

PHARMACOGENETIC AND TUMOUR DRUGS

Elizabeta Topic

Corresponding author's address:
Prof. dr. Elizabeta Topic
Clinical Institute of Chemistry,
School of Medicine University of Zagreb
& Sestre milosrdnice University Hospital,
Vinogradska 29
10 000 Zagreb

One of the main goals to improve chemotherapy in cancer patients is to increase the safety while not reducing the efficacy of therapy. Cancer chemotherapy is mainly empirical with the majority of cytotoxic agents given at a fixed dosage based on either body surface area or weight. These compounds have a narrow therapeutic index, and there is no simple index for monitoring pharmacological effects. Approximately 7% of patients are affected by adverse drug reactions (ADRs), increasing the overall hospital costs by 1.9% and drug costs by 15%. Among other influences, the inter-individual genetic variation has a major impact on drug activity. Genetic variations are the result of multiple mechanisms such as single nucleotide polymorphisms (SNPs) (over 90%), insertion, deletion, tandem repeats and microsatellites. In an attempt to individualize therapy, pharmacogenetics and pharmacogenomics (a polygenic approach to pharmacogenetic studies) are used in search for answers to the hereditary basis for individual differences in drug response.

Drugs used to treat cancer inhibit cell proliferation by several mechanisms. Alkylating agents (e.g., cyclophosphamide, busulfan, carboplatin) readily form covalent bonds with the DNA bases thus introducing crosslinks in the double helix and preventing DNA replication. Anticancer antibiotics (e.g., daunorubicin) intercalating between the DNA base pairs stabilize the DNA-topoisomerase II complex and stop the reversible 'swivelling' at the DNA replication fork, which is required for effective replication of the DNA template. The steroid hormones (e.g., prednisolone) interfere with DNA synthesis and alter intracellular metabolism due to receptor binding. The vinca alkaloids (e.g., vincristine) prevent the formation of the mitotic spindle, whilst the antimetabolites (e.g., methotrexate, 6-mercaptopurine) directly interfere with DNA formation by inhibiting purine and pyrimidine biosynthesis.

This chapter will focus on the impact of genetic polymorphisms, their effects on the activity and response to commonly used anticancer drugs such as mercaptopurine, 5 fluorouracil, cyclophosphamide, platinum agents and camptothecins. The genetic

polymorphisms known to affect responses to anticancer drugs are presented in Table 1.

Table 1. Genetic polymorphisms known to affect responses to anticancer drugs

Gene products	Polymorphism	Affected drugs	Effect of mutations
Thiopurine methyltransferase	Nonsynonymous SNPs, leading to unstable protein	Mercaptopurine, azathioprine, thioguanine	Acute myeloid leukaemia Chronic secondary tumours
Dihydropyrimidine dehydrogenase	Point mutation leading to aberrant splicing	5-Fluorouracil	Possible neurotoxicity and myeloid leukaemia
Glucuronosyl transferase	Varying number of TA repeats in promoter	Irinotecan	Increased risk of diarrhea and myeloid leukaemia
CYP P450 (3A4, 3A5, 2C8)	SNP leading to truncated protein	Docetaxel, paclitaxel	Possible affect on pharmacodynamics of anticancer drugs
Glutathione transferases	Complete deletions; point mutations	Alkylators, topoisomerase II inhibitors, endogenous nucleophiles	Increased sensitivity to toxic and antitumour effects on drugs
Methylene tetrahydrofolate reductase	Point mutation leading to protein instability	Methotrexate	Increased risk of mucositis
Thymidilate synthase	Varying number of tandem repeats in the promoter/enhancer region (TSE)	5-Fluorouracil	Drug resistance

12.1 Polymorphic enzymes in purine/ pyrimidine metabolism

12.1.1 Thiopurine S-Methyltransferase (TPMT)

TPMT is an important enzyme in the biotransformation of so-called thiopurine drugs such as mercaptopurine, thioguanine and azathioprine, commonly used in the treatment of acute lymphoblastic leukaemia, autoimmune disorders, and inflammatory bowel disease. These pro-drugs undergo metabolic activation to form active thioguanine nucleotides, the cytotoxic form of the drug being inactivated by TPMT catalyzing the S-methylation and forming of inactive metabolites. TPMT activity is inherited as an autosomal co-dominant trait and in Caucasians is presented as a trimodal distribution. Eighty-nine percent of the population are wild-type (wt/wt) TPMT with high activity, 11% are heterozygous (wt/mut) for mutations in TPMT with intermediate activity, and 0.3% are homozygous (mut/mut) for variant alleles with low or no activity (Figure 1). A unimodal distribution has been observed in East Asian subjects.

The human TPMT gene is located in chromosome 6 consisting of ten exons, eight of which encode protein. The polymorphic alleles are characterized by SNPs in the open reading frame and are associated with low enzyme activity caused by increased degradation of the mutant protein.

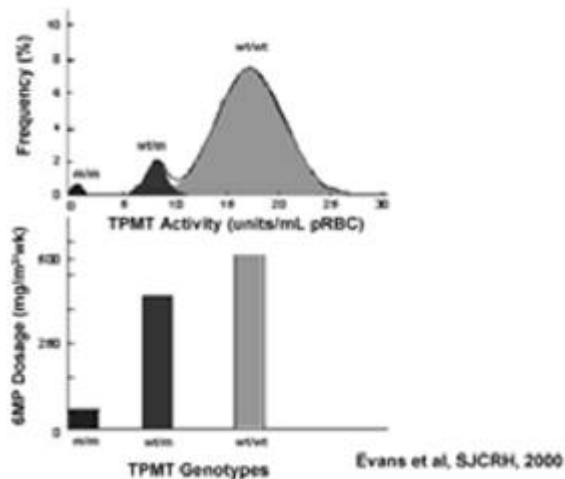


Figure 1. Trimodal TPMT activity distribution in correlation with an inherited trait. Polymorphism in TPMT leads to 3 distinct phenotypes, differing in their mercaptopurine dosage requirement. The three modes of TPMT activity (indicated here in erythrocytes) correspond to 0.3% of the population being homozygous (m/m) for mutations in TPMT, 10% being heterozygous (wt/m) for mutations in TPMT, and 90% being wild-type (wt/wt) TPMT.

At least 10 TPMT alleles have been identified so far. Three of these variant alleles (TPMT*2 (G>C), TPMT*3A (G>A and A>G), and TPMT*3C (A>G)), account for 80%-95% of the low and intermediate enzyme activity cases. In Caucasians TPMT*3A is the most prevalent variant allele (3.2%-5.7%), while TPMT*2 and TPMT*3C alleles are present in low frequency (0.2%-0.8%). Variant allele TPMT*3C is more common in the Asian, African, and African-American populations (5.4%-7.6%).

Patients heterozygous for these alleles have intermediate TPMT activity and tolerate approximately 65% of standard mercaptopurine dosage, while patients homozygous for the variant TPMT alleles are at a high risk of severe, sometimes life-threatening toxicity, requiring significant reduction in drug doses (1/10 to 1/15 of the standard dose).

More recently, a polymorphic locus consisting of a 17- or 18-base-pair repeat element (variable number tandem repeats, VNTR) has been identified within the promoter region of the TPMT gene. VNTR lengths varied from three to nine repeats but the potential clinical significance of the VNTR polymorphism remains unclear.

A major factor responsible for individual variation in toxicity and therapeutic efficacy of thiopurine drugs is the genetically determined level of TPMT activity. There is an inverse relationship between TPMT activity and thioguanine nucleotide levels in erythrocytes. A series of clinical reports has confirmed the association between genetically low TPMT activity and thiopurine drug toxicity. Studies in children with acute lymphoblastic leukaemia have shown that all homozygous, TPMT-deficient patients develop dose-limiting hematopoietic toxicity if treated with conventional doses of thiopurines, whereas most but not all patients with a heterozygous TPMT phenotype have intermediate tolerance to thiopurine therapy.

Pharmacogenetic profile

Genotyping for TPMT*2, TPMT*3A and TPMT*3C can predict TPMT status in 80%-95% of patients. The most common genotyping techniques have involved PCR-based mutation directed assay, with allele-specific primers or mutation-sensitive enzymes. However,

techniques are changing rapidly, so DNA chip technology offers an opportunity to detect all known inactivating mutations with almost complete predictive power. Thus, TPMT is a clear example of a clinically significant genetic polymorphism where prospective genotyping might allow individualization of drug therapy and thereby maximize efficacy and minimize toxicity. TPMT genotyping or phenotyping is now being used in major centers for dose optimization, in order to reduce the likelihood of adverse drug reaction in children with acute lymphocytic leukaemia.

12.1.2 Dihydropyrimidine Dehydrogenase (DPD)

5-fluorouracil (5-FU) is the most frequent chemotherapy drug used in combination therapy to treat a wide variety of malignancies of the gastrointestinal tract, breast, and head and neck. Dihydropyrimidine dehydrogenase (DPD) catabolizes 80%-90% of 5-FU dose to the inactive 5,6-dihydro-5-fluorouracil. Decreased DPD activity is associated with a more than fourfold risk of severe or fatal toxicity from standard doses of 5FU. A genetic polymorphism caused by mutations in the DPD gene results in DPD enzyme with partial to absolute loss of activity.

The DPD gene is located to chromosome 1 and consists of 23 exons. At least 20 polymorphisms in the DPD gene have been reported so far, however, many of these polymorphisms have not been definitely associated with altered DPD activity. Similarly, not all toxicity to 5FU from reduced DPD activity can be explained by the currently known polymorphisms.

The most frequent mutation in patients with partial or complete DPD deficiency is allele DPD*2A causing G>A splice site transition and skipping of exon 14, resulting in a truncated protein. Patients heterozygous for this polymorphism have low DPD activity and toxicity to 5FU. The frequency of the DPD*2A allele in Caucasian populations is 0.9%. Approximately 3% of the population carry heterozygous mutations that inactivate DPD and 0.1% are homozygous for inactivating mutations. Family studies in paediatric patients with DPD-deficiency phenotypes and in cancer patients having moderate to severe toxicity after 5-FU treatment show poor genotype-phenotype concordance. Nearly 25% of cancer patients who experienced grade 3-4 toxicity following 5-FU treatment were heterozygotes for the DPD*2A allele. Yet, there is still some controversy about 5-FU toxicity and a molecular basis for reduced DPD activity.

Pharmacogenetic profile

It seems that genotyping tests for DPD mutations have low sensitivity in identifying high-risk patients, and as yet no single test has been validated as a tool to individualize 5-FU therapy.

Currently, the apparently high false-negative rate for DPD as a predictor for severe 5-FU toxicity restricts the testing of DPD*2A to either research studies or as a component of a panel of oncology-related pharmacogenetic markers.

12.2 Polymorphic drug metabolizing enzymes

12.2.1 UDP-Glucuronosyltransferases 1A1 (UGT1A1)

Irinotecan, a water-soluble camptothecin analog, is used in the treatment of colorectal, lung and other solid tumours. A combination of 5-FU/irinotecan is a common frontline therapy for

colorectal cancer. Irinotecan is converted to an active metabolite, SN-38, which inhibits topoisomerase I to exert antitumour activity. SN38 is conjugated by UGT1A1, the major UGT1 isoform involved in SN-38 glucuronidation, to the inactive SN-38 glucuronide (SN-38G).

Currently more than 30 UGT isoforms encoded by the UGT gene family have been classified into two families of proteins termed UGT1 and UGT2. The gene complex encoding the UGT1 family of enzymes is located on chromosome 2 and involves at least 12 alternative versions of exon 1, each with its own promoter.

UGT1A1 promoter polymorphisms result in reduced UGT1A1 expression and activity. The variable number of (TA) repeats in TATA box in the promoter ranges from five to eight copies. Six repeats, (TA)₆ allele, represents the most common allele. Up to 33% of Caucasians have a variant allele containing seven repeats (TA)₇ (UGT1A1*28), which leads to a 30% reduction in UGT1A1 gene expression. Homozygosity for this variant promoter occurs in 0.5%-19% of Caucasians, and the black and Asian populations. Transcriptional activity of the UGT1A1 gene is inversely related to the number of (TA) repeats in the TATA box. The frequency of the (TA)₇ repeats is higher in black subjects, intermediate in Caucasians, and lower in Asians. Reduced UGT1A1 is linked to a high risk (about fourfold) of severe toxicity from irinotecan treatment, including dose limiting diarrhea and neutropenia. Significant associations between patients with the UGT1A1*28 allele and reduced UGT1A1 expression, and consequently reduced SN38 glucuronidation have been shown in several studies.

In vitro studies showed that the SN-38 glucuronidation rates in human liver microsomes were significantly lower in homozygotes for (TA)₇ allele (7/7) and heterozygotes (6/7) than in homozygotes for (TA)₆ allele (6/6). However, UGT1A1 polymorphism is not the sole predictor of irinotecan clearance because of irinotecan complex metabolism and elimination (involving carboxylesterase, CYP3A4, and transporters).

Pharmacogenetic profile

Assessment of the presence of the UGT1A1*28 allele in patients prior to irinotecan treatment may predict individuals at risk of severe toxicity from irinotecan, allowing the choice of lower doses or alternative therapy. Dose reductions may be necessary in patients homozygous or heterozygous for the (TA)₇ allele.

12.2.2 Cytochrome P450s

At least 30 human CYP isozymes have been identified, but most drugs used in cancer chemotherapy are metabolized by CYP3A, CYP1A, CYP2B, and CYP2C isoforms. Of the many variant CYP alleles identified to date, their function as related to drug metabolism is known for only a minority. The most abundant CYP expressed in the liver, accounting for 60% of the CYP activity, and in intestinal wall is CYP3A4. Twenty-five variant alleles for CYP3A4 have been reported to date, however, despite significant interindividual variation genotype/phenotype studies have not shown concordance in the expression levels and activity.

CYP3A5 expression is polymorphic, with readily detectable expression levels in about 30% of livers and low to undetectable levels in the rest. An SNP, A>G, in intron 3 of the CYP3A5 gene (CYP3A5*3) results in the production of an aberrantly spliced mRNA that encodes a truncated protein product with no CYP3A5 activity. In individuals with at least one CYP3A5*1 allele (wild-type), CYP3A5 is the major contributor to the total CYP3A activity. Data are limited regarding the activity of CYP3A5 toward the large number of known CYP3A4 substrates. However, since CYP3A4 is

involved in the metabolism of a majority of anticancer drugs, CYP3A5 polymorphisms could affect the pharmacodynamics of agents that are metabolized by both enzymes. CYP3A4 and CYP3A5 catalyze the initial oxidation (before cyclization) of docetaxel, a semisynthetic compound closely related to the taxane paclitaxel, used in the treatment of breast and ovarian cancers.

CYP2C8 is the primary enzyme involved in paclitaxel metabolism and its expression is polymorphic. Six variant alleles have been identified with varying allele frequencies among ethnic groups. CYP2C8*2 is found only in African-Americans with a frequency of 0.18%, whereas CYP2C8*3 occurs primarily in Caucasians, with an allele frequency of 0.13%. *In vitro* studies have demonstrated that recombinant CYP2C8*3 is less efficient in paclitaxel metabolism than the wild-type allele.

Pharmacogenetic profile

The contribution of the CYP3A polymorphism to the effect of anticancer drugs has not been elucidated, but because almost half of all anti-cancer drugs are CYP3A substrates, polymorphisms in CYPs are likely to affect the pharmacodynamics of anticancer drugs. The polymorphism of CYP2C8 may have important clinical consequences in individuals homozygous for the CYP2C8*3 allele. Well-designed studies incorporating large-scale sequencing projects, along with complementary laboratory investigations and studies of transcript variants and proteomics, are needed to understand the basis for the interindividual variability in CYP metabolism.

12.2.3 Glutathione S-transferase P1 (GSTP1)

Glutathiones play a role in detoxifying, and consequently in protecting cells from alkylating agents and products of reactive oxidation. The pi-class of human GSTP1 has been found to catalyze glutathione conjugation of reactive metabolites from cyclophosphamide, a drug commonly used in the treatment of breast cancer and other solid tumours. GSTP1 also detoxifies platinum compounds, including oxiplatin, a relatively new chemotherapy drug used in combination with 5FU for the treatment of advanced colorectal cancer. GSTP1 polymorphisms have also been linked to the efficacy and toxicity of cancer chemotherapy.

A SNP in the GSTP1 gene causing an isoleucine to valine substitution at amino acid codon 105 is associated with reduced GSTP1 activity compared to the isoleucine allele. The frequency of this polymorphism in Caucasian population is about 33%. This SNP has been correlated with response to cyclophosphamide chemotherapy treatment in breast cancer patients.

Homozygotes for the valine (low activity) allele have a relative risk of 0.3 and heterozygotes of 0.8 for survival compared with patients homozygous for the isoleucine (high activity) allele.

Pharmacogenetic profile

Currently, studies are mainly focused on the effect of SNPs in GSTP1 on the risk of cancer. Further research on the association of GSTP1 SNPs with response to alkylating agents and platinum drugs will provide information on the usefulness of prescreening patients for GSTP1 genotype prior to treatment.

12.3 Polymorphic enzymes in folate metabolism

12.3.1 Methylenetetrahydrofolate reductase (MTHFR)

Methotrexate is a folic acid antagonist that is commonly used to treat leukaemia, lymphomas and breast cancer. It inhibits several enzymes included in folate metabolism, which is crucial for nucleotide and amino acid synthesis. MTHFR is responsible for maintenance of normal levels of reduced folate and homocysteine, and lack of MTHFR leads to neurologic and vascular diseases. A common genetic MTHFR polymorphism C>A has been shown to be predictive of oral mucositis following methotrexate treatment in patients undergoing bone marrow transplantation. Patients homozygous for variant TT (~10%), or heterozygous for CT genotype (40%) have reduced MTHFR activity as well as lower folate levels than those with a CC genotype. A common genetic polymorphism of G>A transition in exon 1 is also associated with altered folate level, and studies are under way to investigate whether this polymorphism affects methotrexate transport *in vitro* or *in vivo*. Low MTHFR activity may increase or reduce tolerance to chemotherapy.

Pharmacogenetic profile

Although MTHFR genotyping can be suggested in patients undergoing combined anticancer therapy, more studies are needed to define the relationship between MTHFR polymorphisms and toxicities induced by antifolate/fluoropyrimidine therapy. Assessment of the presence of MTHFR**T* allele in patients prior to administration of folic acid antagonists may predict tolerance to chemotherapy.

12.4 Polymorphic drug target

Polymorphisms in drug targets are also an important area for pharmacogenetic studies, since over-expression or under-expression of drug targets could also lead to resistance or toxicity to standard chemotherapy regimens.

12.4.1 Thymidylate synthase

The main target for 5-FU is thymidylate synthase (TS). TS catalyzes the conversion of deoxy-uridine monophosphate (dUMP) to deoxy-thymidine monophosphate and is the only *de novo* source of intracellular thymidylate for DNA synthesis. Inhibition of the enzyme results in deoxythymidine triphosphate depletion and subsequent chromosome breaks and cell death. TS is an important target for the cancer chemotherapy drug 5-FU and TS inhibitors such as raltitrexed, and its overexpression has been linked to their resistance. Cellular sensitivity to 5-FU is related to alterations in the TS level and varies considerably among various tumours. The sensitivity of various tumour types to 5-FU-based chemotherapy is inversely related to TS expression. *In vivo* and *in vitro* studies have shown that lower TS activity is associated with a better antitumour response to 5-FU.

Three polymorphisms have been described in the TS gene. A polymorphic 28 bp tandem repeat in the promoter enhancer region (TSER) has been extensively characterized in multiple world populations. The polymorphism varies from two (TSER*2) to nine (TSER*9) copies of the tandem repeat, with TSER*2 and TSER*3

being the most common alleles. *In vitro* studies have demonstrated that TSER*3 has a higher TS expression than TSER*2.

The frequency of the TSER*3/*3 genotype and allele frequency are similar in Caucasian and Southwest Asian subjects (38%) but higher in the Chinese and Japanese (67%).

Of 24 patients with metastatic colorectal cancer receiving 5-FU infusions, only 22% of nonresponders had the *2/*2 genotype compared with 40% of the responders. In another study of 50 patients with disseminated colorectal cancer, individuals with *2/*2 genotype (in the tumour and normal tissue DNA) had a 50% response rate to 5-FU compared with 9% and 15% in those with *3/*3 and *2/*3 genotypes, respectively, suggesting that the response to chemotherapy was genetically regulated in part by the VNTR TS polymorphisms. Patients with the TSER*3/*3 genotype derived less survival benefit ($p < 0.18$) from 5-FU-based adjuvant chemotherapy, compared with surgery alone, than those with TSER*2/*2 or TSER*2/*3 genotypes ($p < 0.005$). Children with acute lymphoblastic leukaemia treated with methotrexate, homozygous for TSER*3/*3 genotype experienced shorter event-free survival than those homozygous for TSER*2/*2 or heterozygous for TSER*2/*3 genotypes ($p < 0.005$). Methotrexate glutamates inhibit TS, and overexpression of TS is a potential mechanism for the development of resistance in patients with the TSER*3/*3 genotype. These observations suggest that TS gene polymorphisms, by altering TS expression and activity, influence response to chemotherapy in various malignancies.

Recently, a SNP within the second repeat of the TSER*3 allele (3RG allele), which may also affect TS expression, has been described. A study in 208 colorectal cancer patients and 675 controls found a 1.3-fold risk of colorectal cancer for patients with the 3RG allele, implying that the polymorphism may increase the effect of the repeat polymorphism in the TSER.

A third polymorphism in TS gene is a 6 bp deletion located 447 bp downstream from the stop codon. The frequency of allele with deletion is 27% in Caucasians. Recent study results indicate a significant association of deletion allele with a decreased response to 5-FU chemotherapy.

Pharmacogenetic profile

The TSER genotype would be used in conjunction with other TS gene variants and as part of a multiple gene profile in order to better individualize therapy. A large-scale assessment of the role of each TS polymorphism, individually and as a haplotype, is now required to determine whether prospective assessment is warranted in patients prior to 5FU-containing chemotherapy treatment.

12.5 Conclusion

Concerning the real potency of cytotoxic drugs, their very narrow therapeutic index and use at maximal tolerated doses render anticancer agents a high-risk treatment for patients who differ from the average population. Identification of heritable differences responsible for either the occurrence of toxicity or lack of efficacy will allow for the unpredictable and undesirable consequences of cancer treatment to reduce, because adjusting only the dose by

