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PharmGKB summary: Gemcitabine Pathway

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Introduction

Gemcitabine (2', 2'-difluoro 2'deoxycytidine, dFdC) is a cancer drug of the anti-metabolite class. It is a deoxycytidine analog that interferes with DNA synthesis by incorporating into elongating DNA, and indirectly interferes with DNA replication by inhibiting the nucleoside salvage pathway. Gemcitabine is a "first line treatment" in various types of solid tumors including pancreatic, non-small cell lung cancer (NSCLC), breast cancer, and some blood cancers, such as non-Hodgkin's lymphoma [1]. Gemcitabine is administered intravenously, or with injection, and it can be administered alone, or in combination with other antimetabolites such as fluorouracil, DNA damaging agents such as cisplatin, or various other chemotherapeutic agents [2]. Although the pharmacokinetic pathway of gemcitabine is similar to other deoxycytidine analogs it has certain attributes that render it a more broadly efficacious anti-metabolite [3].

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Conflict of Interest
The authors have no conflicts of interest.

Pharmacokinetics

Gemcitabine is a hydrophilic molecule, and three nucleoside transporters mediate most of its uptake into cells: SLC29A1 SLC28A1, and SLC28A3 (see Figure 1). Gemcitabine is also a pro-drug that requires serial phosphorylation by multiple kinases to become pharmacologically active. Deoxycytidine kinase (DCK) catalyzes the initial, and rate-limiting monophosphorylation of gemcitabine to gemcitabine monophosphate (dFdCMP). Phosphorylation to gemcitabine di- (dFdCDP) and tri- phosphate (dFdCTP) is catalyzed by UMP/CMP kinase (CMPK1) and nucleoside-diphosphate kinase (NDPK, NME), respectively. The majority (~ 90%) of intracellular gemcitabine is inactivated by deamination by cytidine deaminase (CDA) to form 2'2' difluorodeoxyuridine (dFdU). Additional inactivation steps include deamination of gemcitabine monophosphate by deoxycytidine deaminase (DCTD) and dephosphorylation of dFdCMP by cytosolic 5' nucleotidases (NT5C) [3](see Figure 1).

Pharmacodynamics

Gemcitabine diphosphate (dFdCDP) depletes a cells deoxyribonucleotide (dNTP) pools via inhibition of ribonucleotide reductase 1 (RRM1). RRM1 is a sub-unit of an enzyme complex that catalyzes the formation of deoxyribonucleotides (dNTPs) from ribonucleotides (rNTPs) through the nucleoside salvage pathway [4, 5]. Gemcitabine triphosphate (dFdCTP) integrates into elongating DNA, and prevents base-excision repair by allowing for a native dNTP to be added next to it, which is termed "masked chain termination". This irreparable error leads to inhibition of DNA synthesis, and eventually apoptosis [6, 7]. Decreasing dNTP pools increasingly favor gemcitabine uptake, and low concentrations of native deoxycytidines (dCTP) promote DCK activity, and inhibit DCTD activity. The attribute of "self-potentiation" is part of why gemcitabine is so widely used as part of a "first-line" chemotherapy treatment.

Pharmacogenomics

Given the widespread use of gemcitabine, its narrow therapeutic index, and inter-individual variability in patient response it is reasonable to expect that pharmacogenomics could be used to specifically tailor gemcitabine dosage to patients. Small study sizes, heterogeneity of the samples, including of the types and stages of cancers, and differences in chemotherapy make comparing patient responses to gemcitabine between groups very difficult.

Currently, intrinsic and acquired resistance to gemcitabine has been attributed to variation in the expression of genes involved in the transport, activation, and inactivation of gemcitabine, as well as its molecular target. Although gemcitabine is generally well-tolerated, hematological toxicities such as neutropenia are commonly reported. A certain percentage of patients also experience serious, and life-threatening complications after administration of gemcitabine. Decreased activity of the principal inactivating enzyme, CDA, is reported to be associated with toxicity, but further studies are needed to validate these findings [8]. Nucleoside diphosphate kinase (NDPKs) are less intensively studied than other genes in the gemcitabine pathway, although there are indications that alterations in their expression may affect gemcitabine resistance and prognoses in some patients [9, 10]

Variants in Transporters

Solute Carrier Family 29

The SLC29A1 transporter (also called the nitrobenzylthioinosine sensitive human equalibrative nucleoside transporter, or hENT1) is a widely expressed, plasma membrane, facilitated diffusion nucleoside transporter, with a broad selectivity for purines and pyrimidines. Kinetic studies show that the SLC29A1 transporter is the primary mediator of gemcitabine uptake into the cell and that cells that are *SLC29A1* null are highly resistant to gemcitabine. SLC29A2 also has a broad affinity for purines and pyrimidines, but its gene expression is most heavily concentrated in skeletal muscle. Structurally, SLC29A1 and SLC29A2 share 46% amino acid sequence identity, and the sequences with the highest homology are their respective transmembrane helices, loops, and hydrophobic terminals [11].

Multiple studies, including a recent meta-analysis, provide strong evidence of a positive relationship between increased *SLC29A1* expression and improved survival outcomes in cancer patients treated with gemcitabine [12-15]. *SLC29A1* is not highly polymorphic, however, and no genetic variants in the coding region of *SLC29A1* have shown significant associations with patient response to gemcitabine. Most reported associations are with genetic variants in non-coding regions. Table 1 summarizes several association studies investigating SNPs in *SLC29A1*, but none show consistent associations with patient outcomes. There are very few studies investigating associations between SNPs in *SLC29A2* with patient response to gemcitabine, and like *SLC29A1*, no genetic variants in *SLC29A2* are consistently associated with patient response to gemcitabine. One paper does report a trend of association between decreased protein levels of SLC29A2, and decreased incidence of complete remission in patients with acute myeloid leukemia (AML) when treated with mitoxantrone and gemcitabine [16]. Further evidence of association is needed to validate the relevance of *SLC29A2* for pharmacogenomic studies of gemcitabine.

Solute Carrier Family 28

SLC28A1 and SLC28A3 nucleoside transporters (also called the human concentrative nucleoside transporters, hCNTs) have a high affinity for pyrimidines, and although the SLC28A2 transporter preferentially transports purines it is also capable of transporting pyrimidines. SLC28A1 and SLC28A2 transporters are enriched in the epithelial cells of the kidney, intestine, and liver, and SLC28A3 is widely distributed in various tissues. The tissue distribution of SLC28A1 and SLC28A2 suggests that they play critical roles in the systemic absorption of nucleosides and nucleoside analogs [11].

Expression of *SLC28A1* and *SLC28A2* varies by tissue type, between individuals, and between cancerous and non-cancerous cells [17]. Cells derived from resected pancreatic tumors, and other cancerous cells tend to have lower *SLC28A1* expression compared to non-cancerous cells [17, 18]. In addition, cells with lower levels of SLC28A1 are shown to be resistant to gemcitabine, but they can be re-sensitized with constitutive expression of *SLC28A1* [18] Most reported polymorphisms in *SLC28A* genes are in coding regions, and not in regulatory regions [11]. Despite being more polymorphic than *SLC29A* genes, no

SNPs in *SLC28A1*, or *SLC28A2* are definitively associated with patient outcomes, or risk of toxicity in patients treated with gemcitabine[19-22]. A recent study reports that the *C* allele at rs7867504 is associated with decreased formation of gemcitabine triphosphate in patients with cancer[23]. Tables 2,3, and 4 summarize the results of pharmacogenomic studies investigating genetic variants of *SLC28A* genes and associations with outcomes in cancer patients treated with gemcitabine.

Variants in Metabolizing Enzymes

Cytidine deaminase

Ninety percent of the gemcitabine that comes into a cell is deaminated by cytidine deaminase (CDA) to 2'2' difluorodeoxyuridine (dFdU), which is much less toxic than gemcitabine. A common concern with cancer drugs is the need to balance the toxicity that is intended for cancer cells with the incidental toxicity that occurs in non-cancerous cells. Decreased expression of *CDA* is associated with increased efficacy of gemcitabine, but an increased risk of toxicity [24]. Three SNPs in *CDA* have been investigated in multiple pharmacogenomic association studies, the results of which are summarized in Table 5. The associations between these SNPs with patient response are inconsistent, or require further investigation.

Neutropenia is the most frequently reported adverse effect of gemcitabine, and risk of neutropenia is predicted to be associated with decreased CDA enzymatic activity. rs2072671 (A>C) changes the protein sequence of CDA by substituting a lysine residue for a glycine (Lys27Gln). At least five *in vitro* studies report that the A allele of rs2072671 is associated with decreased CDA enzymatic activity, or decreased CDA expression, compared to the *C* allele, although one *in vitro* study reports no association between the *C* allele and CDA enzymatic activity [25-29]. If the A allele at rs2072671 is associated with lower enzymatic activity of CDA relative to the C allele, then patients with the AA genotype should have a higher risk of neutropenia, or toxicity compared to patients with the *CC* genotype. Two studies support this prediction and report an increased risk of neutropenia associated with the AA genotype [25, 30]. However, three studies report an increased risk of toxicity, including neutropenia associated with the C allele compared to the A allele [22, 31, 32] and one reports no association with neutropenia [19]. In a study that reports no association between serum CDA enzymatic activity with the C allele, an AC genotype patient died after co-administration of gemcitabine and cisplatin. The patient's serum CDA enzymatic activity (measured as units of activity/mg of CDA protein) was 75% lower than the reference mean of the cohort [33], suggesting that CDA activity, perhaps more than individual SNPs, can predict risk of toxicity in patients administered gemcitabine. Associations between rs2072671 with survival outcomes, or response to gemcitabine are similarly inconclusive. Two studies report no association between rs2072671 with patient outcomes [19, 21], and one reports an association between the A allele with improved outcomes in patients with non-small cell lung cancer (NSCLC) [25].

Unlike, rs2072671, rs1048977 (C>T) is a synonymous variant (Thr145Thr). Investigations of this SNP have yielded inconclusive results regarding its effect on CDA enzymatic activity. One study reports that the TT genotype is associated with increased expression of

CDA in peripheral mononuclear blood cells (PBMCs) from healthy volunteers, which would be expected to enhance CDA enzymatic activity [27]. An independent study, however, reports no association between rs1048977 and enzymatic activity of CDA when assessed in whole blood from healthy volunteers [28]. Two studies report no association between the C allele and patient outcomes [21, 22]. Another reports an association between the CC genotype with improved response to gemcitabine, including a longer median time to progression, but no association with risk of hematologic toxicity [19]. Finally, a study investigating possible associations between SNPs with changes in gemcitabine pharmacokinetics reports that there is an association between the CC and CT genotypes with increased clearance of dFdU, the product of gemcitabine metabolism by CDA [34].

The rs60369023 (G >A) variant, which has only been reported in Korean and Japanese patients [8, 21, 33, 35], changes an alanine residue to a threonine (Ala70Thr). One *in vitro* study reports that the A allele is associated with decreased CDA enzymatic activity, and a second *in vitro* study reports that the A allele is associated with decreased CDA activity towards AraC, another deoxycytidine analog, but not towards gemcitabine [26, 36]. Although this variant has not been extensively studied the evidence of an association between it and CDA enzymatic activity is compelling. In a small study of 6 Japanese cancer patients, the only person that was homozygous for the A allele suffered from severe neutropenia and abnormally high plasma levels of gemcitabine, and abnormally low levels of dFdU [37]. In another study of over 200 Japanese cancer patients, the A allele was associated with decreased dFdU levels, and elevated AUC and C_{max} levelsof gemcitabine. Within the same cohort, the only patient that was homozygous for the A allele had abnormally high plasma levels of gemcitabine, and abnormally low plasma levels of dFdU. In addition, when platinum or fluorouracil were co-administered with gemcitabine, the A allele became significantly associated with neutropenia. Finally, the same study also quantified CDA enzymatic activity in a subset of patients (n=120), and reports that the A allele correlated with reduced enzymatic activity, providing further evidence of an association between the A allele with an increased risk of neutropenia, especially for patients co-administered platinum or fluorouracil [38]

Deoxycytidine Kinase

Gemcitabine is monophosphorylated by deoxycytidine kinase (DCK) to form 2′2′ difluorodeoxycytidine 5′ mono-phosphate (dFdCMP, gemcitabine monophosphate). In cancer cell lines prolonged exposure to gemcitabine correlates with decreased expression of *DCK*, and is associated with acquired resistance to gemcitabine [39, 40]. *In vivo* studies show an association between decreased DCK protein levels with shorter progression free survival in patients treated with gemcitabine [14, 41, 42]. Despite some well-established correlations between DCK protein levels with resistance to gemcitabine, no SNPs in *DCK* have shown a consistent association with patient response, or risk of toxicity in clinical studies [21, 22, 26, 43]. The results of those studies are summarized in Table 6.

One study reports that the TT genotype at rs4694362 (C>T), an intronic SNP, is not significantly associated with patient outcomes, but that it is associated with increased risk of neutropenia in pancreatic cancer patients [22]. A second study reports that the T allele is

associated with a more rapid disease progression, but only when it is part of a haplotype with seven other SNPs [20]. A third study reports that there is no association between the T allele with patient survival in metastatic breast cancer patients [21].

Despite a lack of association between variants in *DCK* and patient response to gemcitabine, there are well-established associations between variants in *DCK* and response to Ara-C, which is metabolized by DCK in a similar way to gemcitabine [44]. One study reports an association between the GT haplotype at rs80143932 and rs2306744 and a greater incidence of complete remission after the first induction of AraC with relapse free survival for six months after treatment [43]. The frequency of this haplotype is high in Asian patients and low in Caucasians (15.6% and 2%, respectively), and is also associated with increased *DCK* expression *in vitro* as well as *in vivo* [43, 45]. Although it has not yet been studied in patients receiving gemcitabine the associations between this haplotype with response to AraC may inform future investigations with gemcitabine.

Deoxycytidylate deaminase

Deoxycytidylate deaminase (DCTD) deaminates, and inactivates, gemcitabine monophosphate to difluorodeoxyuridine monophosphate (dFdUMP). The few studies investigating whether there are associations between genetic variants in *DCTD* and esponse to gemcitabine are summarized in Table 7. rs35932500 (T>C) is associated with decreased DCTD activity *in vitro*, but this has not been tested in clinical studies. One pharmacokinetic study reports significant associations between two SNPs in the 3' UTR of with formation of dFdCTP [34]. A synonymous coding SNP, rs4742 (A>C), has also been evaluated in patients receiving gemcitabine, but it has not shown any significant association with either risk of neutropenia, or with patient response[19, 22].

Cytidine mono-phosphate kinase 1

Cytidine mono-phosphate kinase 1 (CMPK1) phosphorylates gemcitabine monophosphate to form 2'2'-difluorodeoxycytidine 5' diphosphate (dFdCDP, gemcitabine diphosphate). dFdCDP inhibits the activity of ribonucleotide reductase, which replenishes cellular dNTP pools via the nucleoside salvage pathway. Table 8 summarizes the reported associations between SNPs in *CMPK1* with patient response to gemcitabine. The reported associations between patient survival and SNPs in *CMPK1* are inconclusive and require further investigation. The CC genotype rs1044457(C>T), a 3'UTR SNP, is associated with decreased formation of dFdCTP in patients with a variety of solid tumors [34], and a study of pancreatic cancer patients reports that the *CC* genotype is associated with improved overall survival, and a longer time to tumor and disease progression [20]. A third study in lung cancer patients reports no association between the CC genotype in rs1044457 and patient response [46].

5' Nucleotidases

5' nucleotidase (NT5C) 2 and 3 catalyze the de-phosphorylation of gemcitabine mono-phosphate back to gemcitabine [1]. Increased expression of *NT5C* genes are associated with resistance to nucleoside analogs such as AraC and gemcitabine, and increased expression is

predicted to also be associated with decreased survival in patients [47, 48]. Little is known about which specific genetic variants in *NT5C* or *NT5C3* are responsible for variability in gene expression, much less which specific variants could influence patient outcomes. One study reports positive associations between specific genetic variants in genes that are involved in gemcitabine metabolism, including *NT5C2*, and *NT5C3*, with various pharmacokinetic endpoints including gemcitabine clearance, and dFdCTP formation clearance (the fraction of gemcitabine that forms gemcitabine triphosphate) [34]. The study reports that three SNPs in *NT5C2* (rs1926029, rs3740387, rs11598702), and two SNPs in *NT5C3* (rs3570117, rs6946062), are associated with inter-individual differences in gemcitabine pharmacokinetics. It is worth noting, however, that this exploratory study of 37 SNPs did not do corrections for multiple hypothesis testing, thus the reported associations merit further investigation.

Variants in drug targets

Ribonucleotide Reductase 1

Ribonucleotide reductase (RNR) catalyzes the synthesis of 2' deoxyribonucleotides (dNTPs) from ribonucleotides. RNR is a complex that consists of a large and small subunit, and each sub-unit is composed of a ribonucleotide reductase homo-dimer. The large sub-unit is a homo-dimer of ribonucleotide reductase 1 (RRM1), and the small sub-unit is a homo-dimer of ribonulceotide reductase 2 (RRM2). RRM1 can also form an active complex with a homo-dimer of p53 inducible RRM2b, which is an isoform of RRM2 [49]. Gemcitabine diphosphate inhibits RRM1 by binding to its active site. This depletes a cell of intracellular dNTPs because RRM1 maintains cellular dNTP pools via the nucleoside salvage pathway. Less is known about the association between genetic variants in *RRM2* and *RRM2b* gene expression, and gemcitabine resistance *in vitro* [24, 50]. In contrast, RRM1 has been thoroughly studied in regards to the effects of gemcitabine.

In addition to maintaining cellular dNTP pools, RRM1 also acts as a tumor suppressor. High levels of expression are associated with reduced cell migration *in vitro*, which has been confirmed in a syngeneic mouse model where RRM1 was also found to mediate the inhibition of cell migration, invasion, and metastasis, through PTEN, a critical tumor-suppressor [51, 52]. An *in vitro* study in a lung cancer cell line reports that prolonged gemcitabine exposure correlates with increased expression of *RRM1*, and gemcitabine resistance [51]. The association between high levels of RRM1 and gemcitabine resistance has been confirmed in multiple independent studies, which also show that gemcitabine resistant cells can be re-sensitized by siRNA knockdown of *RRM1* [53-55]. Despite some conflicting results [56], current evidence supports a correlation between lower baseline *RRM1* expression levels with increased time to tumor progression, and increased overall survival in patients treated with gemcitabine [49, 57, 58].

One of the most important discoveries regarding RRM1 is that higher baseline levels of RRM1 are correlated with improved outcome in cancer patients that are not treated with gemcitabine. In a study of non-small cell lung cancer (NSCLC) patients who were treated with surgery only, patients with higher baseline RRM1 protein levels in resected tumor samples had better outcomes than patients with lower baseline RRM1 protein levels [59].

Lower baseline RRM1 protein levels in tumors predicted improved response in patients treated with gemcitabine, but higher baseline RRM1 protein levels predicted improved response in patients treated with surgery only [60]. In patients treated with surgical resection, the TT genotype at rs11030918 (T>C) is associated with increased progression-free survival when it is part of a haplotype with a second *RRM1* SNP, rs12806698 (C>A) [61].

The results of association studies investigating genetic variants in *RRM1* with patient response to gemcitabine are summarized in Table 9. Two studies report that rs9937 (A>G) is associated with an increased risk of toxicity (hematological, and neurological toxicity), but its association with patient survival is unclear. One study reports that the G allele (when part of a haplotype with the A allele at rs1042858) is associated with a decreased risk of hematologic toxicity, but lower overall survival in breast cancer patients receiving gemcitabine monotherapy [62]. Another study reports that the AA genotype is associated with shorter progression free survival, and an increased risk of neutropenia [22]. A third study reports that the AA genotype is associated with an increased risk of neurotoxicity, but is not associated with survival outcomes in metastatic breast cancer patients [21]. All three studies provide some evidence of association between the A allele with an increased risk of adverse reactions, but the associations with survival are inconsistent. Although rs11030918 (T>C) is reported to be associated with survival in patients treated with resection, it is not associated with survival in patients administered gemcitabine. One study reports an association between the T allele at rs11030918 with an increased risk of neurotoxicity (when it is part of a haplotype with three other SNPs), and a second study reports an association between the C allele and a decreased incidence of nausea and vomiting [21, 22]. Given all the known biological functions of RNR in the development of cancer it is important to consider how the types of malignancy and the types of combination chemotherapies could result in the inconsistent associations that are reported here.

Conclusions and Future Directions

In this review, we have summarized the results of pharmacogenomic studies investigating patient variability in response to gemcitabine. None of the SNPs covered here are consistently associated with any clinically relevant phenotypes, which could be due to one of many factors. Heterogeneity of the patients, including differences in the type of malignancy, the stage of malignancy, the type of combination chemotherapy, and differences in the ethnic background of patients, makes comparing the results between studies very difficult. In addition many studies reported here include less than one hundred patients, so if the effect size of an individual SNP is small, true associations may be missed.

As an example, the *C* allele at rs2072671 (A>C) in *CDA* changes the amino acid sequence of the protein. Because of its critical role in detoxifying gemcitabine alterations in the protein sequence might increase a patient's risk of an adverse reaction to gemcitabine (e.g. neutropenia). In the five studies that investigated associations between rs2072671 with risk of neutropenia, four report an association and one does not. Two report an association between increased risk of neutropenia with the A allele [25, 30], and two report an association with the C allele [32]. All patients were treated with gemcitabine, but some were

also treated with radiotherapy, paclitaxel, erlotinib, platinum, or were sometimes treated with gemcitabine alone. In the study that reports no association between rs2072671 (A>C) with risk neutropenia, patients were a mix of Chinese, Malay, or Indian [19]. Only one of the five studies had more than 100 patients [22].

Variability of gene expression, and protein levels of SLC29A1, CDA, and RRM1 present the most compelling associations with patient response to gemcitabine. Two recent meta-analyses (12 studies, that included 875 pancreatic cancer patients, and 16 studies that included 632 pancreatic cancer patients), both concluded that a high level of SLC29A1 protein is prognostic of improved survival outcomes in patients administered gemcitabine compared to patients with low levels of SLC29A1 protein [12, 63]. Although more evidence is needed, it appears that low CDA enzymatic activity is a good predictor of whether patients are more likely to experience gemcitabine-associated toxicity [30, 33, 37, 38]. High baseline protein levels of RRM1 are also good predictors of not only whether patients are likely to develop resistance to gemcitabine, but whether they might derive more benefit from forgoing gemcitabine treatment in favor of surgical resection with curative intent [49].

In summary, gemcitabine pharmacogenomic studies investigating single nucleotide variants have yielded little in terms of clinically actionable findings. Fortunately, some relevant associations have come from association studies that investigate expression levels, or protein levels of key genes and proteins, respectively, of genes in the pharmacokinetic and pharmacodynamic pathways of gemcitabine. In addition, further research efforts extending beyond the known pharmacodynamic and pharmacokinetic pathways have uncovered additional biology associated with gemcitabine efficacy, and additional genes outside these pathways have already been identified to play a role in gemcitabine response [64, 65]. Ultimately, more research is needed to discover clinically relevant single nucleotide variants to improve upon current gemcitabine therapies for cancer patients.

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Abbreviations

dFdU	2’2’ difluorodeoxyuridine
dFdUMP	2’2’ difluorodeoxyuridine-5’- monophosphate
NDP	ribonucleotide diphosphate
dNDP	2’- deoxynucleotide diphosphate
dNTP	2’- deoxynucleotide triphosphate

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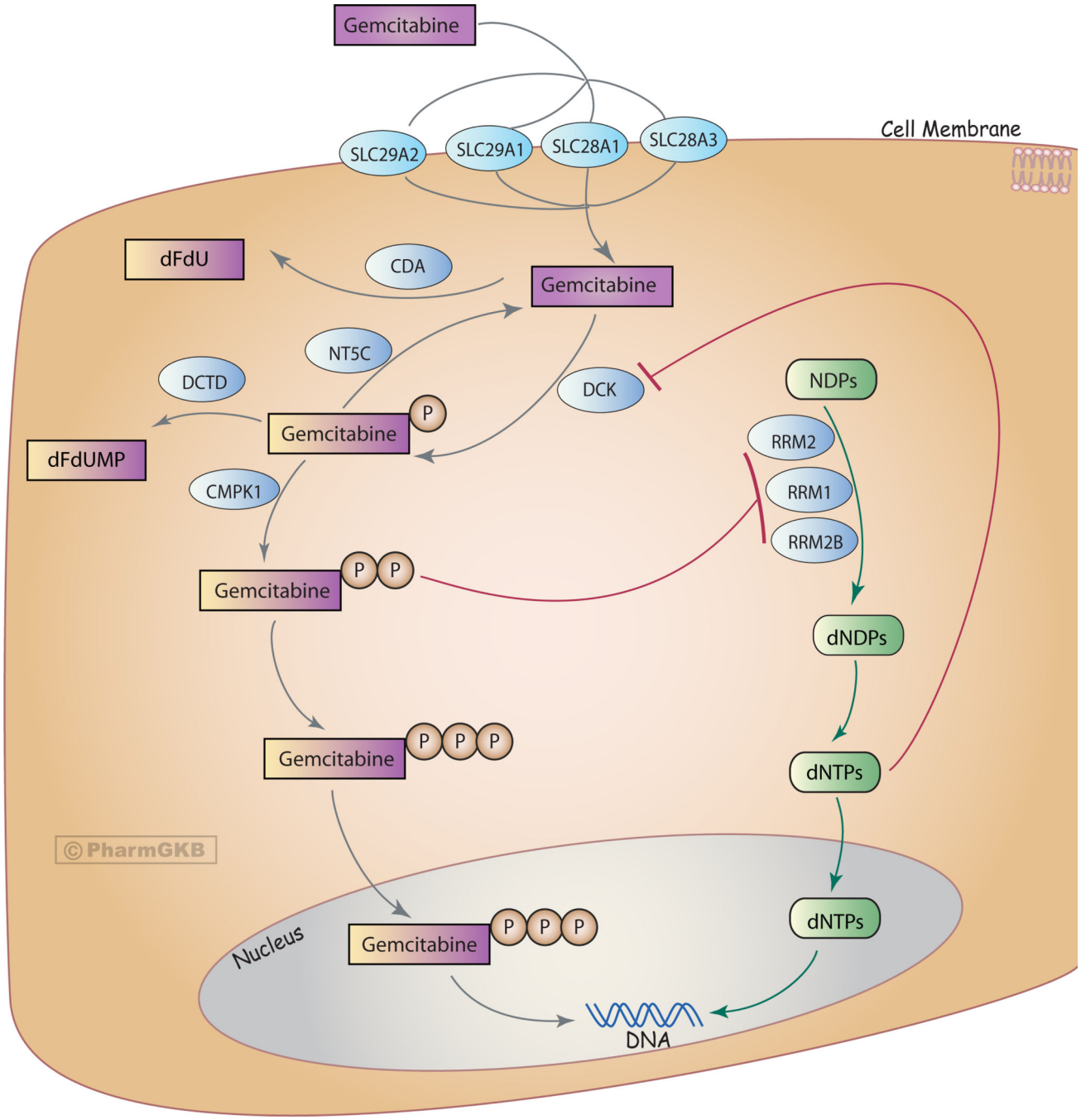

Figure 1.

Stylized cells depicting the metabolism and mechanism of action of gemcitabine. A fully interactive version is available at PharmGKB: http://www.pharmgkb.org/pathway/PA2036.

Table 1

Single nucleotide polymorphisms in *SLC29A1* and related phenotypes

rsID	Location/Effect	Findings	PMID
rs760370	201A>G	The A allele is associated with shorter overall survival compared with the G allele in people with metastatic breast cancer treated with a combination of gemcitabine and paclitaxel; this SNP is part of a haplotype with the rs747199 G allele.	24361227
rs760370	201A>G	The GG genotype is associated with worse tumor response compared to genotypes AA and AG in people with pancreatic cancer when treated with a combination of gemcitabine and radiotherapy, or platinum.	20665488
rs747199	−706G>C	The G allele is associated with lower overall survival compared with the C allele, in people with metastatic breast cancer treated with a combination of gemcitabine and paclitaxel; this SNP is part of a haplotype with rs760370 A allele.	24361227
rs747199	−706G>C	Both the G and C allele show a trend of association with increased expression of *SLC29A1* when part of a 3 SNP haplotype with rs731780 (C) and rs70914 (G) (*in vitro*).	16609362
rs731780	−1345 C>G	The C allele is associated with increased expression of *SLC29A1* when part of a 3 SNP haplotype with rs70914 (G) and rs747199 (G, or C); *in vitro*	16609362
rs70914	−1050G>A	The G allele is associated with increased expression of *SLC29A1* when part of a 3 SNP haplotype with rs731780 (C) and rs747199 (G, or C); *in vitro.*	16609362
rs9394992	29+913C>T	The CT and TT genotypes are associated with increased risk of neutropenia, but are not associated with response compared to genotype CC in people with pancreatic cancer when treated with gemcitabine and radiotherapy, or platinum.	20665488
rs3778504	−52+1681G>A	The AA and AG genotypes are associated with increased time to progression compared to genotype GG in people with cancer.	22838950

All alleles reported in the table are on the positive strand. Variants marked by an * are the complement of what is reported in the study.

Abbreviations: SNP (single nucleotide polymorphism).

Table 2

Single nucleotide polymorphisms in *SLC28A1* and related phenotypes

rsID	Location/Effect	Findings	PMID
rs2242047	1528C>T (Arg51Cys)	The CC genotype is associated with slower tumor progression compared to genotype TT in people with pancreatic cancer when treated with a combination of gemcitabine and erlotinib, or fluoropyrimidines.	22838950
rs2242047	1528C>T (Arg51Cys)	The CC genotype is not associated with response, or risk of hematologic toxicity compared to genotypes CT and TT in people with NSCLC when treated with a combination of gemcitabine and carboplatin.	18538445
rs2242046	+1561 G>A (Asp521Asn)	The GG genotype is associated with a decreased risk of hematologic toxicity compared to genotypes AG and AA in people with NSCLC, but is not associated with response to gemcitabine.	18538455

All alleles reported in the table are on the positive strand. Variants marked by an * are the complement of what is reported in the study.

Abbreviations: NSCLC (non-small cell lung cancer).

Table 3

Single nucleotide polymorphisms in *SLC28A2* and related phenotypes

rsID	Location/Effect	Findings	PMID
rs1060896	225C>A (Ser75Arg)	The CC genotype is associated decreased risk of hematologic toxicity (higher neutrophil counts, but no difference in platelet counts), and decreased response (shorter median survival time, but no difference in time to progression) compared to genotypes AA and AC in people with NSCLC when treated with a combination of gemcitabine and carboplatin. This SNP was found to be in strong LD with rs11854484.	18538445
rs1060896	225C>A (Ser75Arg)	The AA genotype is not associated with response compared to genotypes AC and CC in people with pancreatic cancer when treated with a combination of gemcitabine and radiotherapy, or platinum.	20665488
rs11854484	65C>T (Pro22Leu)	The CC genotype is associated decreased risk of hematologic toxicity (higher neutrophil counts, but no difference in platelet counts), and decreased response (shorter median survival time, but no difference in time to progression) compared to genotypes CC and CT in people with NSCLC when treated with a combination of gemcitabine and carboplatin. This SNP was found to be in strong LD with rs1060896.	18538445

All alleles reported in the table are on the positive strand. Variants marked by an * are the complement of what is reported in the study.

Abbreviations: SNP (single nucleotide polymorphism), NSCLC, (non-small cell lung cancer) LD (linkage disequilibrium).

Table 4

Single nucleotide polymorphisms in *SLC28A3* and related phenotypes

rsID	Location/Effect	Findings	PMID
rs7867504	267T>C (Thr89=)	The C allele is associated with decreased formation of dFdCTP compared to the T allele in people with cancer.	24300978
rs7867504	267T>C (Thr89=)	The CC and CT genotypes are associated with longer overall survival as compared to the TT genotype in people with metastatic breast cancer treated with a combination of gemcitabine and paclitaxel.	24361227
rs7867504	267T>C (Thr89=)	The CC genotype is not associated with tumor progression compared to genotypes CT and TT in people with pancreatic cancer when treated with a combination of gemcitabine and radiotherapy, or platinum.	20665488

All alleles reported in the table are on the positive strand. Variants marked by an * are the complement of what is reported in the study.

Abbreviations: dFdCTP (gemcitabine triphosphate).

Table 5

Single nucleotide polymorphisms in *CDA* and related phenotypes

rsID	Location/Effect	Phenotype	PMID
rs2072671	79A>C (Lys27Gln)	The C allele was not associated with CDA enzymatic activity compared to the A allele in patients with cancer when treated with gemcitabine and cisplatin. There was a toxic death case (severe hematological toxicity including leukopenia) in the single individual with the AC genotype, who also had CDA enzymatic activity less than 75% of the average of the cohort.	17885621
rs2072671	79A>C (Lys27Gln)	The AC and CC genotypes are associated with increased risk of severe neutropenia compared to genotype AA in people with late stage, or metastatic cancer (NSCLC, bladder, cholangiocellular carcinoma *etc*.). Some patients received gemcitabine monotherapy, others combination therapy with cisplatin.	22546611
rs2072671	79A>C (Lys27Gln)	The C allele is associated with increased frequency of nausea and vomiting compared to the A allele in people with mesothelioma that were treated with a combination of gemcitabine and platinum compounds.	22134350
rs2072671	79A>C (Lys27Gln)	The AA and AC genotypes are associated with increased risk of severe hematologic toxicity compared to genotype CC in people with pancreatic cancer that are treated with gemcitabine, but not in patients treated with fluorouracil.	21625252
rs2072671	79A>C (Lys27Gln)	The AA genotype is associated with a decreased risk of neutropenia compared to the AC and CC genotypes in people with pancreatic cancer when treated with a combination of gemcitabine and radiotherapy, or platinum.	20665488
rs2072671	79A>C (Lys27Gln)	The AA genotype is associated with increased risk of neutropenia or thrombocytopenia, and improved survival, including longer time to progression compared to genotypes AC and CC in people with stage III or IV NSCLC treated with gemcitabine and cisplatin. The A allele is also associated with decreased CDA enzymatic activity (*in vitro*).	18347182
rs2072671	79A>C (Lys27Gln)	The AA genotype is not associated with increased risk of neutropenia, or response compared to the AC and CC genotypes in people with NSCLC when treated with a combination gemcitabine and carboplatin.	18538445
rs2072671	79A>C (Lys27Gln)	The A allele is not associated with patient survival, or neurotoxicity compared to the C allele in people with metastatic breast cancer treated with a combination of gemcitabine and paclitaxel.	24361227
rs2072671	79A>C (Lys27Gln)	Genotype AA is associated with decreased activity of CDA in whole blood from healthy volunteers as compared to genotypes AC and CC (*in vitro*).	23651026
rs2072671	79A>C (Lys27Gln)	Genotype CC is associated with increased expression of CDA in PBMCs (*in vitro*).	21521023
rs2072671	79A>C (Lys27Gln)	The A allele is associated with decreased enzymatic activity compared to the C allele in cytoplasmic extracts from RBCs (*in vitro*).	18600531
rs2072671	79A>C (Lys27Gln)	The A allele is associated with decreased enzymatic activity compared to the C allele in in cells transfected with DNA from healthy Caucasian and African-American healthy volunteers (*in vitro*).	16551864
rs1048977	435C>T (Thr145=)	The TT genotype is associated with increased expression of CDA in peripheral mononuclear blood cells from healthy volunteers (*in vitro*)	21521023
rs1048977	435C>T (Thr145=)	The CC genotype is not associated with enzymatic activity of CDA in whole blood compared to genotypes CT and TT (*in vitro*).	23651026
rs1048977	435C>T (Thr145=)	The CC genotype is not associated with response compared to genotypes CT and TT in people with pancreatic cancer when treated with a combination of gemcitabine and radiotherapy, or platinum.	20665488
rs1048977	435C>T (Thr145=)	The C allele is not associated with response compared to the T allele in people with metastatic breast cancer treated with a combination of gemcitabine and paclitaxel.	24361227
rs1048977	435C>T (Thr145=)	The CC and CT genotypes are associated with increased elimination clearance of dFdU, and formation of dFdCTP when compared to the TT genotype in people with cancer.	22838949
rs1048977	435C>T (Thr145=)	The CC genotype is associated with increased response, but is not associated with hematological toxicity compared to genotypes CT and TT in people with NSCLC when treated with a combination of gemcitabine and carboplatin.	18538445

rsID	Location/Effect	Phenotype	PMID
rs60369023	208G>A (Ala70Thr)	The AA genotype is associated with severe risk of toxicity compared to genotype GG. This is a case study of a single patient with pancreatic cancer who was treated with a combination of gemcitabine and cisplatin.	15814642
rs60369023	208G>A (Ala70Thr)	The A allele is associated with decreased clearance of gemcitabine, and increased risk of neutropenia compared to the G allele in people with cancer when gemcitabine is combined with platinum or fluorouracil therapy.	17194903
rs60369023	208G>A (Ala70Thr)	The A allele is not associated with increased risk of neurotoxicity compared to the G allele in people with metastatic breast cancer when treated with gemcitabine and paclitaxel.	24361227
rs60369023	208G>A (Ala70Thr)	The A allele is not associated with catalytic activity of recombinant CDA protein towards gemcitabine (*in vitro*).	23230131
rs4655226	155-3137C>T	Genotype CC is associated with increased clearance of dFdU compared to genotype CT in people with cancer.	22838949

All alleles reported in the table are on the positive strand. Variants marked by an * are the complement of what is reported in the study.

Abbreviations: NSCLC (non-small cell lung cancer), CDA (cytidine deaminase), dFdU (2', 2' difluorodeoxyuracil), dFdCTP (gemcitabine triphosphate), PBMC (peripheral blood mononuclear cells).

Table 6

Single nucleotide polymorphisms in *DCK* and related phenotypes

rsID	Location/Effect	Findings	PMID
rs4694362	IVS6 – 1205C>T	The TT genotype was associated with an increased risk of neutropenia, but is not associated with response compared to the CT and TT genotypes in people with pancreatic cancer when treated with a combination of gemcitabine and radiotherapy, or platinum.	20665488
rs4694362	IVS6 – 1205C>T	The T allele is not associated with response compared to the C allele in people with metastatic breast cancer treated with a combination of gemcitabine and paclitaxel.	24361227
rs4694362	IVS6 −1205C>T	The T allele is associated with increased disease progression compared to the C allele when part of a haplotype (GGCCGTGT in rs3925058, rs7543016, rs4600090, rs9436883, rs35687416, rs1044457, rs7684954, rs4694362) in people with pancreatic cancer.	22838950
rs12648166	207+9846A>G	Genotype AA is not associated with response compared to genotypes AG and GG in people with pancreatic cancer when treated with a combination of gemcitabine and radiotherapy, or platinum.	20665488
rs12648166	207+9846A>G	The A allele is not associated with response compared to the G allele in people with breast cancer treated with a combination of gemcitabine and paclitaxel.	24361227
rs66878317	70A>G (Ile24Val)	The AG and GG genotypes are associated with increased clearance of gemcitabine compared to the AA genotypes in purified proteins (*in vitro*).	23230131
rs3775289	IVS1 −1110T>C	Genotype CC is not associated with response, or risk of neutropenia in people with NSCLC compared to genotypes CT and TT in people with NSCLC.	18538455
rs2306744	−201C>T	The T allele is associated with increased *DCK* expression both *in vitro* and *in vivo*, when part of a haplotype with the G allele of rs80143932 in people with AML. The two SNPs were found to be in strong LD. *	15564883
rs80143932	−360C>G	The G allele is associated with increased *DCK* expression both *in vitro* and *in vivo*, when part of a haplotype with the T allele of rs2306744 in people with AML. The two SNPs were found to be in strong LD.*	15564883

All alleles reported in the table are on the positive strand. Variants marked by an * are the complement of what is reported in the study.

Abbreviations: SNP (single nucleotide polymorphism), NSCLC (non-small cell lung cancer), AML (acute myeloid leukemia), DCK (deoxycytidine kinase), LD (linkage disequilibrium).

Table 7

Single nucleotide polymorphisms in *DCTD* and related phenotypes

rsID	Location/Effect	Findings	PMID
rs4742	348T>A, T>C, T>G (315 T>C) (Val105=)	Genotype TT is not associated with risk of neutropenia, or response to gemcitabine compared to genotypes CT and CC in people with NSCLC when treated with a combination gemcitabine and carboplatin.	18538445
rs4742	348T>A, T>C, T>G (315 T>C) (Val105=)	Genotype CC is not associated with response compared to genotypes CT and TT in people with pancreatic cancer when treated with a combination of gemcitabine and radiotherapy, or platinum.	20665488
rs12507552	C>T (458+759G>A)	The TT genotype is associated with decreased survival compared to the CT and TT genotypes in people with pancreatic cancer. When the T allele is part of a haplotype (CGATAACA in rs12507552, rs3886768, rs13147196, rs13137332, rs13116598, rs6823077, rs11730323) it is associated with increased tumor progression.	22838950
rs35932500	*T>C (172, 205A>G) (Asn69Asp, Asn58Asp)	The C allele is associated with decreased enzyme activity of DCTD compared to the T allele. The effect was seen in cells transfected with DNA from healthy Caucasian volunteers but not in cells transfected with DNA from healthy African-American volunteers (*in vitro*).	16551864
rs1130902	*506G>A	The AA and AG genotypes were associated with increased formation clearance of dFdCTP compared to the GG genotype in people with cancer.	22838949
rs6834938	*1739G>A	The CC and CT genotypes were associated with increased formation clearance of dFdCTP compared to the TT genotype in people with cancer.	22838949

All alleles reported in the table are on the positive strand. Variants marked by an * are the complement of what is reported in the study.

Abbreviations: dFdCTP (gemcitabine triphosphate), NSCLC (non-small cell lung cancer), DCTD (deoxycytidylate deaminase).

Table 8

Single nucleotide polymorphisms in *CMPK1* and related phenotypes

rsID	Location/Effect	Findings	PMID
rs1044457	*360C>T	The CC genotype was associated with improved overall survival, and a longer time to disease and tumor progression compared to the TT genotype in people with pancreatic cancer when treated with a combination of gemcitabine and erlotinib, or fluoropyrimidines.	22838950
rs1044457	*360C>T	The CC genotype is not associated with response to gemcitabine compared to genotype TT in people with NSCLC treated with gemcitabine and cisplatin.	21642870
rs1044457	*360C>T	The CC genotype is associated with decreased formation clearance of gemcitabine triphosphate compared to genotype TT in people with cancer.	22838949
rs4492666	171+1057A>C	The CC genotype is associated with shorter survival time compared to the AC and AA genotypes in people with NSCLC.	21642870
rs4492666	171+1057A>C	The A allele is not associated with response to gemcitabine compared to the C allele in people with breast cancer treated with a combination of gemcitabine and paclitaxel.	24361227
rs11211524	321-928A>C (172-928A>C)	The A allele is not associated with response to gemcitabine compared to allele C in people with breast cancer treated with a combination of gemcitabine and paclitaxel.	24361227
rs11211524	321-928A>C (172-928A>C)	The CC genotype is associated with decreased response compared to genotypes AA and AC in people with NSCLC and treated with a combination of gemcitabine and cisplatin.	21642870
rs35687416	240G>T (Gln80His)	The T allele is associated with improved overall survival and time to progression compared to the G allele in people with pancreatic cancer.	22838950
rs3925058	−1995G>A	The G allele is not associated with a moderate increase in time to progression compared to the A allele in people with pancreatic cancer.	22838950
rs7543016	22G>C (Gly8Arg)	The G allele is associated with an increased time to progression compared to the C allele in people with pancreatic cancer.	22838950

All alleles reported in the table are on the positive strand. Variants marked by an * are the complement of what is reported in the study.

Abbreviations: NSCLC (non-small cell lung cancer).

Table 9

Single nucleotide polymorphisms in *RRM1* and Related Phenotypes

rsID	Location/Effect	Findings	PMID
rs183484	1082C>A (Arg284Arg)	The CC genotype is not associated with overall survival compared to the genotypes AC and AA in people with NSCLC given a combination of gemcitabine and cisplatin.	21642870
rs183484	1082C>A (Arg284Arg)	The AC genotype is moderately associated with a decrease in progression free survival compared to genotypes AA and CC in people with pancreatic cancer; four SNPs in this study, including rs183484, were predictive of a decrease in progression free survival when using 0 or 1 variant as a reference. The other variants included rs2072671, rs760370, rs9937.	20665488
rs1042927	*316C>A	The C allele is associated with a decrease in overall survival compared to the A allele in people with mesothelioma.	22134350
rs1042927	*316C>A	The A allele is associated with an increased likelihood of neurotoxicity, but not patient response compared to the C allele in people with metastatic breast cancer that are treated with a combination of gemcitabine and paclitaxel; this SNP is part of a four SNP haplotype that includes rs1042927, rs11030918, rs9937, and rs720106.	24361227
rs11030918	−756T>C (−524T>C)	The TT genotype is not associated with risk of hematologic toxicity, or response to gemcitabine in people with NSCLC compared to genotypes CC and CT when treated with a combination of gemcitabine and cisplatin.	18538445
rs11030918	−756T>C (−524T>C)	The C allele is associated with a decreased occurrence of nausea and vomiting compared to the T allele in people with mesothelioma.	22134350
rs11030918	−756T>C (−524T>C)	The TT genotype is not associated with overall survival compared to the CT and CC genotypes in people with NSCLC given a combination of gemcitabine and carboplatin.	21642870
rs11030918	−756T>C (−524T>C)	The T allele is associated with increased risk of neurotoxicity compared to the C allele in people with breast cancer; this SNP is part of a four SNP haplotype that includes rs1042927, rs11030918, rs9937, and rs720106.	24361227
rs9937	2455 A>G (Thr741Thr)	The G allele is associated with a decreased risk of neutropenia and leukopenia compared to the A allele when part of a haplotype with the A allele at rs1042858 in people with breast cancer. The G allele is also associated with shorter progression-free survival, and lower overall survival when part of a haplotype with the A allele at rs1042858.	17602053
rs9937	2455 A>G (Thr741Thr)	The AA genotype is associated with shorter progression-free survival and increased risk of neutropenia when compared to genotypes AG and GG in people with pancreatic cancer when treated with a combination of gemcitabine and radiotherapy, or platinum.	20665488
rs9937	2455 A>G (Thr741Thr)	The AA genotype is associated with increased risk of neurotoxicity compared to the AG and GG genotypes in people with metastatic breast cancer when treated with a combination of gemcitabine and paclitaxel; this SNP is part of a four SNP haplotype that includes rs1042927, rs11030918, rs9937, and rs720106. This variant was not associated with overall, or progression-free survival.	24361227
rs1042858	42G>A (2464G>A) (A744A)	The AA genotype is not associated with progression free survival compared to genotypes AG and GG in people with pancreatic cancer when treated with a combination of gemcitabine and radiotherapy, or platinum.	20665488
rs1042858	42G>A (2464G>A) (A744A)	The A allele is associated with a decreased risk of neutropenia, leukopenia, shorter progression free survival, and lower overall survival compared to the G allele in people with breast cancer when part of a haplotype with rs9937 G allele.	17602053
rs1042858	42G>A (2464G>A) (A744A)	The AA genotype is not associated with progression free survival compared with the genotypes AG and GG in people with NSCLC.	20226083
rs12806698	(−37C>A) −269C>A	The CC genotype is not associated with hematological toxicity, survival, or tumor response compared to the AC or AA genotypes in people with NSCLC when treated with a combination of gemcitabine and carboplatin.	18538445
rs12806698	(−37C>A) −269C>A	The CC genotype is not associated with overall survival compared with genotypes AC and AA in people with NSCLC.	21642870

rsID	Location/Effect	Findings	PMID
rs12806698	(−37C>A) −269C>A	The CC genotype is moderately associated with better overall survival and progression free survival compared to the genotypes AC and AA when part of a haplotype with genotype TT rs11030918 in people with lung cancer.	15639717
rs12806698	(−37C>A) −269C>A	The AA genotype is associated with decreased incidence of nausea and vomiting compared to the genotypes AC and AA in people with mesothelioma.	22134350
rs12806698	(−37C>A) −269C>A	The AC genotype is associated with improved tumor response compared with genotypes AA and CC in people with NSCLC when part of a haplotype with rs11030918 CT, although overall and progression free survival did not differ between groups.	18483375
rs12806698	(−37C>A) −269C>A	The AC genotype is associated with longer progression free survival compared to the AA and CC genotypes in people with NSCLC.	20226083
rs720106	792+287T>C	The T allele is associated with increased risk of neurotoxicity compared to the C allele. This SNP is part of a four SNP haplotype that includes rs1042927, rs11030918, rs9937, and rs720106	24361227

All alleles reported in the table are on the positive strand. Variants marked by an * are the complement of what is reported in the study.

Abbreviations: SNP (single nucleotide polymorphism), NSCLC (non-small cell lung cancer).