Association between genetic polymorphisms in SLC19A1, SLCO1B1 and SLCO1B3 genes predicts survival and toxicity in North Indian lung cancer patients undergoing platinum-based doublet chemotherapy

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Abstract

Abstract Aims: SLC transporters are expressed in lungs and are essential membrane proteins responsible for the transport of wide range of chemotherapeutic drugs. Polymorphisms in SLC19A1, SLCO1B1, and SLCO1B3 gene in North Indian lung cancer patients are investigated. Methods: A total of 610 lung cancer patients undergoing platinum-based chemotherapy were recruited in the study. Polymorphisms of SLC19A1 (G80A), SLCO1B1 (A388G, T521C) and SLCO1B3 (A1683-5676G) in North Indian lung cancer patients were assessed and statistical analysis were carried out. Results: Our data revealed that patients harboring mutant genotype (AA) for SLC19A1 G80A polymorphism had higher MST as compared patients with wild type (GG) genotype (MST=9.33 versus 8.23). ADCC patients with mutant genotype (AA) showed better survival outcomes for SLC19A1 G80A (MST=9.4 versus 8.8, HR=0.6; p=0.04). In SCLC, SLC19A1 G80A polymorphism revealed increased survival in the patients harboring mutant genotype (AA) (MST=9.6 months versus 7.6 months, p=0.04). For SLCO1B3 polymorphism, patients administered with carboplatin/cisplatin and docetaxel showed inferior survival outcomes in subjects carrying heterozygous alleles (AG) (MST=2.9 months versus 9.6 months, p=0.006, HR=14.01). For anemia, SLCO1B1 T521C showed that patients with heterozygous genotype (TC) had a reduced risk of developing anemia (OR=0.44, 95% CI=0.20-0.96; p=0.04). Patients with SLCO1B1 A388G polymorphism harboring AG alleles was associated with a lower incidence of thrombocytopenia (OR=0.41, 95% CI=0.20-0.82; p=0.01). Patients with heterozygous (AG) genotype (OR=0.35, 95% CI=0.17-0.72; p=0.002) for A1683-5676G polymorphism showed lower incidence of nephrotoxicity. Conclusion: Genotyping of SLC polymorphism is crucial for predicting survival and toxicity in lung cancer patients undergoing platinum-based chemotherapy.

Association between genetic polymorphisms in *SLC19A1, SLCO1B1* and *SLCO1B3* genes predicts survival and toxicity in North Indian lung cancer patients undergoing platinum-based doublet chemotherapy

Running head: SLC polymorphism and clinical outcomes in Lung cancer patients

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What is already known about this subject

SLC transporters are known mediators of drug disposition that facilitates 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.

What this study adds

SLC19A1, SLCO1B1 , and *SLCO1B3* polymorphisms are associated with altered activity of these transporters and affects overall survival as well as toxicity levels in lung cancer patients.

Genotyping for the *SLC* polymorphism could be a valuable tool for predicting which lung cancer patients will benefit the most from platinum-based doublet chemotherapy.

Abstract

Aims: SLC transporters are expressed in lungs and are essential membrane proteins responsible for the transport of wide range of chemotherapeutic drugs. Polymorphisms in *SLC19A1* , *SLCO1B1* , and *SLCO1B3* gene in North Indian lung cancer patients are investigated.

Methods: A total of 610 lung cancer patients undergoing platinum-based chemotherapy were recruited in the study. Polymorphisms of *SLC19A1* ($G^{80}A$), *SLCO1B1*($A^{388}G$, $T^{521}C$) and*SLCO1B3* ($A^{1683\text{-}5676}G$) in North Indian lung cancer patients were assessed and statistical analysis were carried out.

Results: Our data revealed that patients harboring mutant genotype (AA) for *SLC19A1* $G^{80}A$polymorphism had higher MST as compared patients with wild type (GG) genotype (MST=9.33 versus 8.23). ADCC patients with mutant genotype (AA) showed better survival outcomes for *SLC19A1* $G^{80}A$ (MST=9.4 versus 8.8, HR=0.6; p=**0.04**). In SCLC, *SLC19A1* $G^{80}A$ polymorphism revealed increased survival in the patients harboring mutant genotype (AA) (MST=9.6 months versus 7.6 months, p=**0.04**). For SLCO1B3 polymorphism, patients administered with carboplatin/cisplatin and docetaxel showed inferior survival outcomes in subjects carrying heterozygous alleles (AG) (MST=2.9 months versus 9.6 months, p=**0.006, HR=14.01**). For anemia, SLCO1B1 T521C showed that patients with heterozygous genotype (TC) had a reduced risk of developing anemia (OR=0.44, 95% CI=0.20-0.96; p=**0.04**). Patients with

SLCO1B1 $A^{388}G$ polymorphism harboring AG alleles was associated with a lower incidence of thrombocytopenia (OR=0.41, 95% CI=0.20-0.82; p=**0.01**). Patients with heterozygous (AG) genotype (OR=0.35, 95% CI=0.17-0.72; p=**0.002)** for $A^{1683\text{-}5676}G$ polymorphism showed lower incidence of nephrotoxicity.

Conclusion: Genotyping of *SLC* polymorphism is crucial for predicting survival and toxicity in lung cancer patients undergoing platinum-based chemotherapy.

Keywords: Lung cancer, *SLC* transporter, survival, platinum-based chemotherapy, polymorphism, Toxicity

Introduction

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, and the median survival time is even less than tenmonths. 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 cancer are administered platinum-based doublet chemotherapy. Even though cisplatin or carboplatin is effective when coupled with non-platinum-based chemotherapy medications such as paclitaxel, pemetrexed, 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 such as age, concurrent therapy, drug interactions, and the nature of the disease can impact chemotherapy outcomes, there are plenty of inter-individual differences in drug response due to sequence variants of genes that encode drug transporters.[3]

Drug transporters are known mediators of drug disposition, facilitating the influx of substrates into cells and the efflux of drugs and metabolites from cells.Solute carrier (SLC) transporters are the most prominent family of the membrane proteins accountable for the uptake 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 (Figure 1).[5] SLC transporters are expressed in many tumors 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 esophageal 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 theessential 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 (Sutherland et al., 2020).[6] Various genetic polymorphisms are described in the *SLCO1B1* gene; among them, themost 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 lower expression of OATP1B1 protein and significantly reduced transport activity compared to the wild type (TT) genotype.[13-16] Another variant of OATP is an intronic region mutation of $SLCO1B3A^{1683\text{-}5676}G$(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 $A^{1683\text{-}5676}G$ variant 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 North Indian lung cancer patients. 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.

Methods

Sample collection and study design

Patients with lung cancer who visited the authors' institute's lung cancer clinic (Post Graduate Institute of Medical Education and Research, PGIMER) were included in the current study. The study enrolled newly diagnosed individuals with histologically or cytologically proven NSCLC and SCLC for three years. The institute's ethical committee approved the study. The inclusion criteria of the lung cancer patients meeting all the following requirements were eligible for enrollment: (i) Diagnosis of lung cancer confirmed by cytology or histology (ii) Stage IIIB - IV or IIIA (not scheduled for surgery). (iii) Untreated and intent to treat with definitive platinum-based chemotherapy [platinum agent (Cisplatin or Carboplatin) in combination with either docetaxel, paclitaxel, gemcitabine, pemetrexed, or irinotecan]. (iv) An Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0-2. (v) At least one bi-dimensionally measurable lesion, according to the RECIST criteria. (vi) Adequate organ function, defined as absolute neutrophil count > 1500/µl, platelet count>100,000/µl, and levels of creatinine, liver enzyme, and alanine aminotransferase (ALT) less than two times the upper limits of normal (ULN).(vii) Written informed consent was obtained. The exclusion criteria included (i) Non-Bronchogenic tumors. (ii) Primary pleural tumors – mesothelioma (iii) Sarcomas (iv) Metastatic tumors to lungs or pleural (v) Early-stage (resectable) lung cancer.

All of the participants signed an informed consent form. 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, tumor histology, and smoking status were all recorded. CBC was done tendays after the first chemotherapy session, and any additional cycles if needed. CBC, renal function, and liver tests were performed before each treatment cycle and 3-4 weeks following the last chemotherapy round. The paper was reported using the STROBE checklist.

Chemotherapy regimen

Docetaxel (75 mg/m2), irinotecan (100 mg/m2), pemetrexed (500 mg/m2), or paclitaxel (175 mg/m2) were given as a 1-hour infusion, followed by iv. infusion of cisplatin (70 mg/m2) for over 3 hours. Four cycles of chemotherapy were given before a tumor response assessment, as is the usual procedure at PGIMER. Before the end of the four cycles, the tumor response was assessed, and if there was undesirable toxicity or clinic-radiological symptoms of cancer development, chemotherapy was stopped if necessary. Subjects who exhibited an objective response to chemotherapy were given two more treatment cycles (i.e., maximum

of 6 cycles). Response Evaluation Criteria in Solid Tumors (RECIST) criteria measure tumor response. The common toxicity criteria (CTC) version 3.0 was implemented to record and classify adverse events. PGIMER's established procedures were used to manage any negative effects. We assessed the following hematological toxicity parameters: leukopenia, anemia, thrombocytopenia, and neutropenia. Diarrhea, nausea, and constipation were investigated for gastrointestinal toxicity. Patients with febrile neutropenia or grade 3 or higher 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. Follow-up was done every three weeks during chemotherapy and every three months for the rest of the research. Overall survival was calculated from chemotherapy to death or the last follow-up date.

Genotyping of SLC variants

The genomic DNA was extracted from 3-4 ml of blood using the phenol-chloroform extraction technique. The genotype of variations was determined using the PCR-PFLP method as previously mentioned.[10,19,20] For amplifying a DNA fragment for the *SLC19A1* ($G^{80}A$, rs1051266) variant following sets of primers were used: forward primer 5'- AGTGTCACCTTCGTCCCCTC-3' and reverse primer 5'- CTCCCGCGTGAAGTTCTT-3'. For *SLCO1B1* $A^{388}G$ in exon 4 (rs2306283), the set of primers used was forward primer 5'-ATAATGGTGCAAATAAAGGGG-3'and reverse primer 5'-ACTATCTCAGGTGATGCTCTA-3' and for *SLCO1B1* $T^{521}C$ (rs4149056) variant, following sets of primers were used: forward primer 5'-TTGTCAAAGTTTGCAAAGTG-3' and reverse primer 5'-GAAGCATATTACCCATGAGC-3'. For amplifying a DNA fragment for the*SLCO1B3* ($A^{1683\text{-}5676}G$, rs11045585) variant following sets of primers were used: forward primer 5'-GTGGGTAAAAGGCAGGTAAATG-3' and reverse primer 5'-GAATTCAAACATCTC ACTGTGCTC-3'. The PCR mixture of 20 µl comprised of 1X PCR buffer, 0.5 µM of forward and reverse primer, 1.5 mM $MgCl_2$, 100 µg/ml bovine serum albumin (BSA), 200 µM dNTPs, 1U Taq polymerase (DNAzyme, Thermo Scientific), and 200 ng of DNA. PCR conditions used for the mixture were: 95@C for 5 min and 94@C for 30s (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 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. 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), variant alleles were identified as AA (162,68 bp), and heterozygous alleles produced both bands (162,125,68,37 bp) (**Figure2a**).[10] For$A^{388}G$, the PCR product of 214 bp was checked on 2.0% agarose gel and then digested 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 heterozygous displayed all the bands**(Figure-2b)** . 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 variant alleles were identified as CC (189, 20 bp) (**Figure2c**).[19] For$A^{1683\text{-}5676}G$, a PCR product of 286 bp was digested with 5U of *Rsa I* restriction enzyme (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, and heterozygous displayed all the bands **(Figure-2d)** .[20]Digested fragments were separated on 8 % Native- polyacrylamide gel electrophoresis (PAGE) and were stained with the ethidium bromide and visualized under 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.

Statistical analysis

The study focused on the North Indian population and included age, gender, and smoking behavior information. The Chi-square goodness-of-fit test was applied to see if the cases followed Hardy-Weinberg equilibrium. The odds of lung carcinoma risk were studied using MedCalc Statistical Software (version 14.8.1 MedCalc Software, Ostend, Belgium). Descriptive data are presented as median, mean ± standard deviation (SD), or number/ percentages. The odds ratio (OR) with the 95 % confidence interval (CI) evaluated using the logistic regression analysis for any toxicity. All the patients' overall survival (OS) was calculated from day one of chemotherapy to death or the last follow-up date. The univariate Kaplan-Meir method assessed OS time

using log Rank p-value and median OS time. After adjusting for other factors, multivariate Cox regression analysis was used to assess the independent effect of polymorphism on overall survival. In all analyses, a p-value of <0.05 was considered 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 hematological toxicity such as neutropenia, anemia, 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 has been systematically recorded during treatment.

Results

Patient characteristics and clinical predictors

Demographic characteristics of the case study group consisted of gender, age, smoking status, Tumor, node, metastasis (TNM) staging, pack-years, and various other clinical factors that are illustrated in **Table 1.** At the time of diagnosis, the lung cancer 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 of25.58+-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 carcinomasubjects 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. A total of 419 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.

Genotypic distribution of SLC variants

For *SLC19A1* $G^{80}A$ polymorphism, 195(31.9%) patients were homozygous for the GG genotype, 131 (21.4%) patients were homozygous for the AA genotype, whereas 284 (46.5%) patients were heterozygous (GA) **(Table 2).**While for *SLCO1B1* $A^{388}G$ polymorphism, 152 (24.9%) patients were harboring homozygous AA

genotype, 297 (48.6%) were heterozygous AG , and 161 (26.3%) were homozygoteGG 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. For the *SLCO1B3* $A^{1683\text{-}5676}G$ variant, 545 (89.34%) patients were harboring homozygous AA genotype, 64 (10.4%) were heterozygousAG , and 1 (0.16%) were homozygote GG variant. The genotypic frequencies for *SLC19A1* $G^{80}A$ was (χ2=2.08,df=2; p=0.14). The genotypic frequencies of *SLCO1B1* $A^{388}G$ (χ2=0.41,df=2; p=0.52) and *SLCO1B1* $T^{521}C$ polymorphism was (χ2=1.13, df=2; p=0.28) whereas for *SLCO1B3* $A^{1683\text{-}5676}G$ genotypic frequency was χ2=0.38,df=2; p=0.53. Therefore, genotypic distribution for these four polymorphic sites followed the Hardy-Weinberg equilibrium with a p-value> 0.05, as shown in **table 2.** So, these samples could be a Mendelian population in a state of genetic equilibrium.

Association of SLC genotype with overall survival

The correlation of the *SLC* polymorphisms with survival in 593 lung cancer samples was investigated. After three years, 83.1% (493) of patients had died, and 16.8% (100) had survived. The association of the*SLC* SNPs with lung cancer survival was investigated using both univariate and multivariate analysis after adjusting for smoking status, gender, age, tumor stage, ECOG,KPS, and histology. This analysis used four alternative models (co-dominant, dominant, recessive, and additive) to determine the relationship between *SLC* polymorphism and overall survival (OS) in lung cancer patients. As demonstrated in**Table 3** , the findings were presented as the best-fitted model. 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 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 versus 7.9; Log-rank p=0.41; HR=1.10). After applying the Cox regression model, results demonstrated poor survival outcomes in patients carrying mutant (GG) alleles when compared with patients carrying wild (AA) allele (HR=1.40, 95% I=1.0-1.97; p=**0.04**), as shown in **figure 3.** For*SLCO1B1* $T^{521}C$ polymorphism, 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.

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 versus 7.97; p=0.58). However, only one patient harbored 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.

Association of SLC polymorphism with histology and overall survival

We further assessed the role of SLC variants in predicting median survival of lung carcinoma patients based on histological subtypes. On analysis, we noted that ADCC patients harboring mutant genotype (AA) showed better survival outcomes compared to the ADCC subjects with wild-type genotype (GG) for *SLC19A1* $G^{80}A$ (MST=9.4 versus 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 harboring the mutant genotype (GG) showed poor survival outcomes when compared to combined wild type and heterozygous genotype (AA+AG) (MST=6.8 months versus 7.5 months; log-rank p=0.42). After multivariate analysis, the overall death risk of patients carryingGG 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 analyzing the association of*SLC19A1* $G^{80}A$ polymorphism with SCLC histology, our results 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 versus 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 4**). 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 prognostic significance for *the SLCO1B1* $A^{388}G$, $T^{521}C$, and *SLCO1B3* $A^{1683\text{-}5676}G$ polymorphism (**Supplementary Table 1).**

Association of SLC polymorphism with the chemotherapeutic regimen 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**Supplementary Table 2.** 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 versus 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**) as shown in **Supplementary Table 2** .

In the case of lung cancer subjects receiving carboplatin/cisplatin along with irinotecan, paclitaxel, or pemetrexed, no substantial correlation was seen between overall survival and *SLC19A1, SLCO1B1,* and *SLC19A1* polymorphism **Supplementary Table 2** .

Association of SLC polymorphisms with gender and OS

In lung carcinoma patients, 83.2% (404) of males and 82.4% (89) of females were dead at the time of analysis. 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 versus 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, ECOG, 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*SLCO1B1*$A^{388}G$ 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 versus 7.7 months; HR=1.35, 95% CI=1.01-1.81; p=**0.04**) as shown in **Supplementary Table 3** . 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)

Association of SLC polymorphism and chemotherapy response

Correlation between *SLC* polymorphism and the effectiveness of response rate and clinical benefits were studied using univariate logistic regression. **Supplementary Table 4** 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 none of the SLC polymorphisms might predict chemotherapeutic response or clinical benefit in a significant way.

Association of SLCgenotype with tumor 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 tumor extension (T3 versus T4), and metastasis (positive versus negative) as shown in **Supplementary Table 5.** 18.7% of lung cancer patients diagnosed with T4 tumor extension

carried mutant alleles for SLC19A1 $G^{80}A$ genotype compared to 30.3% patients in T3 tumor extension **(Table 5).** A decreased odds of developing T4 was observed in mutant genotype (AA) as compared to the patients categorized in T3 tumor extension (AOR=0.42, 95% CI=0.2-0.89; p=**0.02**). Similarly, when the comparison was made between the patients with T3 and T4 size in *SLCO1B1* $T^{521}C$, a decreased odds towards lung carcinoma was observed in the heterozygous (TC) genotype of 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, combining the two risk genotypes (TC +CC), increased risk for lung cancer was not observed in T4 compared to patients with T3 tumor 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 tumor size and its progression.

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 6a** 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 were a total of four-terminal nodes found. *SLC19A1* $G^{80}A$ 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 6b** . 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**Supplementary Table 6** . 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.

Association between SLC genotypes and toxicity

One of the current research goals was to look into 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) versus 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.

All chemotherapy-related adverse effects (AEs) were recorded for each treatment cycle. In hematological toxicities, we evaluated anemia, leukopenia, absolute neutropenia, and thrombocytopenia. In anemia, a total of 406 patients' toxicity data was available **(Table 6a)** . The table depicts that 25.1% of patients were categorized in grade 0 toxicity, and74.8% individuals were categorized in grade 1-4 toxicity. As observed for *SLCO1B1* $T^{521}C$ polymorphism, 7.14% of individuals harbored single copy mutant allele (TC).**Supplementary Table 8** 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) versus no/low-grade (grade 0-2) toxicity, we observed that 26.6% of patients in grade 3-4 ane-

mia 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). We did not find any association between the SLC polymorphisms other hematological toxicities such as leukopenia and other AEs due to chemotherapy (**Supplementary Table 7**).

For analysis of absolute neutrophil count (ANC), we compared intermediate/severe toxicity (grades 2-4) versus no/low-grade (grades 0-1) toxicity as shown in **Supplementary Table 9** . A total of 367 patients' toxicity data was available for ANC. For *SLCO1B1* $A^{388}G$ polymorphism, 16% (3) patients harbored the mutant genotype (GG). **Supplementary Table 9** depicts subjects with mutant genotype (GG) of$A^{388}G$ had a reduced risk of developing neutropenia toxicity (OR=0.42, 95% CI=0.10-1.76, log-rank-p=0.23) when compared to the individuals with wild type genotype (AA). After adjusting with confounding, factors like age, gender, regimen, and performance status, the decreased risk was consistent in mutant genotype (GG) as compared to wild-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 analyzed the impact of SLC variants in causing thrombocytopenia. As shown in**Table 7,** we compared low/intermediate/severe toxicity (grades 1-3) versus 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) 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 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**).

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) versus no/low grade (grades 1-2) toxicity**.** As shown in **table 6a** , data of 366 patients were available to assess the associationbetween 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**) as shown in **table 7** .

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 6b).** 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 8** . 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) versus 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 (** T**able 8)** . We did not find any association between the SLC polymorphisms, other gastrointestinal toxicities such as diarrhea and anorexia, and other AEs due to chemotherapy (**Supplementary Table 10**).

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. Given the effect of these polymorphisms on SLC transporter activity, it is essential to look into their role in lung cancer, as their expression levels could control the extent and duration of chemotherapeutic drug inflow, affecting patients' responses. Hence, understanding and minimizing inter-individual variation in drug responsiveness, toxicity, and sensitivity 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 influencing survival rates and their correlation with toxicity in lung cancer patients undergoing platinum-based chemotherapy treatment. Our results from North Indian lung carcinoma patients suggest that mutant genotype (AA) of $SLC19A1 G^{80}A$polymorphism 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.[21] A study by Liu*et al* . did not find any significant association for$RFC v G^{80}A$ polymorphism with lung cancer susceptibility.[22] However, we can explain the increased survival trend in lung cancer patients with mutant 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,[23]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 better survival in the patients with wild-type genotype (GG) in comparison to the mutant genotype (AA) of $SLC19A1\ G^{80}A$polymorphism.[24] 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 mutant 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)* .[25] 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.[26] 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.[27] *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.[28] These disparate results could be ascribed to ethnicity and clinical characteristics of patients, such as the methodology used to detect the expression of SLC and heterogeneity in tumor histology, all of which could play a decisive role.

Our results for $SLCO1B1\ T^{521}C$ polymorphism showed reduced survival in patients with mutant 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.[29] Due to this reduced activity of the transporter, patients harboring the variant allele (C) of$T^{521}C$ polymorphismmay have reduced inflow of drugs, which can explain the lower survival in lung cancer patients. On the contrary, Feng *et al.* has shown better survival in gastric cancer patients having mutant *(CC)* and heterozygous *(TC)*genotype.[30]

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. I t mediates folate uptake in the cell and transports antifolate chemotherapeutic agents. RFC1 is substantially expressed in lung cancer patients.[31] It has been reported that in adenocarcinoma cells, RFC1 proteins are overexpressed.[32]

Furthermore, studies have linked downregulation of the RFC1 protein to impaired drug transport, developing resistance.[24,33]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 results did not show any association of $G^{80}A$ polymorphism with the survival of the patients undergoing pemetrexed and cisplatin/carboplatin treatment. Our results align with the various studies on NSCLC.[34-36] 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.[37] 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.[38] 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.

We have also evaluated whether *SLC* polymorphisms were associated with toxicity among platinum-based chemotherapy-treated lung cancer patients. Our results showed that patients 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.[39] 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.[40] This may explain the reduced risk of developing 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 risk of anemia. The heterozygous genotype *(GA)* of $G^{80}A$polymorphism showed a substantial protective effect from hematological toxicity-anemia. Generally, folate is required for RBC production and is a typical target of chemotherapeutic agents.[41] These drugs impede folate metabolism, and 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 anemia. However, our results suggest that the heterozygous genotype *(GA)* of $G^{80}A$ polymorphism is associated with a reduced risk of developing anemia in lung cancer patients.

Our results showed a reduced risk of developing nephrotoxicity in patients harboring heterozygous genotype (AG) on comparing severe toxicity (grade 3-5) with the absence of any/ intermediate toxicity (grade 1-2). Mechanistically, the kidney is the main organ involved in eliminating platinum-based chemotherapeutic drugs.[42]Many drugs, particularly those with a propensity for nephrotoxicity, are eliminated in the urine by active tubular secretion in addition to glomerular filtration.[43] *In vitro* studies have shown that *OATP1B1* and *OATP1B3* transport cisplatin and carboplatin, and the toxicity of these drugs in human tumor cells was enhanced with increased *OATP1B3* mRNA expression.[4] Hilgendorf *et al.,* have reported low mRNA expression of *SLCO1B3* in the kidney.[44]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 cancer patients. We observed that heterozygous genotype of *SLC19A1* $G^{80}A$ and*SLCO1B1* $A^{388}G$ polymorphisms were associated with protective effects from gastrointestinal toxicity.Due to a scarcity of research, determining the actual incidence rate of chemotherapy-induced constipation across all cancer patients is challenging. Some studies state that *SLCO1B1* polymorphisms are correlated with the increased risk of gastrointestinal toxicity in acute lymphoblastic leukemia.[45] 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.[46,47] Several factors, including the patient's characteristics, dose schedule, the type of chemotherapy, and regimen employed, influence the severity and extent of chemotherapy-induced toxicity. 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 lung cancer. This could be because of public awareness or its similarity to other diseases like tuberculosis.Many patients who receive chemotherapy at an advanced stage of cancer cannot achieve the treatment's endpoint, weakening the research's scope on chemotherapy response.The majority of the people who came to PGIMER for treatment were from rural areas in Northern India.Since it was difficult for them to commute from their homes, many patients in remote areas did not regularly visit the hospital. Some patients left the clinic before or after six chemotherapy cycles, while others did not.

Due to the shortage of clinical data could not conduct progression-free survival analysis on the current study group.On the other hand, our study has several advantages, including enrollingmany lung cancer patients.Second, patients on platinum-based chemotherapy regimens were included, and everyone was treated at the same hospital.Third, patients were enrolled, and clinical parameter data was obtained separately, regardless of the information of SLC polymorphism.

Conclusion

In the present study, we have investigated the significance of four SLC polymorphisms on the survival of North Indian lung carcinoma patients undergoing platinum-based chemotherapy regimens. Briefly, 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.

When adopting this technique in the clinic, significant caution should be exercised to better and personalize therapeutic choices to predict prognosis and therapy outcomes. Our study evaluates 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 north Indian lung cancer patients.It is well understood that individual toxicities are determined by the platinum-based chemotherapy regimen used. In the future, further studies should be explored to analyze the impact of these SLC polymorphisms on the activity of SLC transporter in order to identify the involvement of a specific polymorphism in the pharmacokinetics, pharmacological activity, and toxicity of chemotherapeutic agents. Such insights could lead to pharmacogenetically enhanced and individualized drug dosing, correspondingto pharmacokinetic discrepancies in chemotherapeutic drugs between individuals.

Competing interests

All of the authors of this manuscript report that they have no conflict of interest

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Author contributions

P.S. collected information, performed molecular and statistical analyses, and wrote the first paper draft; S.S. assisted molecular analyses and reviewed the final version of the paper and acted as the corresponding author; N.S. provided with the blood samples and clinical data of the patients from PGIMER, Chandigarh.

Data availability statement

All the data generated or analyzed during this study are included in this article. Further queries can be directed to corresponding author.

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Figure legends

Figure 1: Schematic model of SLC transporters in organs necessary for drug distribution,

absorption and elimination

Figure 2a: 6.0% Native-PAGE of PCR products for the detection of SLC19A1 G80A polymorphism. Lane 1: Marker (M) (100 bp); Lane 2: Undigested PCR product; Lane 3, 6, 9: Homozygous wild (GG) genotype; Lane 4,7,10: Heterozygous (GA) genotype; Lane 5,8: Homozygous variant (AA) genotype

Figure 2b: 6.0% Native-PAGE of PCR products for the detection of *SLCO1B1* A388G polymorphism. Lane 1: Marker (M) (100 bp); Lane 2: Undigested PCR product; Lane 5, 8: Homozygous wild (AA) genotype; Lane 4,7,10: Heterozygous (AG) genotype; Lane 3,6,9: Homozygous variant (GG) genotype

Figure 2c: 6.0% Native-PAGE of PCR products for the detection of *SLCO1B1* T521C polymorphism. Lane 1: Marker (M) (100 bp); Lane 2,3,4: Homozygous wild (TT) genotype; Lane 5,8: Heterozygous (TC) genotype; Lane 6: Homozygous variant (GG) genotype

Figure 2d: 6.0% Native-PAGE of PCR products for the detection of *SLCO1B3* A1683-5676G polymorphism. Lane 1: Marker (M) (100 bp); Lane 2: Undigested PCR product; Lane 3,5,10: Homozygous wild (AA) genotype; Lane 4,6,9: Heterozygous (AG) genotype; Lane 7: Homozygous variant (GG) genotype

Figure 3: Kaplan Meier curves illustrating the association between overall survival in different genotypes of *SLCO1B1 (A388G)* polymorphism in lung cancer patients. (Note: AA represents wild-type genotype, AG represents heterozygous genotype, and GG represents homozygous for mutant genotype)

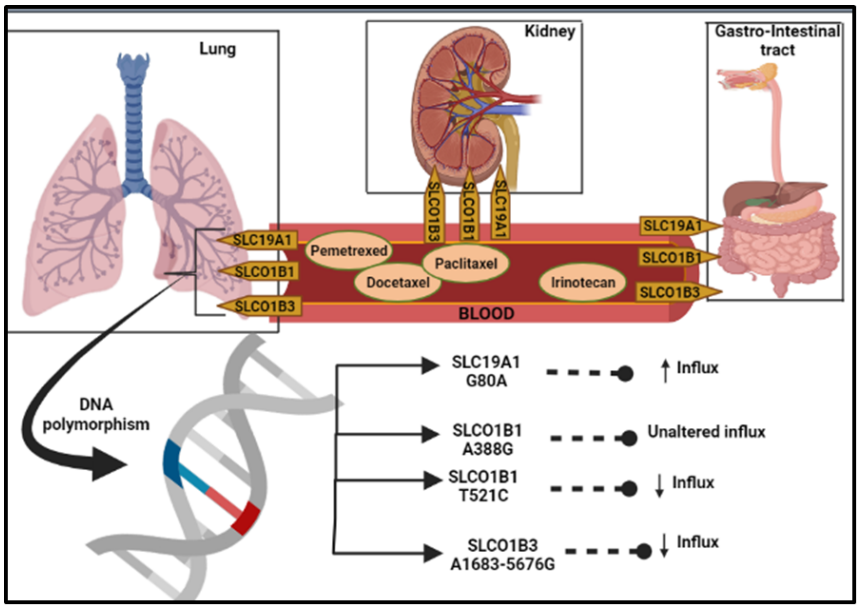
Figure 4: Kaplan Meier curves illustrating the association between overall survival in different genotypes of *SLC19A1 (G80A)*polymorphism in SCLC patients. (Note: GG represents wild-type genotype, GA represents heterozygous genotype, and AA represents homozygous for mutant genotype)

Figure 5: Kaplan Meier curves illustrating the association between overall survival in different genotypes of *SLCO1B3 (A1683-5676G)* polymorphism in patients administered with docetaxel cisplatin/carboplatin. (Note: AA represents wild-type genotype and AG represents heterozygous genotype)

Figure 6a: Recursive partitioning method (STREE) explaining correlation of *SLC* SNPs with overall survival in lung carcinoma patients

Figure 6b: Overall survival according to different nodes identified by survival tree analysis.

HR, hazard ratio; MST, median survival time



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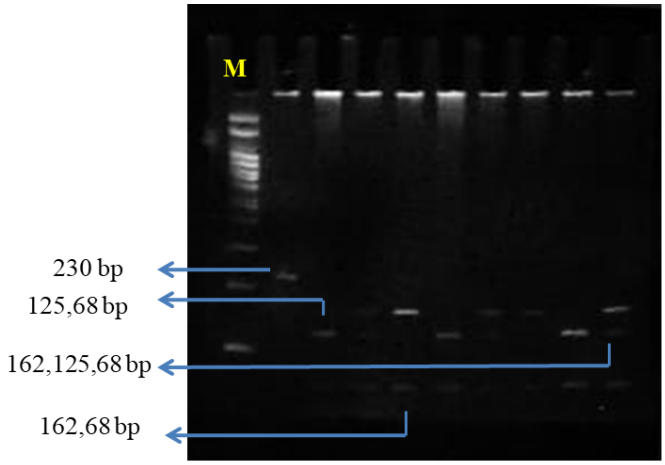

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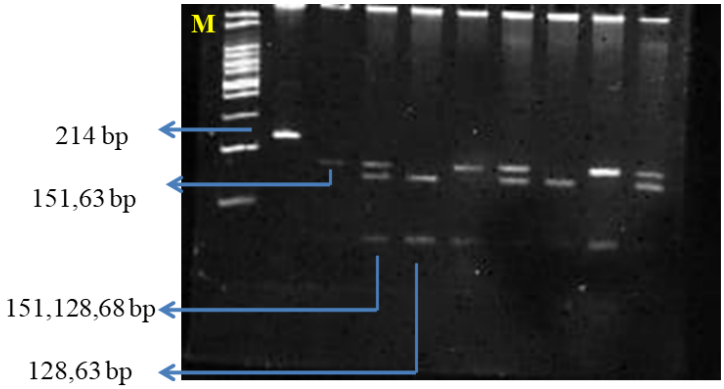

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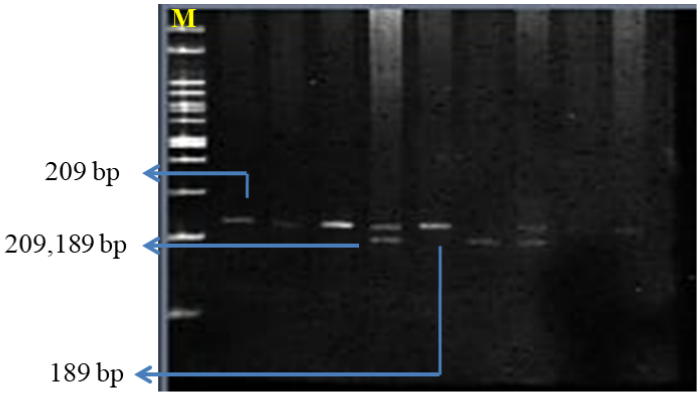

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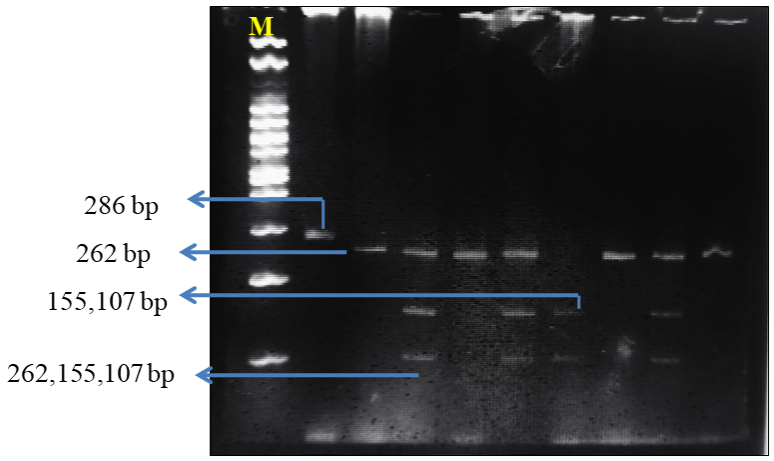

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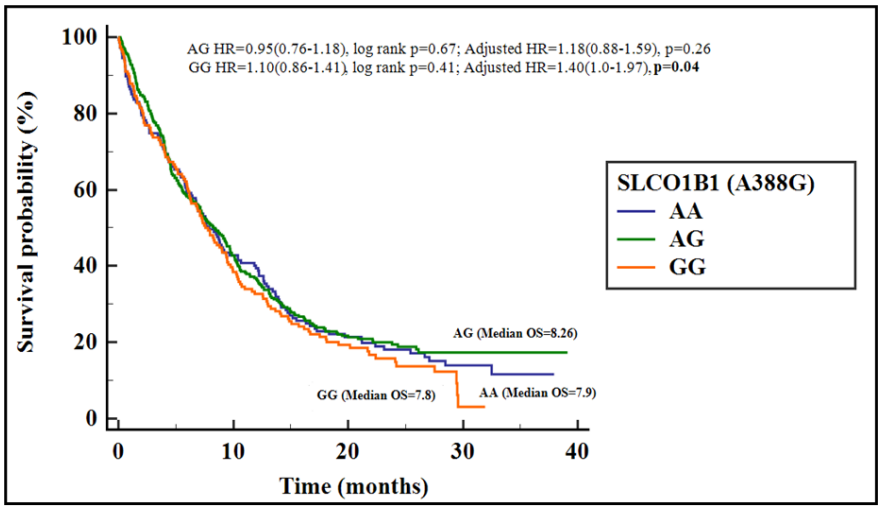

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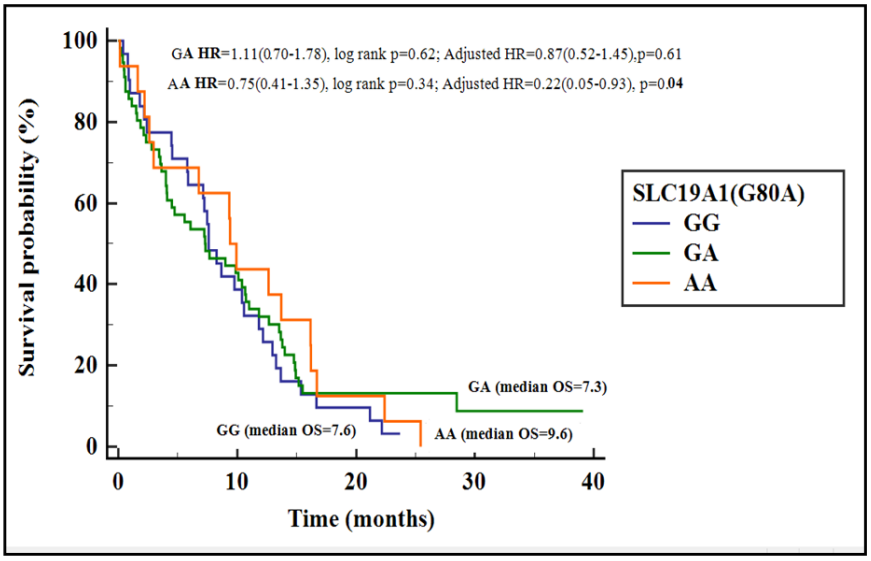

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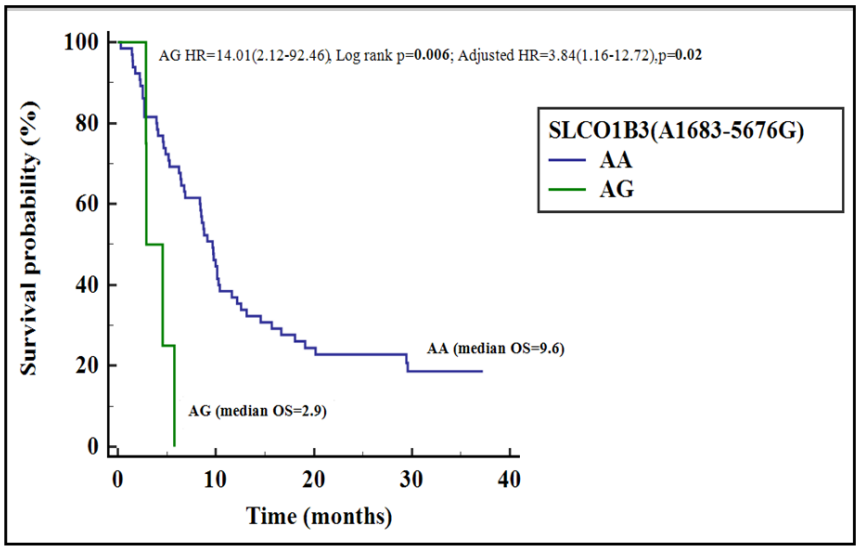

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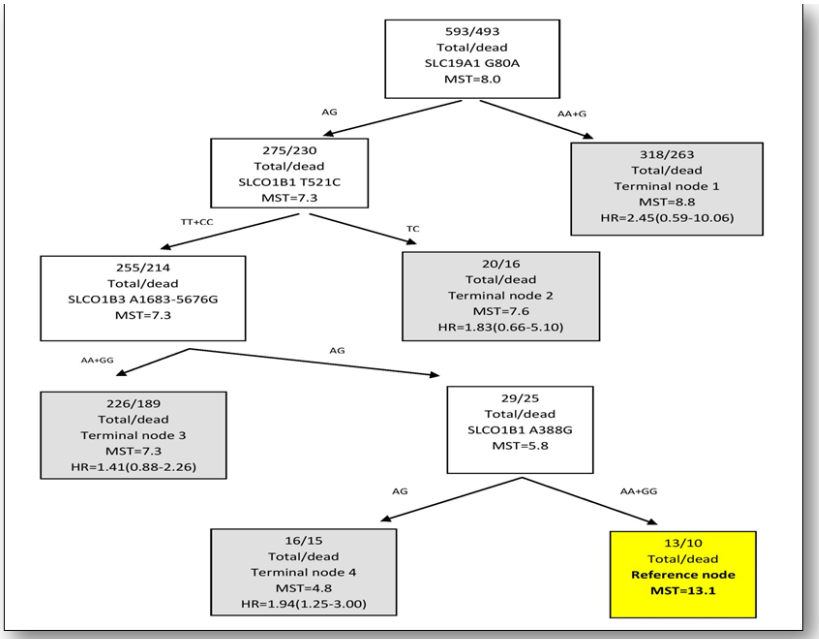

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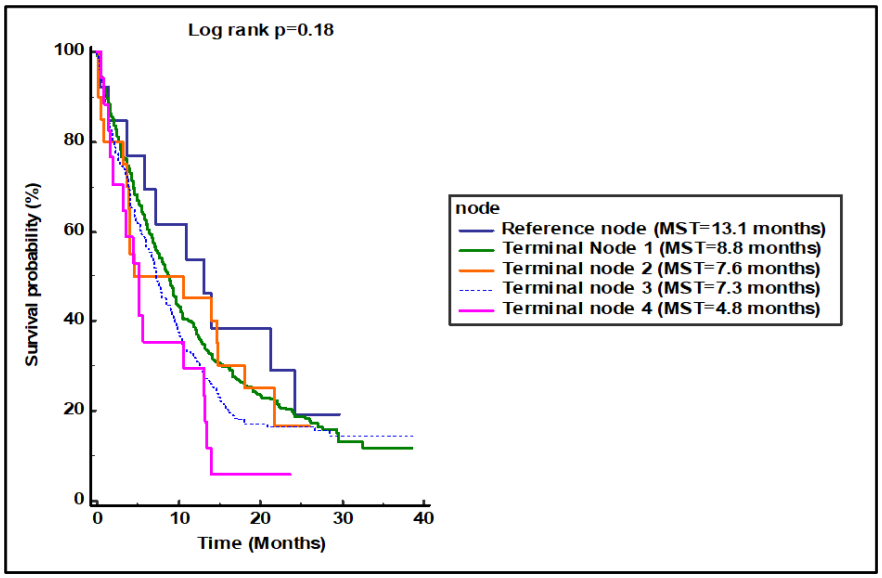

Figure 6b: Overall survival according to different nodes identified by survival tree analysis. HR, hazard ratio; MST, median survival time

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