxicity

Before the initiation of chemotherapy, blood samples were collected for genetic studies. Platinum-based drug such as carboplatin and cisplatin (70 mg/m^2) was infused in patients for over 3-h as first-line chemotherapy, followed by the combination of second-line nonplatinum drugs, that is, docetaxel (75 mg/m^2), irinotecan (100 mg/m^2), pemetrexed (500 mg/m^2), or paclitaxel (175 mg/m^2). Four chemotherapy cycles were administered before a tumor response assessment, which is common practice at the clinic PGIMER. The tumor response was evaluated just before the end of the four cycles, and if there were any unfavorable toxicity or clinic-radiological signs of cancer development, chemotherapy was discontinued, if necessary. Subjects who responded objectively to chemotherapy received two additional treatment cycles (i.e., maximum of 6 cycles). The evaluation of tumor response was carried out using RECIST criteria. The common toxicity criteria version 3.0 was used to record and categorize AEs. To evaluate adverse effects, PGIMER's established protocol was used. The toxicity data for 407 of the 502 subjects in the study was available.

Chemotherapy-associated-AEs were assessed for patients after one/or two/or three/or four chemotherapeutic cycles. The following hematological toxicity parameters were evaluated: leukopenia, anemia, thrombocytopenia, and neutropenia. For assessing nephrological toxicity, glomerular filtration rate was determined. The gastrointestina GI toxicity of diarrhea, nausea, and constipation was investigated. Patients with febrile neutropenia or GI adverse effects of grade 3 or higher were admitted to the hospital if outpatient treatment was not accessible or beneficial. Other side effects of the chemotherapy were also documented. During chemotherapy, patients were checked every 3 weeks and every 3 months for the rest of the study. Overall survival was calculated from the start of chemotherapy until death or the end of the last follow-up period.

2.3 | Genotyping of ABC variants

The phenol-chloroform separation technique was used to isolate genomic DNA from 3 to 4 ml of blood. Each SNP was genotyped using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. PCR-RFLP for the four SNPs of *ABCB1* $C^{1236}T$ (rs1128503), $C^{3435}T$ (rs1045642), $G^{2677}A$ (rs2032582), and $G^{2677}T$ (rs2032582) were investigated, as reported earlier by, Rustemoglu et al. and Panczyket al.[22,23]

For the *ABCC1* gene, PCR-RFLP of the two polymorphisms $G^{2168}A$ (rs4148356) and $G^{3173}A$ (rs41410450) were performed as explained previously.[24] The genotyping for *ABCC2* $G^{4544}A$ (rs8187710) polymorphism was done as initially reported by Daly et al.[25] The *ABCG2* variant, $C^{421}A$ (rs2231142), was genotyped using allele-specific PCR (AS-PCR) assay. The genotyping method and primers used were based on a previous study by Zheng et al.[26]

PCR (25 µl) reaction constituted X1 PCR buffer, 0.5 µM forward and reverse primers, 200 µM dNTPs, 100 µg/ml bovine serum albumin, 1 U Taq polymerase (DNAzyme II; Thermo Scientific), and 200 ng DNA. As stated previously, the amplified products were digested using restriction enzymes. The digested product was separated on a polyacrylamide gel or an agarose gel and stained with ethidium bromide to ascertain the restriction patterns. Two researchers double-checked the information to ensure no errors, and 20% of the samples were chosen randomly to verify that the results were 100% accurate.

2.4 | Statistical analysis

The research was based on the North Indian population and included age, gender, and smoking behavior data. The risk of lung carcinoma was investigated using the MedCalc Statistical Software (version 14.8.1 MedCalc Software). Descriptive data is represented as median, mean ± standard deviation, or number/percentage. Logistic regression analysis was performed for any toxicity, and the odds ratio (OR) with 95% confidence intervals (CIs) were calculated. After adjusting for other variables, logistic regression analysis was performed to examine the independent influence of polymorphism on AEs. A p Value of <0.05 was considered significant in all observations.

Toxicity in lung cancer patients was determined using the National Cancer Institute Criteria 3.0 (http://ctep.cancer.gov). Hematological toxicity received special consideration, including neutropenia, anemia, leukopenia, thrombocytopenia, GI toxicity, nephrotoxicity, and hepatotoxicity. Grade 3 or 4 toxicities were defined as severe toxicity. Treatment was deferred when there was severe hematologic toxicity until the patient recovered to a grade 1 or 0. With GI toxicity in grades 3 and 4, the doses of each drug were lowered by 25%. Platinum was reduced by 25% if creatinine clearance fell below 59–41 ml min^{-1}. Platinum was discontinued if creatinine clearance fell below 40 ml min-^{1}. Throughout treatment, clinical data was meticulously recorded.

3 | RESULTS

3.1 | Demographic characteristics of patients

Demographic characteristics of the case group, comprising age, gender, tumor size (T), smoking status, lymph node invasion (N), pack-years, metastases (M), and tumor staging, are summarized in Table 1 and Supporting Information: Table 1. 502 patients with lung cancer were

TABLE 1 Demographic characteristics

Variable	Total (*N* = 502)	Cases, *n* (%)
Gender	502	
Male		401 (79.8)
Female		101 (20.1)
Age	502	60 ± 9.67
		30–86
Smoking status	502	
Smokers		396 (78.8)
Nonsmokers		106 (21.1)
Pack years	502	
Mean ± SD		22.81 ± 23.52
Performance status	502	
KPS (below 60)		88 (17.5)
KPS (70–80)		256 (50.9)
KPS (90–100)		138 (27.4)
Unknown		20 (3.98)
ECOG (0–1)		220 (43.8)
ECOG (2)		187 (37.2)
ECOG (3–4)		76 (15.1)
Unknown		19 (3.7)
Histological types	495	
SQCC		207 (41.8)
ADCC		205 (41.4)
SCLC		76 (15.3)
Others		7 (1.41)
Chemotherapy regimen	370	
Pemetrexed cis/carboplatin		113 (30.5)
Irinotecan cis/carboplatin		45 (12.1)
Docetaxel cis/carboplatin		46 (12.4)
Paclitaxel cis/carboplatin		86 (23.2)
Gemcitabine cis/carboplatin		31 (8.37)
Gefitinib		15 (4.05)
Etoposide		17 (4.59)
Others		17 (4.59)

enrolled to study polymorphism in *ABCB1*, *ABCC1*, *ABCC2*, and *ABCG2* genes. The Hardy-Weinberg equilibrium test for all the SNPs has been highlighted in Supporting Information: Table 2. The patients' mean age was 60.67 ± 9.67 (30–86). Men outnumbered women by 79.8% (401) –20.1% (101) of the enrolled patients. Smokers comprised 78.8% of the population, much higher than nonsmokers (21.1%). The average pack-year for smokers was 22.81 ± 23.52. When lung cancer patients were classified based on the TNM staging, 32.2% were diagnosed with stage III, while 58.1% were detected with stage IV lung cancer. Patients with tumor sizes T1 and T2 exhibited low rates of 6.17% and 8.7%, respectively, but patients with T3 and T4 had high rates of 18.32% and 60.1%, respectively. 54.5% had already developed metastasis during diagnosis, whereas 42.8% had no metastatic involvement. Lymph node involvement was found in 13.7% for N0, 7.7% for N1, 43.8% for N2, 31.8% for N3, and 0.19% for N4 patients. On histological examination, 41.4% were revealed to have adenocarcinoma (ADCC), 41.8% of patients had squamous cell carcinoma, and 15.3% had SCLC. Furthermore, the cases were classified by the ECOG and Karnofsky's status (KPS). 220 (43.8%) patients had ECOG scores range of 0–1, 187 (37.2%) people had ECOG scores of 2, and 76 (15.1%) people had ECOG score of 3–4. KPS values of less than 60 were found in 88 participants, with a score of 70–80 in 256 patients and 90–100 in 138 individuals. Lung carcinoma patients were given platinum-based therapy (cisplatin/carboplatin), 113 patients undergoing pemetrexed therapy, 45 getting irinotecan, 86 receiving paclitaxel, 46 getting docetaxel, 31 having gemcitabine, 15 with gefitinib, 17 receiving etoposide, and 17 getting other regimens.

The current research's objective was to investigate the onset of toxicity in lung cancer patients who received platinum-based doublet chemotherapy with paclitaxel, pemetrexed, docetaxel, and irinotecan. We used three potential analytic strategies to see how ABC variants affected different toxicity levels. In the first analysis, individuals with any toxicity grade (grades 1–4) were compared to those with no toxicity (grade 0); in the second and third assessments, patients with intermediate/severe toxicity (grades 2–4) were compared to those with no/low-grade (grade 0–1) toxicity, and high-grade toxicity (grades 3–4) versus no/low/intermediate toxicity (grades 0-2) to see whether the ABC polymorphisms, that is, *ABCB1* ($C^{1236}T$, $C^{3435}T$, *and* $G^{2677}T/A$), *ABCC1* ($G^{3173}A$ *and* $G^{2168}A$), *ABCC2* ($G^{4544}A$), and *ABCG2* ($C^{421}A$) were associated to severe toxicity. Cases were then classified into different categories of toxicity in this manner.

3.2 | Association between ABC polymorphisms and hematological toxicity

All chemotherapy-related AEs were recorded for each treatment cycle. We assessed leukopenia, anemia, thrombocytopenia, and absolute neutropenia in hematological toxicity. In anemia, among 407 patients, 67.8% of individuals had grade 1–2 toxicity, 7.12% of patients had grade 3–4 toxicity, and 25% showed no toxicity. Among 405 patients, 10.8% showed low/intermediate toxicity (grade 1–2) in leukopenia, while 2.22% of patients had high toxicity (grade 3–4). In the case of thrombocytopenia, 13.5% of patients had grade 1–2 toxicity, while only one patient showed grade 3–4 toxicity. Among 366 patients, 6.55% of individuals showed grade 1–2 neutropenia, whereas 2.45% had severe neutropenia (grade 3–4) (Table 2A). Upon assessing the effect of different ABC polymorphisms, our results showed that 21.2% of patients harbored a single copy of mutant allele (*GA*) for *ABCC1* $G^{3173}A$ polymorphism. Table 3 shows that when patients with low/intermediate/severe toxicity

TABLE 2a Groups of hematological with CTC grades in lung cancer patients

Toxicity	Adverse events	*n*	Toxicity profiles Grade 0, *n* (%)	Grade 1–2, *n* (%)	Grade 3–4, *n* (%)
Hematological toxicity	Anemia	407	102 (25.0)	276 (67.8)	29 (7.12)
	Leukopenia	405	352 (86.9)	44 (10.8)	9 (2.22)
	Thrombocytopenia	399	344 (86.2)	54 (13.5)	1 (0.25)
	Neutropenia	366	333 (90.9)	24 (6.55)	9 (2.45)

(Grade 1–4) were compared with no toxicity grade (grade 0), then patients harboring heterozygous (*GA*) genotype had an increased risk of developing leukopenia as compared to the wild type (*GG*) genotype of *ABCC1* $G^{3173}A$ polymorphism (OR = 1.88, 95% CI = 1.0–3.56, $p = 0.04$). None of the other *ABCB1* ($C^{1236}T$, $C^{3435}T$, and $G^{2677}T/A$), *ABCC1* ($G^{2168}A$), *ABCC2* ($G^{4544}A$), and *ABCG2* ($C^{421}A$) were associated with the risk of developing leukopenia (Supporting Information: Table 3).

A total of 407 patients' toxicity data was available in the case of anemia (Table 2a). Table 3 shows that 25.1% of individuals were classified in grade 0 toxicity, and 74.9% of patients were categorized in low/intermediate/severe toxicity (grades 1–4). As observed for *ABCC1* $G^{3173}A$ polymorphism, 18.6% of patients harbored single copy mutant allele (*GA*). Table 3 shows that individuals with the heterozygous (*GA*) genotype had an increased risk of developing anemia (OR = 1.24, 95% CI = 0.70-2.19; $p = 0.4$) when compared to the patients with a wild-type genotype (*GG*). After adjusting with different confounding, factors like gender, age, performance status, and regimen, 2.7 folds increased risk was observed in heterozygous genotype (*GA*) as compared to wild-type genotype (*GG*) (adjusted odds ratio [AOR] = 2.70, 95% CI = 1.06–6.88; $p = 0.03$). In the case of the dominant model, increased risk in combined genotype *(GA + AA)* for *ABCC1* $G^{3173}A$ polymorphism was noted with the odds of 2.85 as compared to the wild type (*GG*) (AOR = 2.85, 95% CI = 1.12–7.23, $p = 0.02$). For *ABCC2* $G^{4544}A$ polymorphism, when individuals with severe toxicity (grades 3–4) were compared to no/low-grade (grade 0–2) toxicity, our results showed that 7.1% of individuals had grade 3–4 anemia. Individuals harboring one copy of the mutant (*GA*) allele showed an increased risk of developing anemia (OR = 4.24, 95% CI = 1.10–16.38; $p = 0.03$) as compared to patients having both copies of the wild-type allele (*GG*). When adjusted with various confounding factors such as age, regimen, performance status, and gender, the heterozygous (*GA*) genotype showed a 5.63-fold increased risk of developing anemia as compared to the wild-type (*GG*) genotype (AOR = 5.63, 95% CI = 1.13–28.04, $p = 0.03$). However, we did not find any correlation between the ABC polymorphisms and other hematological toxicities (Supporting Information: Table 3).

3.3 | Association between ABC polymorphisms and nephrotoxicity

Chemotherapeutic drugs are excreted through the kidneys and are nephrotoxic; thus, renal functions are monitored during chemotherapy. Data of 397 patients were available to evaluate the nephrotoxicity. Among 397 patients, 81.1% were categorized as low/intermediate toxicity, whereas 4.28% of patients were classified in severe toxicity grade, and the other 14.6% showed no nephrotoxicity (Table 2B). We have evaluated chemotherapy-induced nephrotoxicity by comparing intermediate/severe toxicity (grades 3–5) versus no/low grade (grades 1–2) toxicity. We compared low/intermediate/severe (grades 2–5) toxicity to no (grade 1) toxicity in chemotherapy-induced nephrotoxicity. As illustrated in Table 4, lung cancer patients with heterozygous (*GA*) genotype for *ABCB1* $G^{2677}A$ polymorphism showed a lower incidence of developing

TABLE 2b Groups of graded nephrological toxicity with CTC grades in lung cancer patients

Toxicity	Adverse events	*n*	Toxicity profiles Grade 1, *n* (%)	Grade 2–3, *n* (%)	Grade 4–5, *n* (%)
Nephrotoxicity	GFR	397	58 (14.6)	322 (81.1)	17 (4.28)

TABLE 2c Gastrointestinal toxicity with CTC grades in lung cancer patients

Toxicity	Adverse events	*n*	Toxicity profiles Grade 1, *n* (%)	Grade 2–4, *n* (%)
Gastrointestinal toxicity	Diarrhea	135	62 (45.9)	73 (54.0)
	Constipation	99	30 (30.3)	69 (69.6)
	Anorexia	121	7 (5.78)	114 (94.2)
	Nausea-vomiting	118	54 (45.7)	64 (54.2)

TABLE 2d Groups of graded hepatotoxicity with CTC grades in lung cancer patients

Toxicity	Adverse events	*n*	Toxicity profiles Grade 0, *n* (%)	Grade 1–2, *n* (%)	Grade 3–4, *n* (%)
Hepatotoxicity	SGOT	317	242 (76.3)	74 (23.3)	1 (0.31)
	SGPT	317	180 (56.7)	132 (41.6)	5 (1.57)
	ALP	307	153 (49.8)	144 (46.9)	10 (3.25)

Abbreviations: ALP, alkaline phosphatase; CTC, common toxicity criteria; GFR, glomerular filtration rate; SGOT, serum glutamate oxaloacetate transaminase; SGPT, serum glutamate pyruvate transaminase.

TABLE 3 Association of *ABC* polymorphism with Hematological toxicity

Hematological toxicity						
Leukopenia						
Genotype rs41410450 ABCC1 (G3173A)	**Grade of toxicity 0, *n* (%) *N* = 352**	**Grade of toxicity 1–4, *n* (%) *N* = 53**	**OR (95% CI)**	***p***	**AOR (95% CI)**	***p***
GG	276 (78.4)	36 (67.9)	1.00 (Reference)	-	1.00 (Reference)	-
GA	69 (19.6)	17 (32.0)	1.88 (1.0–3.56)	**0.04**	1.72 (0.75–3.90)	0.19
AA	7 (1.98)	0 (0)	-	-	-	-
GA + AA	76 (21.59)	17(32.0)	1.71 (0.91–3.22)	0.09	1.61 (0.71–3.62)	0.25
Anemia						
Genotype rs41410450 ABCC1 (G3173A)	**Grade of toxicity 0, *n* (%) *N* = 102**	**Grade of toxicity 1–4, *n* (%) *N* = 305**	**OR (95% CI)**	***p***	**AOR (95% CI)**	***p***
GG	83 (81.3)	232 (76.0)	1.00 (Reference)	-	1.00 (Reference)	
GA	19 (18.6)	66 (21.6)	1.24 (0.70–2.19)	0.45	2.70 (1.06–6.88)	**0.03**
AA	0 (0)	7 (2.2)	-	-	-	-
GA + AA	19 (18.6)	73 (23.9)	1.37 (0.78–2.41)	0.26	2.85 (1.12–7.23)	**0.02**
Genotype rs8187710 ABCC2 (G4544A)	**Grade of toxicity 0–2, *n* (%) *N* = 378**	**Grade of toxicity 3–4, *n* (%) N = 29**	**OR (95% CI)**	***p***	**AOR (95% CI)**	***p***
GG	368 (97.3)	26 (89.6)	1.00 (Reference)		1.00 (Reference)	
GA	10 (2.6)	3 (10.3)	4.24 (1.10–16.38)	**0.03**	5.63 (1.13–28.04)	**0.03**

Note: Data were calculated by unconditional logistic regression and adjusted for age, gender, performance status, and regimen. A $p < 0.05$ was considered statistically significant. Bold values are statistically significant.

Abbreviations: AOR, adjusted odds ratio; OR, crude odds ratio, 95% CI, 95% confidence interval.

nephrotoxicity when compared to individuals with wild-type genotype (*GG*) (OR = 0.37, 95% CI = 0.17–0.77; $p = 0.008$). For *ABCC1* $G^{3173}A$ polymorphism, 85.3% of patients were categorized as low/intermediate and severe, whereas 14.6% showed no renal toxicity. In the dominant model, the combined heterozygous and mutant genotype (*GA* + *AA*) were associated with a reduced risk of nephrotoxicity with the odds of 0.54 as compared to the wild-type genotype (*GG*) (OR = 0.54, 95% CI = 0.29–0.99, $p = 0.04$). We did not find any correlation between the *ABCB1* ($C^{1236}T$, $C^{3435}T$, *and* $G^{2677}T$), *ABCC1*($G^{2168}A$), *ABCC2* ($G^{4544}A$), and ABCG2 ($C^{421}A$) polymorphisms and nephrotoxicity related AEs due to chemotherapy (Supporting Information: Table 4).

3.4 | Association between ABC polymorphisms and GI toxicity

In the GI toxicity, we assessed diarrhea, constipation, anorexia, and nausea/vomiting. Among 135 patients, 45.9% of individuals had grade 1 toxicity, and 54.0% of patients had grade 2–4 toxicity. In constipation, among 99 patients, 30.3% showed grade 1 toxicity, while 69.6% patients had low/intermediate/high toxicity (grade 2–4). In the case of anorexia, 5.78% of patients had grade 1 toxicity, while 94.2% showed grade 2–4 toxicity. Among 118 patients, 45.7% of individuals did not suffer from nausea/vomiting, whereas 54.2% experienced grade 2–4 toxicity (Table 2C). Upon evaluating the effect of different ABC polymorphisms, our results showed that 8.88% of patients harbored a single copy of mutant allele (*CA*) for *ABCG2* $C^{421}A$ polymorphism. Table 5 shows that when patients with low/intermediate/severe toxicity (grade 2–4) were compared with no toxicity grade (grade 1), then patients harboring heterozygous (*CA*) genotype had a lower incidence of having diarrhea as compared to the wild type (*CC*) genotype of *ABCG2* $C^{421}A$ polymorphism (OR = 0.25, 95% CI = 0.06–0.97, $p = 0.04$).

In diarrhea, when the comparison was made between intermediate/severe toxicity (grades 3–4) versus no/low grade (grade 1–2) toxicity, our results for *ABCC1* $G^{3173}A$ polymorphism showed that patients with one copy of mutant allele (*GA*) were associated with high odds of having diarrhea (OR = 1.42, 95% CI = 0.55–3.65, $p = 0.4$). When adjusted with different covariates such as age, regimen, gender, and performance status 2.78, a fold increased risk of having diarrhea was observed in the heterozygous genotype (*GA*) compared to the wild-type genotype (AOR = 2.78, 95% CI = 0.94–8.21, $p = 0.04$). Similarly, in the dominant model, the increased risk was noted in the combined (*GA* + *AA*) genotype as compared to the wild-type (*GG*) genotype (AOR = 2.82, 95% CI = 0.99–8.04, $p = 0.04$).

Anorexia can be caused by cancer, chemotherapy, radiation, or several other factors and is a typical side effect of cancer treatment. Chemotherapy and radiation therapy have several adverse effects that can cause anorexia and weight loss, including nausea and vomiting. In the case of anorexia, when patients with grade-1 toxicity were compared with individuals with grade 2–4 toxicity for *ABCC1* $G^{3173}A$

TABLE 4 Association of *ABC* polymorphism with Nephrotoxicity

Nephrotoxicity						
Genotype rs1128503 ABCB1 (C1236T)	**Grade of toxicity 1, *n* (%) *N* = 58**	**Grade of toxicity 2–5, *n* (%) *N* = 339**	**OR (95% CI)**	***p***	**AOR (95% CI)**	***p***
CC	31 (53.4)	184 (54.2)	1.00 (Reference)		1.00 (Reference)	
CT	15 (25.8)	106 (31.2)	1.19 (0.61–2.30)	0.6	1.84 (0.68–4.99)	0.22
TT	12 (20.6)	49 (14.4)	0.68 (0.32–1.43)	0.31	0.79 (0.29–2.15)	0.65
CT + TT	27 (46.5)	155 (45.7)	0.96 (0.55–1.69)	0.9	1.28 (0.59–2.79)	0.5
Genotype rs1045642 ABCB1 (C3435T)	**Grade of toxicity 1, *n* (%) *N* = 58**	**Grade of toxicity 2–5, *n* (%) *N* = 339**	**OR (95% CI)**	***p***	**AOR (95% CI)**	***p***
CC	11 (18.9)	62 (18.2)	1.00 (Reference)		1.00 (Reference)	
CT	22 (37.9)	144 (42.4)	1.16 (0.53–2.54)	0.7	1.33 (0.45–3.90)	0.59
TT	25 (43.1)	133 (39.2)	0.94 (0.43–2.03)	0.8	0.86 (0.31–2.32)	0.76
CT + TT	47 (81.0)	277 (81.7)	1.04 (0.51–2.13)	0.9	1.0 (0.39–2.56)	0.9
Genotype rs2032582 ABCB1 (G2677A)	**Grade of toxicity 1, *n* (%) *N* = 58**	**Grade of toxicity 2–5, *n* (%) *N* = 339**	**OR (95% CI)**	***p***	**AOR (95% CI)**	***p***
GG	46 (79.3)	309 (91.1)	1.00 (Reference)		1.00 (Reference)	
GA	12 (20.6)	30 (8.8)	0.37 (0.17–0.77)	**0.008**	0.41 (0.15–1.11)	0.07
Genotype rs2032582 ABCB1 (G2677T)	**Grade of toxicity 1, *n* (%) *N* = 58**	**Grade of toxicity 2-5, *n* (%) *N* = 339**	**OR (95% CI)**	***P***	**AOR (95% CI)**	***p***
GT	26 (44.8)	179 (52.8)	1.00 (Reference)		1.00 (Reference)	-
TT	32 (55.1)	160 (47.1)	1.37 (0.78–2.40)	0.26	0.65 (0.29–1.41)	0.27
Genotype rs41410450 ABCC1 (G3173A)	**Grade of toxicity 1, *n* (%) *N* = 58**	**Grade of toxicity 2–5, *n* (%) *N* = 339**	**OR (95% CI)**	***p***	**AOR (95% CI)**	***p***
GG	39 (67.2)	268 (79.0)	1.00 (Reference)	-	1.00 (Reference)	
GA	17 (29.3)	66 (19.4)	0.56 (0.30–1.06)	0.07	0.52 (0.22–1.22)	0.13
AA	2 (3.4)	5 (1.4)	0.36 (0.06–1.94)	0.23	0.38 (0.03–4.17)	0.43
GA + AA	19 (32.7)	71 (20.9)	0.54 (0.29–0.99)	**0.04**	0.56 (0.24–1.32)	0.18
Genotype rs8187710 ABCC2 (G4544A)	**Grade of toxicity 1, *n* (%) *N* = 58**	**Grade of toxicity 2–5, *n* (%) *N* = 339**	**OR (95% CI)**	***p***	**AOR (95% CI)**	***p***
GG	56 (96.5)	329 (97.0)	1.00 (Reference)	-	1.00 (Reference)	-
GA	2 (3.4)	10 (2.9)	0.85 (0.18–3.98)	0.83	1.77 (0.17–17.7)	0.62
Genotype rs2231142 ABCG2 (C421A)	**Grade of toxicity 1, *n* (%) *N* = 58**	**Grade of toxicity 2–5, *n* (%) *N* = 339**	**OR (95% CI)**	***p***	**AOR (95% CI)**	***p***
CC	53 (91.3)	290 (85.5)	1.00 (Reference)		1.00 (Reference)	
CA	5 (8.6)	49 (14.4)	1.79 (0.68–4.7)	0.23	1.35 (0.37–4.85)	0.64

Note: Data were calculated by unconditional logistic regression and adjusted for age, gender, performance status, and regimen. A $p < 0.05$ was considered statistically significant. Bold values are statistically significant.

Abbreviations: AOR, adjusted odds ratio; OR, crude odds ratio; 95% CI, 95% confidence interval.

polymorphism, a lower incidence of anorexia was observed in patients with one copy of mutant allele (*GA*) as compared to patients having both copies wild-type alleles (*GG*) (AOR = 0.20; 95% CI = 0.04–0.97, $p = 0.04$ (Table 5). In case of nausea and vomiting, patients harboring heterozygous genotype (*CT*) for *ABCB1* $C^{1236}T$ variant were associated with a reduced risk of having nausea/vomiting as compared to the wild type (*CC*) genotype (OR = 0.35, 95% CI = 0.15–0.81, $p = 0.01$). Upon adjusting with different covariates, the lower incidence of nausea/vomiting was consistent in the heterozygous genotype (*CT*) as compared to the wild-type (*CC*) genotype (AOR = 0.30, 95% CI = 0.11–0.79, $p = 0.015$). A decrease of having nausea/vomiting was also observed in the mutant genotype (*TT*) as compared to the wild type (*CC*) (AOR = 0.23, 95% CI = 0.06–0.81, $p = 0.02$). Similarly, in the dominant model, the combined genotype (*CT* + *TT*) showed a lower incidence of nausea as compared to

TABLE 5 Association of *ABC* polymorphism with gastrointestinal toxicity

Diarrhea						
Genotype rs2231142 ABCG2 (C421A)	**Grade of toxicity 1, *n* (%) *N* = 62**	**Grade of toxicity 2–4, *n* (%) *N* = 73**	**OR (95% CI)**	***p***	**AOR (95% CI)**	***p***
CC	53 (85.4)	70 (95.8)	1.00 (Reference)	-	1.00 (Reference)	-
CA	9 (14.5)	3 (4.1)	0.25 (0.06–0.97)	**0.04**	0.25 (0.05–1.10)	0.06
Genotype rs41410450 ABCC1 (G3173A)	**Grade of toxicity 1–2, *n* (%) *N* = 106**	**Grade of toxicity 3–4, *n* (%) *N* = 29**	**OR (95% CI)**	***p***	**AOR (95% CI)**	***p***
GG	81 (76.4)	19 (65.5)	1.00 (Reference)		1.00 (Reference)	
GA	24 (22.6)	8 (27.5)	1.42 (0.55–3.65)	0.46	2.78(0.94–8.21)	**0.04**
AA	1 (0.9)	2 (6.8)	-	-	-	-
GA + AA	25 (23.5)	10 (34.4)	1.70 (0.70–4.14)	0.23	2.82(0.99–8.04)	**0.04**
Anorexia						
Genotype rs41410450 ABCC1 (G3173A)	**Grade of toxicity 1, *n* (%) *N* = 7**	**Grade of toxicity 2–3, *n* (%) *N* = 114**	**OR (95% CI)**	***p***	**AOR (95% CI)**	***p***
GG	3 (42.8)	87 (76.3)	1.00 (Reference)	-	1.00 (Reference)	
GA	4 (57.1)	24 (21.0)	0.20 (0.04–0.98)	**0.04**	0.43 (0.06–2.94)	0.39
AA	0 (0)	3 (2.6)	-	-	-	-
GA + AA	4 (57.1)	27 (23.6)	0.23 (0.04–1.10)	0.06	0.46 (0.06–3.07)	0.42
Constipation						
Genotype rs1045642 ABCB1 (C3435T)	**Grade of toxicity 1, *n* (%) *N* = 30**	**Grade of toxicity 2, *n* (%) *N* = 69**	**OR (95% CI)**	***p***	**AOR (95% CI)**	***p***
CC	3 (10)	14 (20.2)	1.00 (Reference)		1.00 (Reference)	
CT	17 (56.6)	22 (31.8)	0.27 (0.06–1.12)	0.07	0.17 (0.03–0.97)	**0.04**
TT	10 (33.3)	33 (47.8)	0.70 (0.16–2.9)	0.63	0.61 (0.10–3.63)	0.58
CT + TT	27 (90)	55 (79.7)	0.43 (0.11–1.64)	0.22	0.39 (0.09–1.70)	0.21
Nausea/vomiting						
Genotype rs1128503 ABCB1 (C1236T)	**Grade of toxicity 1, *n* (%) *N* = 54**	**Grade of toxicity 2–3, *n* (%) *N* = 64**	**OR (95% CI)**	***p***	**AOR (95% CI)**	***p***
CC	20 (37.0)	41 (64.0)	1.00 (Reference)	-	1.00 (Reference)	-
CT	22 (40.7)	16 (25)	0.35 (0.15–0.81)	**0.01**	0.30 (0.11–0.79)	**0.015**
TT	12 (22.2)	7 (10.9)	0.28 (0.09–0.83)	**0.02**	0.23 (0.06–0.81)	**0.02**
CT + TT	34 (62.9)	23 (35.9)	0.33 (0.15–0.70)	**0.003**	0.27 (0.11–0.66)	**0.0039**

Note: Data were calculated by unconditional logistic regression and adjusted for age, gender, performance status, and regimen. A $p < 0.05$ was considered statistically significant. Bold values are statistically significant.

Abbreviations: AOR, adjusted odds ratio; OR, crude odds ratio, 95% CI, 95% confidence interval.

the wild-type genotype (*CC*) (AOR = 0.27, 95% CI = 0.11–0.66, $p = 0.003$), as shown in Table 5.

Additionally, we assessed GI toxicity (constipation) by comparing individuals with no toxicity (grade 1) grade to patients with intermediate-high toxicity (grade 2). Lung carcinoma patients with the heterozygous genotype (*CT*) for *ABCB1* $C^{3435}T$ variant had a lower incidence of constipation when compared to the individuals harboring wild-type genotype (*CC*) (OR = 0.27, 95% CI = 0.06–1.12, $p = 0.07$). The reduced risk was consistent when we adjusted with different covariates such as age, gender, performance status, and regimen in the heterozygous (*CT*) genotype as compared with the wild type (*CC*) genotype (AOR = 0.17, 95% CI = 0.03–0.97, $p = 0.04$) as shown in Table 5. We did not find any correlation between other ABC polymorphisms and GI-related AEs due to chemotherapy (Supporting Information: Table 5).

3.5 | Association between ABC polymorphisms and hepatotoxicity

An unanticipated reaction typically causes Chemotherapy-induced hepatotoxicity. The enzyme levels of Serum glutamate oxaloacetate

TABLE 6 Association of ABC polymorphism with hepatotoxicity

AST						
Genotype rs41410450 ABCC1 (G3173A)	**Grade of toxicity 0, *n* (%) *N* = 242**	**Grade of toxicity 1–3, *n* (%) *N* = 75**	**OR (95% CI)**	***p***	**AOR (95% CI)**	***P***
GG	198 (81.8)	53 (70.6)	1.00 (Reference)		1.00 (Reference)	
GA	38 (15.7)	21 (28)	2.06 (1.11–3.81)	**0.02**	1.76 (0.76–4.07)	0.18
AA	6 (2.4)	1 (1.3)	0.62 (0.07–5.28)	0.6	1.77 (0.12–24.49)	0.66
GA + AA	44 (18.1)	22 (29.3)	1.86 (1.03–3.3)	**0.03**	1.78 (0.79–4.02)	0.16
ALT						
Genotype rs1128503 ABCB1 (C1236T)	**Grade of toxicity 0, *n* (%) *N* = 180**	**Grade of toxicity 1–3, *n* (%) *N* = 137**	**OR (95% CI)**	***p***	**AOR (95% CI)**	***p***
CC	107 (59.4)	70 (51.0)	1.00 (Reference)	-	1.00 (Reference)	-
CT	42 (23.3)	51 (37.2)	1.85 (1.11–3.08)	**0.01**	1.93 (0.98–3.81)	**0.04**
TT	31 (17.2)	16 (11.6)	0.78 (0.40–1.54)	0.49	0.83 (0.34–2.03)	0.69
CT + TT	73 (40.5)	67 (48.9)	1.40 (0.89–2.19)	0.13	1.46 (0.8–2.65)	0.20
Genotype rs2032582 ABCB1 (G2677T)	**Grade of toxicity 0, *n* (%) *N* = 180**	**Grade of toxicity 1–3, *n* (%) *N* = 137**	**OR (95% CI)**	***p***	**AOR (95% CI)**	***p***
GT	86 (47.7)	82 (59.8)	1.00 (Reference)		1.00 (Reference)	
TT	94 (52.2)	55 (40.1)	0.61 (0.39–0.96)	**0.03**	0.46(0.25–0.85)	**0.01**
ALP						
Genotype rs1128503 ABCB1(C1236T)	**Grade of toxicity 0, n(%) N = 153**	**Grade of toxicity 1-3, n(%) N = 154**	**OR[1] (95% CI)**	***p***	**AOR[2](95% CI)**	***p***
CC	94 (61.4)	76 (49.3)	1.00 (Reference)	-	1.00 (Reference)	-
CT	36 (23.5)	54 (35.0)	1.85 (1.10–3.11)	**0.01**	1.93 (0.98–3.83)	**0.04**
TT	23 (15.0)	24 (15.5)	1.29 (0.67–2.46)	0.43	1.37 (0.59–3.16)	0.45
CT + TT	59 (38.5)	78 (50.6)	1.63 (1.03–2.57)	**0.03**	1.67 (0.92–3.02)	0.08

Note: Data were calculated by unconditional logistic regression and adjusted for age, gender, performance status, and regimen. A $p < 0.05$ was considered statistically significant.

Abbreviations: ALP, alkaline phosphatase; AOR, adjusted odds ratio; OR, crude odds ratio; SGOT, serum glutamate oxaloacetate transaminase; SGPT, serum glutamate pyruvate transaminase; 95% CI: 95% confidence interval.

transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), and alkaline phosphatase (ALP) are the primary components of the LFT. On categorizing patients based on SGOT levels, 74 patients were classified in grades 1–2, while only one patient was categorized in grades 3–4, while 242 patients were classified in grades 0 (Table 2D). The analysis of SGOT to *ABCC1* $G^{3173}A$ showed statistical differences. Our results revealed an increased risk of liver injury in the heterozygous genotype of the *ABCC1* $G^{3173}A$ variant compared to the wild-type genotype (OR = 2.06, 95% CI = 1.11–3.81, $p = 0.02$). In the dominant model, the combined genotype (*GA* + *AA*) showed a high risk of liver damage as compared to the individuals having wild-type genotype (*GG*) (OR = 1.86, 95% CI = 1.03–3.3, $p = 0.03$) as shown in Table 6.

On categorizing patients based on SGPT levels for *ABCB1* $C^{1236}T$ polymorphism, our results demonstrated an increased risk of liver injury in patients harboring heterozygous genotype (*CT*) as compared to the wild-type genotype (*CC*) (OR = 1.85, 95% CI = 1.11–3.08, $p = 0.01$). Upon adjusting with different covariates, the heterozygous genotype (*CT*) showed an increased risk of 1.93-fold as compared to the wild-type genotype (*CC*) (AOR = 1.93, 95% CI = 0.98–3.081, $p = 0.04$).

For *ABCB1* $G^{2677}A$ polymorphism, mutant genotype (*AA*) was associated with a lower incidence of liver toxicity as compared to the heterozygous genotype (*GA*) (OR = 0.61, 95% CI = 0.39–0.96, $p = 0.03$). Upon adjusting with various covariates such as age, gender, performance status, and regimen, the mutant genotype was still associated with reduced risk compared to the wild-type genotype (*GG*) (AOR = 0.46, 95% CI = 0.25–0.85, $p = 0.01$) as shown in Table 6. We did not find any correlation between other ABC polymorphisms and hepatic related AEs due to chemotherapy (Supporting Information: Table 6).

4 | DISCUSSION

ABC transporters regulate the outflow of the chemotherapeutic agents across the biological membranes in different organs. These membrane proteins are essential in transporting the essential

biological substrates across the cell membranes, such as anticancer drugs'[27] and can thus influence chemotherapy treatment outcomes. Also, genetic variants in the ABC transporter genes have been reported to alter the therapeutic outcome of patients treated with various chemotherapeutic drugs.[28–30] Genetic mutations may cause individual variability in the pharmacotherapy and metabolism of the efflux transporters, potentially resulting in population-specific drug transport disparities. The interplay between genetic, physiological, and environmental factors results in variation in a population pharmacokinetic characteristics of chemotherapeutic agents. Many theories have been proposed regarding the association between overexpression of ABC transporters and multidrug resistance. However, few theories have been reported regarding the role of these ABC gene polymorphisms in correlation with carcinoma patients' toxicity. Therefore, comprehending and mitigating inter-individual variation in drug receptivity, sensitivity, and toxicity, including its vital role in determining the most effective therapy option for patients with lung cancer, is essential and clinically relevant.

We have investigated the association of ABC polymorphisms and toxicity in patients with lung cancer undergoing platinum-based chemotherapy. According to our findings, patients with at least one mutant allele (GA) in *ABCC1* $G^{3173}A$ had an increased risk of developing anemia and leukopenia. Mechanistically, chemotherapeutic agents target folate to produce RBCs.[31] Chemotherapeutic drugs affect folate metabolism, so reducing folate causes aberrant DNA synthesis that promotes genomic instability, contributing to the notion that a deficiency of folic acid hampers RBC maturation, leading to anemia.[32] Another explanation could be that, in various investigations, *ABCC1* overexpression has been correlated with drug resistance in lung carcinoma cells.[33,34] Reports have shown that overexpression of the ABCC1 transporter gene may correlate to decreased drug availability and drug resistance.[35] Yin et al. showed that the *ABCC1* $G^{3173}A$ (Arg1058Gln) mutation had been related to decreased chemotherapeutic drug resistance.[24] Many other factors are responsible for modulating a patient's clinical outcome, such as type of mutation and interindividual variation, which are indisputable.

Also, our unpublished data regarding the assessment of survival and other clinical outcomes of lung cancer patients having *ABCC1* $G^{3173}A$ polymorphism has shown higher survival in patients having at least one mutant allele (GA) as compared to the wild-type (GG) genotype (MST = 8.57 vs. 8.37). In addition, our results demonstrated better response in patients with $G^{3173}A$ polymorphism and undergoing platinum-based chemotherapy. Patients with *ABCC1* $G^{3173}A$ mutation can probably retain more drugs than wild-type (GG) patients, because of which a better prognosis and response to chemotherapy have been noted. However, more drug bioavailability to a cell is correlated to AEs. It is plausible that this is the reason for high toxicity in patients with *ABCC1* $G^{3173}A$ polymorphism. We found the strongest correlation between *ABCC1* $G^{3173}A$ mutations and hematological toxicity. The mechanism elucidating alteration in the transport of the cytotoxic chemotherapy impacts erythropoiesis remains unknown. These mutations could potentially affect intracellular doses of the delivered chemotherapeutic agent in cell type-specific methods. Another explanation is that some cells are susceptible to unavoidable variations in a dose of a specific metabolite. Also, it is plausible that the effect on erythropoiesis could be due to a specialized involvement of the damaged gene during erythropoiesis.[35]

Our results showed a reduced risk of developing nephrotoxicity in patients with*ABCB1* $G^{2677}A$ and *ABCC1* $G^{3173}A$ mutation. The *ABCB1* gene is expressed in the proximal tubule, collecting duct of cells, and thick loop of Henle in the kidney.[36] The kidney is the main organ engaged in clearing platinum-based chemotherapy drugs.[37] Various drugs with the propensity of causing nephrotoxicity are excreted in the urine by active renal secretion and glomerular filtration.[38] Walsh et al. have reported overexpression of the *ABCB1* gene in renal carcinoma.[39]

Furthermore, MRP1 overexpression has also been associated with multidrug resistance in lung, ovarian, breast, kidney, and pancreatic cancer.[40–43] Mohammad et al. reported that overexpression of drug transporter is responsible for the increased drug efflux in cancer.[44] So, keeping these facts in view, our results suggest that *ABCB1* $G^{2677}A$ and *ABCC1* $G^{3173}A$ polymorphism correlated with the protective effect from nephrotoxicity in cancer patients undergoing platinum-based treatment doublet chemotherapy.

The GI system is the first line of protection against drugs and other xenobiotics in the human body. The expression of *ABCB1*, *ABCC1*, and *ABCG2*have been reported at the apical surface of epithelial cells in the intestine.[45] Our results showed that people with one mutant allele for *ABCB1* $C^{1236}T$ and $C^{3435}T$ polymorphism correlated with the protective effect of GI toxicity. Nausea/vomiting is caused by cisplatin as an emetic agent and causes such side effects in 90% of patients who are not administered with antiemetics (50 mg/m^2 or above).[46] Due to high p-glycoprotein (P-gp) levels in the GI tract and overexpression of MDR1 in the carcinoma cells, the efflux capacity of the various chemotherapeutic drugs such as taxanes and vinca alkaloids is increased.[47] Chemotherapeutic drugs are probably eliminated faster, thus not showing nausea or vomiting-like side effects. A report by Qian et al. showed lower odds of GI toxicity in patients with at least one allele for ABCB1 C3435T variant in NSCLC patients undergoing platinum-based chemotherapy; however, substantial significance was not achieved in any model.[48]

Our results also demonstrated the protective effect of GI toxicity in people harboring heterozygous genotype for *ABCG2* $C^{421}A$ polymorphism, and Delou et al. reported that the *ABCG2* gene is highly expressed in the GI tract.[49] Our results for *ABCC1* $G^{3173}A$ showed that patients having heterozygous genotype (GA) undergoing platinum-based chemotherapy were associated with an increased risk of chemotherapy-induced diarrhea. Understanding the exact incidence of chemotherapy-induced toxicity in lung cancer patients is difficult due to the lack of studies. The severity and degree of chemotherapy-induced toxicity are determined by many parameters, such as the patient's attributes, regimen type, type of chemotherapy, and dose schedule.

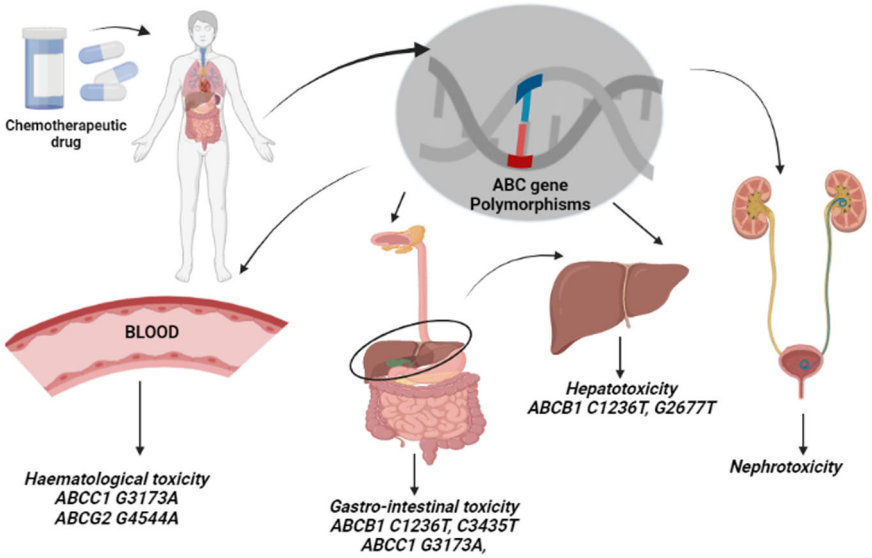


FIGURE 1 Illustration summarizing the main ABC gene polymorphisms that predict toxicity in lung cancer patients. ABC, ATP-binding cassette.

A drug-induced hepatic injury is an AE of grave concern because of its severe implications. Hepatic toxicity of platinum is characterized by elevated transaminases, bilirubin, and ALP.[6] Cisplatin causes direct DNA strand breakage in the basal epithelium, producing reactive oxygen species and causing cellular injury. Oxidative stress is a hallmark of mitochondrial damage and plays a significant role in this toxicity.[50] Oxidative damage to the mitochondrial cell reduces the efficacy of oxidative phosphorylation and, in the presence of Ca^{2+}, causes a transition in mitochondrial permeability.[51] Damage to the plasma membrane and protein leakage is the leading causes of high ALT levels in the serum.[52] Despite significant research over the previous few decades, the basic mechanisms of chemotherapeutic drug induce damage remains a mystery.

On the other hand, genetic mutations in an individual are crucial in developing hepatic injury.[53] Our results demonstrated a high risk of hepatotoxicity in lung patients with *ABCB1* $C^{1236}T$ and *ABCC1* $G^{3173}A$ variation. P-gp is expressed in the canalicular membrane of the liver, where it aids biliary drug excretion.[54] Reports have suggested the crucial role of ABCB1 in a specific form of liver toxicity.[53] Additionally, studies have suggested low levels of MRP1 expression in a healthy human liver.[45] Reduced expression of MRP1 in the liver probably reduces the drug efflux from the cell, and the concentration of the drug in the cell increases, leading to hepatic toxicity.

Our study has particular strengths, such as the current investigation has been carried out in a large sample size compared to the other studies, which can increase the reliability of the results. Another study's strength is that all patients were administered platinum-based doublet chemotherapy. The patient recruitment and other clinical parameters were collected independently, irrespective of the associated polymorphism. The current investigation had drawbacks, including a limited sample size of SCLC patients and a reduced incidence of toxicity. In our study, few patients had grade 3–4 toxicity, while most patients had intermediate or low-grade toxicity (grade 1–2). Most of the patients were in stage III and 1 V of lung cancer. Some study participants experienced significant adverse effects that prompted delaying or terminating the treatment. Hence, it is imperative to conduct multiple center studies with many cases.

5 | CONCLUSION

Toxicity from chemotherapy has been documented in a few studies on other malignancies, but the reports regarding the association of platinum-based doublet chemotherapy in patients with lung cancer having ABC gene polymorphism are scanty and conflicting. An illustration summarizing the key findings of the current investigation showing the correlation of *ABC* gene polymorphisms with toxicity in lung cancer patients is depicted in Figure 1. Our results showed a strong association between *ABCC1* $G^{3173}A$ and *ABCG2* $G^{4544}A$ in causing anemia in lung cancer patients who underwent platinum-based doublet chemotherapy. We also report a correlation between *ABCB1* $C^{1236}T$, $C^{3435}T$, and *ABCC1* $G^{3173}A$ polymorphism in predicting GI toxicity. Additionally, *ABCB1* $C^{1236}T$ polymorphisms had a strong association in causing hepatic toxicity in lung cancer patients undergoing platinum-based doublet chemotherapy. To the best of our knowledge, no one has reported the correlation of ABC gene polymorphisms with hematological, GI, hepatic, and nephrotoxicity, specifically in lung cancer of the North Indian population. Future studies should be conducted to determine the impact of *ABC* polymorphisms' in predicting toxicity in lung cancer patients undergoing chemotherapy treatment. Such insights might favor personalized treatment strategies that can enhance therapeutic efficacy and minimize adverse effects caused by chemotherapeutic drugs.

AUTHOR CONTRIBUTIONS

Parul Sharma collected information, performed molecular and statistical analyses, and wrote the first paper draft. Siddharth Sharma assisted molecular analyses and reviewed the final version of the paper and acted as the corresponding author. Navneet Singh

provided with the blood samples and clinical data of the patients from PGIMER, Chandigarh.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All the data generated or analyzed during this study are included in this article. Further queries can be directed to the corresponding author.

ETHICS STATEMENT

The study was reviewed and approved by the institutional review board of the Post Graduate Institute of Medical Education & Research, Chandigarh, India (Approval No. IEC-04/2018-884). This study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

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REFERENCES

[1] R. L. Siegel, K. D. Miller, A. Jemal, *CA Cancer J. Clin.* **2019**, *69*(1), 7.
[2] R. S. Herbst, J. V. Heymach, S. M. Lippman, *N. Engl. J. Med.* **2008**, *359*, 1367.
[3] W. Liu, Y. Wang, J. Luo, H. Yuan, Z. Luo, *Front. Oncol.* **2020**, *9*, 1573.
[4] S. Novello, F. Barlesi, R. Califano, T. Cufer, S. Ekman, M. G. Levra, K. Kerr, S. Popat, M. Reck, S. Senan, G. V. Simo, J. Vansteenkiste, S. Peters, *Ann. Oncol.* **2016**, *27*, v1.
[5] Y. Shi, Y. Sun, *Thorac. Cancer* **2015**, *6*, 10.
[6] S. Cao, C. Wang, H. Ma, R. Yin, M. Zhu, W. Shen, J. Dai, Y. Shu, L. Xu, Z. Hu, H. Shen, *Sci. Rep.* **2015**, *5*(1), 11556.
[7] Y. Xiong, B. Y. Huang, J. Y. Yin, *Med. Oncol.* **2017**, *34*, 48.
[8] S. S. Hampras, L. Sucheston, J. Weiss, M. R. Baer, G. Zirpoli, P. K. Singh, M. Wetzler, R. Chennamaneni, J. G. Blanco, L. Ford, K. B. Moysich, *Int. J. Mol. Epidemiol. Genet.* **2010**, *1*(3), 201.
[9] H. Kato, N. Sassa, M. Miyazaki, M. Takeuchi, M. Asai, A. Iwai, Y. Noda, M. Gotoh, K. Yamada, *Cancer Chemother. Pharmacol.* **2016**, *78*(4), 855.
[10] E. De Mattia, G. Toffoli, J. Polesel, M. D'Andrea, G. Corona, V. Zagonel, A. Buonadonna, E. Dreussi, E. Cecchin, *Pharmacogenet. Genomics* **2013**, *23*(10), 549.
[11] J. Y. Han, H. S. Lim, Y. K. Yoo, E. S. Shin, Y. H. Park, S. Y. Lee, J. E. Lee, D. H. Lee, H. T. Kim, J. S. Lee, *Cancer* **2007**, *110*(1), 138.
[12] L. Cortejoso, M. I. García, P. García-Alfonso, E. González-Haba, F. Escolar, M. Sanjurjo, L. A. López-Fernández, *Cancer Chemother. Pharmacol.* **2013**, *71*(6), 1463.
[13] J. Pan, J. Han, J. Wu, L. Sheng, H. Huang, Q. Yu, *Respiration* **2008**, *75*(4), 380.
[14] S. Chen, X. Huo, Y. Lin, H. Ban, Y. Lin, W. Li, B. Zhang, W. W. Au, X. Xu, *Int. J. Hyg. Environ. Health* **2010**, *213*(2), 140.
[15] P. Riera, A. Artigas-Baleri, J. Salazar, A. Sebio, A. C. Virgili, M. J. Arranz, D. Páez, *Front. Pharmacol.* **2020**, *11*, 973.
[16] J. R. Choi, J. O. Kim, D. R. Kang, J. Y. Shin, X. H. Zhang, J. E. Oh, J. Y. Park, K. A. Kim, J. H. Kang, *Cancer Res. Treat.* **2015**, *47*(3), 509.
[17] L. Elens, D. Tyteca, N. Panin, P. Courtoy, D. Lison, J. B. Demoulin, V. Haufroid, *Pharmacogenet. Genomics* **2011**, *21*(12), 884.
[18] J. A. Sprowl, V. Gregorc, C. Lazzari, R. H. Mathijssen, W. J. Loos, A. Sparreboom, *Clin. Pharmacol. Ther.* **2012**, *91*(6), 1022.
[19] P. C. Cha, T. Mushiroda, H. Zembutsu, H. Harada, N. Shinoda, S. Kawamoto, R. Shimoyama, T. Nishidate, T. Furuhata, K. Sasaki, K. Hirata, Y. Nakamura, *J. Hum. Genet.* **2009**, *54*(10), 572.
[20] G. Cusatis, V. Gregorc, J. Li, A. Spreafico, R. G. Ingersoll, J. Verweij, V. Ludovini, E. Villa, M. Hidalgo, A. Sparreboom, S. D. Baker, *J. Natl. Cancer. Inst.* **2006**, *98*(23), 1739.
[21] M. Chaplin, J. J. Kirkham, K. Dwan, D. J. Sloan, G. Davies, A. L. Jorgensen, *PLoS Med.* **2020**, *17*(9), e1003344.
[22] A. Rustemoglu, G. Gumus-Akay, N. Karakus, S. Yigit, S. Sahin, T. Tasliyurt, *Xenobiotica* **2014**, *44*(10), 933.
[23] M. Panczyk, E. Balcerczak, S. Piaskowski, K. Jamroziak, G. Pasz-Walczak, M. Mirowski, *Int. J. Colorectal Dis.* **2009**, *24*(8), 895.
[24] J. Y. Yin, Q. Huang, Y. Yang, J. T. Zhang, M. Z. Zhong, H. H. Zhou, Z. Q. Liu, *Pharmacogenet. Genomics.* **2009**, *19*(3), 206.
[25] A. K. Daly, G. P. Aithal, J. B. S. Leathart, R. A. Swainsbury, T. S. Dang, C. P. Day, *Gastroenterology* **2007**, *132*(1), 272.
[26] C. Zheng, H. Yang, Q. Wang, H. Rao, Y. Diao, *Turk. J. Med. Sci.* **2016**, *46*(2), 361.
[27] J. I. Fletcher, M. Haber, M. J. Henderson, M. D. Norris, *Nat. Rev. Cancer* **2010**, *10*, 147.
[28] K. Kiyotani, T. Mushiroda, M. Kubo, H. Zembutsu, Y. Sugiyama, Y. Nakamura, *Cancer Sci.* **2008**, *99*, 967.
[29] J. R. Choi, J. O. Kim, D. R. Kang, J. Y. Shin, X. H. Zhang, J. E. Oh, J. Y. Park, K. A. Kim, J. H. Kang, *Cancer Res. Treat.* **2015**, *47*(3), 509.
[30] S. C. Chew, O. Singh, X. Chen, R. D. Ramasamy, T. Kulkarni, E. J. D. Lee, E. H. Tan, W. T. Lim, B. Chowbay, *Cancer Chemother. Pharmacol.* **2011**, *67*(6), 1471.
[31] K. Robien, A. Boynton, C. M. Ulrich, *Pharmacogenomics* **2005**, *6*, 673.
[32] W. H. Schreurs, J. Odink, R. J. Egger, M. Wedel, P. F. Bruning, *Int. J. Vitam. Nutr. Res.* **1985**, *55*(4), 425.
[33] N. Triller, P. Korošec, I. Kern, M. Košnik, A. Debeljak, *Lung Cancer* **2006**, *54*(2), 235.
[34] J. J. Yeh, N. Y. Hsu, W. H. Hsu, C. H. Tsai, C. C. Lin, J. A. Liang, *Lung* **2005**, *183*(3), 177.
[35] D. Theile, P. Wizgall, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2021**, *394*(8), 1621.
[36] S. Ernest, E. Bello-Reuss, *Kidney Int. Suppl.* **1998**, *65*, 11.
[37] H. Burger, W. J. Loos, K. Eechoute, J. Verweij, R. H. J. Mathijssen, E. A. C. Wiemer, *Drug Resist. Updates* **2011**, *14*(1), 22.
[38] R. Ho, R. Kim, *Clin. Pharm. Ther.* **2005**, *78*(3), 260.
[39] N. Walsh, A. Larkin, S. Kennedy, L. Connolly, J. Ballot, W. Ooi, G. Gullo, J. Crown, M. Clynes, L. O'Driscoll, *BMC Urol.* **2009**, *9*(1), 6.
[40] V. O. Poku, S. H. Iram, *PeerJ* **2022**, *10*, e12594.
[41] M. A. Barrand, T. Rhodes, M. S. Center, P. R. Twentyman, *Eur. J. Cancer* **1993**, *29*, 408.
[42] A. Larkin, L. O'Driscoll, S. Kennedy, R. Purcell, E. Moran, J. Crown, M. Parkinson, M. Clynes, *Int. J. Cancer* **2004**, *112*, 286.
[43] J. Li, Z.-N. Li, Y.-J. Du, X.-Q. Li, Q.-L. Bao, P. Chen, *Clin. Lung Cancer* **2009**, *10*, 414.
[44] I. S. Mohammad, W. He, L. Yin, *Biomed. Pharmacother.* **2018**, *100*, 335.
[45] E. M. Leslie, R. G. Deeley, S. P. C. Cole, *Toxicol. Appl. Pharmacol.* **2005**, *204*(3), 216.
[46] K. M. Doherty, *Clin. J. Oncol. Nurs.* **1999**, *3*(3), 113.
[47] R. El-Awady, E. Saleh, A. Hashim, N. Soliman, A. Dallah, A. Elrasheed, G. Elakraa, *Front. Pharmacol.* **2017**, *7*, 535.
[48] C. Y. Qian, Y. Zheng, Y. Wang, J. Chen, J. Y. Liu, H. H. Zhou, J. Y. Yin, Z. Q. Liu, *Cancer Commun.* **2016**, *35*(1), 1.

[49] M. A. Delou, G. Lopes, A. M. Capella, *Hypertension* **2009**, *54*(2), 210.
[50] H. M. Hassan, L. H. Al-Wahaibi, M. A. Elmorsy, Y. F. Mahran, *Drug Des. Dev.Ther.* **2020**, *14*, 2335.
[51] J. B. A. Custódio, C. M. P. Cardoso, M. S. Santos, L. M. Almeida, J. A. F. Vicente, M. A. S. Fernandes, *Toxicology* **2009**, *259*(1–2), 18.
[52] M. R. McGill, *EXCLI J.* **2016**, *15*, 817.
[53] E. Ulzurrun, C. Stephens, F. Ruiz-Cabello, M. Robles-Diaz, P. Saenz-López, H. Hallal, G. Soriano, E. Roman, M. C. Fernandez, M. I. Lucena, R. J. Andrade, *PLoS One* **2014**, *9*(4), e94675.
[54] M. Yu, W. Zhang, L. Qin, L. Tian, C. Zhou, *Anat. Rec.* **2010**, *293*(7), 1167.

SUPPORTING INFORMATION

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ORIGINAL ARTICLE

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Polymorphisms in solute carrier genes (*SLC19A1*, *SLCO1B1*, and *SLCO1B3*) predicts survival and toxicity in North Indian lung cancer patients undergoing platinum-based doublet chemotherapy

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Abstract

What is Known and Objective: Solute Carrier (SLC) transporters are known mediators of drug disposition that facilitate the influx of substrates and various chemotherapeutic agents into cells. Polymorphisms in the *SLC19A1*, *SLCO1B1*, and *SLCO1B3* gene influence the prognosis in the cancer patients, but little is known about their role in lung cancer in Asians. So, the current study aims to investigate the polymorphisms in *SLC19A1*, *SLCO1B1*, and *SLCO1B3* genes in Northern Indian lung cancer patients.

Methods: Patients with lung cancer who had a confirmed histology and cytology diagnosis were enrolled in the study. *SLC* polymorphisms were assessed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) for variations in *SLC19A1* ($G^{80}A$), *SLCO1B1* ($A^{388}G$, $T^{521}C$), and *SLCO1B3* ($A^{1683\text{-}5676}G$).

Results and Discussion: Our results showed that mutant genotype for *SLC19A1* $G^{80}A$ polymorphism had higher median survival time (MST) compared to wild genotype. ADCC patients with mutant genotype showed better survival compared to wild genotype for *SLC19A1* $G^{80}A$. SCLC patients $G^{80}A$ polymorphism showed increased survival in patients with mutant genotype ($p = 0.04$). In *SLCO1B3*, $A^{1683\text{-}5676}G$ patients carrying heterozygous alleles and administered with platinum and docetaxel showed inferior survival ($p = 0.006$). In $T^{521}C$ variant, patients with carrier genotype had reduced chances of developing anaemia ($p = 0.04$). Patients with *SLC19A1* and *SLCO1B3* variants showed lower incidence of thrombocytopenia and nephrotoxicity.

What is New and Conclusion: Our findings imply that *Solute Carrier gene* polymorphisms modulate the overall survival in lung cancer patients undergoing platin-based doublet chemotherapy, also these polymorphisms have a modifying impact on the associated adverse events/toxicity.

KEYWORDS

lung carcinoma, overall survival, platinum-based doublet chemotherapy, polymorphism, *SLC* transporters, toxicity

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1 | WHAT IS KNOWN AND OBJECTIVE

Lung carcinoma is the second most common type of cancer (accounting for 13% of all occurrences) and the leading cause of carcinoma fatalities (23% of the total cases).[1] It only has a 15% 5-year survival rate, besides the median survival time (MST) is even less than 10 months. Moreover, regardless of histological type, half of all lung cancer patients develop metastatic or advanced malignancy (stages III and IV) at the time of diagnosis. Patients with advanced lung carcinoma are given platinum-based chemotherapy. Even though platinum-based drugs, such as cisplatin/carboplatin, are effective when coupled with non-platinum-based chemotherapy medications, for example, pemetrexed, paclitaxel, irinotecan, or gemcitabine, significant diversity in treatment response has been reported.[2] Different individuals react to the same drug in different ways. Although many genetic and environmental factors for instance age, drug interactions, concurrent therapy, and the nature of the disease can impact chemotherapy outcomes, there are plenty of inter-individual differences in the drug response due to sequence mutations of genes that encode drug transporters.[3]

Drug transporters are well-known mediators of drug disposition, facilitating the influx of substrates into the cells and the out flow of drugs as well as metabolites from cells. Solute carrier (SLC) transporters are the most prominent family of the membrane proteins accountable for the uptake and transport of numerous endogenous and xenobiotic compounds. These transporters are expressed widely in the human body, but notably in the epithelia of essential organs such as the liver, gut, kidney, and lung.[4] These transporters are also involved in multiple physiological processes, such as the cellular uptake of nutrients, xenobiotics, and absorption of chemotherapeutic drugs.[5] SLC transporters are usually expressed in many tumours and differentially in malignant and non-malignant tissues.[6] Among the SLC transporter family members, polymorphism in *SLC19A1*, *SLCO1B1*, and *SLCO1B3* are reported widely in different carcinomas, and outcomes have sparked curiosity in studying the function of these transporters in cancer progression.[6]

SLC19A1 encodes reduced folate carrier protein (RFC) and facilitates the movement of antifolate drugs used in cancer chemotherapy. The efficacy of chemotherapeutic drugs is associated with the activity and levels of SLC transporters in both cancer and normal tissues.[7] The RFC gene is highly polymorphic in nature and among the various SNP variants in the *SLC19A1* gene, polymorphism $G^{80}A$ (Arg27His, rs1051266) is usually studied in different clinical conditions. This variant has been extensively researched for its role in transport uptake and its correlation to cancer risk, treatment response, and toxicity. *G to A* polymorphism results in the substitution of arginine amino acid with histidine and thus leads to the alteration in the *SLC19A1* transporter structure, affecting its function,[8] thus modifying drug bioavailability and influencing the therapeutic outcome. Reports in the past have revealed a significant association of mutant genotype (*AA*) of $G^{80}A$ polymorphism with an elevated risk of oesophageal and gastric carcinoma, respectively.[9] Increased influx of the methotrexate drug has also been reported in patients with mutant genotype (*AA*) because $G^{80}A$ mutation may be responsible for the increased transporter activity.[10] Adjei et al. concluded that *SLC19A1* seems to predict survival differences in pemetrexed-treated NSCLC patients.[11]

Solute carrier organic anion transporting polypeptides (OATP) is another SLC drug transporter that mediates the cellular influx of various chemotherapeutic drugs into the cell. Among these transporters, *SLCO1B1* (*OATP1B1*) and *SLCO1B3* (*OATP1B3*) are the essential proteins localized at the basolateral membrane of the hepatocytes.[12] Overexpression of *SLCO1B1* and *SLCO1B3* has been reported in ovarian, prostate, breast, and lung cancer.[6] Various genetic polymorphisms are described in the *SLCO1B1* gene; among them, the most relevant SNPs related to drug disposition are $A^{388}G$ (rs2306283) and $T^{521}C$ (rs4149056). Reports have suggested some controversial results with the $A^{388}G$ variant as some studies have reported that $A^{388}G$ is significantly correlated with the increased expression of *SLCO1B1*, assuring increased transporter activity, while others have demonstrated unaltered transporter function.[13–15] On the other hand, variant $T^{521}C$ has been correlated with reduced expression of OATP1B1 protein and significantly lower transport activity compared to the wild type (*TT*) genotype.[13–16] Another variant of OATP is an intronic region mutation of *SLCO1B3* $A^{1683\text{-}5676}G$ (rs11045585) associated with the docetaxel-induced leukopenia.[17] *SLCO1B3* is a highly polymorphic gene that displays notable allele frequencies variations among various ethnic populations. Polymorphism in the *SLCO1B3* gene has been correlated with the altered OATP activity that often leads to drug-associated adverse events. Yamada et al. reported that the increased rate of the $A^{1683\text{-}5676}$Gvariant might contribute to the reduced *SLCO1B3* function that may alter therapeutic efficacy.[18]

Our research aimed to determine the role of *SLC19A1*, *SLCO1B1*, and *SLCO1B3* gene polymorphism in lung cancer patients of North India. The findings could help us better understand the molecular mechanisms underlying altered OATP expression, anticancer drug transport, cancer development, and therapy efficiency and see if these transporters can be harnessed as potential diagnostic and predictive molecular markers. To the best of our knowledge, no such studies evaluating the role of these polymorphisms in lung cancer have been undertaken in the North Indian lung cancer patients.

2 | METHODS

2.1 | Procurement of sample and study strategy

Lung carcinoma patients who went to the researchers' institute's lung carcinoma hospital (Post Graduate Institute of Medical Education and Research, PGIMER) were involved in the current study. Participants having confirmed histology and cytology for lung cancer (SCLC/NSCLC) were enrolled in the study for a 3-year period. The institute's ethical committee approved the study (Approval No. IEC-04/2018-884). The inclusion criteria were set to enrol lung cancer patients meeting all the ensuing requirements: (i) Lung carcinoma detection verified by histology or cytology (ii) Stage IIIB—IV or IIIA (not scheduled for surgery). (iii) Patients who were not treated and

had intent to treat with definitive platin-based chemotherapy (Cisplatin/Carboplatin) in combination with either docetaxel, paclitaxel, gemcitabine, irinotecan or pemetrexed. (iv) Patients having an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2. (v) As per Response Evaluation Criteria in Solid Tumours (RECIST) standards, patients having at least one bi-dimensionally quantifiable lesion[19] (vi) Acceptable organ function, such as absolute neutrophil count greater than 1500/μl, platelet count greater than 100,000/μl, and creatinine levels, liver enzymes such as alanine aminotransferase (ALT) <2-times the ULN (upper limits of normal). (vii) Written permission was taken from the patients. The exclusion measures contained (i) patients with non-bronchogenic tumours, (ii) primary pleural tumours—mesothelioma, (iii) sarcomas (iv), metastatic tumours to lungs or pleural, (v) early-stage (resectable) lung cancer.

At the beginning of this study, the patients had renal function tests, a complete blood count (CBC), a contrast-enhanced computed tomography (CT) scan of the thorax (which also encompassed the upper abdomen), liver function tests, and a chest radiograph. The demographic characteristics of the participants (gender, age, and performance status), disease stage, tumour histology, and smoking habits were all recorded. CBC was done 10 days after the first chemotherapeutic session, and any additional cycles if needed. CBC, renal function, and liver tests were performed before each treatment cycle till the 3–4 weeks following the final chemotherapy round. The paper was reported using the STROBE checklist.

2.2 | Chemotherapy regimen

Docetaxel (75 mg/m^2), irinotecan (100 mg/m^2), paclitaxel (175 mg/m^2), or pemetrexed (500 mg/m^2), were administered for 1-h infusion preceded by *iv*. Infusion of a drug cisplatin (70 mg/m^2) for over 3 h. Four chemotherapeutic cycles were given before a tumour response assessment, as is the usual procedure at PGIMER. Prior to the end of the four chemotherapy cycles, the assessment of tumour response was done, and if there were any clinic-radiological symptoms or adverse events (AEs) of cancer development, chemotherapy was halted if necessary. Subjects who exhibited an objective response to chemotherapy were given two more treatment cycles (six cycles maximum). The assessment of tumour response was assessed by RECIST criteria measure tumour response.[19] The common toxicity criteria (CTC) version 3.0 was implemented to collect and classify toxicity-related AEs.[20] PGIMER's established procedures were used to manage any negative effects. We assessed the following haematological toxicity parameters: leukopenia, anaemia, thrombocytopenia, and neutropenia. Diarrhoea, nausea, and constipation were investigated for gastrointestinal toxicity. Patients having febrile neutropenia or else grade 3 or more severe gastrointestinal adverse effects were admitted to the hospital if outpatient treatment was not available or effective. Any other side effects from the chemotherapy were also noted. During chemotherapy, follow-up was conducted every 3 weeks and every 3 months for the rest of the research. From chemotherapy treatment to the death or the last follow-up date was used to compute overall survival.

2.3 | Genotypic evaluation of SLC variants

The genomic DNA was isolated from 3 to 4 ml of blood utilizing the phenol-chloroform extraction technique. The genotype of variations was determined using the PCR-PFLP method as previously mentioned.[10,21,22] For amplifying a DNA fragment for the *SLC19A1* ($G^{80}A$, rs1051266) polymorphisms, the ensuing sets of primers were utilized such as 5′-AGTGTCACCTTCGTCCCCTC-3′ (sense) and 5′-CTCCC GCGTGAAGTTCTT-3′ (anti-sense). For *SLCO1B1* $A^{388}G$ in exon 4 (rs2306283), the set of primers used was forward primer 5′-ATAATGGTGCAAATAAAGGGG-3′and reverse primer 5′-ACTATCTC AGGTGATGC TCTA-3′ and for *SLCO1B1* $T^{521}C$(rs4149056) polymorphism, subsequent sets of primers were utilized such as 5′-TTGTCAAAGTTTG-CAAAGTG-3′ (sense) and 5′-GAAGCATATTAC-CCATGAGC-3′ (anti-sense). For amplifying a DNA fragment for the *SLCO1B3* ($A^{1683\text{-}5676}G$, rs11045585) the primers were 5′-GTGGGTA-AAAGGCAGGTAA ATG-3′ (sense) and 5′-GAATTCAAACATCTCACTGTGCTC-3′ (anti-sense). The PCR master mix of 20 μl comprised of 1X PCR buffer, 1.5 mM $MgCl_2$, 100 μg/mL bovine serum albumin (BSA), 200 μM dNTPs, 1U Taq polymerase (Thermo Scientific), 0.5 μM of forward primer and reverse primer and 300 ng of DNA. The conditions for PCR used were: 95°C for 5 min and 94°C for 30 s (denaturation), 59°C ($G^{80}A$/rs1051266), 55°C ($A^{388}G$/rs2306283), 60°C ($T^{521}C$/rs4149056) and 57°C ($A^{1683\text{-}5676}G$/rs11045585) for 45 s for annealing trailed by 72°C for 30 s of extension for 28 cycles and 5 min at 72°C for final extension. For *SLC19A1* $G^{80}A$, a PCR product of 230 base pairs (bp) was digested with 5U of *HhaI* restriction enzyme (New England Biolabs) at 37°C. The wild-type alleles were recognized as GG (125, 68, 37 bp), mutant alleles were recognized as AA (162, 68 bp), and carrier alleles (GA) produced all bands (162, 125, 68, 37 bp) (Figure 1a).[10] For $A^{388}G$, the PCR amplicon of 214 bp was resolved on a 1.8% agarose gel followed by digestion with 5U of *TaqI* restriction enzyme (New England Biolabs), respectively at 65°C. The wild type allele (*AA*) yielded bands of 151, 63 bp variant allele (*GG*), were identified by 128, 63, 23 bp, and carrier (AG) showed all the bands as shown in Figure 1b. For $T^{521}C$, a PCR product of 209 bp was digested with 5U of *HhaI* restriction enzyme (New England Biolabs) at 37°C. The wild-type alleles were recognized as TT (209 bp), and mutant alleles were recognized as CC (189, 20 bp) (Figure 1c).[19] For $A^{1683\text{-}5676}G$, a PCR amplicon of 286 bp was subjected to restriction digestion with 5U of *Rsa I* (New England Biolabs) at 37°C. The wild type allele (AA) yielded bands of 262, 24 bp, variant allele (GG) were identified by 155, 107, 24 bp, while heterozygous showed all the fragments (Figure 1d).[22] Fragments digested with respective enzymes were separated on 8% Native-polyacrylamide gel electrophoresis (PAGE) and were subjected to stain with the ethidium bromide (EtBr) and were visualized with the help of UV trans-illuminator. Two separate people looked into the data to eliminate any potential biases, and 20% of the samples were chosen at random, ensuring that the outcomes were 100% reproducible.

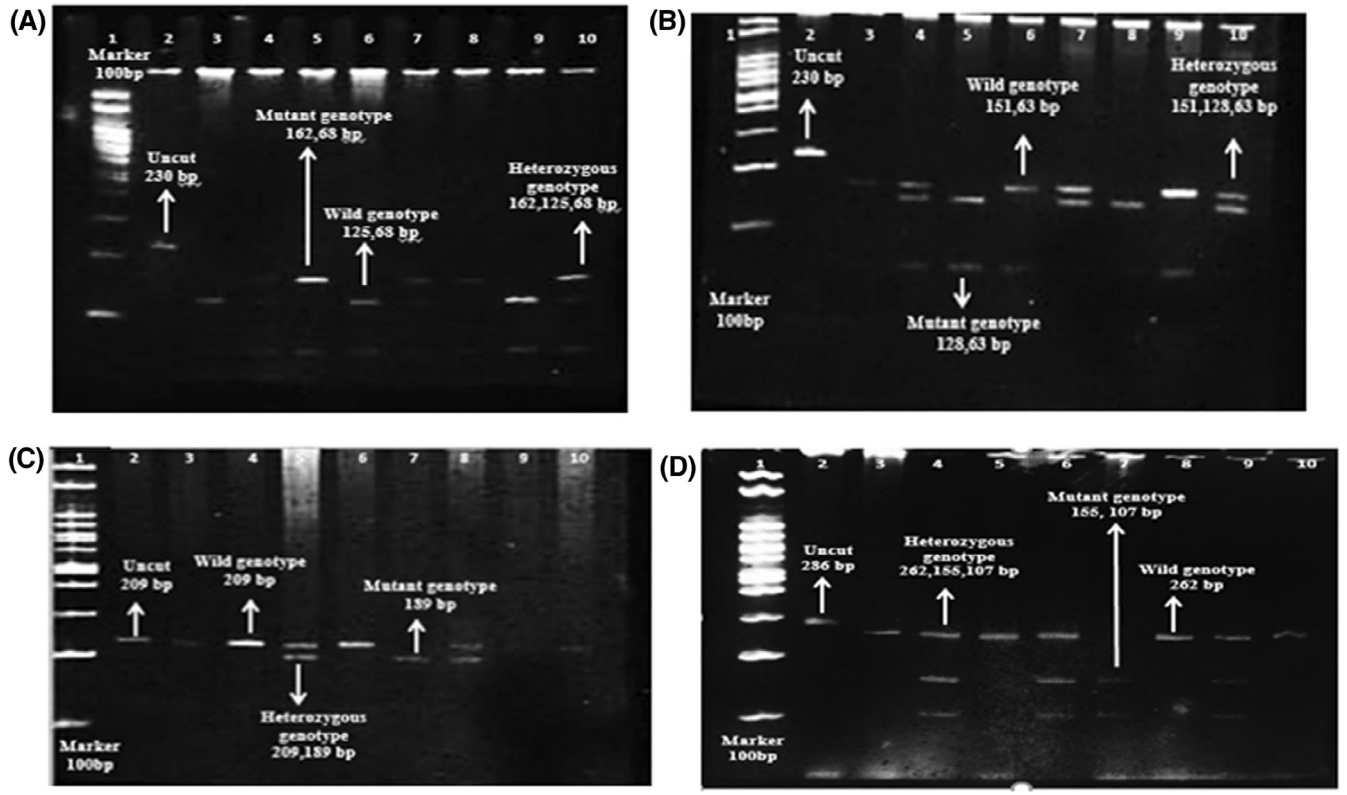


FIGURE 1 (A) Illustration of SLC19A1 $G^{80}A$ polymorphism. Lane 1—Marker (M) (100 bp); Lane 2—PCR amplicon; Lane 3, 6, 9—Wild genotype; Lane 4, 7, 10—Carrier genotype; Lane 5, 8—variant genotype; (B) illustration of SLCO1B1 $A^{388}G$ polymorphism. Lane 1—Marker (M) (100 bp); Lane 2—PCR amplicon; Lane 5, 8—Wild genotype; Lane 4, 7, 10—carrier genotype; Lane 3, 6, 9—variant genotype; (C) illustration of SLCO1B1 $T^{521}C$ polymorphism. Lane 1—Marker (M) (100 bp); Lane 2, 3, 4—wild genotype; Lane 5, 8—carrier genotype; Lane 6—variant genotype; (D) illustration of SLCO1B3 $A^{1683\text{-}5676}G$ polymorphism. Lane 1—Marker (M) (100 bp); Lane 2—PCR amplicon; Lane 3, 5, 10—Wild genotype; Lane 4, 6, 9—Carrier genotype; Lane 7—variant genotype

2.4 | Statistical analysis

The study focused on the patients of lung carcinoma of the North Indian population and consisted age, gender, as well as smoking behaviour information. The Chi-square test was applied to see if the cases followed Hardy–Weinberg equilibrium. The risk of lung carcinoma was studied using MedCalc Statistical Software (version 14.8.1 MedCalc Software, Ostend, Belgium). Descriptive data are presented as median, number/percentages, mean, or standard deviation (SD). The odds ratio (OR) along with the 95% confidence interval (CI) was assessed with the logistic regression analysis for any adverse-events. All the patients' overall survival (OS) was calculated from first day of chemotherapy to demise or else the last followed-up date. The univariate Kaplan-Meier method assessed OS time using log Rank *p* value and median OS time. After adjusting for other factors, multivariate regression analysis was employed to evaluate the independent effect of the polymorphism on OS. In all analyses, a *p* value of <0.05 was chosen to be significant.

Furthermore, the recursive partitioning strategy accounted for high-order gene interactions. This method generates the initial split by considering the critical factor determining the patients' overall survival. Survival analysis trees (STREE) application was used to generate the decision tree (http://c2s2.yale.edu/software/stree). The log-rank approach was used as the splitting method. The generated tree was binary, with each terminal node representing a subset of individuals with a specific genotypic combination, thus displaying a range of survival times and prognosis. Multivariate cox proportional hazard analysis was used to determine the HRs and 95% CIs for every terminal node, adjusted for gender, age, histology, ECOG, KPS, stage, and regimen. Toxicity in the lung cancer patients was assessed according to standard National Cancer Institution Criteria 3.0 (http://ctep.cancer.gov). Maximum attention was paid to haematological toxicity such as neutropenia, anaemia, leukopenia and thrombocytopenia, gastrointestinal toxicity nephrotoxicity. Severe toxicity or severe event consisted of grade 3 or 4 hematologic toxicity and grade 1–4 nephrotoxicity. With severe hematologic toxicity, the subsequent treatment was postponed until recovery to grade 1 or grade 0. With grade 3 and 4 gastrointestinal toxicity, the doses of each drug were reduced by 25%. If creatinine clearance decreases to within the range of 59–41 ml min^{-1}, platinum was reduced by 25%. If creatinine clearance decreases below 40 ml min^{-1}, platinum was stopped. Clinical data have been systematically recorded during treatment.

TABLE 1 Patient characteristics

	SLC SNPs				
Variable	**Total (*N* = 610)**	**Cases, *n* (%)**	**Cases, *n* (%)**	**Total (*N*)**	**Cases, *n* (%)**
Gender	610		**Lymph node**	610	
Male		496 (81.3)	N0		79 (12.9)
Female		114 (18.6)	N1		51 (8.3)
Age	603	60 ± 10.05	N2		255 (41.8)
		29–86	N3		195 (31.9)
Smoking status	609		N4		1 (0.16)
Smokers		485 (79.6)	Unknown		29 (4.75)
Non-smokers		124 (20.3)	**Performance status**	610	
Pack years	600		KPS (below 60)		106 (17.3)
Mean ± SD		25.58 ± 31.88	KPS (70–80)		314 (51.4)
Overall survival	593		KPS (90–100)		168 (27.5)
Dead		493 (83.1)	Unknown		22 (3.6)
Alive		100 (16.8)	ECOG (0–1)		267 (43.7)
TNM staging	610		ECOG (2)		233 (38.1)
I		5 (0.81)	ECOG (3–4)		89 (14.5)
II		19 (3.11)	Unknown		21 (3.4)
III IV Unclassified		168 (27.5) 385 (63.1) 33 (5.40)	**Histological types** SQCC ADCC SCLC Others	600	245 (40.8) 244 (40.6) 104 (17.3) 7 (1.17)
Tumour size	610		**Chemotherapy regimen**	419	
Tx		21 (3.44)	Pemetrexed cis/carboplatin		130 (31.0)
T1		36 (5.90)	Irinotecan cis/carboplatin		71 (16.9)
T2		54 (8.8)	Docetaxel cis/carboplatin		70 (16.7)
T3		122 (20)	Paclitaxel cis/carboplatin		87 (20.7)
T4		347 (56.8)	Gemcitabine cis/carboplatin		29 (6.92)
Unknown		30 (4.91)	Gefitinib		15 (3.57)
Metastasis	610		Etoposide		17 (4.05)
M1		309 (50.6)	**Chemotherapy response**	315	
Unknown		29 (4.75)	CR/PR		133 (42.2)
			SD/PD		182 (57.7)

3 | RESULTS

3.1 | Patient characteristics and clinical predictors

Patients characteristics of the case study group consisted of gender, age, smoking status, Tumour, node, metastasis (TNM) staging, pack-years, and various other clinical factors that are showed in Table 1. At the time of diagnosis, the lung carcinoma patients had an average age of 60.10 years (range 29–86). Among 610 patients, 496 (81.3%) males and 114 (18.6%) females were present in the case group. Smokers accounted for 79.6% of the group, more significant than non-smokers (20.3%). Smokers had an average pack-year of 25.58 ± 31.88. TNM staging was used to classify the patients: 0.81% had stage I lung cancer, 3.11% had stage II lung cancer, 27.5% had stage III lung cancer, and 63.1% had stage IV lung carcinoma. The lung cancer patients T3 and T4 exhibited high frequencies of 20% and 56.8%, whereas patients with tumour size T1 and T2 had incidence of 5.90% and 8.8%, respectively. TNM data revealed that 44.5% of patients had no metastasis (M0), whereas 50.6% had distant metastatic involvement (M1).Regarding lymph node involvement, the incidence of patients with no lymph node (N0) was 12.9% of, whereas N1, N2, N3, and N4 lymph node involvement was found in 8.3%, 41.8%, 31.9%, and 0.16% of all cases, respectively. Histologically, squamous cell carcinoma (SQCC) patients made up 40.8% of lung cancer diagnoses, adenocarcinoma (ADCC) patients made up 40.66%, and small-cell lung cancer (SCLC) patients made up 17.3%. KPS and ECOG indicated the individuals' performance status and was used to classify the cases. There were 168 individuals (27.5%) with KPS scores of 100–90,

TABLE 2 Results of genetic equilibrium test

Genotype, rs1051266, SLC19A1 (G80A)	No. patients (%)	χ^2	*p* Value
GG	195 (31.9)		
GA	284 (46.5)	2.08	0.14
AA	131 (21.4)		
GA + AA	415 (68.0)		
Genotype, rs2306283, SLCO1B1 (A388G)	**No. patients (%)**	χ^2	***p* Value**
AA	152 (24.9)		
AG	297 (48.6)	0.41	0.52
GG	161 (26.3)		
AG + GG	458 (75.0)		
Genotype, rs4149056, SLCO1B1 (T521C)	**No. patients (%)**	χ^2	***p* Value**
TT	563 (92.2)		
TC	45 (7.3)	1.13	0.28
CC	2 (0.32)		
TC + CC	47 (7.7)		
Genotype, rs11045585, SLCO1B3 (1683-5676A > G)	**No. patients (%)**	χ^2	***p* Value**
AA	545 (89.34)		
AG	64 (10.4)	0.38	0.53
GG	1 (0.16)		
AG + GG	65 (10.6)		

314 (51.4%) with KPS scores of 80–70, and 106 people (17.3%) with KPS scores of less than 60 in our study. As per the ECOG score, 43.7% (267) of the people had ECOG score 0–1, 38.1% (233) had ECOG score of 2, and 14.5% (89%) of the people had ECOG score 3–4. A total of 419 patients received carboplatin or cisplatin-based therapy, with 130 patients receiving pemetrexed, 71 patients receiving irinotecan, 87 patients receiving paclitaxel, 70 patients receiving docetaxel, 29 patients receiving gemcitabine, 17 patients receiving etoposide, and 15 patients receiving gefitinib.

3.2 | Genotypic distribution of SLC polymorphism

For *SLC19A1* $G^{80}A$ polymorphism, 195 (31.9%) patients were having *GG* genotype, 131 (21.4%) patients were having *AA* genotype, while 284 (46.5%) patients were carriers (*GA*) (Table 2). While for *SLCO1B1* $A^{388}G$ polymorphism, 152 (24.9%) patients were having *AA* genotype, 297 (48.6%) were heterozygous *AG*, and 161 (26.3%) were homozygote *GG* variant whereas, for *SLCO1B1* $T^{521}C$, 563 (92.2%) patients were homozygous for *TT* genotype, 45 (7.3%) patients harboured heterozygous (TC) genotype and 2 (0.32%) individuals had homozygous (*CC*) genotype. For the *SLCO1B3* $A^{1683\text{-}5676}G$ variant, 545 (89.34%) patients were having *AA* genotype, 64 (10.4%) were carriers *AG*, and 1 (0.16%) were having *GG* variant. The genotypic frequencies for *SLC19A1* $G^{80}A$ was ($\chi^2 = 2.08$, $df = 2$; $p = 0.14$). The genotypic frequencies of *SLCO1B1* $A^{388}G$ ($\chi^2 = 0.41$, $df = 2$; $p = 0.52$) and *SLCO1B1* $T^{521}C$ polymorphism was ($\chi^2 = 1.13$, $df = 2$; $p = 0.28$) whereas for *SLCO1B3* $A^{1683\text{-}5676}G$ genotypic frequency was $\chi^2 = 0.38$, $df = 2$; $p = 0.53$. Therefore, genotypic distribution for these four polymorphisms were in agreement with Hardy–Weinberg equilibrium (p value > 0.05), as shown in Table 2.

3.3 | Association of SLC genotype with overall survival

The correlation of the *SLC* polymorphisms with survival in 593 lung cancer samples was investigated. After 3 years, 83.1% (493) of patients had died, and 16.8% (100) had survived. The correlation of survival with *SLC* SNPs were examined using univariate Kaplan-Meier and multivariate regression analysis after making adjustments for smoking habits, gender, tumour stage, age, ECOG, histology, KPS. This analysis used four alternative models (Dominant, co-dominant, additive, and recessive) to determine the relationship between *SLC* polymorphism and OS in lung carcinoma patients (Table 3). Lung carcinoma patients who were harbouring mutant genotype (*AA*) for *SLC19A1* $G^{80}A$ polymorphism had a higher MST as compared to the subjects carrying wild type (*GG*) genotype (MST = 9.33 versus 8.23, $p = 0.4$). The *SLC19A1* $G^{80}A$ polymorphism did not show any significant difference in survival in any of the models used.

For *SLCO1B1* $A^{388}G$, in the co-dominant model, we observed very marginal lower survival in patients carrying both mutant (*GG*) alleles as compared to the patients who were carrying both wild-types (*AA*) alleles (MST = 7.8 vs. 7.9; Log-rank $p = 0.41$; HR = 1.10). After using regression model, our outcomes demonstrated reduced survival outcomes in patients carrying variant (*GG*) alleles in comparison to patients carrying reference (*AA*) allele (HR = 1.40, 95% CI = 1.0–1.97; $p = 0.04$), as revealed in Figure 2. For *SLCO1B1* $T^{521}C$ polymorphism,

TABLE 3 Univariate and multivariate analysis for *SLC* variants

Genotype, rs1051266, SLC19A1 (G80A)	CASES, *n* (%), *N* = 593	DEAD, *n* (%), *N* = 493	ALIVE, *n* (%), *N* = 100	Univariate analysis			Multivariate analysis	
				MST (months)	Log-rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
GG	194 (32.71)	161 (32.65)	33 (33)	8.23	-	1.0 (reference)	-	-
GA	276 (46.54)	231 (46.85)	45 (45)	7.27	0.33	1.10 (0.90–1.34)	1.11 (0.85–1.45)	0.42
AA	123 (20.74)	101 (20.48)	22 (22)	9.33	0.55	0.92 (0.72–1.18)	0.87 (0.61–1.23)	0.44
Dominant model								
GG	194 (32.71)	161 (32.65)	33 (33)	8.23	-	1.0 (reference)	-	-
GA + AA	399 (67.28)	332 (67.34)	67 (67)	7.93	0.64	1.04 (0.86–1.26)	1.00 (0.78–1.29)	0.94
Recessive model								
GG + GA	470 (79.25)	392 (79.51)	78 (78)	7.6	-	1.0 (reference)	-	-
AA	123 (20.7)	101 (20.48)	22 (22)	9.33	0.23	0.88 (0.71–1.08)	0.79 (0.58–1.08)	0.14
Additive model								
GG + AA	317 (53.45)	262 (53.14)	55 (55)	8.8	-	1.0 (reference)	-	-
GA	276 (46.54)	231 (46.85)	45 (45)	7.27	0.15	1.13 (0.95–1.36)	1.16 (0.92–1.48)	0.20
Genotype, rs2306283, SLCO1B1 (A388G)	**CASES, *n* (%), *N* = 593**	**DEAD, *n* (%), *N* = 493**	**ALIVE, *n* (%), *N* = 100**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
AA	147 (24.78)	124 (25.15)	23 (23)	7.9	-	1.0 (reference)	-	
AG	290 (48.90)	234 (47.46)	56 (56)	8.26	0.67	0.95 (0.76–1.18)	1.18 (0.88–1.59)	0.26
GG	156 (26.30)	135 (27.38)	21 (21)	7.8	0.41	1.10 (0.86–1.41)	1.40 (1.0–1.97)	**0.04**
Dominant model								
AA	147 (24.78)	124 (25.15)	23 (23)	7.9	-	1.0 (reference)	-	
AG + GG	446 (75.21)	369 (74.84)	77 (77)	7.97	0.9	1.00 (0.81–1.22)	1.27 (0.96–1.69)	0.08
Recessive model								
AA + AG	437 (73.69)	358 (72.61)	79 (79)	8.26	-	1.0 (reference)	-	
GG	156 (26.30)	135 (27.38)	21 (21)	7.8	0.24	1.12 (0.92–1.38)	1.23 (0.95–1.60)	0.11
Additive model								
AA + GG	303 (51.09)	259 (52.53)	44 (44)	7.8	-	1.0 (reference)	-	
AG	290 (48.90)	234 (47.46)	56 (56)	8.26	0.31	0.91 (0.76–1.08)	1.02 (0.80–1.29)	0.8
Genotype, rs4149056, SLCO1B1 (T521C)	**CASES, *n* (%), *N* = 593**	**DEAD, *n* (%), *N* = 493**	**ALIVE, *n* (%), *N* = 100**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
TT	548 (92.41)	453 (91.88)	95 (95)	7.97	-	1.0 (reference)		
TC	43 (7.25)	38 (7.70)	5 (5)	7.47	0.57	1.10 (0.78–1.55)	0.95 (0.61–1.48)	0.84
CC	2 (0.33)	2 (0.40)	0	5.16	0.98	0.98 (0.24–3.93)	0.77 (0.10–5.69)	0.80
Dominant model								
TT	548 (92.41)	453 (91.88)	95 (95)	7.97	-	-	-	-
TC + CC	45 (7.58)	40 (8.11)	5 (5)	7.47	0.58	1.09 (0.78–1.53)	0.94 (0.61–1.44)	0.79
Genotype, rs11045585, SLCO1B3 (1683-5676A > G)	**CASES, *n* (%), *N* = 593**	**DEAD, *n* (%), *N* = 493**	**ALIVE, *n* (%), *N* = 100**	**Univariate analysis**			**Multivariate analysis**	
				MST (months)	**Log rank *p***	**Unadjusted HR[a]**	**Adjusted HR[b] (95% CI)**	***p***
AA	529 (89.20)	437 (88.64)	92 (92)	7.97	-	-	-	-
AG	63 (10.62)	56 (11.35)	7 (7)	7.6	0.52	1.09 (0.82–1.46)	0.89 (0.61–1.31)	0.58
GG	1 (0.16)	0	1 (1)	-	-	-	-	-
				7.97	-	1.0 (reference)	-	

(Continues)

TABLE 3 (Continued)

Genotype, rs11045585, SLCO1B3 (1683-5676A > G)	CASES, *n* (%), *N* = 593	DEAD, *n* (%), *N* = 493	ALIVE, *n* (%), *N* = 100	Univariate analysis			Multivariate analysis	
				MST (months)	Log rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
Dominant model								
AA	529 (89.20) 64 (10.79)	437 (88.64) 56 (11.35)	92 (92) 8 (8)	7.6	0.67	1.06 (0.79–1.41)	0.86 (0.58–1.26)	0.44
Additive model								
AA + GG	530 (89.37)	437 (88.64)	93 (93)	8.02	-	1.0 (reference)	-	-
AG	63 (10.62)	56 (11.35)	7 (7)	7.6	0.50	1.10 (0.82–1.47)	0.90 (0.61–1.33)	0.62

Note: Bold value represents that *p* value of <0.05 is considered statistically significant.
[a]Unadjusted Hazards ratio for Kaplan-Meier analysis.
[b]Hazards ratio for age, sex, smoking, stage, regimen, KPS, ECOG.

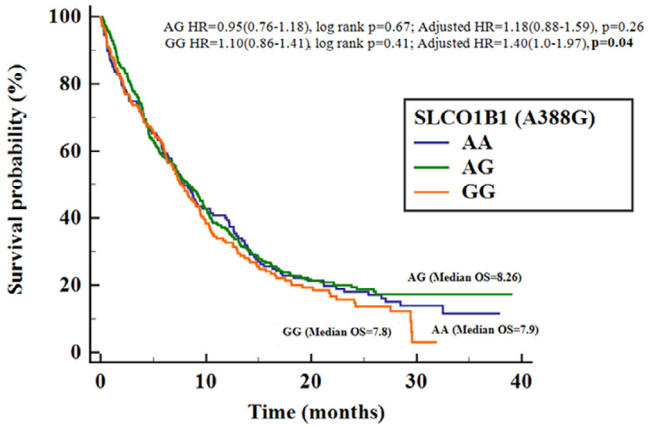


FIGURE 2 Correlation between overall survival in different genotypes of *SLCO1B1* (*A388G*) polymorphism in lung cancer patients. Wild-type genotype (AA), carrier genotype (AG), and mutant genotype (GG)

we observed lower OS in the lung carcinoma patients who had variant genotype (CC) as compared to the patients carrying wild-type genotype (TT) (MST = 5.16 vs. 7.97; $p = 0.80$). However, no significant differences in terms of survival were observed for the *SLCO1B1* $T^{521}C$ variant.

For the *SLCO1B3* $A^{1683\text{-}5676}G$ variant, the patients having heterozygous allele (*AG*) showed lower median survival in lung carcinoma patients as compared to the individuals harbouring wild type (AA) genotype (MST = 7.6 vs. 7.97; $p = 0.58$). However, only one patient harboured mutant genotype, so the survival differences were not calculated for this group. No prognostic implication was found for $A^{1683\text{-}5676}G$ polymorphism for OS in lung cancer patients.

3.4 | Elucidation of SLC variants with OS and histology

Th effect of SLC variants in predicting median survival of lung carcinoma patients based on histology subcategories was also assessed. On analysis, we noted that ADCC patients having variant genotype (*AA*) had better survival as compared to the ADCC subjects with reference-type genotype (*GG*) for *SLC19A1* $G^{80}A$ (MST = 9.4 vs. 8.8, Log-rank $p = 0.2$) as shown in Table 4. After applying the cox regression model, there was a decreased hazard ratio for lung cancer patients ($HR^1 = 0.60$, 95% CI = 0.36–1.00, $p = 0.04$).

For *SLCO1B1* $A^{388}G$, patients diagnosed with SQCC and having the variant genotype (*GG*) showed reduced survival when compared to combined reference type and heterozygous genotypes (*AA* + *AG*) (MST = 6.8 months versus 7.5 months, log-rank $p = 0.42$). After multivariate regression study, the overall death risk of patients carrying *GG* genotype at $A^{388}G$ was 1.54 times higher than individuals with *AA* + *AG* genotype (HR = 1.54, 95% CI = 0.99–2.39; $p = 0.05$). However, on analysing the correlation of *SLC19A1* $G^{80}A$ variations with SCLC histology, our results depicted increased OS in the patients having variant genotype (*AA*) as compared to the SCLC participants having reference genotype (*GG*) (MST = 9.6 vs. 7.6 months, $p = 0.34$). After multivariate analysis, such patients had a low death ratio than SCLC subjects carrying the wild genotype (*GG*) (HR = 0.22, 95% CI = 0.05–0.93, $p = 0.04$, Table 4, Figure 3). Similar results were also obtained when the recessive model was applied (HR = 0.30, 95% CI = 0.11–0.84, $p = 0.02$). None of the other SCLC patients showed substantial survival differences for the *SLCO1B1* $A^{388}G$, $T^{521}C$, and *SLCO3B3* $A^{1683\text{-}5676}G$ polymorphism (Table S1).

3.5 | Correlation of SLC variation with the OS and chemotherapy regimen

The lung carcinoma patients in the study were administered platinum-based doublet chemotherapy in addition to pemetrexed, paclitaxel, irinotecan, and docetaxel. Therefore, we chose to investigate the modifying effects of *SLC* polymorphism in addition its relationship to chemotherapy, and patients' survival, as shown in Table S2. All lung malignancy patients received carboplatin/cisplatin-based therapy along with second-line chemotherapy treatment, for example, docetaxel, pemetrexed, irinotecan, or paclitaxel. For *SLCO1B3* $A^{1683\text{-}5676}G$

TABLE 4 Association of *SLC* SNPs on overall survival according to histology

Histology-ADCC								
				Univariate analysis			Multivariate analysis	
Genotype, rs1051266, SLC19A1 (G80A)	CASES, *n* (%), *N* = 231	DEAD, *n* (%), *N* = 186	ALIVE, *n* (%), *N* = 45	MST (months)	Log rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
GG	83 (35.93)	70 (37.63)	13 (28.88)	8.8	-	1.0 (reference)	-	-
GA	95 (41.12)	77 (41.39)	18 (0.4)	7.37	0.82	1.03 (0.74–1.43)	0.91 (0.58–1.44)	0.71
AA	53 (22.94)	39 (20.96)	14 (31.11)	9.4	0.22	0.78 (0.53–1.15)	0.60 (0.36–1.00)	**0.04**
Dominant model								
GG	83 (35.93)	70 (37.63)	13 (28.88)	8.8	-	1.0 (reference)	-	-
GA + AA	148 (64.06)	116 (62.36)	32 (71.11)	8.3	0.69	0.94 (0.69–1.27)	0.76 (0.52–1.12)	0.18
Recessive model								
GG + GA	178 (77.05)	147 (79.03)	31 (68.88)	7.9	-	1.0 (reference)	-	-
AA	53 (22.94)	39 (20.96)	14 (31.11)	9.4	0.16	0.78 (0.56–1.09)	0.70 (0.44–1.10)	0.13
SCLC								
				Univariate analysis			Multivariate analysis	
Genotype, rs1051266, SLC19A1 (G80A)	CASES, *n* (%), *N* = 103	DEAD, *n* (%), *N* = 95	ALIVE, *n* (%), *N* = 8	MST (months)	Log rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
GG	31 (30.09)	30 (31.57)	1 (12.5)	7.6	-	1.0 (reference)	-	-
GA	56 (54.36)	49 (51.57)	7 (87.5)	7.3	0.62	1.11 (0.70–1.78)	0.87 (0.52–1.45)	0.61
AA	16 (15.53)	16 (16.84)	-	9.6	0.34	0.75 (0.41–1.35)	0.22 (0.05–0.93)	**0.04**
Dominant model								
GG	31 (30.09)	30 (31.57)	1 (12.5)	7.6	-	1.0 (reference)	-	-
GA + AA	72 (69.90)	65 (68.42)	7 (87.5)	9	0.47	0.85 (0.54–1.33)	0.56 (0.30–1.04)	0.07
Recessive model								
GG + GA	87 (84.46)	79 (83.15)	8 (100)	7.6	-	1.0 (reference)	-	-
AA	16 (15.53)	16 (16.84)	-	9.6	0.69	0.89 (0.53–1.51)	0.30 (0.11–0.84)	**0.02**
SQCC								
				Univariate analysis			Multivariate analysis	
Genotype, rs2306283, SLCO1B1 (A388G)	CASES, *n* (%), *N* = 243	DEAD, *n* (%), *N* = 201	ALIVE, *n* (%), *N* = 42	MST (months)	Log rank *p*	Unadjusted HR[a]	Adjusted HR[b] (95% CI)	*p*
AA	59 (24.27)	50 (24.87)	9 (21.42)	6.73	-	1.0 (reference)	-	
AG	119 (48.97)	95 (47.26)	24 (57.14)	8.43	0.33	0.84 (0.59–1.19)	1.06 (0.65–1.74)	0.80
GG	65 (26.74)	56 (27.86)	9 (21.42)	6.86	0.79	1.05 (0.71–1.54)	1.68 (0.93–3.03)	0.08
Dominant model								
AA	59 (24.27)	50 (24.87)	9 (21.42)	6.73	-	1.0 (reference)	-	
AG + GG	184 (75.72)	151 (75.12)	33 (78.57)	7.8	0.56	0.90 (0.65–1.26)	1.26 (0.80–1.98)	0.31
Recessive model								
AA + AG	178 (73.25)	145 (72.13)	33 (78.57)	7.5	-	1.0 (reference)	-	
GG	65 (26.74)	56 (27.86)	9 (21.42)	6.8	0.42	1.13 (0.82–1.56)	1.54 (0.99–2.39)	0.05

Note: Bold value represents that *p*-value of <0.05 is considered statistically significant.
[a]Unadjusted Hazards ratio for Kaplan-Meier analysis.
[b]Hazards ratio for age, sex, smoking, stage, regimen, KPS, ECOG.

polymorphism, our results showed that patients who were given carboplatin/cisplatin along with the docetaxel and having heterozygous alleles (*AG*) showed inferior survival outcomes as compared to the patients having reference type genotype (*AA*) (MST = 2.9 vs. 9.6 months, Log-Rank $p = 0.006$, HR = 14.01). Upon adjusting with multivariate factors such as age, gender, stage, smoking, histology, and PS, we observed a high hazard or death ratio in the patients having heterozygous genotype (*AG*) as compared to the subjects harbouring wild type genotype (AA) (HR = 3.84, 95% CI = 1.16–12.72; $p = 0.02$, Figure 4) as shown in Table S2.

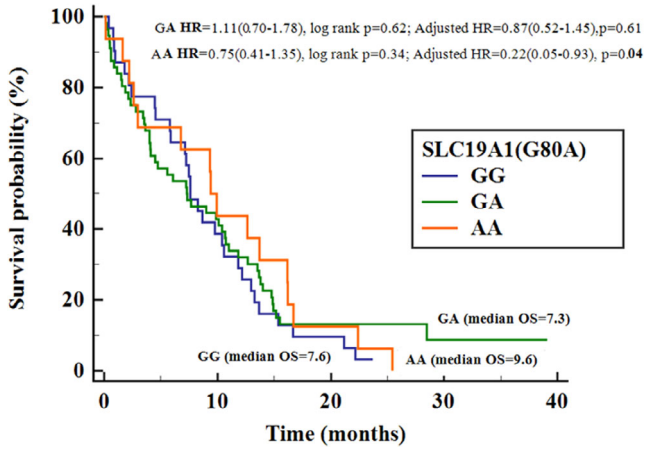


FIGURE 3 Correlation between overall survival in different genotypes of *SLC19A1* (*G80A*) polymorphism in SCLC patients. Wild-type (GG) genotype, carrier (GA) genotype, and mutant (AA) genotype

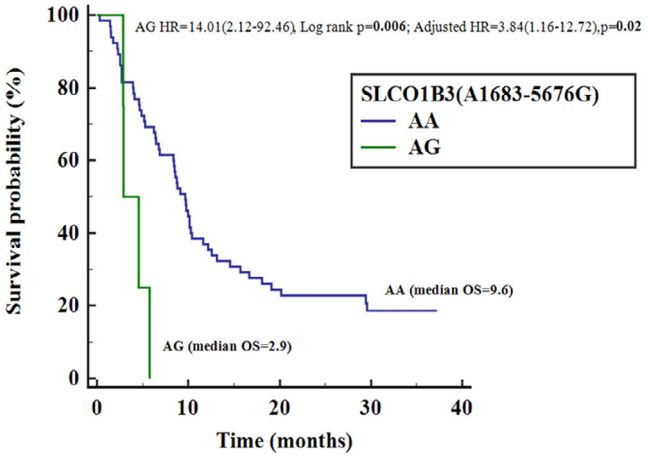


FIGURE 4 Correlation between overall survival in different genotypes of *SLCO1B3* (*A1683-5676G*) polymorphism in patients administered with docetaxel cisplatin/carboplatin. Wild-type (AA) genotype and heterozygous (AG) genotype

Lung carcinoma subjects undergoing carboplatin/cisplatin along with irinotecan, paclitaxel, or pemetrexed treatment showed no substantial correlation between OS and *SLC19A1*, *SLCO1B1*, and *SLC19A1* polymorphism Table S2.

3.6 | Relationship of SLC polymorphisms with OS and gender

Males having *SLC19A1* $G^{80}A$ polymorphism and possessing heterozygous genotype (*GA*) showed reduced OS as compared to the patients harbouring reference-type alleles (*GG*) (MST = 7.13 vs. 7.9 months; HR = 1.1; log-rank $p = 0.38$). After adjusting with multiple factors, the death risk increased to 1.26 times in heterozygous genotype (*GA*) as compared to the wild type (*GG*) genotype (HR = 1.26, 95% CI = 0.99–1.61; $p = 0.05$). Also, for *SLCO1B1A*^{388}*G* polymorphism, in the co-dominant model, lower overall survival was observed in the males having mutant genotype (*GG*) as compared to the subjects harbouring reference type (AA) genotype (MST = 7.57 versus 7.7 months; HR = 1.35, 95% CI = 1.01–1.81; $p = 0.04$) as shown in Table S3. However, we did not find any significant association of SLC polymorphism in females with overall survival. We also evaluated the survival differences in lung cancer patients according to age; however, no significant association was found between SLC polymorphisms and overall survival (data not shown).

3.7 | Relationship between SLC polymorphism and chemotherapeutic response

Correlation between *SLC* polymorphism and the effectiveness of response rate (RR) and clinical benefits were studied using logistic regression. Table S4 shows the relationship between *SLC* polymorphism and treatment responses. The individuals showing stable and progressive disease were categorized as non-responders, whereas subjects demonstrating complete or partial response were classified as good responders. Our outcomes showed no association between *SLC* polymorphisms according to the chemotherapeutic response to platinum-based doublet therapy. As a result, we concluded that SLC polymorphisms may not predict the chemotherapy response in a significant way.

3.8 | Correlation between SLC genotype and tumour characteristics

In order to assess the correlation between *SLC* variants and the clinic-pathological parameters, the patients were bifurcated based on cancer stage (stage III versus stage IV), lymph node involvement (Nx + N0 + N1 versus N2 + N3 + N4), primary tumour extension (T3 vs. T4), and metastasis (positive versus negative) as revealed in Table S5. 18.7% of lung carcinoma patients diagnosed with T4 tumour extension carried mutant alleles for SLC19A1 $G^{80}A$ genotype compared to 30.3% patients in T3 tumour extension (Table 5). A decreased-odds of developing T4 was noted in variant genotype (*AA*) when compared to the patients categorized in T3 tumour extension (AOR = 0.42, 95% CI = 0.2–0.89; $p = 0.02$). In the same lines, when the patients with T3 were compared with individuals with T4 size in *SLCO1B1* $T^{521}C$, a decreased odds towards lung carcinoma was noted in the carrier (*TC*) genotype of T4 as compared to the patients with T3 tumour extension (AOR = 0.29, 95% CI = 0.11–0.77, $p = 0.01$). Using the dominant model, combining the two risk genotypes (*TC* + *CC*), high risk for lung carcinoma was not observed in T4 compared to patients with T3 tumour extension (AOR = 0.34, 95% CI = 0.13–0.88; $p = 0.02$). Therefore, our results show that these genotypes did not show any relationship with the tumour size and its progression.

TABLE 5 Relationship of different *SLC* genotypes with clinic-pathological parameters

	Tumour size			
Genotype, rs1051266, SLC19A1 (G80A)	**T3 (122), *n* (%)**	**T4 (347), *n* (%)**	**AOR (95% CI)[a]**	***p*[b]**
GG	35 (28.68)	101 (29.10)	1.0 (reference)	
GA	50 (40.98)	181 (52.16)	1.19 (0.62–2.29)	0.58
AA	37 (30.32)	65 (18.73)	0.42 (0.20–0.89)	**0.02**
GA + AA	87 (71.31)	246 (70.89)	0.86 (0.48–1.55)	0.62
Genotype, rs2306283, SLCO1B1 (A388G)	**T3 (122), *n* (%)**	**T4 (347), *n* (%)**	**AOR (95% CI) [a]**	***p*[b]**
AA	31 (25.40)	80 (23.05)	1.0 (reference)	
AG	61 (50)	164 (47.26)	0.74 (0.37–1.46)	0.39
GG	30 (24.59)	103 (29.68)	1.15 (0.52–2.53)	0.72
AG + GG	91 (74.59)	267 (76.94)	0.86 (0.45–1.64)	0.66
Genotype, rs4149056, SLCO1B1 (T521C)	**T3 (122), *n* (%)**	**T4 (347), *n* (%)**	**AOR (95% CI) [a]**	***p*[b]**
TT	109 (89.34)	323 (93.08)	1.0 (reference)	
TC	12 (9.83)	23 (6.62)	0.29 (0.11–0.77)	**0.01**
CC	1 (0.81)	1 (0.28)	-	-
TC + CC	13 (10.65)	24 (6.91)	0.34 (0.13–0.88)	**0.02**
Genotype, rs11045585, SLCO1B3 (1683-5676A > G)	**T3 (122), *n* (%)**	**T4 (347), *n* (%)**	**AOR (95% CI)[a]**	***p*[b]**
AA	111 (90.98)	312 (89.91)	1.0 (reference)	
AG	11 (9.01)	34 (9.79)	1.41 (0.54–3.69)	0.47
GG	0	1 (0.28)	-	-
AG + GG	11 (9.01)	35 (10.08)	1.46 (0.56–3.82)	0.43

Note: Bold value represents that *p*-value of <0.05 is considered statistically significant.
[a]Adjusted Odds ratios, 95% confidence intervals and their corresponding *p*-values were calculated by logistic regression analysis after adjusting for age, gender, smoking and regimen.
[b]Two-sided χ^2 test for either genotype distribution or allelic frequencies between the responders and non-responders. The number in bold indicates the significant values in the table.

3.9 | High-order gene–gene interactions using STREE

Multiple interaction analyses involving various genes implicated in SLC transporters add a new dimension to assess the high-order parametric interactions within them and their impact on the overall survival of lung cancer patients. The recursive partitioning strategy was adopted to generate a survival tree in this study, demonstrating various SLC SNPs' roles in prognosis. Figure 5 shows the tree generated by the STREE software. The log-rank approach was used to generate the tree out of all the methods available. There was a total of four-terminal nodes found. *SLC19A1G*^{80}*A* was the most crucial factor as it is a root node influencing the prognosis of patients. Terminal node 5 with the highest MST was used as a reference node (13.1 months). In addition, each node's HR is mentioned. The survival curves and the MST associated with each terminal node are illustrated in Figure 5b. It shows a difference between the median survival time of different nodes and the reference node (log-rank *p* 0.18), though a significant correlation was not observed. The findings of the Cox regression analysis for every terminal node are summarized in Table S6. Significant differences were observed in the median survival times when terminal node 4 was compared to the reference node (4.8 months versus 13.1 months; HR = 2.24, 95% CI = 0.99–5.02; $p = 0.05$). After adjusting with different covariates, including age, gender, stage, smoking, histology, regimen, ECOG, and KPS, the death risk was 1.94 times as compared to the reference node (HR = 1.94; $p = 0.002$). A similar trend was noted in all the other nodes; however, significant differences were not achieved.

3.10 | Relationship between SLC polymorphism and toxicity

One of the research goals was to look into the emergence of AEs in lung carcinoma patients given platinum-based dual chemotherapy with pemetrexed, docetaxel, irinotecan, and paclitaxel. The odds ratio (OR) as well as 95% CI for different grades of toxicity following therapy in participants with each genotype were calculated using univariate regression analysis. Adjusted ORs were also evaluated using a multivariate regression model and adjustment was made with age, gender, regimen, and PS. We utilized three possible analysis approaches to understand how SLC variations affects toxicity levels at different grades. We made a comparison among patients with any toxicity grade, that is, grades 1 to 4 to no toxicity grade, that is, grade

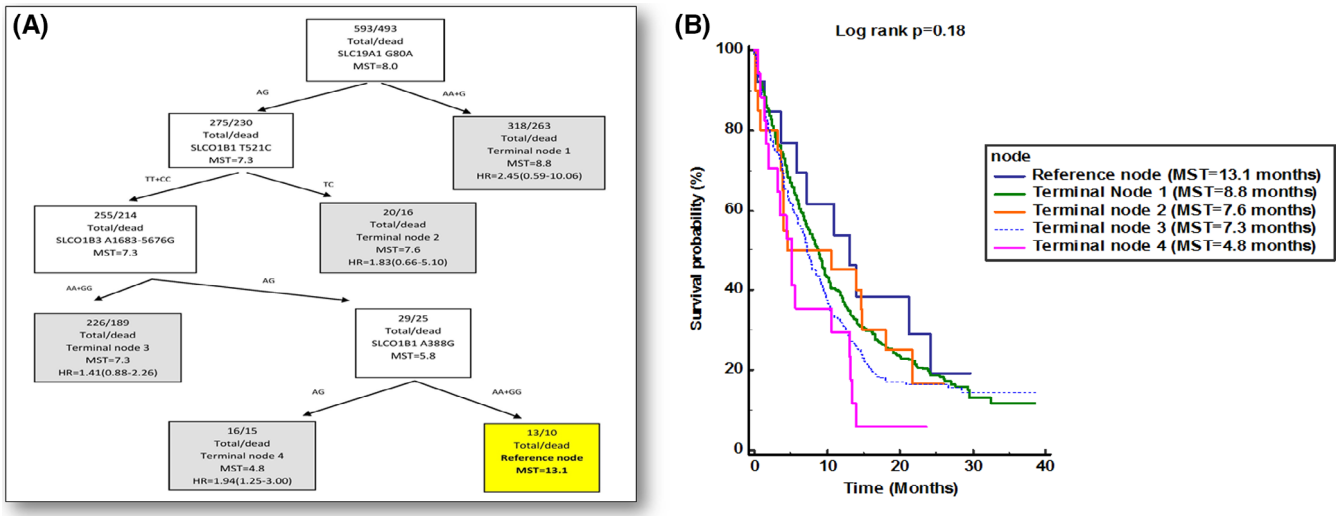


FIGURE 5 (A) Recursive partitioning strategy (STREE) elucidating relationship of *SLC* SNPs with overall survival in lung cancer patients. (B) Overall survival conferring to different nodes found by survival tree analysis. HR, hazard ratio; MST, median survival time.

TABLE 6 Haematological and nephrotoxicity with CTC grades in lung cancer patients

			Toxicity profiles		
Toxicity	**Adverse events**	***n***	**Grade 0, *n* (%)**	**Grade 1–2, *n* (%)**	**Grade 3–4, *n* (%)**
Haematological toxicity	Anaemia	406	102 (25.1)	274 (66.9)	30 (7.38)
	Leukopenia	404	349 (86.3)	45 (11.1)	10 (2.47)
	Thrombocytopenia	398	342 (85.9)	55 (13.8)	1 (0.25)
	Neutropenia	367	333 (90.7)	24 (6.53)	10 (2.72)
Nephrotoxicity	GFR	366	-	179 (48.9)	187 (51.09)

0, also, individuals with grades 2–4 to those with grade 0–1 toxicity were compared and in the final assessment grades 3–4 were compared with grades 0–2 to see if the *SLC* variations were linked to high toxicity.

Chemotherapy-related AEs were recorded for each chemotherapy cycle. In haematological toxicities, we evaluated anaemia, leukopenia, absolute neutropenia, and thrombocytopenia. The toxicity data of 406 people with anaemia could be obtained (Table 6). The table depicts that 25.1% of patients were categorized in grade 0 toxicity; and 74.8% individuals were categorized in grade 1–4 toxicity. As observed for *SLCO1B1* $T^{521}C$ polymorphism, 7.14% of individuals had single copy variant allele (*TC*). Table S8 displays that subjects with the carrier genotype (*TC*) had a reduced risk of developing anaemia (OR = 0.44, 95% CI = 0.20–0.96; $p = 0.04$) when compared to the patients with a wild type genotype (*TT*). For *SLC19A1* $G^{80}A$ polymorphism, on comparing individuals with grades 3–4 versus grade 0–2 toxicity, we detected that 26.6% of patients in grade 3–4 anaemia having carrier (*GA*) genotype showed a low odds of anaemia (OR = 0.35, 95% CI = 0.14–1.74; $p = 0.02$) as compared to the reference type genotype (*GG*). No association among the SLC polymorphisms other leukopenia and other adverse events due to chemotherapy as depicted in Table S7.

For analysis of absolute neutrophil count (ANC), we compared grades 2–4 vs. grades 0–1 toxicity events as shown in Table S9. For *SLCO1B1* $A^{388}G$ polymorphism, 16% (3) patients harboured the mutant genotype (*GG*). Table S9 depicts subjects with mutant genotype (*GG*) of $A^{388}G$ had a reduced odd of having neutropenia toxicity (OR = 0.42, 95% CI = 0.10–1.76, log-rank $p = 0.23$) when compared to the individuals with reference (*AA*) genotype. When we did the adjustment with various factors like age, gender, PS, and regimen, the decreased risk was consistent in mutant genotype (*GG*) as compared to reference-type genotype (*AA*) (AOR = 0.17, 95% CI = 0.02–1.04; $p = 0.05$).

Chemotherapy-induced thrombocytopenia is a potentially lethal consequence resulting in chemotherapy dosage delays, reductions, or discontinuance. Keeping this complication in view, we have analysed the impact of SLC variants in causing thrombocytopenia. As shown in Table 7, we compared grades 1–3 versus grade 0 toxicity. For thrombocytopenia, there were 367 patients with available toxicity data, of which 32.1% (18) patients with *SLCO1B1* $A^{388}G$ polymorphism harbouring a single copy of mutant allele (*AG*) was associated with a lower incidence of thrombocytopenia when compared to the patients carrying wild type alleles (*AA*) (OR = 0.41, 95% CI = 0.20–0.82; $p = 0.01$). After adjusting with multiple variables, the reduced risk in

TABLE 7 Association of *SLC* polymorphism with haematological toxicity and nephrotoxicity

Haematological toxicity						
Thrombocytopenia						
Genotype, rs1051266, SLC19A1 (G80A)	**Grade of toxicity 0, *n* (%), *N* = 342**	**Grade of toxicity 1–3, *n* (%), *N* = 56**	**OR[1] (95% CI)**	***p***	**AOR[2] (95% CI)**	***p***
GG	107 (31.2)	13 (23.21)	1.00 (reference)	-	1.00 (reference)	
GA	167 (48.8)	28 (50)	1.38 (0.68–2.78)	0.36	1.21 (0.52–2.82)	0.64
AA	68 (19.8)	15 (26.7)	1.81 (0.81–4.05)	0.14	1.65 (0.62–4.34)	0.30
GA + AA	235 (68.7)	43 (76.7)	1.50 (0.77–2.91)	0.22	1.32 (0.60–2.90)	0.48
Genotype, rs2306283, SLCO1B1 (A388G)	**Grade of toxicity 0, *n* (%), *N* = 342**	**Grade of toxicity 1–3, *n* (%), *N* = 56**	**OR[1] (95% CI)**	***p***	**AOR[2] (95% CI)**	***p***
AA	77 (22.5)	19 (33.9)	1.00 (Reference)		1.00 (Reference)	
AG	177 (51.7)	18 (32.1)	0.41 (0.20–0.82)	**0.01**	0.35 (0.14–0.85)	**0.02**
GG	88 (25.7)	19 (33.9)	0.87 (0.43–1.77)	0.71	0.75 (0.30–1.81)	0.52
AG + GG	265 (77.4)	37 (66.0)	0.56 (0.30–1.03)	0.06	0.50 (0.23–1.07)	0.07
Genotype, rs4149056, SLCO1B1 (T521C)	**Grade of toxicity 0, *n* (%) *N* = 342**	**Grade of toxicity 1–3, *n* (%), *N* = 56**	**OR[1] (95% CI)**	***p***	**AOR[2] (95% CI)**	***p***
TT	318 (92.9)	53 (94.6)	1.00 (Reference)	-	1.00 (Reference)	
TC	24 (7.01)	3 (5.35)	0.75 (0.21–2.57)	0.64	0.75 (0.16–3.57)	0.72
CC	0	0	-	-	-	-
Genotype, rs11045585, SLCO1B3, (1683-5676A > G)	**Grade of toxicity 0, *n* (%), *N* = 342**	**Grade of toxicity 1–3, *n* (%), *N* = 56**	**OR[1] (95% CI)**	***p***	**AOR[2] (95% CI)**	***p***
AA	300 (87.7)	49 (87.5)	1.00 (reference)		1.00 (reference)	
AG	41 (11.9)	7 (12.5)	1.04 (0.44–2.46)	0.91	1.07 (0.37–3.03)	0.89
GG	1 (0.29)	0	-	-	-	-
AG + GG	42 (12.2)	7 (12.5)	1.02 (0.43–2.4)	0.96	1.05 (0.37–2.96)	0.92
Nephrotoxicity						
Genotype, rs1051266, SLC19A1 (G80A)	**Grade of toxicity 1, *n* (%), *N* = 47**	**Grade of toxicity 2–5, *n* (%), *N* = 319**	**OR[1] (95% CI)**	***p***	**AOR[2] (95% CI)**	***p***
GG	15 (31.9)	101 (31.6)	1.00 (reference)	-	1.00 (reference)	
GA	26 (55.3)	153 (47.9)	0.87 (0.44–1.73)	0.69	0.45 (0.16–1.30)	0.14
AA	6 (12.7)	65 (20.3)	1.60 (0.59–4.35)	0.34	0.96 (0.25–3.63)	0.9
GA + AA	32 (68.0)	218 (68.3)	1.01 (0.52–1.95)	0.9	0.62 (0.24–1.62)	0.33
Genotype, rs2306283, SLCO1B1 (A388G)	**Grade of toxicity 1, *n* (%), *N* = 47**	**Grade of toxicity 2–5, *n* (%) *N* = 319**	**OR[1] (95% CI)**	***p***	**AOR[2] (95% CI)**	***p***
AA	15 (31.9)	81 (25.3)	1.00 (reference)		1.00 (reference)	
AG	17 (36.1)	157 (49.2)	1.71 (0.81–3.6)	0.15	1.41 (0.48–4.13)	0.52
GG	15 (31.9)	81 (25.3)	-	-	-	-
AG + GG	32 (68.0)	268 (84.0)	1.37 (0.70–2.67)	0.34	1.31 (0.49–3.47)	0.58
Genotype, rs4149056, SLCO1B1 (T521C)	**Grade of toxicity 1, *n* (%), *N* = 47**	**Grade of toxicity 2–5, *n* (%), *N* = 319**	**OR[1] (95% CI)**	***p***	**AOR[2] (95% CI)**	***p***
TT	43 (91.4)	297 (93.1)	1.00 (reference)	-	1.00 (reference)	
TC	4 (8.51)	22 (6.89)	0.79 (0.26–2.42)	0.68	0.53 (0.10–2.71)	0.44
CC	0	0	-	-	-	-
Genotype, rs11045585, SLCO1B3 (1683-5676A > G)	**Grade of toxicity 1, *n* (%), *N* = 47**	**Grade of toxicity 2–5, *n* (%), *N* = 319**	**OR[1] (95% CI)**	***p***	**AOR[2] (95% CI)**	***p***
AA	40 (85.1)	281 (88.0)	1.00 (Reference)		1.00 (Reference)	

(Continues)

TABLE 7 (Continued)

Genotype, rs11045585, SLCO1B3 (1683-5676A > G)	Grade of toxicity 1, *n* (%), *N* = 47	Grade of toxicity 2–5, *n* (%), *N* = 319	OR[1] (95% CI)	*p*	AOR[2] (95% CI)	*p*
AG	6 (12.7)	38 (11.9)	0.90 (0.35–2.26)	0.82	1.71 (0.36–8.1)	0.49
GG	1 (2.12)	0	-	-	-	-
AG + GG	7 (14.8)	38 (11.9)	0.77 (0.32–1.84)	0.56	1.08 (0.29–4.07)	0.90
Genotype, rs1051266, SLC19A1 (G80A)	**Grade of toxicity 1–2, *n* (%) *N* = 179**	**Grade of toxicity 3–5, *n* (%) *N* = 187**	**OR[1] (95% CI)**	***p***	**AOR[2] (95% CI)**	***p***
GG	59 (32.9)	57 (30.4)	1.00 (reference)		1.00 (reference)	
GA	90 (50.2)	89 (47.5)	1.02 (0.64–1.63)	0.92	1.13 (0.58–2.19)	0.70
AA	30 (16.7)	41 (21.9)	1.41 (0.78–2.56)	0.25	1.29 (0.53–3.14)	0.56
GA + AA	120 (67.0)	130 (69.5)	1.12 (0.72–1.74)	0.61	1.18 (0.64–2.18)	0.59
Genotype, rs2306283, SLCO1B1 (A388G)	**Grade of toxicity 1–2, *n* (%), *N* = 179**	**Grade of toxicity 3–5, *n* (%), *N* = 187**	**OR[1] (95% CI)**	***p***	**AOR[2] (95% CI)**	***p***
AA	46 (25.6)	50 (26.7)	1.00 (reference)		1.00 (reference)	
AG	84 (46.9)	90 (48.12)	0.83 (0.51–1.35)	0.46	1.41 (0.67–2.93)	0.35
GG	49 (27.3)	47 (25.1)	0.74 (0.42–1.30)	0.30	1.34 (0.60–2.97)	0.46
AG + GG	133 (74.3)	137 (73.2)	0.80 (0.51–1.26)	0.34	1.37 (0.70–2.70)	0.35
Genotype, rs4149056, SLCO1B1 (T521C)	**Grade of toxicity 1–2, *n* (%) *N* = 179**	**Grade of toxicity 3–5, *n* (%) *N* = 187**	**OR[1] (95% CI)**	***p***	**AOR[2] (95% CI)**	***p***
TT	168 (93.8)	172 (91.9)	1.00 (reference)	-	1.00 (reference)	-
TC	11 (6.14)	15 (8.02)	1.33 (0.59–2.98)	0.48	1.04 (0.33–3.24)	0.94
CC	0	-	-	-	-	-
Genotype, rs11045585, SLCO1B3 (1683-5676A > G)	**Grade of toxicity 1–2, *n* (%) *N* = 179**	**Grade of toxicity 3–5, *n* (%) *N* = 187**	**OR[1] (95% CI)**	***p***	**AOR[2] (95% CI)**	***p***
AA	147 (82.1)	174 (93.0)	1.00 (Reference)	-	1.00 (Reference)	-
AG	31 (17.31)	13 (6.95)	0.35 (0.17–0.72)	**0.002**	0.41 (0.16–1.07)	0.06
GG	1 (0.55)	0	-	-	-	-
AG + GG	32 (17.8)	13 (6.95)	0.34 (0.17–0.67)	**0.002**	0.38 (0.15–0.98)	**0.04**

Note: OR[1] (Crude odds ratio), 95% CI (95% confidence interval), AOR[2] (adjusted Odds ratio) data were calculated by unconditional logistic regression and adjusted for age, gender, performance status, and regimen. A *p* value <0.05 was considered statistically significant. Bold value represents that *p*-value of <0.05 is considered statistically significant.

TABLE 8 Gastrointestinal toxicity with CTC grades in lung cancer patients

Toxicity	Adverse events	*n*	Toxicity profiles	
			Grade 1, *n* (%)	Grade 2–4, *n* (%)
Gastrointestinal toxicity	Diarrhoea	133	61 (45.8)	72 (54.1)
	Constipation	101	31 (30.6)	70 (69.3)
	Anorexia	122	8 (6.55)	114 (93.4)
	Nausea-vomiting	116	53 (45.6)	63 (54.3)

heterozygous genotype (*AG*) for causing thrombocytopenia was consistent when compared to the reference-type genotype (*AA*) (AOR = 0.35, 95% CI = 0.14–0.85; $p = 0.02$).

Renal functions are assessed during chemotherapy as chemotherapeutic drugs are eliminated via the kidneys and nephrotoxic. We have evaluated chemotherapy-induced nephrotoxicity by comparing grades 3–5 versus grades 1–2 toxicity. As depicted in Table 6, data of 366 patients were available to assess the association between the SLC polymorphisms and nephrotoxicity. Patients having lung carcinoma with carrier (*AG*) genotype for $A^{1683\text{-}5676}G$ polymorphism showed a lower incidence of nephrotoxicity when compared to the patients with wild type genotype (*AA*) (OR = 0.35, 95% CI = 0.17–0.72; $p = 0.002$). When confounding characteristics were taken into account, the reduced risk of nephrotoxicity in heterozygous and mutant genotype (*AG* + *GG*) was consistent when compared to reference-type genotype (*AA*) (AOR = 0.38, 95% CI = 0.15–0.98; $p = 0.04$) as shown in Table 7.

TABLE 9 Association of *SLC* polymorphism with Gastrointestinal toxicity

Constipation						
Genotype, rs1051266, SLC19A1 (G80A)	**Grade of toxicity 1, *n* (%), *N* = 31**	**Grade of toxicity 2, *n* (%), *N* = 70**	**OR[1] (95% CI)**	***p***	**AOR[2] (95% CI)**	***p***
GG	4 (12.9)	25 (35.7)	1.00 (reference)		1.00 (reference)	
GA	20 (64.5)	27 (38.5)	0.21 (0.06–0.71)	**0.01**	0.17 (0.03–0.87)	**0.03**
AA	7 (22.5)	18 (25.7)	0.41 (0.10–1.61)	0.20	0.24 (0.03–1.85)	0.17
GA + AA	27 (87.0)	45 (64.2)	0.26 (0.08–0.84)	**0.02**	0.22 (0.05–0.97)	**0.04**
Genotype, rs2306283, SLCO1B1 (A388G)	**Grade of toxicity 1, *n* (%), *N* = 31**	**Grade of toxicity 2, *n* (%), *N* = 70**	**OR[1] (95% CI)**	***p***	**AOR[2] (95% CI)**	***p***
AA	8 (25.8)	15 (20.8)	1.00 (reference)		1.00 (reference)	
AG	16 (51.6)	36 (51.4)	1.2 (0.42–3.39)	0.73	3.87 (0.91–16.48)	**0.04**
GG	7 (22.5)	19 (27.1)	1.44 (0.42–4.90)	0.55	2.22 (0.51–9.64)	0.28
AG + GG	23 (74.1)	55 (78.5)	1.27 (0.47–3.42)	0.62	2.87 (0.82–10.04)	0.09
Genotype, rs4149056, SLCO1B1 (T521C)	**Grade of toxicity 1, *n* (%), *N* = 31**	**Grade of toxicity 2, *n* (%), *N* = 70**	**OR[1] (95% CI)**	***p***	**AOR[2] (95% CI)**	***p***
TT	29 (93.5)	67 (95.7)	1.00 (reference)		1.00 (reference)	
TC	2 (6.45)	3 (4.28)	0.64 (0.10–4.09)	0.64	1.15 (0.10–12.6)	0.9
CC	0	-	-	-	-	-
Genotype, rs11045585, SLCO1B3 (1683-5676A > G)	**Grade of toxicity 1, *n* (%), *N* = 31**	**Grade of toxicity 2, *n* (%), *N* = 70**	**OR[1] (95% CI)**	***p***	**AOR[2] (95% CI)**	***p***
AA	28 (90.3)	63 (87.5)	1.00 (reference)		1.00 (reference)	
AG	3 (9.67)	7 (10)	1.03 (0.24–4.30)	0.96	1.47 (0.26–8.26)	0.65
GG	0	-	-	-	-	-
Nausea-vomiting						
Genotype, rs1051266, SLC19A1 (G80A)	**Grade of toxicity 1, *n* (%), *N* = 53**	**Grade of toxicity 2–3, *n* (%), *N* = 63**	**OR[1] (95% CI)**	***p***	**AOR[2] (95% CI)**	***p***
GG	17 (32.0)	21 (33.3)	1.00 (reference)	-	1.00 (reference)	-
GA	25 (47.1)	30 (47.6)	0.97 (0.42–2.23)	0.94	0.97 (0.37–2.55)	0.95
AA	11 (20.7)	12 (19.0)	0.88 (0.31–2.49)	0.81	0.64 (0.19–2.12)	0.47
GA + AA	36 (67.9)	42 (66.6)	0.94 (0.43–2.05)	0.88	0.95 (0.38–2.32)	0.91
Genotype, rs2306283, SLCO1B1 (A388G)	**Grade of toxicity 1, *n* (%), *N* = 53**	**Grade of toxicity 2–3, *n* (%), *N* = 63**	**OR[1] (95% CI)**	***p***	**AOR[2] (95% CI)**	***p***
AA	14 (26.4)	15 (23.8)	1.00 (reference)		1.00 (reference)	
AG	23 (43.3)	37 (58.7)	1.50 (0.61–3.67)	0.37	1.39 (0.49–3.97)	0.53
GG	16 (30.1)	11 (17.4)	0.64 (0.22–1.84)	0.41	0.22 (0.05–1.03)	0.05
AG + GG	39 (73.5)	48 (76.1)	1.14 (0.49–2.66)	0.74	0.86 (0.32–2.28)	0.77
Genotype, rs4149056, SLCO1B1 (T521C)	**Grade of toxicity 1, *n* (%), *N* = 53**	**Grade of toxicity 2–3, *n* (%), *N* = 63**	**OR[1] (95% CI)**	***p***	**AOR[2] (95% CI)**	***p***
TT	49 (92.4)	60 (95.2)	1.00 (reference)		1.00 (reference)	
TC	4 (7.54)	3 (4.76)	0.61 (0.13–2.86)	0.53	0.79 (0.14–4.46)	0.79
CC	0	-	-	-	-	-
Genotype, rs11045585, SLCO1B3 (1683-5676A > G)	**Grade of toxicity 1, *n* (%), *N* = 53**	**Grade of toxicity 2–3, *n* (%), *N* = 63**	**OR[1] (95% CI)**	***p***	**AOR[2] (95% CI)**	***p***
AA	45 (84.9)	55 (87.3)	1.00 (reference)		1.00 (reference)	
AG	8 (15.0)	8 (12.6)	0.81 (0.28–2.35)	0.70	0.60 (0.17–2.10)	0.43
GG	0	-	-	-	-	-

Note: OR[1]: Crude odds ratio, 95% CI: 95% confidence interval, AOR[2]: Adjusted Odds ratio data were calculated by unconditional logistic regression and adjusted for age, gender, performance status, and regimen. A *p* value <0.05 was considered statistically significant. Bold value represents that *p*-value of <0.05 is considered statistically significant.

Additionally, we investigated adverse events in the gastrointestine by comparing patients with grade-1 to individuals with grade 2 toxicity. We evaluated diarrhoea, anorexia, constipation, and nausea-vomiting (Table 8). Lung cancer patient with the carrier genotype (*AG*) for *SLC19A1* $A^{388}G$ polymorphism has a low risk of having constipation as compared to the patients harbouring reference-type genotype (*AA*) (AOR = 0.17, 95% CI = 0.03–0.87, $p = 0.03$) as shown in Table 9. The reduced risk was consistent when we compared the combined heterozygous and mutant (*AG* + *GG*) genotype with the wild type (*AA*) genotype (AOR = 0.22, 95% CI = 0.05–0.97; $p = 0.04$). For *SLCO1B1* $A^{388}G$, lung cancer patients with carrier genotype (AG) showed 3.87-fold increased odds of having constipation (AOR = 3.87, 95% CI = 0.91–16.48; $p = 0.04$) as compared to the patients with reference type genotype (*AA*).

In nausea or vomiting, the comparison was made between grades 2–3 vs. grade 1 toxicity. Among 116 patients, 54.3% had grade 2–3 nausea or vomiting. For *SLCO1B1* $A^{388}G$ variation, a lower incidence of nausea/vomiting was observed in patients with variant genotype (*GG*) as compared to patients with reference-type genotype (*AA*) (AOR = 0.22; 95% CI = 0.05–1.03, $p = 0.05$) (Table 9). No correlation was found among the *SLC* polymorphisms, other gastrointestinal toxicities (Table S10).

4 | DISCUSSION

SLC transporters mediate the flow of chemotherapeutic drugs across biological membranes in various organs, and the various single nucleotide polymorphisms alter this inflow of drugs. Genetic polymorphisms can cause individual variations in the metabolism and pharmacotherapy of these transporters, which may create population-specific disparities in drug transport. In a population, heterogeneity in the pharmacokinetic profile of chemotherapeutic drugs results from multiple interactions between genetic, environmental, and physiological factors. In the past, many theories have been put forth to explain the observed discrepancies regarding several therapeutic drugs that may increase or inhibit the function or expression of solute carrier protein, thus altering the phenotypic activity of the SLC transporters. Specified the effect of such variations on SLC transporter activity, it is essential to look into their role in lung carcinoma, as their expression levels could control the extent and duration of chemotherapeutic drug inflow, affecting patients' responses. Hence, understanding and minimizing inter-individual disparity in drug receptiveness, toxicity, and its critical role in identifying the efficient treatment option for lung carcinoma patients is therefore imperative and clinically meaningful.

We have attempted to investigate the significance of *SLC* polymorphisms in affecting prognosis and its correlation with adverse events in lung carcinoma patients undergoing platinum-based chemotherapeutic treatment. The statistical analysis in North Indian lung carcinoma patients points that variant genotype (*AA*) of *SLC19A1* $G^{80}A$ variation is correlated with increased survival. Our results align with Collin et al. who found no correlation of $G^{80}A$ polymorphism in recessive, dominant, or additive models in prostate cancer.[23] A study by Liu et al. did not find any significant association for *RFC*v$G^{80}A$ polymorphism with lung cancer susceptibility.[24] However, we can explain the increased prognostic trend in lung carcinoma patients with variant genotype (*AA*) of *SLC19A1*$G^{80}A$ polymorphism. The *A* allele of $G^{80}A$ might have a greater influx propensity for the substrates, resulting in improved bioavailability and chemotherapeutic drug intake,[25] thereby leading to better survival outcomes in the individuals with the mutant genotype (*AA*). On the contrary to our outcomes, Jabeen et al. has reported increased survival in the patients with reference-type genotype (*GG*) in comparison to the mutant genotype (*AA*) of *SLC19A1* $G^{80}A$ polymorphism.[26] These incongruent results could be attributed to the differences in clinical characteristics and ethnicity of patients.

In patients with *SLCO1B1*$A^{388}G$ polymorphism, our results demonstrated that the patients with variant genotype (*GG*) had the same median survival time as the wild-type genotype (*AA*). Our results are in concordance with *Lee* et al. who put forth that *SLCO1B1* $A^{388}G$ is associated with an unaltered transport function, so there would be no modification in the drug influx, which could be the likely cause for no difference in median survival time between mutant (*GG*) and wild type genotypes (*AA*).[27] On the contrary, Sissung et al. stated that $A^{388}G$ polymorphism is correlated with the altered SLCO1B1 transport function, leading to modifications in the transmembrane domain structure.[28] While Liutkevicie et al. put forth that the *G* allele of $A^{388}G$ is associated with the increased SLCO1B1 function, proposing increased functional activity of the transporter.[29] *SLCO1B1* polymorphism has been reported by numerous researchers, who have linked the $A^{388}G$ mutation to an increase, decrease, or no effect on the transporter activity.[30]

Our results for *SLCO1B1* $T^{521}C$ polymorphism showed reduced survival in patients with variant genotype (*CC*) as compared to both homozygous (*TT*) and heterozygous genotype (*TC*), though the substantial significance between $T^{521}C$ polymorphism and overall survival was not achieved. Studies in the past have shown that the *C* allele of $T^{521}C$ is correlated with decreased activity of SLCO1B1.[31] Due to this reduced activity of the transporter, patients harbouring the variant allele (*C*) of $T^{521}C$ polymorphism may have reduced inflow of drugs, which can explain the lower overall survival in lung carcinoma patients. On the contrary, Feng et al. has shown better survival in gastric carcinoma patients having mutant (*CC*) and heterozygous (*TC*) genotype.[32]

We have also assessed the impact of SLC polymorphism on overall survival based on histology. Our findings demonstrated that ADCC patients with the mutant genotype (*AA*) of the *SLC19A1* $G^{80}A$ polymorphism had a better prognosis ($p = 0.04$). Mechanistically, the RFC1 protein is encoded by the main influx transporter *SLC19A1*. It mediates folate uptake in the cell and transports antifolate chemotherapeutic agents. RFC1 is substantially expressed in lung cancer patients.[33] It has been reported that in adenocarcinoma cells, RFC1 proteins are overexpressed.[34]

Furthermore, studies have linked downregulation of the RFC1 protein to impaired drug transport, developing resistance.[26,35] The overexpression of RFC1 in ADCC patients explains our results with better survival in the mutant genotype (*AA*) of *SLC19A1* $G^{80}A$

polymorphism. Even though *SLC19A1* is the most common antifolate uptake transporter, our outcomes did not show any relationship of $G^{80}A$ polymorphism in association with survival of the patients undergoing pemetrexed and cisplatin/carboplatin treatment. Our results align with the various studies on NSCLC.[36–38] Our findings also revealed that patients with SCLC who had the mutant genotype (*AA*) of *SLC19A1* $G^{80}A$ polymorphism had a better prognosis ($p = 0.04$). To the best of our knowledge, none of the previous studies have looked at the role of the *SLC19A* $G^{80}A$ polymorphism in SCLC, as most of the research has focused on its impact on NSCLC. Based on our findings, we believe that the *SLC19A1* $G^{80}A$ polymorphism may improve overall survival in SCLC patients.

As per our results, patients who were given docetaxel along with cisplatin/carboplatin and were heterozygous carriers (*AG*) for the *SLCO1B3* ($A^{1683\text{-}5676}G$) polymorphism had a significantly shorter survival time as compared to the wild type (*AA*) genotype. No supporting studies were found because the pharmacogenetics of *SLCO1B3* is little understood, and previous in vivo and in vitro studies gave inconsistent results regarding the functional effects of the *SLCO1B3* polymorphisms. Chew et al. revealed that the *SLCO1B3* ($A^{1683\text{-}5676}G$) variant allele is related to altered docetaxel disposition.[39] However, our investigation did not find any patients with mutant genotype (*GG*) for *SLCO1B3* ($A^{1683\text{-}5676}G$) polymorphism administered docetaxel and cisplatin/carboplatin therapy, and we could not assess the role of its variant genotype on the survival of lung cancer patients. Chew et al. have mentioned that inter-individual variability in docetaxel disposition is influenced by *SLCO1B3* pharmacogenetics.[40] However, more research is needed to investigate the functional characterization of *SLCO1B3*-associated docetaxel transport, which may help researchers better understand the molecular basis for docetaxel disposal disposition.

SLC polymorphisms and its association with toxicity among platinum-based chemotherapeutic-treated lung carcinoma patients. Our results showed that subjects with at least one mutant allele (*AG*) in *SLCO1B1* $A^{388}G$ had a significantly lower risk of developing thrombocytopenia. Leiri et al. have reported that the presence of the *G* allele at the codon 388 can modulate the activity of SLCO1B1.[41] The systemic concentration of the chemotherapeutic drug is strongly dependent on the activity of SLCO1B1. An increase in the SLCO1B1 activity and subsequent increase in the hepatic clearance of the chemotherapeutic drug may lead to reduced systemic exposure and hence less toxicity.[42] This may explain the reduced odds of having thrombocytopenia in the heterozygous (*AG*) genotype of $A^{388}G$, as the occurrence of chemotherapy-induced thrombocytopenia varies according to the treatment used. Our findings showed a significant association of *SLC19A1* $G^{80}A$ polymorphism with a reduced odd of having anaemia. The carrier genotype (*GA*) of $G^{80}A$ polymorphism showed a substantial protective effect from haematological toxicity-anaemia. Generally, folate is required for RBC production and is a targeted via chemotherapeutic agents.[43] Such drugs impede the folate metabolism, then a deficiency of folate causes DNA synthesis abnormalities, which leads to genomic instability leading to the hypothesis that a lack of folic acid inhibits RBC maturation, resulting in anaemia. However, our outcomes suggest that the carrier genotype (*GA*) of $G^{80}A$ variation is correlated with a reduced odd of having anaemia in lung cancer patients.

Our results showed a low risk of having nephrotoxicity in patients having carrier genotype (*AG*) on comparing grade 3–5 with grade 1–2. Mechanistically, the kidney is the main organ involved in eliminating platinum-based chemotherapeutic drugs.[44] Many drugs, particularly those with a propensity for nephrotoxicity, are eliminated in the urine by active tubular secretion in addition to glomerular filtration.[45] In vitro studies have shown that *OATP1B1* and *OATP1B3* transport cisplatin and carboplatin, and the toxicity of these drugs in human tumour cells was enhanced with increased *OATP1B3* mRNA expression.[4] Hilgendorf et al., have reported low mRNA expression of *SLCO1B3* in the kidney.[46] Also, it has been reported that the $A^{1683\text{-}5676}G$ variant may lead to the reduced activity of *SLCO1B3* that might vary the therapeutic efficacy.[18] These findings explain that *SLCO1B3* has reduced expression in the kidney, leading to reduced drug uptake and thereby showing a reduced risk of causing nephrotoxicity.

Gastrointestinal (GI) toxicity is a typical adverse effect of chemotherapy treatments in lung carcinoma patients. We observed that carrier genotype of *SLC19A1* $G^{80}A$ and *SLCO1B1* $A^{388}G$ polymorphisms were associated with protective effects from gastrointestinal toxicity. Some studies state that *SLCO1B1* polymorphisms are correlated with the increased risk of gastrointestinal toxicity in acute lymphoblastic leukaemia.[47] In the case of *SLC19A1*, *Lima* et al. found that *G* carriers of $G^{80}A$ polymorphism were linked to chemotherapy-associated gastrointestinal toxicity,[10] whereas various other studies did not find any correlations with the toxicity.[48,49] Therefore, these conflicting data of *SLC* polymorphism and chemotherapy-related gastrointestinal toxicity need to be clarified.

It is essential to mention that the present study has certain limitations since carcinoma is often detected at a late stage; our study only includes patients with advanced stage III and IV lung cancer. This could be because of community alertness or its similarity to other diseases like tuberculosis. Many patients who receive chemotherapy at an advanced stage III and IV of cancer cannot achieve the treatment's endpoint, weakening the research's scope on chemotherapy response. The majority of the people who visited PGIMER clinic for the treatment were from rural areas in Northern India. As it was difficult for them to travel from their region, many patients in remote areas did not regularly visit the hospital.

Due to the shortage of clinical data could not conduct progression-free survival (PFS) analysis on the present study group. On the other hand, our study has several advantages, including enrolling many lung carcinoma patients. Second, patients on platinum-based doublet chemotherapeutic regimens were included, and everyone was treated at the PGIMER hospital only. Third, patients were enrolled, and clinical parameter data was obtained separately, regardless of the information of SLC polymorphism.

5 | WHAT IS NEW AND CONCLUSION

The current research depicts the significance of four SLC polymorphisms in the survival of North Indian lung carcinoma patients who

underwent platinum-based doublet chemotherapeutic regimens. We used genotyping analysis to see if *SLC* polymorphism causes differences in patient clinical outcomes. Our findings imply that SLC polymorphisms are essential in regulating SLC gene expression, altering the transporter activity, and affecting overall survival in lung cancer patients. To the best of our knowledge, this is the first study to evaluate the role of *SLC* polymorphism in North Indian lung cancer patients. According to our findings, genotyping for the *SLC* polymorphism could be a valuable tool for predicting which lung cancer patients will benefit the most from platinum-based chemotherapy. However, a review of previous papers reveals a plethora of contradictory findings of the activity of these transporters. Nonetheless, more research into the role of SLC polymorphisms is needed before a meaningful conclusion can be reached.

Our study assesses the survival differences in lung cancer patients with *SLC* polymorphisms. Surprisingly, our findings demonstrate a link between *SLC* polymorphism and a lower risk of toxicity in northern Indian patients with lung carcinoma. It is well understood that individual toxicities are determined by the platinum-based chemotherapy regimen used.

AUTHOR CONTRIBUTIONS

Parul Sharma performed molecular as well as statistical analysis, collected information, and also wrote the paper; Siddharth Sharma helped molecular analyses as well as studied the final version draft of the research paper and also represented as the corresponding author; whereas Navneet Singh kindly provided with the patients' blood samples besides the clinical data from PGIMER, Chandigarh.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All the data generated from this research is presented within the manuscript. Further queries can be directed to the corresponding author.

ETHICS STATEMENT

The current study followed the Declaration of Helsinki as well as Good Clinical Practice standards. The PGIMER's institutional review board in Chandigarh, India, gave their approval to the study (Approval No. IEC-04/2018-884). Written informed consent was duly signed by the patients.

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REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin*. 2020;70:7-30.
2. Lima A, Seabra V, Martins S, Coelho A, Araújo A, Medeiros R. Thymidylate synthase polymorphisms are associated with the therapeutic outcome of advanced non-small cell lung cancer patients treated with platinum-based chemotherapy. *Mol Biol Rep*. 2014;41(5):3349-3357.
3. Choi JR, Kim JO, Kang DR, et al. Genetic variations of drug transporters can influence on drug response in patients treated with docetaxel chemotherapy. *Cancer Res Treat: Off J Korean Cancer Assoc*. 2015;47(3):509-517.
4. Lancaster CS, Sprowl JA, Walker AL, Hu S, Gibson AA, Sparreboom A. Modulation of OATP1Btype transporter function alters cellular uptake and disposition of platinum chemotherapeutics. *Mol Cancer Ther*. 2013;12(8):1537-1544.
5. Lin L, Yee SW, Kim RB, Giacomini KM. SLC transporters as therapeutic targets: emerging opportunities. *Nat Rev Drug Discov*. 2015;14(8):543-560.
6. Sutherland R, Meeson A, Lowes S. Solute transporters and malignancy: establishing the role of uptake transporters in breast cancer and breast cancer metastasis. *Cancer Metas Rev*. 2020;39(3):919-932.
7. Abdel-Haleem AM, el-Zeiry MI, Mahran LG, et al. Expression of RFC/-SLC19A1 is associated with tumor type in bladder cancer patients. *PLoS One*. 2011;6(7):e21820.
8. Chango A, Emery-Fillon N, De Courcy GP, et al. A polymorphism (80G->A) in the reduced folate carrier gene and its associations with folate status and homocysteinemia. *Mol Genet Metab*. 2000;70(4):310-315.
9. Wang L, Chen W, Wang J, et al. Reduced folate carrier gene G80A polymorphism is associated with an increased risk of gastroesophageal cancers in a Chinese population. *Eur J Cancer*. 2006;42:3206-3211.
10. Lima A, Bernardes M, Sousa H, et al. SLC19A1 80G allele as a biomarker of methotrexate-related gastrointestinal toxicity in Portuguese rheumatoid arthritis patients. *Pharmacogenomics*. 2014;15(6):807-820.
11. Adjei AA, Salavaggione OE, Mandrekar SJ, et al. Correlation between polymorphisms of the reduced folate carrier gene (SLC19A1) and survival after pemetrexed-based therapy in non-small cell lung cancer: a north central cancer treatment group-based exploratory study. *J Thorac Oncol*. 2010;5(9):1346-1353.
12. Roth M, Obaidat A, Hagenbuch B. OATPs, OATs and OCTs: the organic anion and cation transporters of the SLCO and SLC22A gene superfamilies. *Br J Pharmacol*. 2012;165(5):1260-1287.
13. Tirona RG, Leake BF, Merino G, Kim RB. Polymorphisms in OATP-C. identification of multiple allelic variants associated with altered transport activity among European and African-Americans. *J Biol Chem*. 2001;276(38):35669-35675.
14. Iwai M, Suzuki H, Ieiri I, Otsubo K, Sugiyama Y. Functional analysis of single nucleotide polymorphisms of hepatic organic anion transporter OATP1B1 (OATP-C). *Pharmacogenetics*. 2004;14(11):749-757.
15. Shitara Y et al. Clinical significance of organic anion transporting polypeptides (OATPs) in drug disposition: their roles inhepatic clearance and intestinal absorption. *Biopharm Drug Dispos*. 2013;34:45-78. doi: 10.1002/bdd.1823
16. Nozawa T, Nakajima M, Tamai I, et al. Genetic polymorphisms of human organic anion transporters OATP-C (SLC21A6) and OATP-B (SLC21A9): allele frequencies in the Japanese population and functional analysis. *J Pharmacol Exp Ther*. 2002;302(2):804-813.
17. Kiyotani K, Mushiroda T, Kubo M, Zembutsu H, Sugiyama Y, Nakamura Y. Association of genetic polymorphisms in SLCO1B3 and ABCC2 with docetaxel-induced leukopenia. *Cancer Sci*. 2008;99(5):967-972.
18. Yamada A, Maeda K, Kiyotani K, Mushiroda T, Nakamura Y, Sugiyama Y. Kinetic interpretation of the importance of OATP1B3 and MRP2 in docetaxel-induced hematopoietic toxicity. *CPT Pharmacomet Syst Pharmacol*. 2014;3(7):1-10.
19. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009;45(2):228-247.
20. Trotti A, Colevas AD, Setser A, et al. CTCAE v3.0: development of a comprehensive grading system for the adverse effects of cancer treatment. *Semin Radiat Oncol*. 2003;13(3):176-181.

21. Liu J, Long J, Zhang S, FangX LY. Polymorphic variants of SLCO1B1 in neonatal hyperbilirubinemia in China. *Ital J Pediatr*. 2013;39(1):1-5.
22. Nagy A, Szalai R, Magyari L, Bene J, Toth K, Melegh B. Extreme differences in SLCO1B3 functional polymorphisms in Roma and Hungarian populations. *Environ Toxicol Pharmacol*. 2015;39(3):1246-1251.
23. Collin SM, Metcalfe C, Zuccolo L, et al. Association of folate-pathway gene polymorphisms with the risk of prostate cancer: a population-based nested case-control study, systematic review, and meta-analysis. *Cancer Epidemiol Biomarkers Prev*. 2009;18(9):2528-2539.
24. Liu H, Jin G, Wang H, WuW LY, Qian J, Sun W. Association of polymorphisms in one-carbon metabolizing genes and lung cancer risk: a case-control study in Chinese population. *Lung Cancer*. 2008;61(1):21-29.
25. Lima A, BernardesM AR, Monteiro J, SousaH MR, Seabra V. SLC19A1, SLC46A1 and SLCO1B1 polymorphisms as predictors of methotrexate-related toxicity in Portuguese rheumatoid arthritis patients. *Toxicol Sci*. 2014;142(1):196-209.
26. Jabeen S, Holmboe L, Alnæs GIG, Andersen AM, Hall KS, Kristensen VN. Impact of genetic variants of RFC1, DHFR and MTHFR in osteosarcoma patients treated with high-dose methotrexate. *Pharm J*. 2015;15(5):385-390.
27. Lee HH, Ho RH. Interindividual and interethnic variability in drug disposition: polymorphisms in organic anion transporting polypeptide 1B1 (OATP1B1; SLCO1B1). *Br J Clin Pharmacol*. 2017;83:1176-1184. doi:10.1111/bcp.13207
28. Sissung TM, Troutman SM, Campbell TJ, et al. Transporter pharmacogenetics: transporter polymorphisms affect normal physiology, diseases, and pharmacotherapy. *Discov Med*. 2012;13:19-34.
29. Liutkeviciene R, Vilkeviciute A, Slavinskaite A, Petrauskaite A, Tatarunas V, Kriauciuniene L. Evaluation of serum SLCO1B1 levels and genetic variants of SLCO1B1 rs4149056 and rs2306283 in patients with early and exudative age-related macular degeneration. *Gene*. 2018;676:139-145.
30. Niemi M, Pasanen MK, Neuvonen P. Organic anion transporting polypeptide 1B1: a genetically polymorphic transporter of major importance for hepatic drug uptake. *Pharmacol Rev*. 2011;63:157-181. doi:10.1124/pr.110.002857
31. Gong IY, Kim RB. Impact of genetic variation in OATP transporters to drug disposition and response. *Drug Metab Pharmacokinet*. 2013;28:4-18.
32. Feng W, Liu X, Zhao X, et al. Influence of SLCO1B1 in gastric cancer patients treated with EOF chemotherapy. *Oncol Lett*. 2018;16(4):4489-4497.
33. Li Y, Gan S, Ren L, et al. Multifaceted regulation and functions of replication factor C family in human cancers. *Am J Cancer Res*. 2018;8(8):1343-1355.
34. Nunez MI, Behrens C, Woods DM, et al. High expression of folate receptor alpha in lung cancer correlates with adenocarcinoma histology and mutation. *J Thorac Oncol*. 2012;7(5):833-840.
35. Gorlick R, Goker E, Trippett T, et al. Defective transport is a common mechanism of acquired methotrexate resistance in acute lymphocytic leukemia and is associated with decreased reduced folate carrier expression. *Blood*. 1997;89:1013-1018.
36. Tiseo M, Giovannetti E, Tibaldi C, et al. Pharmacogenetic study of patients with advanced non-small cell lung cancer (NSCLC) treated with second-line pemetrexed or pemetrexed-carboplatin. *Lung Cancer*. 2012;78:92-99.
37. Smit EF, Burgers SA, Biesma B, et al. Randomized phase II and pharmacogenetic study of pemetrexed compared with pemetrexed plus carboplatin in pretreated patients with advanced non-small-cell lung cancer. *J Clin Oncol*. 2009;27:2038-2045.
38. Goricar K, Kovac V, Dolzan V. Polymorphisms in folate pathway and pemetrexed treatment outcome in patients with malignant pleural mesothelioma. *Radiol Oncol*. 2014;48(2):163-172.
39. Chew SC, Sandanaraj E, Singh O, et al. Influence of SLCO1B3 haplotype-tag SNPs on docetaxel disposition in Chinese nasopharyngeal cancer patients. *Br J Clin Pharmacol*. 2012;73(4):606-618.
40. Chew SC, Singh O, Chen X, et al. The effects of CYP3A4, CYP3A5, ABCB1, ABCC2, ABCG2 and SLCO1B3 single nucleotide polymorphisms on the pharmacokinetics and pharmacodynamics of docetaxel in nasopharyngeal carcinoma patients. *Cancer Chemother Pharmacol*. 2011;67:1471-1478.
41. Ieiri I, Higuchi S, Sugiyama Y. Genetic polymorphisms of uptake (OATP1B1, 1B3) and efflux (MRP2, BCRP) transporters: implications for inter-individual differences in the pharmacokinetics and pharmacodynamics of statins and other clinically relevant drugs. *Expert Opin Drug Metab Toxicol*. 2009;5(7):703-729.
42. Bins S, Lenting A, El Bouazzaoui S, et al. Polymorphisms in SLCO1B1 and UGT1A1 are associated with sorafenib-induced toxicity. *Pharmacogenomics*. 2016;17(14):1483-1490.
43. Robien K, Boynton A, Ulrich CM. Pharmacogenetics of folate-related drug targets in cancer treatment. *Future Med*. 2005;6:673-689.
44. Burger H, Loos WJ, Eechoute K, Verweij J, Mathijssen RH, Wiemer EA. Drug transporters of platinum-based anticancer agents and their clinical significance. *Drug Resist Update*. 2011;14(1):22-34.
45. Ho RH, Kim RB. Transporters and drug therapy: implications for drug disposition and disease. *Clin Pharm Ther*. 2005;78(3):260-277.
46. Hilgendorf C, Ahlin G, Seithel A, Artursson P, Ungell AL, Karlsson J. Expression of thirty-six drug transporter genes in human intestine, liver, kidney, and organotypic cell lines. *Drug Metab Dispos*. 2007;35(8):1333-1340.
47. Treviño LR, Shimasaki N, Yang W, et al. Germline genetic variation in an organic anion transporter polypeptide associated with methotrexate pharmacokinetics and clinical effects. *J Clin Oncol*. 2009;27(35):5972-5978.
48. Plaza-Plaza JC, Aguilera M, Cañadas-Garre M, et al. Pharmacogenetic polymorphisms contributing to toxicity induced by methotrexate in the southern Spanish population with rheumatoid arthritis. *Omics: J Integr Biol*. 2012;16(11):589-595.
49. Owen SA, Hider SL, Martin P, Bruce IN, Barton A, Thomson W. Genetic polymorphisms in key methotrexate pathway genes are associated with response to treatment in rheumatoid arthritis patients. *Pharmacogenomics J*. 2013;13(3):227-234.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Review

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Pharmacogenomics

ATP binding cassette transporters and cancer: revisiting their controversial role

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The expression of ATP-binding cassette transporter (ABC transporters) has been reported in various tissues such as the lung, liver, kidney, brain and intestine. These proteins account for the efflux of different compounds and metabolites across the membrane, thus decreasing the concentration of the toxic compounds. ABC transporter genes play a vital role in the development of multidrug resistance, which is the main obstacle that hinders the success of chemotherapy. Preclinical and clinical trials have investigated the probability of overcoming drug-associated resistance and substantial toxicities. The focus has been put on several strategies to overcome multidrug resistance. These strategies include the development of modulators that can modulate ABC transporters. This knowledge can be translated for clinical oncology treatment in the future.

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The transport of various molecules across the lipid membrane is a vital function of a living organism. Specific transporters mediate this process, and the most prominent transporter gene family in this is ATP binding cassette (ABC) family. These transporters bind with ATP and use this energy to translocate different molecules such as amino acids, sugars, metal ions, proteins, peptides and hydrophobic compounds across the plasma membrane [1]. ABC transporters in humans are localized on the plasma membrane and export various drugs, conjugates of a drug and metabolites out of the cells [2]. The superfamily of ABC transporter encodes proteins that are bifurcated on the basis of the organization of nucleotide-binding domain, amino acid sequence, phylogenetic analysis and structural similarity of the gene. These transporters remain classified into seven subfamilies (ABCA–ABCG) so far, 49 subtypes, with a pseudogene of ABC transporters have been recognized in humans [3]. Some of the ABC transporters play a vital role in various cellular processes, such as maintaining homeostasis, cell division, lipid trafficking and antigen processing [4].

ABC transporters are expressed in different tissues such as the intestine, lung, liver, brain and kidney, and play a vital role in the absorption, distribution and drug excretion [5]. The presence of ABC transporters in organs and tissues can reduce the level of some drugs, thus diminishing or abolishing their efficiency [6]. Overexpression of some ABC transporters in different tissues has been found to generate multidrug resistance (MDR). MDR is the principal mechanism by which numerous cancers get resistant to chemotherapeutic drugs. The various mechanisms that cause MDR include altered drug targets, reduced drug uptake, increased drug efflux by the drug transporters, anticancerous drug sequestration in lysosomes [7]. The primary MDR mechanism is increased efflux rate of anticancer drug from the cancerous cells by ATP transporters. Members of the ABC family, such as ABCB, ABCC, and ABCG, are the leading players in the MDR development [8].

MDR is one of the leading clinical obstacles in cancer chemotherapy and overcoming this hurdle will be of great help in providing treatment for various infectious diseases [9]. Most patients develop resistance to various drugs in the hospital, which causes healthcare costs to skyrocket. The center for disease control and prevention reports that nearly 2.8 million people are identified with severe infectious diseases each year due to drug resistance and around 35,000 patients die due to treatment failure [10]. Some life-threatening illnesses caused by herpes simplex virus,

influenza and HIV are resistant and hard to treat. Like this, cancer cells are initially sensitive to even particular anticancer drugs and later become resistant to various anticancer drugs [11]. Even though the drug combination might effectively direct cancerous cells to apoptosis, this strategy leads to undesired toxicity [12].

ABC transporter expression has not only been concerned with cancer cell resistance to anticancer therapy, but it also alters the disposition of the chemotherapeutic drugs and their associated toxicity. With the understanding of drug efflux in MDR, the development of various inhibitors to the ABC transporters will act as an adjunct therapy. This review article presents an orderly overview of the functional mechanism and structure of ABC transporters. The article also highlights various ABC proteins involved in drug absorption, distribution metabolism, toxicity and resistance. The article emphasizes the importance of several substrates and inhibitors of the transporters and some clinical trials concerning inhibition of ABC transporters. Actual cases studies and the various advanced technologies have been discussed to counter MDR The current understanding of MDR in the cancer cells in this review article will benefit the clinical therapies in the future.

ABC transporters

ABC transporters: an overview

ABC transporters are among the main ATP-driven pumps that derive energy by ATP hydrolysis, thereby helping ABC pumps to translocate substrates across the cells against the electrochemical gradient [13]. ABC proteins are the most prominent family of transmembrane transporters. These proteins are known to have a wide diversity of expression. As ABC transporters depend on ATP to efflux proteins, the hydrolysis of ATP is vital to facilitate the translocation of different substrates against the concentration gradient from the intracellular region to the extracellular region [14]. Members of this large transporter family play a crucial role in numerous cellular processes. One example of this is the role of ABC transporters in multidrug resistance in cancer cells which will be discussed later in this review [15]. The role of ABC proteins is not only restricted to the transport of different substrates, but it also transports a variety of hydrophobic compounds and peptides across the membrane [16].

Structure & mechanism of action

The structure of functional ABC protein consists of the association of four characteristic domains, in other words, two transmembrane domains (TMD) and two nucleotide-binding domains (NBD). The NBDs are much conserved all through the different ABC transporters. NBDs are positioned in the cytoplasm and are involved in energy transfer for transporting the substrates across the membrane. The TMDs contain six transmembrane α helices and provide substrate specificity. ABC transporters can either be full transporters (TMD1-NBD1-TMD2-NBD2) or half transporters (TMD-NBD) [17]. The half transporter should form a homodimer or heterodimer to be a functional transporter. The motifs identified in NBD are walker A and walker B, A-loop, D-loop, Q-loop, H-loop and consensus sequence LSSGQ [18]. NBD has two subdomains, in other words, a catalytic core and α helical domain. The former contains Walker A motif (conserved glycine motif) and Walker B domain (involved in binding and hydrolysis of ATP). The α helical domain contains the LSGGQ motif (C-loop), also known as the ABC signature motif involved in nucleotide binding. Upon dimerization via interacting with the D loop, NBDs form the site for ATP binding and hydrolysis. One subunit of Walker A and B motifs and the other subunit of Q-loop form the ATP binding site to favor binding and hydrolysis of two ATP molecules. NBDs are separated in the absence of nucleotides but come close together upon ATP binding; however, hydrolysis leads to the disruption of the dimer interface. NBDs are attached to the intracellular region of TMDs, forming a close and functional connection between the two domains. The NBD binds to ATP and, the ATP hydrolysis provides energy for the transport of a substrate. The specific NBD-TMD interaction provides the transmission gear for the conformational changes caused by substrate binding and ATP hydrolysis [19]. Figure 1 illustrates the detailed mechanism of the ABC transporters.

Localization, expression & physiological role of ABC transporters

The human genome carries seven subfamilies of ABC transporters, in other words, ABCA and ABCG [20]. The following section provides a general introduction to the expression/functions of ABC subfamilies. The expression patterns of ABC transporters have revealed that ABC transporters are widely expressed in the small intestine, liver, heart, brain and kidney. These transporters are also found to be expressed in organs that are involved in barrier function (trachea, lung), secretory function (adrenal gland) and tropic function (uterus and placenta) [21]. Table 1 illustrates the family, localization and physiological function of ABC transporters. The role of ABC transporters has

Table 1. Chromosome localization and associated physiological functions of the members of ABC transporters in different tissues/organs.

Gene	Alias	Number of genes	Subfamily	Chromosome location	Function
ABCA	ABC1	12	*ABCA1*	9q31.1	Efflux of cholesterol
			ABCA2	9q34.3	Drug resistance
			ABCA3	16p13.3	Surfactant secretion
			ABCA4	1p21.3	Efflux
			ABCA5	17q24.3	Cholesterol translocation
			ABCA6	17q24.3	
			ABCA7	19p13.3	Phospholipids and cholesterol release
			ABCA8	17q24.3	
			ABCA9	17q24.3	
			ABCA10	17q24.3	
			ABCA11	2q34	
			ABCA12	9q31.1	
ABCB	MDR	11	*ABCB1*	7q21.12	Multidrug resistance
			ABCB2	6p21.3	Peptide transport
			ABCB3	6p21.3	
			ABCB4	7q21.12	Phosphatidylcholine transport
			ABCB5	7p21.1	
			ABCB6	2q35	Transport of Iron
			ABCB7	Xq21-q22	Fe/S cluster transport
			ABCB8	7q36.1	
			ABCB9	12q24.31	
			ABCB10	1q42.13	
			ABCB11	2q24.3	Transportation of bile salt
ABCC	MRP	12	*ABCC1*	16p13.12	Mulitdrug resistance
			ABCC2	10q24.2	Efflux
			ABCC3	17q21.33	Drug resistance
			ABCC4	13q32.1	Nucleoside transport
			ABCC5	3q27.1	
			ABCC6	16p13.12	
			ABCC7	7q31.31	Ion channel
			ABCC8	11p15.1	Sulfonylurea receptor
			ABCC9	12p12.1	ATP channel regulation
			ABCC10	6p21.1	
			ABCC11	16q12.1	
			ABCC12	16q12.1	
ABCD	ALD	4	*ABCD1*	Xq28	Cholesterol transport regulation
			ABCD2	12q11	
			ABCD3	1p22.1	
			ABCD4	14q24.3	
ABCE	OABP	1	*ABCE1*	4q31.31	
ABCF	GGN20	3	*ABCF1*	6p21.1	
			ABCF2	7q36.1	
			ABCF3	3q27.1	
ABCG	White	5	*ABCG1*	21q22.3	Cholesterol transport
			ABCG2	4q22	Toxin efflux, resistance and urine transporter
			ABCG4	11q23	Sterol trafficking
			ABCG5	2p21	Sterol transport
			ABCG8	2p21	

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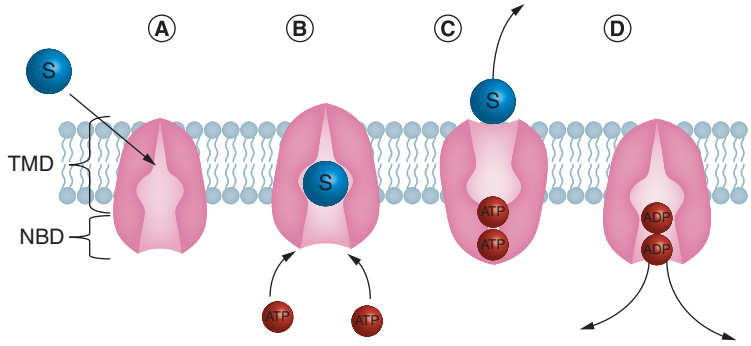


Figure 1. Detailed mechanism of substrate transport by ATP-binding cassette transporter.(A) Binding of ligand to TMD pocket induces conformational change in the NBD region. **(B)** Binding of the two ATP molecules in the NBD region. **(C)** Release of energy takes place by formation of closed NBD dimer leading to conformational change in TMD region. **(D)** ATP hydrolysis prompts dissociation of closed NBD dimer leading to conformational change in TMD region. Lastly, ADP and phosphate releases thereby restoring ABC transporter for opening of NBD dimer and ready for further cycle. NBD: Nucleotide-binding domain; S: Substrate; TMD: Transmembrane domain.

been mediated in normal fetal development, and these transporters play an active role in the nutrient distribution and metabolites exchange across the placenta. In adults, the activity of ABC transporters is correlated with circadian rhythm. Reports suggest a broad range of expression of ABC transporters in human tissue.

ABCA subfamily

The ABCA subfamily contains 12 full transporters, in other words, *ABCA1–ABCA13*, with ABCA11 as a transcribed pseudogene. Out of all the subfamilies of ABC transporters, this subfamily comprises the most prominent ABC proteins. Categorization of the ABCA family has been based on its phylogenetic analysis and is divided into two subgroups. The first subgroup is recognized as '*ABCA6*-like' transporters and includes *ABCA5–ABCA10*. Based on this, it has been reported that their genes form a cluster on chromosome 17q24. These transporters are well characterized by high amino acid sequence identity and have a small size ranging between 1543 and 1642 amino acids as compared with other ABCA transporters [22]. The second subgroup contains seven ABCA transporters, in other words, *ABCA1–ABCA4*, *ABCA7, ABCA12* and *ABCA13*, clustered on six different chromosomes. *ABCA1* is positioned on chromosome 9q31.1 and is involved in cholesterol and phospholipid removal from the cells out upon high-density lipoproteins particles [23]. In humans, mutations in *ABCA* genes lead to monogenetic diseases such as ABCA1 to HDL deficiency and Tangier disease. *ABCA3* mutations are associated with neonatal surfactant deficiency. *ABCA4* mutation is linked with the chorioretinal disorder;*ABCA12* mutations are connected with Harlequin ichthyosis [24–27]. A study in the past has reported *ABCA2* and *ABCA3* involvement in multidrug resistance development [28]. *ABCA1* and *ABCA3* are the well-characterized transporters in the human lungs. *ABCA1* is involved in controlling phospholipid and cholesterol transportation in alveolar type II cells. Reports have suggested that *ABCA7* is a homolog of *ABCA1* and has high expression in the lung tissue of a mouse. Some other complex diseases associated with ABCA transporters are (*ABCA1*) atherosclerosis, (*ABCA1*, *ABCA2*, *ABCA5* and *ABCA7*) Alzheimer's disease, and (*ABCA3*) pediatric interstitial lung disease [29,30].

ABCB subfamily

The subfamily of ABCB transporter constitutes 11 members; among them, 7 are half transporters and 4 are full transporters. ABCB proteins are localized in diverse membrane compartments, such as endoplasmic reticulum, plasma membrane, mitochondrial and lysosomal membranes. The *ABCB1* (MDR1) gene is positioned on chromosome 7q21.12 [31]. MDR1 encodes for P-glycoprotein (P-gp), which is involved in cell defense against xenobiotics. P-gp leads to the transportation of several hydrophobic substrates and anti-cancerous drugs such as vinblastine, doxorubicin and etoposide [32]. *ABCB1* is expressed in the liver and intestine, the organs that are mainly involved in excretion. The expression of P-gp has also been reported in the lungs, at the apical side of ciliated collecting ducts or ciliated epithelial cells [33]. P-gp plays a crucial role in developing multidrug resistance in cancerous cells due to its broad substrate recognition and high transport capacity. *ABCB2*/peptide transporter 1 (TAP1) and *ABCB3*/TAP2 are half transporters and play a significant role in the adaptive immune system [34]. They are involved in heterodimer formation to ship antigenic peptides from cytoplasm to endoplasmic reticulum (ER) lumen. *ABCB4*/MDR3 and

ABCB11/Bile salt export pump (BSEP) have their vital role in phosphatidylcholine transportation and transport of bile salts, respectively. Their mutation is accountable for progressive familial intrahepatic cholestasis [35]. The remaining transporters, *ABCB6-ABCB10*, are half transporters, localized in mitochondria, and are involved in iron metabolism. *ABCB6* is the first ABC transporter positioned in the outer mitochondrial membrane and imports porphyrin [36]. *ABCB7* and *ABCB8* transports heme from the mitochondria to the cytoplasm [37]. The function of *ABCB9* is yet unknown, with limited knowledge of its expression in lysosomes.

ABCC subfamily

All the members of the ABCC family are full transporters and share a similarity in their structure. The subfamily comprises 12 members and one pseudogene, ABCC13 [38]. The ABCC genes are designated as multidrug resistance proteins (MRP). *ABCC1-ABCC6* members of this family are active ATP-dependent transporters, *ABCC7*/CFTR regulates the ion channels, and *ABCC8*/SUR1, *ABCC9*/SUR2 modulate the permeability of potassium channels [39,40]. *ABCC1*/MRP1 is expressed in the brain, testis, intestine, and human bronchial epithelial cells and transports several drugs. *ABCC2*/*MRP2* gene is clustered on chromosome 10q24.2 and is named cMOAT (canalicular multispecific organic anion), a transporter in the liver. *ABCC2* is found in the kidney, liver and gut epithelium apical membranes. *ABCC3*/MRP3 provides resistance from anticancer drugs such as teniposide, etoposide and methotrexate (at higher concentrations). The gene is localized in the adrenals, liver, pancreas, gut, kidney and gallbladder [41]. Some human transporters such as MRP4, MRP5, and MRP6 are also called primary active transporters. MRP4 and MRP5 are involved in the efflux of antiviral drugs, nucleoside monophosphate analogs, and drugs used for myeloid leukemia. The mutations in the gene coding for CFTR/*ABCC7* are accountable for causing cystic fibrosis (CF). CFTR is expressed in the normal respiratory mucosa, and CFTR protein functions as a chloride ion channel, which plays a role in exocrine secretions [42]. *ABCC8*/Sulfonylurea receptor 1 (SUR1) has a binding site for sulfonylurea and is commonly used to treat patients suffering from Type 2 diabetes mellitus. This protein is recognized in regulating cell surface expression and regulates the function of ATP-sensitive potassium (KATP) channels in the pancreatic β cells [43].

ABCD subfamily

The ABCD subfamily consists of four half transporters, and they require the other half to form a dimer to be a functional transporter. *ABCD1*, *ABCD2* and *ABCD3* are localized in peroxisomes, and mutations in these genes lead to different peroxisomal disorders [44]. One such severe X-linked disorder is ALD (Adrenoleukodystrophy), a peroxisomal disorder that affects the nervous system. There is a buildup of very-long-chain fatty acids in this disorder, causing damage to the myelin sheath [45].

ABCE & ABCF subfamily

ABCE1 is the only member of the ABCE subfamily, also known as oligoadenylate binding protein (OABP), localized on chromosome 4q31.31 [46]. The ABCF subfamily contains three genes i.e. *ABCF1*/*ABC50*, *ABCF2*, *ABCF3* [47]. These members of ABC subfamilies do not have identified TMDs, but have NBDs, and thus they are not involved in any membrane transportation function.

ABCG subfamily

Five members (*ABCG1-ABCG5* and *ABCG8*) of this subfamily are half transporters. ABCG subfamily has an exclusive domain arrangement in which TMDs are located at C-terminal and NBDs are localized at N-terminal [48]. Being the first member, *ABCG1* has a significant role in lipid metabolism in humans, and its expression is regulated by cholesterol and lipoproteins in macrophages [49]. *ABCG2*/BCRP is a breast cancer resistant protein localized at chromosome 4q22 and is recognized as multidrug transporter that confers drug resistance in cancerous cells. Being a half transporter, BCRP not only provides resistance against toxins but is also involved in resistance against many chemotherapeutic agents such as topoisomerase I inhibitors and mitoxantrone [50]. *ABCG2* is localized at the plasma membrane and removes the excess porphyrin. The other transporters (*ABCG1*, *ABCG4*, *ABCG5*, and *ABCG8*) have a specific role in the translocation of lipids and steroids [51]. The elevated expression of *ABCG4* was reported in non-small-cell lung cancer (NSCLC) tissues, but not in normal lung tissues [52]. Mutations in *ABCG5* and *ABCG8* cause sitosterolemia, which is a rare heritable disorder [53].

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Intracellular distribution of drugs in ABC transporters: absorption, metabolism & toxicity of pharmacological agents

ABC transporters mediate the absorption and metabolism of drugs and are involved in the transportation of drugs across the membrane. These transporters are the decision makers of the drug accumulation in the cells, which is directly associated with drug toxicity, drug–drug interactions and therapeutic efficacy. Besides their participation in drug disposal and its clinical toxicity, these transporters play a crucial role in maintaining cell homeostasis by regulating the transport of different endogenous compounds.

Drug absorption

The small intestine is the main organ of absorption which allows for the availability of nutrients from the diet. Drug absorption from the GI tract is a vital factor in oral bioavailability. Intestinal absorptive cells and enterocytes have a range of influx transporters localized at the luminal/apical membrane to absorb drugs and food components. Though the knowledge of consequent transport of substrate in the bloodstream is little limited, ABC transporters located in the basolateral membrane can assist this step by catalyzing the molecule movement across the membrane [54]. The notable ABC transporters that can influence drug trafficking are *ABCB1*/MDR1/P-gp, *ABCG2*/MXR/BCRP, *ABCC2*/MRP2 and *ABCC4*/MRP4, respectively. The expression level of these transporters differ between the various segments of the intestine [55]. ABCB1, ABCC2 and ABCG2 have high expression in the intestine and act as a rate-limiting blockade to oral drug absorption. *ABCB1* is the most characterized transporter, and studies have reported the role of P-gp in the gut, thus limiting the bioavailability of paclitaxel that is administered orally. It is also claimed that P-gp could expect the same action if any other toxic substance is ingested [56]. P-gp in the intestine eliminates the parenteral drug administered by directly secreting the drug into the intestine lumen [57]. A similar study by *Van et al.* has shown increased paclitaxel bioavailability in treating mice with P-gp blockers by inhibiting the intestinal P-gp [58]. Even though *ABCC2* and *ABCG2* are higher in the intestine than *ABCB1* expression, significantly less data is on hand describing their role in drug absorption. The report in the past has shown an increase in the bioavailability of the food-derived carcinogen PhIP (2-Amino-1-methyl-6-phenylimidazo [4,5-b]pyridine) in the *ABCC2* deficient rats [59]. Another study by *Marchetti et al.* has concluded that erlotinib is a substrate for ABCB1 and ABCG2. Experiments indicated the increase in the bioavailability of erlotinib in triple knockout mice (Bcrp1/Mdr1a/1b-/-) compared with wild-type mice [60].

Drug distribution

To be effective, the absorbed drugs must be transported to the site of action from the administration site. Drugs most often target the central nervous system (CNS) and blood–CNS interface comprises blood endothelial cells (BBB, blood–brain barrier) and choroid plexus. To date, the requirement of lipophilicity for BBB penetration, though several lipophilic drugs demonstrate poor BBB penetration has been reported numerous times. The reason assigned behind this poor permeability of these drugs is the efflux role of ABC transporters. Presently, little is known about intracellular drug distribution modulation in cells over-expressing ABC transporters [61]. There could be numerous reasons concerning both direct and indirect effects [62].

Firstly, the ABC transporter might be expressed on the plasma membrane and may be expressed on vesicular membranes. These transporters are actively involved in drug transportation from the cytoplasm to the vesicles [63,64]. A further reason recommended by the research is that vesicular sequestration is a saturable process that mainly depends on the drug concentration in the cytoplasm [65,66]. A proficient method for modifying the intracellular drug concentration is an expression of an active efflux pump, for example, P-gp. For instance, a previous study has demonstrated how the same intracellular drug concentration can be achieved. In parental HL-60 cells, such as vincristine resistant HL-60 cells that overexpress the functional P-gp, ten-times additional daunorubicin is necessary for the resistant cells' to grow as compared with parental cells [67,68]. Low intracellular concentrations are correlated with drug sequestration in the cytoplasmic vesicles in both resistant and sensitive cells. The vesicular system's capability is surpassed at the enhanced drug levels, resulting in diffused cytoplasmic or nuclear drug distribution. Therefore, in this scenario, the modified intracellular distribution of the drug in the resistant cells is somewhat an outcome of the activity of the ABC efflux pump, which brings the intracellular drug levels down to a concentration that drops the capacity of the vesicular system. Lastly, overexpression of ABC efflux transporters might also influence the activity of different ion channels and intracellular pH, thus affecting the capacity of cytoplasmic organelles for drug sequestration [62,69,70].

Drug metabolism

After the absorption of a drug in an organism, drug metabolism is mediated by several sequential reactions. Drug metabolism occurs through two different phases, in other words, Phase I and II. Phase I reactions include reduction, oxidations, and hydrolysis and are illustrated as functionalization reactions. In this reaction, a new functional group is added to the parent drug (oxidation), followed by modification of the functional group in the parent drug to be more polar (reduction) and unmask the existing functional group (hydrolysis) [71]. Phase II involves the conjugation of partially detoxified metabolites in order to make them less harmful and water-soluble. Phase III eliminates the detoxified molecules carried by the multiple transporters. The intestine is the leading accepted site for significant non-renal removal of numerous drugs. The part of excreted molecules is reabsorbed in enterocytes because of this repeated reabsorption and extrusion; there is an extension in the residence time within the cell, enhancing the possibility of intestinal metabolism [72]. P-gp is a phase 1 drug-metabolizing enzyme present in high levels in enterocytes of the small intestine [73]. P-gp in the small intestine is the primary route for eliminating intravenous or oral administration of the drugs. One such inhibitor and anti-cancerous drug, indinavir (a substrate for MDR1), were applied to the Caco-2 cells, and the reports have confirmed the first-pass metabolism of the indinavir. Indinavir metabolism increased to 34% in the dexamethasone group (inducer of MDR1) compared with 6% in the control group.

In contrast, P-gp did not influence the hepatic metabolism of indinavir. The possible explanation could be the P-gp at the 'exit site' of hepatocytes in the liver. Therefore, P-gp generally shows interaction with the compounds encountered uptake, intracellular distribution, and metabolism. It was evident from this study that MDR1 increased indinavir metabolism [74].

Drug toxicity

Drug toxicity is the occurrence of severe drug-related problems which can have harmful effects on the organism. Toxicity is caused by various mechanisms, mainly by the interaction of drugs and the ABC transporters. The modulation of co-administered drugs and toxicants might change the toxicity profile by inhibiting the efflux action. Some drugs are considered safe and tolerable for the CNS because efflux transporters restrict their entry [65]. The reports suggest CNS toxicity on co-administration of an Ivermectin, a neurotoxic pesticide and MDR1 drug inhibitor [75]. It is worth mentioning that clinically used drugs, including azithromycin, carvedilol, erythromycin, indinavir, quercetin, ritonavir, verapamil and lapatinib, are potent inhibitors of MDR1 [76].

Co-administration of these drugs can increase the plasma exposure of P-gp substrates in the tissues owing to an increase in efficacy and toxicity. Cyclosporin A is used to treat and prevent allograft rejection; however, this leads to nephrotoxicity. Cyclosporin A is a P-gp substrate, and deviation in the expression of P-gp may lead to cyclosporine A accumulation within the tubular cells [77]. Reports have shown that diabetes downregulates the expression of BCRP and MDR1 at the blood–brain barrier (BBB), which alters the permeability of the substrates and drugs across BBB, affecting toxicity on CNS contributing to brain complications [78].

ABC transporters act as a defending shield preventing uptake and assisting in the clearing of toxic substances [79]. ABCC1 and other ABC transporters are involved in the protection of tissue from drug toxicity. Studies have shown that Abcc1-/- mice are very sensitive to the toxicity of etoposide (intravenously administered) in testicular tubules and oropharyngeal mucosal layer compared with wild-type mice [80]. Mdr1a-/-: Abcc1-/- combined deficiency leads to the dramatic increase in sensitivity toward intraperitoneally administered vincristine (by 128 folds), as well as to etoposide (by 3–5 folds). While Mdr1a-/- and Abcc1-/- deficiencies alone demonstrated 16 and fourfold increase in vincristine toxicity, respectively (Johnson *et al.*, 2001). Reports have shown doxorubicin cardiotoxicity in pediatric cancer patients because of ROS overproduction. An experiment in a mouse model revealed that Mrp1 loss potentiated the cytotoxicity induced by doxorubicin [81]. MRP1 and MRP3 are involved in eliminating various toxicological agents and their conjugates from the epithelial tissues. MRP2 helps in the hepatobiliary excretion of the GSH conjugates of arsenic and its derivates [82]. Besides this, MRP2 also transports various pre-carcinogens and food-derived carcinogens. BSEP/ABCB11 is a bile salt transporter and an antagonist endothelin receptor drug; bosentan inhibits BSEP, which leads to the accumulation of bile salts inducing liver damage [83].

Substrates & inhibitors of ABC transporters

ABC transporters are involved in translocating various substrates across intra- and extracellular membranes, including sterols, lipids and drugs. This section focuses on the four transporters: P-gp, MRP1, MRP2 and ABCG2. It is probable that other transporters of ABC are also clinically significant, but their association is not reported so

Table 2. Detailed list of substrates, tissue location and expression in cancer of ABC transporters.

Gene	Tissue location	Cancer expression	Endogenous substrates	Exogenous substrates
ABCA1	Kidney, nervous system, blood brain barrier and liver	Serous ovarian cancer, pancreas cancer	Cisplatin	Sphingomyelin, phosphatidylserine, phosphatidylcholine
ABC B1	Lung, blood–brain barrier, kidney, liver, small intestine, placenta	Colorectal, liver, renal cancer, Liver cancer, colorectal cancer, lung cancer	Docetaxel, Paclitaxel, vinblastine, vincristine, etoposide, actinomycin D, methotrexate, mitomycin C, colchicine, teniposide, anthracyclines	Bile acids, bilirubin, lipids, steroids, platelet activating factor
ABCB5	Placenta, lung, testes, melanocytes, mammary tissue	Lung, liver, ovarian, leukemia cells, thyroid	5-fluorouracil, Doxorubicin, camptothecin, mitozantrone, irinotecan, topotecan	IL-1 beta
ABCC1	Testes, lung	Head and neck lymphoma, renal thyroid cancer, endometrial glioma, melanoma	Etoposide, SN-38, irinotecan, vinca alkaloids, methotrexate. antineoplastic agents, mitoxantrone, colchicines, imatinib	Sphingosine-1-phosphate, Leukotriene C4, lysophosphatidylinositol, glutathione disulphide, glutathione
ABCC3	Lung, brain, kidney, intestine, liver, colon, testes prostate	Lung, cervical, liver, colorectal, pancreatic, ovarian cancer	Vincristine, etoposide, cisplatin, doxorubicin, teniopside, methotrexate	Glutathione
ABCC4	Lung, Liver, expressed widely	Prostate, liver, lung, brain, blood, colon, endometrial cancer	SN-38, irinotecan, topotecan, 6-Mercaptopurine, methotrexate	Folate, GSH conjugates, steroid, prostaglandins
ABCG2	Lung, intestine, liver, breast, colon	Lung, liver, cervical, testes, melanoma, breast cancer	Imatinib, Mitoxantrone, flavopiridol, anthracyclinstopotecan, methotrexate	Amyloid–β peptides glutathione, androgens

far. In general, *ABCB1*/P-gp exports a wide range of drugs, including immunosuppressive agents, HIV protease inhibitors, chemotherapeutic drugs, antihistamines, analgesics, pesticides, antibiotics and calcium channel blockers [84]. A detailed list of substrates, tissue location, and expression of ABC transporters is summarized in Table 2. All P-gp substrates are amphipathic and it has been hypothesized that P-gp substrates get inserted in the inner leaflet before being flipped to the outer leaflet of the cell membrane. Only the amphipathic molecules will have appropriate membrane insertion properties [85]. Reversal agents inhibit the transportation of P-gp mediated substrates, often termed as P-gp inhibitors. They inhibit P-gp function by disturbing the ATP hydrolysis, competing for the binding site, or altering the expression of P-gp. Most of the inhibitors, for example, quinidine, verapamil and cyclosporine A, are P-gp substrates; this suggests that they work as competitive inhibitors [86].

MRP1 confers resistance to antineoplastic drugs including vincristine, etoposide, teniposide, vinblastine, daunorubicin, doxorubicin, irinotecan (SN-38) [87]. Antivirals (ritonavir and saquinavir), toxicants (chlorpropham, aflatoxin B1, methoxychlor), and heavy metals (trivalent antimony and arsenite) are also substrates of MRP1 [88]. MRP1 transports amphipathic organic anions conjugated with sulfate, glucuronide, or glutathione (GSH) [89]. The potential inhibitors of MRP1 are not developed until now, though various MRP1 inhibitors have been illustrated. Probenecid, benzbromarone, S-decylglutathione, MK571, and sulfinpyrazone are examples of the inhibitors [90]. Though most of them enter the cells poorly, it is hard to get adequate intracellular concentration for effective inhibition. Inhibitors of P-gp such as PSC 833, cyclosporine penetrates the cell and inhibits MRP1 with poor specificity and lower affinity [91].

MRP2 mediates the translocation of various organic anions, most of which are sulfate, glucuronate, or GSH conjugates. Substrates of MRP 2 are in similarity with MRP1; however, the complete overlap is not there. The point of differentiation between MRP2 and MRP1 is that the over-expression of the former is associated with cisplatin resistance [92]. MRP2 substrates are also competitive inhibitors utilizing inside-out membrane vesicles such as MK571, LTC4, Fluorescein, and methotrexate. Nonetheless, these compounds do not penetrate the normal cells satisfactorily to attain inhibition [93]. The compounds that inhibit the activity of MRP2 to a greater or lesser extent are indomethacin, cyclosporin A, rifampicin, and montelukast. However, they might also show interaction with MRPs, P-gp, or BSEP [94].

The substrates of BCRP are doxorubicin, SN-38, topotecan, and mitoxantrone. BCRP substrates are identified by vesicular or cellular transport assays or cytotoxicity assays, comprising a wide range of anti-cancerous drugs, toxins, xenobiotics, and some antibiotics. Other substrates of BCRP are drugs such as cimetidine, prazosin, rosuvastatin, glyburide, vitamin K3, vitamin B2, and various chemical carcinogens [95]. It is worth noting that BCRP substrates substantially overlap with other ABC transporters such as MRPs and P-gp. An effective inhibitor of BCRP, elacridar is also an inhibitor of P-gp [96]. Other inhibitors of P-gp, such as valspodar, tariquidar, cyclosporine A and reserpine, also inhibit the activity of BCRP. Various TKI (Tyrosine kinase inhibitors), such as imatinib, erlotinib,

nilotinib, gefitinib, and lapatinib, inhibits BCRP mediated drug resistance. These TKIs are the substrates of BCRP, demonstrating that all these compounds might work as the competitive inhibitors of BCRP [97].

Multidrug resistance in cancer

The most efficient treatment for cancer patients with hematological malignancies or metastatic tumors is chemotherapy. However, these cancer cells can develop resistance to many anti-cancerous drugs. Chemotherapy resistance is the main problem in the effective treatment of numerous tumor types. Resistance may occur before the drug treatment as innate/primary resistance or can develop some time following the exposure as acquired resistance. Prolonged exposure to a particular chemotherapeutic agent might lead to the development of resistance to various other structurally distinct compounds [98]. Decreased cellular uptake of the drug at the molecular level is hard to overcome. Cellular resistance such as imatinib resistance is correlated with a point mutation in the *bcr-abl* gene. These mutations change the essential amino acids that are involved in the imatinib binding to the kinase domain. Alterations in enzymatic or protein expression can contribute to drug resistance [99]. For instance, the plasma membrane's decreased levels of uptake proteins may alter the cisplatin and methotrexate accumulation [100]. The range of these cell resistance mechanisms indicates the complexity of the drug resistance. Various mechanisms in the drug resistance, for example, drug delivery by excessive metabolism, poor absorption, poor penetration to specific sites, alteration in the apoptotic pathway, mutation of the target, environmental changes, or increased expression of drug efflux pumps, have been reported [101]. One of the most effective MDR mechanisms is an elevated efflux rate of the anti-cancerous drug from the cancerous cells by the ABC transporters. Three ABC proteins are overexpressed in the tumor cells, MDR1, MRP1, and BCRP, and seven other transporters are known to cause resistance to at least a single agent [102].

It is challenging to detect MDR1 in cancerous cells, and it is not suprising that our understanding of the levels of expressions from one tumor to another is still incomplete. Regardless of this, cancers are primarily chemoresistant, such as renal cell, hepatocellular cancers, and adrenocortical cancers have consistently demonstrated the expression of MDR1 [103]. Though, the wide range intractability of the tumors specifies that MDR1 cannot only explain resistance. Tumors that have a low level of expression at baseline, for example, small cell lung cancer (SCLC), leukemia, and breast cancer, develop over-expression of these markers during relapse on top of being previously exposed to chemotherapy [104,105]. The significant resistance is conferred by the over-expression of MDR1 to various cationic and neutral chemotherapeutic substrates, including epipodophyllotoxins (e.g., teniposide and etoposide), taxanes (e.g., docetaxel and paclitaxel), vinca alkaloids (e.g., vincristine and vinblastine), antibiotics, TKIs (nilotinib, and imatinib) [84]. The accumulation of paclitaxel in the brain and GI tract of the Mdr1 knockout mice demonstrates the role of MDR1 in preventing the elimination of paclitaxel into bile and passing the BBB, respectively [106]. A previous study reported the negative association coefficient of MDR1 expression with paclitaxel sensitivity profile, indicating that over-expression of MDR1 efflux pump can lead to paclitaxel resistance [107]. The over-expression of MDR1 can be because of a number of reasons such as amplification and increase in the transcription of the *MDR1* gene, alteration in the translational efficiency of MDR1, mutations in *MDR1* gene, or production of hybrid genes by chromosomal rearrangements in MDR1 gene [108–110]. Role of ABCG2 and ABCC2 has been suggested in the paclitaxel treatment, though significant association to paclitaxel efficacy has not been reported.

There is a significant overlap between MDR1 and MRP1 resistance profiles. The overexpression of MRP1 transporter provides resistance to a broad range of anti-cancerous drugs, for example, Vinca alkaloids, anthracyclines, camptothecins, epipodophyllotoxins, methotrexate, mitoxantrone and saquinavir [94]. However, unlike MDR1 and MRP7, MRP1 does not provide resistance to taxanes, a vital component of the MRP1 resistance profile. MRP1 confers resistance to TKIs, such as imatinib in chronic myelogenous leukemia (CML) [111]. Previous reports claim that HEK293 transfection with the ABCC10 gene conferring resistance to many anti-cancerous drugs such as docetaxel, paclitaxel, vinblastine, vincristine, and gemcitabine. Mrp 7 (*ABCC10*) knockout mice are more vulnerable to the lethal effects of paclitaxel, signifying that the MRP7 transporter is vital for viability and protects against xenobiotic toxicity. Except for *ABCC10*, no other member of the ABCC family has been accounted to confer paclitaxel resistance [112]. The over-expression of *ABCG2* has been reported only in subpopulations (also called as side population) of Acute myelogenous leukemia (AML) samples [50]. *ABCG2* overexpression in these subpopulations was found in many other tumors such as breast cancer, neuroblastomas, SCLC, Erwing sarcomas and glioblastomas. These stem cells confer resistance to the chemotherapeutic drugs, thus contributing to relapse [113].

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Single nucleotide polymorphisms in drug resistance ad toxicity

The arrival of pharmacogenetics into the clinic has opened a path to individualized therapy [114,115]. Undeniably, inter-individual variations in the drug response are key reasons causing adverse drug effects and drug treatment failure. To some extent, individual differences in drug plasma level are detected using single nucleotide polymorphism (SNP), thus enlightening discrete phenotypes of the drug-metabolizing enzymes. Nevertheless, drug bioavailability is also reliant on the expression of some drug transporters, for example, ABC transporters. There is an increasing amount of research addressing various genetic polymorphisms in the ABC transporters [116]. Studies among 48 ABC transporters suggest that ABCB1, ABCC1, and ABCG2 are widely studied. Reports suggest that *ABCB1* has over 50 genetic polymorphisms [117]. *Hoffmeyer et al.* first revealed the association of SNP C3435T (a synonymous mutation in exon 26) with the reduced expression of ABCB1 in variant allele (T) as compared with to wild-type allele (C) in the duodenum of patients [118]. The following study claimed that this effect could be because of non-synonymous mutation G2677T/A [119]. *Gerloff et al.* observed no changes in the clearance of digoxin among Caucasian patients having variant or wild type allele [120]. This study has shown an increased expression of *ABCB1*in Caucasian and Japanese patients carrying 3435 variant allele (T) [121]. The authors claimed the role of synonymous mutations in ABCB1 protein folding and its function Studies claims that SNPs C1236T, G2677T and C3435T in the ABCB1 gene sequence result in a protein with drug alteration and modified inhibitor interactions. Data suggest that on alteration of frequent codons to rare codons (in a cluster of the rarecodons), co-translational folding is affected and might result in distorted function [122].

ABCC1 gene is highly conserved; however, few SNPs have been identified in this gene. Reports have shown that patients undergoing anthracycline treatment and harboring G2168A mutation have a higher cardiotoxicity probability than those carrying wild-type (GG) genotype [123]. In another study, breast cancer patients carrying the mutant genotype for G2168A polymorphism revealed more disease-free survival than patients having GG genotype [124]. Another SNP analyzed was rs17501011 (G >A) in patients with colorectal tumors treated with irinotecan, 5-fluorouracil and folinic acid. It is believed that invariant genotype AA/AG's overall survival was low compared with the patients with no SNP (17 months vs 23 months with HR = 2.08 and p = 0.03) [125].

In the *ABCG2* gene, numerous SNPs are associated with response to treatment and toxicity. One such polymorphism, rs3114020, has been correlated with the high risk of fatality in NSCLC patients in the Asian population. This SNP affects the associated gene expression by modifying the ABCG2 binding sites [126]. It has been also reported that rs2231142 SNP (C >A) polymorphism is well correlated with the higher progression-free survival (PFS) in NSCLC patients treated with gemcitabine, especially in those patients having CA/AA genotype as compared with CC genotype (HR -0.48, 95% CI 0.29 to 0.78) and, p = 0.003) [127].

The findings of *ABCC1* show a high rate of SNPs which are similar to those of ABCB1. Studies on *ABCG2* claim a significant effect of the mutations on ABCG2 expression as well as function [115,117], SNPs in ABC transporters in different ethnic populations might affect the pharmacokinetics of the modulators or substrates, indicating that they might play a more significant role than anticipated in the effectiveness of treatment of the patients having cancer [128]. Even though the association of genetic polymorphism and ABC transporters for cancer treatment is being clarified, the overall picture is still very confusing, and the data is conflicting. Nonetheless, increasing studies reporting an association between SNPs and clinical outcomes strengthens the need to persuade future investigations, which will pave the hurdle in understanding the role of genetic variations in drug transport.

ABC transporters as therapeutic targets for improving chemotherapy

It is crucial to overcome the ABC transporters mediated resistance in cancer cells by different methods such as inhibiting the transporters on the cell surface level, blocking the specific signaling pathway that is responsible for amplification as well as overexpression of ABC transporters, and by targeting the transcription factors regulating the expression of these pumps. Consequently, the inhibition of the ABC transporters has been identified as a method to combat resistance to the anti-cancerous drugs [129]. Figure 2 illustrates targeting of multidrug resistance by the help of chemosensitizers.

While laboratory models have shown promising clinical application of the inhibitors to overcome drug resistance, unluckily, applying this knowledge to the clinic was quite tricky. Oncological drugs are developed in three phases, which define the progress of the drugs in clinical development. Initial trials are the Phase I studies which are referred to as dose-defining studies. The purpose is to describe the safety and efficacy of the drug to take it to the next phase. Usually, the trials are being carried out in chronological dose escalation until toxicity levels are noted. Phase II trials search for some indication of the drug's efficacy in the known patient population, whereas the phase

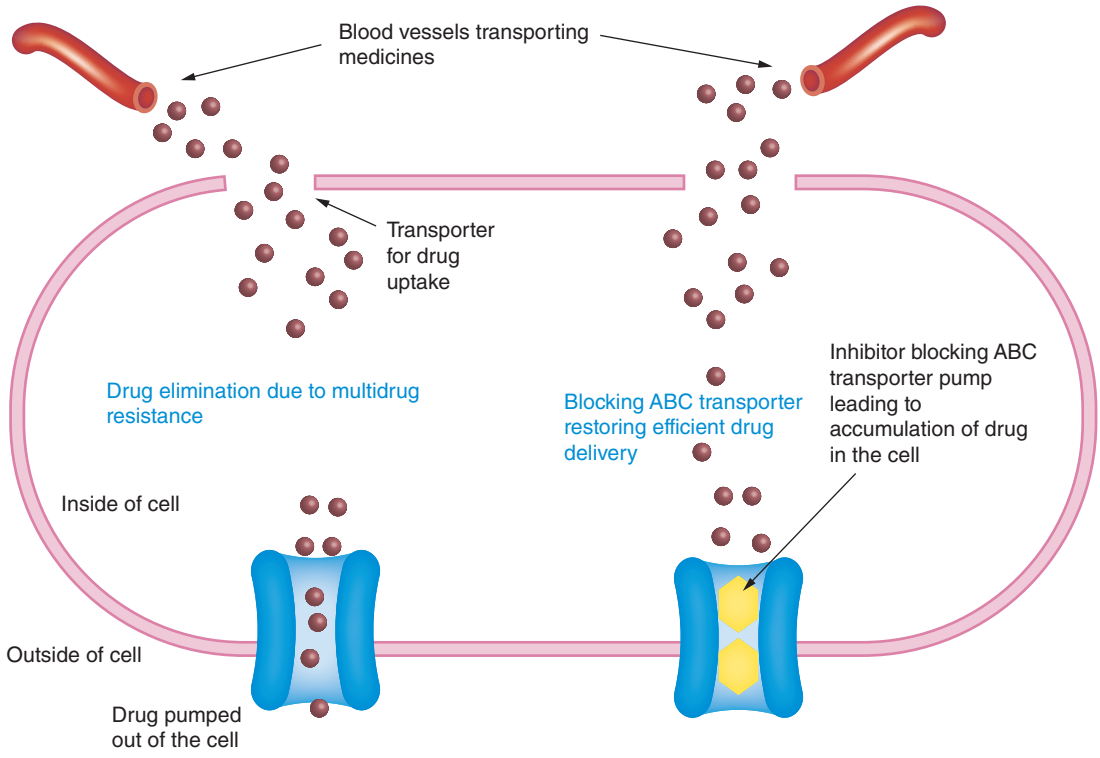


Figure 2. Targeting multidrug resistance by chemosensitizers.

III trials compare both treatments. Clinical trials investigating ABC transporter inhibitors have been categorized into three generations. First-generation ABC inhibitors include the compounds that are already approved by US FDA for medical uses. Second-generation ABC inhibitors consisted of compounds that were developed explicitly as *P-gp* inhibitors with some distinguished pharmacokinetic interactions. Third-generation inhibitors comprise compounds intentionally developed lack significant pharmacokinetic interactions [130].

First-generation MDR inhibitors

First-generation MDR inhibitors were used in clinical trials as functional modulators of P-gp. The earliest trials on P-gp inhibitors included drugs with verapamil [131], quinine, and cyclosporine A [132]; however, they repressed the efflux action of P-gp at the high concentration by competing with the anti-cancerous drugs [133]. The outcome produced by these drugs was not acceptable, as severe adverse effects and toxicity were reported [134]. Verapamil, a phenylalkylamine, is a calcium channel blocker that is utilized for hypertension treatment. A phase II randomized clinical trial in small cell lung cancer (SCLC) patients was administered daily with 480 mg of cyclophosphamide, doxorubicin, vincristine, and etoposide (CAVE) along with the chemotherapy, with hypotension as dose-limiting toxicity for the verapamil. However, at this high dose also, the trial failed to reveal the benefit of verapamil [135,136]. Further examination showed low blood levels of the verapamil as compared with the dose claimed to be efficacious in the preclinical studies [135]. It has been validated that verapamil and quinine increased cardiotoxicity, which prevented adequate dosing to inhibit the activity of P-gp [137,138].

Nifedipine and nicardipine are 'off the shelf' drugs in clinical use and are known to reverse the P-gp mediated resistance, but these agents are toxic at higher concentrations. The drugs mentioned above are classified as the first generation of ABC transporter inhibitors [139]. In brief, first-generation MDR inhibitors experience a low therapeutic window, inadequate efficacy in combinatorial with chemotherapy, and less potential for future development [134].

Second-generation MDR inhibitors

Second-generation MDR inhibitors were developed by chemically modifying first-generation inhibitors to inhibit the ABC transporter activity at micromolar concentrations and improvise first-generation inhibitors' potency. Examples of such chemosensitizers are Dexverapamil, Valspodar, and S9788. In one of the phases, I/II clinical trials

reported, Dexverapamil (R-verapamil) was given in combination with vinblastine in the treatment of renal cell carcinoma as an effort to reverse MDR [140]. The observed toxicity profiles were tolerable compared with the clinical trials using verapamil, along with the majority having mild cardiac-associated side effects. Another phase II study was reported in the past to check the proposition of Dexverapamil in chemoresistance reversal. Twenty-one cancer patients received Dexverapamil at a dose of 10 mg/m^2 along with anthracycline. Among the recruited patients, 2 had stable disease, and 2 had a partial response with a 19% disease control rate, indicating a modest activity, thus signifying the predictable resistance reversal abilities of P-gp inhibition [141].

One such P-gp inhibitor, valspodar (PSC833), is a derivative of cyclosporine A and is more potent than cyclosporine A in inhibiting the activity of P-gp with no immunosuppressive adverse effect [142]. However, valspodar fulfilled the criteria for high-affinity non-toxic inhibitor, this drug presented unexpected pharmacokinetic interactions. Through simultaneous inhibition of cytochrome P450 3A4 (CYP3A4), valspodar hindered the metabolism and elimination of the drug, thereby enhancing the anticancer drug exposure [143,144]. The drug-binding site of P-gp has quite a similarity with the CYP3A4 drug binding site; many numerous anti-cancerous agents are known substrates for both P-gp and CYP3A4. It has been noted that valspodar plays an integral role in reducing CYP3A4 associated liver or intestinal metabolism of anti-cancerous agents and simultaneously blocks Pgp-associated efflux from normal and cancerous tissues. This result in decreased drug metabolism that elevates the exposure of the drug and increases the incidence and severity of adverse events mediated with the anti-tumor therapy. The often noted adverse effects were bone marrow toxicity, together with thrombocytopenia and neutropenia that can be endorsed to elevated areas under the curve (AUC) for chemotherapeutic agents. Anti-cancerous drugs dose was decreased by 25–50% to assure this pharmacokinetic interaction in the valspodar trials [145,146]. One such Phase III trial, Cancer and Leukemia Group (CALGB) used valspodar in formerly reported AML patients of age >60. This study was terminated early because of excessive fatalities in the experimental arm during initiation [146]. Irrespective of these problematical outcomes, a patient's subset with the detectable leukemic drug efflux had significant progress in absolute remission rates and a trend toward better disease-freesurvival [146]. Another appealing outcome was observed in the trial with AML patients of age <60. Upon addition of valspodar to etoposide, daunorubicin, and cytarabine, the study noted quite a benefit in disease-free survival and overall survival in patients' subset of 45 years old/younger [147]. Regrettably, the outcomes were not replicated, and valspodar development has since been ceased.

Another P-gp inhibitor, S9788, is a triazine-amino piperidine derivate that can reverse MDR in cells resistant to chemotherapeutic drugs. In a Phase I trial, 38 patients with colorectal carcinoma and renal cell cancer were given doxorubicin alone (n = 16). Twelve patients out of these received combined treatment with doxorubicin and S9788. The pharmacokinetic analysis revealed MDR reversal at specific concentrations of S9788 in patients at non-toxic doses. However, compared with doxorubicin, treatment with both doxorubicin plus S9788 produced a statistically significant increase in grade 3 and 4 granulocytopenia [148].

Nevertheless, the second-generation ABC transporter inhibitors did not overcome drug resistance, and patients suffered from the various side effects allied with the therapy. Studies were performed to overcome the drawbacks of the first and second generation of ABC inhibitors.

Third-generation MDR inhibitors

The third-generation modulators were developed using quantitative structural-activity relationship and combinatorial chemistry [139]. This class of inhibitors was designed to work at nanomolar concentration, thus yielding specific and potent inhibitors. Modulators such as elacridar, dofequidar, tariquidar (XR9576), laniquidar (R101933), and zosuquidar have shown the improved selectivity and inhibitory effect toward ABC transporters [133,149]. Though no one P-gp inhibitor has shown promise and none of the inhibitors have been approved to treat cancer. However, the clinical development of these novel modulators is worth discussing as it might enhance our understanding to use them as MDR reversal agents in oncological therapy.

Tariquidar

Clinical trials demonstrated good tolerability and minimal side effects of tariquidar in healthy individuals [150]. Unlike cyclosporine A and verapamil, tariquidar inhibits the function of P-gp by binding at the site different from the paclitaxel and vinblastine site. Tariquidar was reported to potentiate the cytotoxicity of various drugs, including etoposide, paclitaxel, and vincristine, at low concentrations ranging from 25 to 80 nM. Tariquidar co-administration potentiates antitumor activity of the doxorubicin with no increase in toxicity in mice [129]. Tariquidar was also administered in combination with carboplatin/paclitaxel in patients with NSCLC, but this

study was terminated owing to the chemotherapy-associated toxicity in tariquidar [151]. A phase I clinical trial was conducted in which combination of tariquidar and vinorelbine showed good tolerability. Most prominently, there were no pharmacokinetic interactions between these two agents, thereby, addressing the main obstacle with the P-gp inhibitors in human studies. Nonetheless, a lack of response was noted in this trial [152].

Another Phase II study was carried out in which tariquidar was combined with the standard chemotherapy (taxanes or anthracyclines) in breast cancer patients. Tariquidar was further added to the existing regimen in order to observe whether drug resistance could be reversed or not. Seventeen patients were involved in this study, and partial response was observed in only one patient (ORR: 6%). 5/17 patients, including the patient with the response, had positive P-gp staining before treatment, thus indicating that the response rate was 20% (n = 5) in P-gp positive patients. Fascinatingly, this study also measured 99MTc (technetium-99m) uptake as a surrogate to check whether the predicted biology effect improves drug influx. The most substantial increase of 99MTc was observed in only one patient with the response, thus indicating that 99MTc might be a practical detector of the patients that can benefit from tariquidar and other p-gp inhibitors. This observable fact led to the sudden development of tariquidar in potential radiotracer together with the radioactive (11C) labeling [153]. This area of research has been quite active for tariquidar and other inhibitors [154,155]. In another Phase I study, combination of tariquidar and docetaxel was administered to lung, cervical and ovarian cancer patients [156]. Only 10% of the patients showed the response in this study. A Phase I study on combination of tariquidar with docetaxel, vinorelbine, and doxorubicin has reported ORR of 10% in adolescents and children with the refractory solid cancer [157]. The clinical trials with tariquidar were similar to other third-generation inhibitors, showing comparatively tolerable safety profiles with significant anti-cancerous efficacy.

Elacridar

Elacridar is a specific P-gp inhibitor that modulates the activity of ATPase [158]. Elacridar efficacy to inhibit P-gp activity and its associated effects in plasma concentrations have been characterized extensively in various studies [159]. Phase I clinical trials using elacridar reported very few side effects and quite appreciable pharmacokinetic properties [160]. In an early phase study, elacridar demonstrated increase in the plasma levels of the paclitaxel that was administered orally, thus inhibiting the intestinal activity of P-gp. Interestingly, on switching to the intravenous paclitaxel in the following cycles, the area under the curve of plasma paclitaxel during oral paclitaxel + elacridar was still lower when compared with the intravenous paclitaxel [161]. In another clinical trial, elacridar was found to significantly increase the oral bioavailability of topotecan to greater than twofolds when used in conjunction with the oral topotecan. However, blood topotecan level was still reduced compared with intravenous topotecan level [162].

Nevertheless, these outcomes pointed to a potential application of the elacridar with oral chemotherapeutic agents for long-term usage. The combination of elacridar with intravenous chemotherapy elucidated that it was safe along with relatively minor side effects in conjunction with doxorubicin in solid tumors [163]. The common toxicity reported was neutropenia. Irrespective of the acceptable safety profile of the early phase studies, elacridar was not further developed in future clinical trials.

Zosuquidar

Zosuquidar is a selective and potent inhibitor of P-gp, having a minimum inhibitory effect on the other MDR proteins such as MRP1 and MRP2 [164]. A phase I trial was carried out to check the safety profile of oral zosuquidar and its efficacy on combination with doxorubicin in advanced malignancies. Overall, the drug was reasonably well-tolerated, but limiting toxicities such as hallucinations, cerebellar dysfunction, and palinopsia were reported. Later, another phase I study was conducted in which combination of intravenous zosuquidar with doxorubicin was administered in patients with tumors [165]. In this case, less frequent and less severe neurotoxicity was reported, suggesting different pharmacokinetic profiles between the intravenous and oral formulation of zosuquidar. It is worth noting that phase I studies were not designed for the efficacy and analysis of these two trials; further they have not shown a striking improvement in the response. Another Phase II study demonstrated the combination of zosuquidar and docetaxel in metastatic breast cancer. Though the combination was safe, the trial was not conclusive as it did not reveal any difference in PFS, OS, or ORR in metastatic breast cancer patients.

A Phase I trial was conducted in patients with acute leukemia, where combination of zosuquidar with standard chemotherapy using cytarabine and daunorubicin was administered to the patients [166]. Sixteen patients were allocated to the study. Reports have shown grade 3/4 adverse events such as arrhythmia, hypokalemia, febrile

Table 3. List of inhibitors targeting ABC transporters.

Inhibitors	Drug	Target	Phase	Outcomes	Adverse events
First generation inhibitors	Verapamil	Calcium channel blocker	II	No clinical relevance	Cardiotoxicity Hypertension
Second generation inhibitors	Dexverapamil (R-verapamil)	Calcium channel blocker	I/II	Disease control rate-19% Objective response rate-9%	Cardiac associated side effects
	S9788	P-gp	I	Future development restricted due to severe outcomes	Cardiac arrhythmia, Grade 3 and 4 granulocytopenia
	Valspodar	P-gp	III	No clinical benefit	N/A
Third generation inhibitors	Elacridar	P-gp, ABCG2	I	Enhanced bioavailability of associated chemotherapy drug	Neutropenia
	Tariquidar	P-gp, MRP1	II	Objective response rate- 5% to 10%	N/A
	Zosuquidar	P-gp	III	No clinical benefit	Toxicity

neutropenia, and respiratory failure. Seven patients demonstrated a complete response with a rate of 43.7% while as per the considered fact standard induction chemotherapy in AML patients has an established ORR of 50–60% [167].

According to the IHC staining, 2/4 patients were reported with the p-gp negative cancers, thus showing 50% complete remission (CR). In comparison, 5/12 patients with the p-gp positive tumors were reported with CR of 41.6%, suggesting that zosuquidar may not provide the estimated efficacy on combination with standard induction chemotherapy to AML. Further, a phase II study was carried out in AML patients over 60 years old. Patients were administered with daunorubicin and cytarabine along with either placebo or zosuquidar [168]. Median 2-year OS and response rate were similar in both the cases (9.4 vs 7.2 months, 23 vs 20%).

MK571, an antagonist of leukotriene D4 (LTD4) receptor and fumitremorgin C (FTC), a fungal toxin, are specific modulators of MRP1 and ABCG2, respectively. FTC and MK571 are modulators for inhibiting ABCG2 and MRP1 mediated transport in the laboratory assays [169,170]. However, no extensive study on the modulators of MRP1 and ABCG2 has been investigated in clinical trials. Some P-gp modulators such as dofequidar (MS-209), biricodar (VX-710), and elacridar (GF120918) have shown to play a role in the inhibition of MRP1 and ABCG2 mediated resistance in clinical studies [171–176]. Though the effect of all these modulators is not yet well established and their safety and efficacy are still under evaluation. The highlighted inhibitors are summarized in Table 3.

FDA-approved drugs in cancer treatment

Previous clinical development history demonstrated that small molecules designed to inhibit P-gp were highly unsuccessful in clinical studies. The early generation of the inhibitors had problems with significant toxicity, whereas new generation inhibitors generally had acceptable safety profiles but limited efficacy. Further investigation was carried out for FDA-approved drugs for their P-gp activity. Two different approaches were initiated in which first, FDA-approved drugs and real-world knowledge points to the safety profile of the drugs, thus narrowing down further development issues. In addition, repurposing the combination of these drugs with present antitumor therapeutics might provide additional effects to elevate the efficacy. In the subsequent section, some of the compounds have been highlighted and reported in conjunction with chemotherapy for anticancer treatment.

Macrolides

The FDA has approved three macrolide antibiotics, in other words, azithromycin, clarithromycin and erythromycin, as P-gp inhibitors. These antibiotics have been supported by various studies in the literature [177–179]. Macrolides, the CYP3A4 inducers, might also hinder pharmacokinetics with different outcomes, as CYP3A4 and P-gp both have a role in drug–drug interaction [180,181]. Indeed, research regarding macrolides on anti-cancerous drugs has been done to clarify if drug effects can be efficaciously delivered [182]. The macrolide clarithromycin has been investigated in various clinical studies with cancer subjects, combined with metronomic chemotherapy. The rationale for these trials was mainly to investigate the CYP3A inhibitor activity of clarithromycin and its impact on the associated drug; however, few have shown promising results with potential for further follow-up in clinical studies.

Chlorpromazine

Phenothiazine drugs, like chlorpromazine, can inhibit P-gp. It possesses antipsychotic as well as anti-emetic properties clinically. Fascinatingly, antipsychotics are drugs that were over-represented in P-gp inhibitors (including haloperidol, paliperidone). Chlorpromazine has EC50 and is tenfold weaker than PSC833 in low micromolar

concentrations [183]. Active research is still ongoing in association with chlorpromazine and its antitumor properties. It has been observed to repress the Hippo pathway associated with YAP signaling and cancer stemness; epigenetic antitumor mechanisms suppress chemoresistant tumor growth and numerous other pathways [184,185]. Phenothiazines can inhibit tumor stem cells. Moreover, numerous novel derivatives are presently being developed toward this aim [186]. It is yet to be seen whether phenothiazines such as chlorpromazine inhibit P-gp activity and will be a positive factor in increasing anticancer activity either as a monodrug or in combination with various other cytotoxic agents.

Phosphodiesterase Inhibitors

Sildenafil and Vardenafil are two approved drugs by FDA that inhibit P-gp activity. Both vardenafil and sildenafil are potent PDE5 inhibitors and are utilized in erectile dysfunction. However, the active role of sildenafil in anticancer treatment has been actively studied [187,188] and has also been anticipated to potentiate cisplatin-associated tumor killing [189,190]. Through various mechanisms that have been proposed for this happening, the role of inhibiting P-gp remains very likely to be explanative. The antitumor role of the PDE5 inhibitors as single-use and in combination with the chemotherapy remains an active research area. In the future, more outcomes could be a ray of hope in this arena.

Progressed strategies to circumvent multidrug resistance

The strategies mentioned above did not successfully identify a compound to overcome cancer-mediated drug resistance. There are numerous possible reasons for the lack of clinical advantage of these trials. In order to overcome these hurdles, alternative methods are required to overcome MDR. Therefore, the research is inclining toward novel methods to overcome MDR by means other than blocking substrate binding sites.

CRISPR-Cas9 system

Many inhibitors are being formulated to overcome MDR by inhibiting ABC transporters. When the inhibitors are delivered along with anti-cancerous drugs, inhibition of ABC transporters was attained to some extent, however; their clinical significance is yet in need of proof. In recent times, clustered regularly interspaced short palindromic repeats-associated Cas9 system (CRISPR-Cas9) is being used as a novel genome-editing tool in various cell types and model organisms. CRISPR-Cas9 system utilizes RNA-guided Cas9 nucleases that form a complex with the sgRNA (short guided RNA) to cleave DNA 3–4 base pairs and introduce the double-stranded breaks in a sequence-specific manner [191]. Moreover, the double-stranded breaks are repaired by nonhomologous end-joining or homologous directed DNA repair. The former repair can lead to the creation and deletion of mutations at the target sites, thereby disrupting the function of genomic elements. Lately, the CRISPR-Cas9 system has been shown to block the expression of P-gp efficiently. Nonetheless, many aspects of the CRISPR-Cas-9 system have further improved to enhance its efficacy [192].

RNA interference (siRNA)

Through MDR reversal by inhibiting the MDR expression, gene synthetic small interfering RNA (siRNAs) is widely being used [193]. siRNAs are used to instigate the cleavage of target mRNAs and are being developed against the mRNA sequences of the ABC transporters. Anti-ABC transporters siRNA binds and encourage degradation of the targeted single-strands of mRNA by adding double strands of the target with a multi-protein cleavage complex [194]. Reduced expression of ABCC4 using RNAi is an example in the drug-resistant gastric tumor cells. As an outcome of this inhibition, efficient inhibition of cell proliferation and enhanced cell sensitivity to chemotherapeutic drugs has been seen [81]. The benefits of siRNA technology are in its ability to interfere with mRNA processing at all developmental stages. The target ABC gene expression could be lowered by tenfold with a high degree of specificity through transfecting siRNA into the cells. Regrettably, many nonspecific silencing cases are mediated with siRNA treatments [195].

MicroRNA interference

One such new approach targets ABC inhibition at the translational level. Numerous miRNAs, such as miR-451, -27a, -1253, -298, -296, inhibits P-gp mediated drug resistance in breast cancer cells [196,197]. As per the above findings, the development of many synthetic siRNAs inhibit the ABC transportater genes [198]. Another approach is the development of monoclonal antibodies (mAb) that target ABC transporters and could enhance the efficiency

of chemotherapeutic drugs. Some antibodies, for example, UIC2, MRK-16, and MRK-17, inhibit the function of P-gp in *in vivo* and *in vitro* models, mostly in combination with other P-gp inhibitors [199]. Using the phage display approach, a single-chain fragment variable (scFv), a recombinant antibody, selectively targets the MRP1 transporter and does not affect any other member of the ABC transporters. MRP1 being targeted selectively helps in reversing MDR in tumors that over-express the MRP1 transporter [200].

Gene therapy

In the past few years, gene therapy has been a feasible strategy to overcome MDR. Though, there are limited *in vitro* and *in vivo* studies that support gene manipulation to abolish or minimize drug resistance. The development of CRISPR-Cas 9 gene-editing technology might provide an exceptional tool to regulate gene expression in the cells [201]. As per reports, 50% restoration of chemosensitivity to doxorubicin was attained in adriamycin-resistant ovarian cancer cells (A2780/ADR) by knocking down the expression of the *ABCB1* gene. A significant decrease in the gene expression of P-gp was found to correlate with enhanced sensitivity of transfected cells to the doxorubicin with the single guided RNAs [202]. CRISPR-Cas 9 gene-editing technique was applied to silence the gene expression of ABCB1 in kidney cells [203]. Hence, Cas 9 mediated interference in drug resistance-associated genes might be a promising method to counteract resistance in cancerous cells. However, whether using such technology in cancer patients will be beneficial or not is yet to be known [204].

Loaded nanoparticles

Combination of nanoparticles with anticancer drugs can be used to overcome MDR by increasing the stability of the anticancer drug, cellular delivery, thereby circumventing the efflux by ABC transporters and specific distribution. One such example of this strategy is paclitaxel-loaded iron oxide nanoparticles (PTX-NPs). The combination of PTX-NPs with ABCG2 mAb leads to the decrease in the multiple myeloma tumor volume, with significant elevation in survival rate when injected into severe combined immunodeficient mice having multiple myeloma tumors [205]. Expression of P-gp was targeted on a combination of RNAi-chemotherapeutic drugs in nanocarriers, thus enhancing the delivery and efficiency of the chemotherapeutic drug. Polymeric nanoparticles, micelles, gold nanoparticles, and liposomal nanoparticles are examples of nanocarriers that overcome ABC transporter-mediated resistance in cancer [206].

Aptamers

Usage of aptamers is also one of the strategies to overcome MDR and target resistant tumors. Aptamers are single-stranded DNA or RNA oligonucleotides with length of 20–60 nucleotides that specifically bind to target molecules with high specificity and affinity [207]. Synthesis of aptamer by *Soldevilla et al.* targets resistant tumors that overexpresses MRP1 through a novel strategy called systematic evolution of ligands by exponential enrichment abbreviated as SELEX. SELEX technique selects aptamer from RNA library and screens the RNA library against MRP1 epitope peptide for the hits, and was counter selected using control peptides. MRP1-CD28, a bivalent aptamer, has dual effects, as it can bind to resistant tumors expressing MRP1 while simultaneously delivering CD28 signals to T lymphocytes [208]. This novel approach has shown success in mice bearing melanoma, in which prolonged survival and reduction in tumor growth were noted.

Conclusion & future perspective

Efflux transporters such as ABCB1/P-gp, ABCC1/MRP1 and ABCG2/BCRP could confer MDR in tumor cells. Also, over-expression of the said transporters in the cancer tissues has been associated with the poor therapy effect in cancer. Conceptually, inhibition of these transporters will enhance the accumulation of the drug and thereby will overcome MDR. Though, despite gathered knowledge on ABC transporters and their multifaceted interplay, clinical studies demonstrating the effect of the ABC transporter inhibitors could not accomplish their promises. The design of the inhibition research will require various considerations. The choice of a particular inhibitor will enhance the probability of unwanted side effects as the inhibitor will target different ABC transporters. However, some transporters may be spared from the toxic effect of the drug. As multiple ABC transporters are responsible for inciting MDR, usage of broad-spectrum inhibitors can be favored, but it may also impact the other physiological functions of these transporters. The evading approach might be an alternative for ABC transporter's inhibition dilemma, and recent studies aim to find those drugs evading primary transporters such as ABCB1, ABCC1 and ABCG2.

Also, MDR reversal approaches have their merits, but they have not translated into clinical applications. Therefore, future directions should combine our present knowledge of clinically significant ABC transporters in different fields such as molecular, computational biology, and biochemistry; in conjunction, we could make novel generation inhibitors for future reversal studies of MDR. This integrated approach might also contribute to personalized therapy in tumor patients and accomplish the most favorable treatment outcomes.

Executive summary

- ABC transporters mediate the movement of various molecules and drugs across the membrane of a living organism.
- The human genome carries seven subfamilies of ABC transporters, in other words, ABCA and ABCG.
- These transporters are the decision-makers behind drug accumulation in cells, which is directly associated with drug toxicity, drug–drug interactions and therapeutic efficacy.
- P-gp, MRP1, MRP2, and ABCG2 are of great clinical significance and export many drugs.
- The intensely studied past reports assure the correlation of ABC transporters in multidrug resistance phenotype, particularly in cancer.
- The Center for Disease Control and Prevention reports that nearly 2.8 million people are identified with severe infectious diseases each year due to drug resistance.
- Inhibition of these transporters will enhance the accumulation of the drug and thereby will overcome MDR.
- Preclinical and clinical trials have investigated the probability of overcoming drug-associated resistance and substantial toxicities.
- First-generation MDR inhibitors used in clinical trials included drugs with verapamil, quinine, and cyclosporine A.
- Second-generation MDR inhibitors inhibit the ABC transporter activity at micromolar concentrations and improvise first-generation inhibitors' potency, and such chemosensitizers are Dexverapamil, Valspodar, and S9788.
- The third-generation modulators were developed using quantitative structural-activity relationship and combinatorial chemistry, and such modulators are elacridar, dofequidar, tariquidar (XR9576) laniquidar (R101933) and zosuquidar.
- Despite gathered knowledge on ABC transporters and their multifaceted interplay, clinical studies demonstrating the effect of the ABC transporter inhibitors could not accomplish their promises.
- Macrolide antibiotics (azithromycin, clarithromycin, erythromycin), chlorpromazine, Phosphodiesterase Inhibitors (Sildenafil and Vardenafil) are the FDA-approved drugs in cancer treatment.
- Progressed strategies such as CRISPR-Cas9 system, RNA interference (siRNA), MicroRNA interference (miRNA), gene therapy, loaded nanoparticles, and aptamers are discussed to circumvent multidrug resistance.
- MDR reversal approaches have their merits, but they were not being translated into accomplishing clinical applications.
- In the future, some integrated approaches might contribute to personalized therapy in tumor patients and accomplish the most favorable treatment outcomes.

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References

1. Vasiliou V, Vasiliou K, Nebert DW. Human ATP-binding cassette (ABC) transporter family. *Hum. Genomics* 3(3), 281 (2009).
2. Goffeau A, De Hertogh B, Baret PV. ABC transporters. *Encycl Biol Chem* 1, 1–5 (2004).
3. Dean M, Hamon Y, Chimini G. The human ATP-binding cassette (ABC) transporter superfamily. *J. Lipid Res.* 42(7), 1007–1017 (2001).
4. Pasello M, Giudice AM, Scotlandi K. The ABC subfamily A transporters: multifaceted players with incipient potentialities in cancer. *Sem cancer biol* 60, 57–71 (2020).
5. Linton KJ. Structure and function of ABC transporters. *Physiology* 22(2), 122–130 (2007).
6. Szakacs G, Varadi A, Özvegy-Laczka C, Sarkadi B. The role of ABC transporters in drug absorption, distribution, metabolism, excretion and toxicity (ADME–Tox). *Drug Discov. Today* 13(9–10), 379–393 (2008).
7. Pokharel D, Roseblade A, Oenarto V, Lu JF, Bebawy M. Proteins regulating the intercellular transfer and function of P-glycoprotein in multidrug-resistant cancer. *Ecancermedicalscience* 11, 768–787 (2017).