Anti-Tumour Treatment

An overview of the relations between polymorphisms in drug metabolising enzymes and drug transporters and survival after cancer drug treatment

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Survival

Summary
A wide interindividual variability in survival after cancer treatment is observed. This is attributable to many factors, including tumour and patient related factors. Genetic polymorphisms in drug metabolising enzymes and drug transporters may be one of these factors. Drug metabolising enzymes are responsible for the activation, inactivation and detoxification of many chemotherapeutic agents. Deficiencies in these enzymes may result in altered exposure (both extracellular and intracellular) to the chemotherapeutic agents, thereby influencing the efficacy of treatment. Drug transporters are important in the uptake and excretion of chemotherapeutic agents. Polymorphisms in drug transporter genes may influence the bioavailability and disposition of these agents.

Studies have shown that variability in survival can (partly) be explained by polymorphisms in genes encoding drug metabolising enzymes and drug transporters. This review will discuss the role of genetic polymorphisms in drug metabolising enzymes and drug transporters in relation to survival after cancer treatment.

The most important polymorphisms shown to influence survival after cancer treatment are polymorphisms in the genes encoding the phase II detoxification enzymes glutathione-S-transferases (GSTs). It appears that GSTM1 null and GSTT1 null have a clear association with longer overall survival in patients with different malignancies who are treated with substrates for
these GSTs (mostly alkylating agents and platinum compounds). Genetic polymorphisms in GSTP1 and GSTA1 are also associated with an increased overall survival in patients with different malignancies.

Most of the current data on the relation between treatment response and pharmacogenetics is derived from retrospective and exploratory studies. Prospective studies will be necessary.

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Introduction

Differences in drug response among patients after cancer treatment are common. This variability in drug response is partly due to variability in pharmacokinetics. Factors responsible for this variability include ethnicity, age, gender, diet, smoking, alcohol consumption, renal and liver function, concomitant disease and co-medication. In many cases, however, genetic factors are shown to have an even greater influence on drug disposition. It is estimated that genetic variability accounts for 20–95% of the variability in therapeutic response and toxic effects. These differences in genetic factors, for instance observed in genes encoding drug metabolising enzymes and drug transporters, can influence the pharmacokinetic and pharmacodynamic profile of anti-cancer drugs, leading to differences in response and development of severe toxicities.

Genetic variation in the human genome is a common phenomenon and approximately 1 out of 1000 basepairs differs between any two individuals. Most of these variations are single nucleotide polymorphisms (SNPs). These single nucleotide differences account for >90% of the genetic variation. Insertions and deletions, tandem repeats and microsatellites account for the remaining 10%. The number of polymorphisms identified in genes encoding drug metabolising enzymes and drug transporters is rapidly increasing, probably leading to a better understanding of the observed variation in efficacy and toxicity of anti-cancer drugs in patients.

Drugs are metabolised by drug metabolising enzymes and drug transporters play a role in the disposition of drug in the body. These can be classified into three main categories. The first category consists of phase I enzymes. These include reductases, oxidases and hydrolases. The cytochrome P450 enzymes (CYPs) belong to this category. Most drugs are metabolised by CYPs either as a route to detoxification or as an activation pathway for an inactive prodrug. The second category is called phase II enzymes. These enzymes usually conjugate phase I products, but can also conjugate other reactive intermediates or the parent compound, with various endogenous moieties such as glucuronic acid, glutathione or sulphate. These enzymes also contribute to the intracellular metabolism of many substrates. The phase II enzymes include UDP-glucuronosyltransferases (UGTs), glutathione-S-transferases (GSTs), and sulfotransferases (SULTs). The last category consists of drug transporters. These transporters are membrane-bound proteins that control drug uptake and excretion. Drug transporters greatly influence the bioavailability and disposition of drugs. Examples of genes that encode these transporters are MDR1 (ABCB1), which encodes P-glycoprotein, and ABCG2, which encodes breast cancer resistance protein (BCRP). Polymorphisms in genes encoding drug metabolising enzymes may decrease the intracellular enzyme concentration, lead to a dysfunctional protein, or may structurally alter the enzyme with consequent changes in enzyme function. Polymorphisms in drug transporter genes can influence the uptake and excretion capability of the protein. Together this may alter the pharmacokinetic and pharmacodynamic profile of a drug. Therefore, polymorphisms in genes encoding proteins involved in drug metabolism and disposition may be important for treatment response after cancer treatment. Especially the influence of polymorphisms on survival after cancer treatment is important since this is the ultimate outcome measure.

This review will focus on the influence of genetic polymorphisms in phase I and II enzymes and drug transporters on survival after cancer treatment. A review of the literature of studies reporting significant relations between survival and polymorphisms in drug metabolising enzymes and drug transporters is provided. Furthermore, the clinical relevance of these polymorphisms in predicting outcome is discussed.

Methods

A literature search was carried out using PubMed for publications concerning the influence of polymorphisms in drug metabolising enzymes and drug transporters on survival. Furthermore, reference lists of publications were screened on other relevant studies. Studies that reported significant relations were included in this review, limiting results to human research published in English.

Phase I enzymes

Cytochrome P450

CYP enzymes are important in the biosynthesis and degradation of endogenous compounds such as steroids, lipids and vitamins. They metabolise many drugs as well as chemicals present in the diet and environment. The CYP enzymes are responsible for the metabolism of over 90% of clinically prescribed drugs. CYPs reduce or alter the pharmacologic activity of many drugs and facilitate their elimination. Three families of encoded proteins, CYP1, CYP2, and CYP3 contribute mainly to the metabolism of drugs.

Individual CYP enzymes each have unique substrate specificity. However, considerable overlap may be present. Thus, drugs may be metabolised by a single CYP enzyme or a variety of CYP enzymes may contribute.

The liver is the major site of CYP mediated metabolism, but the CYP enzymes are also expressed in the enterocytes in the epithelium of the small intestine, kidney and lung.
Genetic polymorphisms can result in inhibition or induction of the involved enzymes. This can markedly influence blood levels of a given drug, leading to under-treatment or toxicity. Differences among patients in drug metabolism in the liver and intestine are common and are often major contributors to differences in drug response and adverse effects.7

Several human CYP enzymes have been correlated with survival after cancer treatment. The effects of polymorphisms in these enzymes and their association with survival are described below. Table 1 gives an overview of studies describing a relation between polymorphisms in CYP genes and survival.

### CYP2D6

The CYP2D6 gene is a well studied member of the CYP superfamily, with over 60 allelic variants described.8 They can be classified based upon their effects on enzyme activity, either to increase, decrease or totally eliminate CYP2D6 activity. CYP2D6 gene duplication exists in the population and this is correlated with ultrarapid metabolism.6 Most of the variation in CYP2D6 activity is explained by a subgroup of CYP2D6 SNPs. The non-functional alleles *3, *4, *5 and *6 are responsible for >98% of CYP2D6 poor metabolisers in Caucasians.8

CYP2D6 is involved in the metabolism of numerous drugs. With respect to anti-cancer drugs, CYP2D6 is involved in the conversion of tamoxifen to the 50–100-fold more potent 4-hydroxytamoxifen and endoxifen. Thus, for treatment with tamoxifen, CYP2D6 activity might be of importance. Polymorphisms in the CYP2D6 gene may lead to less formation of 4-hydroxytamoxifen and endoxifen and therefore result in shorter survival times for cancer patients treated with tamoxifen.

Several studies have indeed found that breast cancer patients, who are carriers of non-functional alleles of CYP2D6, like CYP2D6 *4, *5, *10, and *41, have shorter relapse-free periods, worse event-free survival and lower overall survival10–11 (Table 1). For example, Goetz et al.12 demonstrated that breast cancer patients, who were treated with tamoxifen and who were homozygous for CYP2D6*4 experienced significantly shorter relapse-free time (P = 0.023) and poorer disease-free survival (P = 0.012) compared with women who had at least one wild-type CYP2D6 allele, although results were only significant in univariate analysis.

On the other hand, Wegman et al.13 found that possession of at least one CYP2D6*4 allele leads to an improved survival rate in breast cancer patients treated with tamoxifen. This is in contrast with the results of other studies and with the hypothesis that wild-type homozygous patients are supposed to generate the active metabolite 4-hydroxytamoxifen more readily and thereby have improved response of tamoxifen. However, the results were obtained from a small number of patients, and therefore the association may be a coincidence.

### CYP3A4, CYP3A5

The CYP3A subfamily represents the majority of CYP protein in the human liver and is involved in the metabolism of many drugs. CYP3A4 is the major hepatic CYP3A enzyme and is also present in the intestinal epithelium. CYP3A4 activity shows wide inter-individual variation of up to 40-fold.14

The first genetic CYP3A4 polymorphism described is the promoter variant allele CYP3A4*1B (A-392G) with a large interethnic variation. The allele frequency is 2–9% in Caucasians and 35–67% in African-Americans. The CYP3A4*1B allele is not identified in Asian subjects.6 Although the CYP3A4*1B allele was initially shown to have a 1.5-fold increase in transcription in vitro, other reports indicate no change in enzyme activity.6,15

Although a number of genetic variants have been identified in CYP3A4, it is generally accepted that most of the known SNPs in the coding and 5′-flanking regions of CYP3A4 are not the main determinants for the large interindividual variability of CYP3A4 expression or activity.16

Another important member of the CYP3A subfamily is CYP3A5, which is expressed in only 10–40% of Caucasians. CYP3A5 is expressed in the liver as well as in other organs (prostate, kidney, adrenal, pituitary). A genetic polymorphism in intron 3 of the CYP3A5 gene (A6986G) results in loss of expression of the enzyme, which was named the CYP3A5*3 allele. It appeared that 80% of the Caucasian population and 30% of the African American population are

### Table 1: Association studies of polymorphisms in CYP genes and survival

<table>
<thead>
<tr>
<th>Genes</th>
<th>SNP</th>
<th>Subjects</th>
<th>Treatment</th>
<th>Effect on end point</th>
<th>Risk measure (95% CI)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>CYP2D6</td>
<td>CYP2D6*4</td>
<td>190</td>
<td>TAM</td>
<td>DFS↓</td>
<td>CYP2D6*4 /4 HR = 2.44 (1.22–4.90)</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>CYP2D6*10</td>
<td>21</td>
<td>TAM</td>
<td>TTP↓</td>
<td>CYP2D6*10/*10 HR = 3.68 (1.23–11.04)</td>
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</tr>
<tr>
<td>CYP2D6</td>
<td>CYP2D6*4, *5, *10, *41</td>
<td>206</td>
<td>TAM</td>
<td>EFS↓</td>
<td>CYP2D6*4, *5, *10 or *41 HR = 1.89 (1.10–3.25)</td>
<td>11</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>CYP2D6*4</td>
<td>24</td>
<td>TAM</td>
<td>RFS†</td>
<td>CYP2D6*4 RR = 0.28 (0.11–0.74)</td>
<td>13</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>CYP3A4*1B</td>
<td>85</td>
<td>CP and platinum</td>
<td>OS↓</td>
<td>CYP3A4*1B P = 0.043</td>
<td>18</td>
</tr>
<tr>
<td>CYP3A5</td>
<td>CYP3A5*1</td>
<td>85</td>
<td>CP and platinum</td>
<td>OS↓</td>
<td>CYP3A5*1 HR = 2.6 (P = 0.0035)</td>
<td>18</td>
</tr>
<tr>
<td>CYP3A5</td>
<td>CYP3A5*3</td>
<td>108</td>
<td>TAM</td>
<td>RFS†</td>
<td>CYP3A5*3/3 HR = 0.13 (0.02–0.86)</td>
<td>19</td>
</tr>
</tbody>
</table>

CP, cyclophosphamide; TAM, tamoxifen; DFS, disease-free survival; TTP, time to disease progression; EFS, event-free survival; RFS, recurrence-free survival; OS, overall survival; HR, hazard ratio; RR, relative risk.
homzygous for this inactive CYP3A5 allele and are thus deficient in CYP3A5 activity.6

Because CYP3A4 and CYP3A5 have overlapping substrate specificities, the contribution of each CYP3A4 and CYP3A5 to total CYP3A activity will depend on both the drug under investigation and the individual.5

CYP3A4 and CYP3A5 play a role in the activation of cyclophosphamide to 4-hydroxycyclophosphamide, the major metabolic pathway.17 Petros et al.18 showed that polymorphisms in CYP3A4 and CYP3A5 were associated with a reduced systemic clearance of cyclophosphamide and a significant poorer overall clinical response (Table 1). Median survival of 85 breast cancer patients with the CYP3A4*1B polymorphism was 1.3 years (95% CI 0.6–2.1) compared to 2.7 years (95% CI 1.8–4.1) for patients with both copies of the common allele (P = 0.043). Similar differences in median survival were observed for polymorphisms in CYP3A5. Patients with the wild-type allele CYP3A5*1 had a significantly shorter median survival than patients with both copies of the CYP3A5*3 allele (P = 0.002). These data are opposite to expectations, since CYP3A4*1B and CYP3A5*1 are associated with increased transcriptional activity and presence of CYP3A5 enzyme, respectively. A possible explanation provided by the authors is that women carrying the CYP3A4*1B allele or the CYP3A5*1 allele show lower autoinduction of total CYP3A compared to women carrying the wild-type CYP3A4 allele or CYP3A5*3 alleles, resulting in reduced activation of cyclophosphamide. However, 29 polymorphisms in 17 drug metabolising genes were tested and no adjustments were made to account for the number of statistical tests conducted.

Tamoxifen is metabolised to N-desmethyltamoxifen by CYP3A4 and CYP3A5. N-desmethyltamoxifen is a weak anti-oestrogen and a precursor of the active metabolite endoxifen.19 Wegman et al.19 showed that the genotype of CYP3A5 may contribute to tamoxifen response. They found an improved relapse-free survival in patients homozygous for CYP3A5*3. This is rather unexpected since this genotype represents an inactive form of the enzyme and should therefore not catalyse the formation of the primary metabolite N-desmethyltamoxifen. This outcome gives rise to questions about the hypothesis that genotypes contributing to the biosynthesis of the active metabolites improve the outcome of tamoxifen treatment. However, the metabolism of tamoxifen is complex and the mechanisms responsible for the resistance are therefore unlikely to be explained by a single polymorphism but rather by a combination of several mechanisms.

**NAD(P)H:quinine oxidoreductase 1**

NAD(P)H:quinine oxidoreductase 1 (NQO1, EC 1.6.99.2) is a flavoprotein that has a wide variety of substrates, including quinones and their derivatives. NQO1 catalyzes the two-electron reduction of substrates by using either NADH or NADPH as an electron donor. NQO1 is thought to be of importance in the activation of mitomycin C. Furthermore, NQO1 exerts a detoxifying role through protection from free radicals generated by both drug and carcinogen metabolism.20–22

A genetic polymorphism has been characterized in NQO1(G609T), which results in the conversion of proline to serine in the NQO1 protein. This SNP is named NQO1*2 and results in almost no active enzyme in persons with homozygous variant alleles and reduced activity in persons with heterozygous alleles. Approximately 5% of the Caucasian population are homozygous variant and nearly 40% of Caucasian individuals are heterozygous for the substitution.20,21 Table 2 gives an overview of studies describing a relation between polymorphisms in the NQO1 gene and survival.

Overall, it can be stated that patients treated with substrates for NQO1 and having the NQO1*2 variant have a worse therapeutic outcome than patients with wild-type NQO1.20–22 (Table 2).

Krajinovic et al.22 showed that children with ALL, who were treated with a combination of chemotherapeutic agents, those individuals with the NQO1*2 variant had a significantly shorter event-free survival (P = 0.003) compared to individuals with wild-type NQO1. It can be hypothesized that impaired cell protection from free radicals that have arisen through drug and carcinogen metabolism led to the development of recurrent malignancies.

Other studies have shown that this effect was also seen in patients who received mitomycin C therapy and had the NQO1*2 variant. In patients with peritoneal cancer, who were administered a 2-h heated (40.5 °C) intraperitoneal perfusion with mitomycin C (40 mg) and also in patients with non-small cell lung cancer who received mitomycin C 8 mg/m² iv, repeated every 21 days for a maximum of six courses, those patients with the NQO1*2 variant had a poorer survival than patients with wild-type NQO1.20,21 The lower NQO1 activity, caused by the NQO1*2 variant, results in reduced activation of mitomycin C and diminished cytotoxic activity.

**Phase II enzymes**

**Glutathione-S-transferase**

The GSTs are phase II detoxification enzymes. GSTs catalyse the conjugation of glutathione to a wide range of substrates, including mutagens, carcinogens and chemotherapeutic agents like alkylating agents and anthracyclines.18,23–28

The detoxification capability plays an important role in the protection of the cell from environmental, genotoxic and oxidative stress, but is also associated with drug resistance.25,26,29 The detoxification of reactive oxygen species, which may act as intermediates in the cytotoxicity of many chemotherapeutic agents, may modulate the response to a specific drug, even when the chemotherapeutic agent itself is not a substrate.24

The most important human GST genes are GSTM1, GSTT1, GSTP1 and GSTA1.25,26,30,31

Human GST genes contain different polymorphisms; deletions as well as single nucleotide polymorphisms. Loss of GSTM1 and GSTT1 enzyme function is ascribed to a homozygous deletion resulting in the GSTM1 null and GSTT1 null genotype, respectively. The GSTM1 gene is absent in approximately 50% of the Caucasian population. The GSTT1 gene is absent in approximately 15% of the Caucasian population.23

Four different alleles of the GSTP1 gene, GSTP1*A, *B, *C and *D have been described, arising from nucleotide transi-
tions that change codon 105 from Ile to Val and codon 114 from Ala to Val. The different GSTP1 proteins differ in their ability to metabolise anti-cancer agents. The Val\textsuperscript{105} and the Val\textsuperscript{114} allele are associated with reduced catalytic activity.\textsuperscript{30,32}

The gene GST\textsubscript{A1} has two important alleles: *A and *B. A promoter point mutation leads to decreased promoter activity in carriers of the GST\textsubscript{A1}B allele, thereby reducing its expression.\textsuperscript{24,25,30}

Differences in detoxification of treatment agents or GST-mediated protection against oxidative damage during treatment due to polymorphisms in GST genes can influence survival. Individuals with GST\textsubscript{M1}/T1 null genotypes or GST\textsubscript{A1}/P1 genotypes with reduced activity would experience a higher effective dose of chemotherapeutic agents and/or more reactive oxidant damage to tumour tissue. Therapy might then be more effective in these patients, in which case longer survival for this group would be observed. However, shorter survival for patients with reduced capacity for GST-mediated detoxification might be explained by more severe therapy-related toxicity.

In this section the association between polymorphisms in GST genes and survival after cancer treatment will be discussed for several malignancies. Table 3 gives an overview of studies describing a relation between polymorphisms in GST genes and survival.

### Breast cancer

Chemotherapy regimens for breast cancer frequently consist of GST substrates such as anthracyclines and cyclophosphamide.\textsuperscript{18,29,33,38,39}

Studies in breast cancer patients show that single and combined deletions of GST\textsubscript{M1} and GST\textsubscript{T1} are linked to a better clinical outcome.\textsuperscript{16,28,38,40} (Table 3). Ambrosone et al.\textsuperscript{38} genotyped 251 women with primary breast cancer treated with cyclophosphamide, doxorubicin, 5-fluorouracil and radiotherapy. After adjustment for age, race and stage, GST\textsubscript{M1} and GST\textsubscript{T1}-null genotypes predicted significantly better disease-free survival and overall survival. More studies in breast cancer patients showed similar results.\textsuperscript{18,29,40}

These chemotherapeutic agents as well as radiotherapy generate reactive oxygen species. GST\textsubscript{M1} null and GST\textsubscript{T1} null genotypes have reduced ability to detoxify those species, resulting in improved treatment response.

In contrast, Kristensen et al.\textsuperscript{41} reported a significantly shorter overall survival in breast cancer patients homozygous for the GST\textsubscript{M1} null allele. However, treatment was not reported and no adjustment for other prognostic factors was performed. Therefore, the results of this study should be interpreted with caution.

The less active variants GST\textsubscript{P1} Val\textsuperscript{105} and GST\textsubscript{A1}B have been associated with an increased survival in patients receiving a cyclophosphamide-based chemotherapy regimen.\textsuperscript{42–44} Patients with the GST\textsubscript{P1} Val\textsuperscript{105} variant or the GST\textsubscript{A1}B variant have a reduced ability to detoxify the active cyclophosphamide metabolites and would therefore

### Acute myeloid leukaemia

Standard therapy for patients with acute myeloid leukaemia (AML) consists of an anthracycline (e.g. daunorubicin) combined with agents like cytarabine and etoposide. Anthracyclines are substrates for GSTs.\textsuperscript{33} Studies showed that deletions in two of the most important GST subfamily genes, GST\textsubscript{M1} and GST\textsubscript{T1} influence the outcome of treatment with induction therapy in patients with AML. A study by Voso et al.\textsuperscript{34} showed that patients with AML and GST\textsubscript{M1} null or GST\textsubscript{T1} null genotype had a significantly lower response to anthracycline-based induction therapy (P = 0.04) and a shorter survival (P = 0.04) compared to patients with an undeleted genotype (Table 3). These results were confirmed in a study with Chinese male patients with de novo AML.\textsuperscript{35} Also Barragan et al.\textsuperscript{36} found that the GST\textsubscript{M1} null genotype was associated with a poorer survival in AML patients. The GST enzymes play an important role in the detoxification of chemotherapeutic agents. Polymorphisms resulting in lack of enzymatic activity will lead to reduced detoxification. This reduced detoxification will increase toxicity, but it might also be expected to confer a higher response due to the reduced degradation of chemotherapeutic agents.

In these studies, the poorer survival observed in patients with GST\textsubscript{M1} null or GST\textsubscript{T1} null genotypes might be attributed to increased toxicity, however toxicity data were not available.

A significant relation between an increased risk for death due to toxicity and GST\textsubscript{T1} null genotype in patients with AML was reported by two different groups.\textsuperscript{23,37} Naoe et al.\textsuperscript{37} showed that the GST\textsubscript{T1} null genotype was associated with a worse prognosis in adults, mainly due to increased early death after initial chemotherapy, Davies et al.\textsuperscript{23} found similar results in children. Children with AML and GST\textsubscript{T1} null genotype experienced more toxicity and reduced survival after standard induction therapy compared to children with at least one GST\textsubscript{T1} allele.\textsuperscript{23}

These results show that GST\textsubscript{M1} null or GST\textsubscript{T1} null are associated with a worse prognosis in patients with AML. Although one might expect an improved survival in patients with GST\textsubscript{M1} null or GST\textsubscript{T1} null due to a higher effective dose of chemotherapy, in AML reduced GST activity may lead to a worse prognosis, probably due to an increase in toxic deaths.

### Table 2: Association studies of polymorphisms in NQO1 genes and survival

<table>
<thead>
<tr>
<th>Genes</th>
<th>SNP</th>
<th>Subjects</th>
<th>Disease</th>
<th>Treatment</th>
<th>Effect on end point</th>
<th>Risk measure (95% CI)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NQO1</td>
<td>NQO1*2</td>
<td>109</td>
<td>Peritoneal cancer</td>
<td>Mitomycin C</td>
<td>OS↓</td>
<td>NQO1*2 P = 0.037</td>
<td>20</td>
</tr>
<tr>
<td>NQO1</td>
<td>NQO1*2</td>
<td>27</td>
<td>NSCLC</td>
<td>Mitomycin C</td>
<td>OS↓</td>
<td>NQO1<em>2/2</em>2 P = 0.007</td>
<td>21</td>
</tr>
<tr>
<td>NQO1</td>
<td>NQO1*2</td>
<td>320</td>
<td>ALL</td>
<td>Vincristine, CP, doxorubicin, methotrexate</td>
<td>EFS↓</td>
<td>NQO1*2 HR = 3.6 (1.7–7.4)</td>
<td>22</td>
</tr>
</tbody>
</table>
receive a higher effective dose. Thus, the improved survival for these patients may be attributable to improved treatment efficacy.

It can be concluded that for patients with breast cancer, treated with cyclophosphamide and anthracyclines, the GST genotypes with less or no activity may result in improved survival, possibly due to a better response to chemotherapy.

Ovarian Cancer
Mounting evidence suggests that polymorphisms in GST genes may affect survival in ovarian cancer patients. GSTP1 expression is common in ovarian tumours and high levels of GSTP1 expression have been associated with drug resistance and poor survival in ovarian cancer patients.45-47 Therapy for ovarian cancer commonly includes platinum compounds, which are substrates for the GST enzymes.47-50

Therefore, it is plausible that ovarian cancer patients with GST genotypes with low activity (GSTP1 Ile105/Val105 or Val105/Val105) or deleted GST genotypes (GSTM1 null or GSTT1 null) would have a reduced intracellular metabolism. This could lead to a better response to treatment and a longer overall survival.

Beeghly et al.47 studied disease progression and survival in 215 women with primary invasive epithelial ovarian cancer, who were treated with platinum-based chemotherapy. They reported that women with GSTM1 null and GSTP1 Ile105/Val105 or Val105/Val105 genotype had better progression-free survival and overall survival. This was confirmed by other studies 49-51 (Table 3).

Some studies, however, have reported no relation or opposite associations between GST polymorphisms and survival. Howells et al.48 concluded that patients with both GSTM1 null and GSTT1 null genotypes had a significantly shorter overall survival ($P = 0.001$). However, the results were not adjusted for potential confounders. The association became insignificant when either residual disease or tumour grade was included in a subgroup analysis performed later.52

Overall, it can be concluded that less active GSTP1 variants and the null genotypes of GSTM1 and GSTT1 may result in better progression-free survival and longer overall survival in patients with ovarian cancer, possibly due to decreased detoxification of platinum compounds.

Colorectal cancer
The platinum compound oxaliplatin is an important drug in the treatment of colorectal cancer.53 Inconclusive results have been found in studies investigating the influence of GST polymorphisms in relation to survival in colorectal cancer patients. Stoehlmacher et al.53 demonstrated that the GSTP1 Ile105/Val105 polymorphism was associated with increased survival in colorectal cancer patients who received oxaliplatin/5-fluorouracil chemotherapy. The survival increased with the number of GSTP1 Val105 alleles. Sun et al.54 showed the opposite (Table 3). These conflicting results were considered by the authors to be related to the different characteristics of patients included in the two studies. The patient groups differed in ethnicity, age and treatment. Furthermore, follow-up periods were different.

Polymorphisms in GSTM1 and GSTT1 were not associated with survival or clinical response in 107 patients with colorectal cancer treated with oxaliplatin/5-fluorouracil chemotherapy.53

Lung cancer
Platinum-based treatment is also common for patients with non-small cell lung carcinoma (NSCLC). Lu et al.55 concluded that the GSTP1 Ala114Val polymorphism, which leads to a less active enzyme, significantly increased overall survival ($P = 0.037$) in lung cancer patients treated with platinum-based chemotherapy (Table 3). The GSTP1 Ile105/Val polymorphism was not associated with a better response to treatment or an increase in survival.55-57

The relation between GSTM1 null and overall survival has also been investigated. Sweeney et al. studied 274 men with lung cancer (both non-small cell and small cell histologies) treated with radiotherapy and chemotherapy (not further specified) and found that those who had the GSTM1 null genotype had shorter overall survival. This association was independent of stage at diagnosis and histology, which were strong predictors of survival in this study.57

Other malignancies
Also in gastric and pancreatic cancer, multiple myeloma or Hodgkin’s lymphoma it has been demonstrated that patients harbouring the GSTP1 Val105 allele have a better clinical outcome32,58-60 (Table 3). Hohaus et al.60 showed that the GSTP1 Ile105/Val polymorphism was associated with an improved failure-free survival in patients with Hodgkin’s lymphoma receiving combination chemotherapy consisting of alkylating agents and anthracyclines, known substrates for GSTP1. This was also seen in multiple myeloma patients treated with conventional chemotherapy, however, not in those treated with high-dose chemotherapy.58 The reasons for the differences between conventional and high-dose chemotherapy in effect of GSTP1 polymorphisms on outcome are unknown but may be due to the capacity for high-dose chemotherapy to overcome functional differences between the genotypes. High-dose chemotherapy may lead to cellular depletion of glutathione.61,62 This may obscure any effect of polymorphisms in the GSTP1 gene. Furthermore, differences in GSTP1 specificity for the chemotherapeutic agents used in each arm could have contributed to the differences in effect seen.

In contrast, Lee et al.63 found a poorer prognosis of carriers of the GSTP1 Val105 allele in oesophageal cancer patients treated with platinum-based chemotherapy. The increase in hazard ratio of death with the allelic number of GSTP1 Val105 supported a gene-dose effect on patient survival.

Deletions in GSTM1 and/or GSTT1 have been associated with a longer overall survival and a longer disease-free survival in patients with Hodgkin’s lymphoma or glioma treated with anthracyclines and/or alkylating agents.54,65

Uridine diphosphate glucuronosyltransferase
Glucuronidation is a major pathway for the elimination of hydrophobic endogenous substrates such as steroids and bile acids and numerous xenobiotics including environmental carcinogens and cytotoxics. The enzymes that catalyse these reactions, UDP-glucuronosyltransferases (UGTs), are endoplasmic reticulum-bound transmembrane proteins that display tissue-specific expression patterns, including the
### Table 3  Association studies of polymorphisms in glutathione-S-transferase (GST) genes and survival

<table>
<thead>
<tr>
<th>Genes</th>
<th>SNP</th>
<th>Subjects</th>
<th>Treatment</th>
<th>Effect on end point</th>
<th>Risk measure (95% CI)</th>
<th>Reference</th>
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<td><strong>AML</strong></td>
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<td>GSTM1/T1</td>
<td>GSTM1/T1 null</td>
<td>106</td>
<td>Anthracycline-based</td>
<td>OS↓</td>
<td>GSTM1/T1 null HR = 2.4 (1.2–4.9)</td>
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<td>GSTM1</td>
<td>GSTM1 null</td>
<td>254</td>
<td>Anthracycline-based</td>
<td>OS↓</td>
<td>GSTM1/T1 null P = 0.03</td>
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<tr>
<td>GSTM1/T1</td>
<td>GSTM1 null</td>
<td>83</td>
<td>Anthracycline-based</td>
<td>DFS↓</td>
<td>GSTM1 null RR = 2.43 (1.05–5.58)</td>
<td>36</td>
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<td>GSTT1</td>
<td>GSTT1 null</td>
<td>306</td>
<td>Anthracycline-based</td>
<td>OS↓</td>
<td>GSTT1 null RR = 1.6 (P = 0.02)</td>
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<td>GSTT1 null</td>
<td>193</td>
<td>Anthracycline-based</td>
<td>OS↓</td>
<td>GSTT1 null RR = 1.53 (1.05–2.18)</td>
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<td><strong>Breast cancer</strong></td>
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<td>GSTM1</td>
<td>GSTM1 null</td>
<td>85</td>
<td>CP and cisplatin</td>
<td>OS↑</td>
<td>GSTM1 null P = 0.041</td>
<td>18</td>
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<td>GSTM1 null</td>
<td>239</td>
<td>Not reported</td>
<td>OS↑</td>
<td>GSTM1 null P = 0.036</td>
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<td>GSTM1/T1</td>
<td>GSTM1/T1 null</td>
<td>251</td>
<td>CAF</td>
<td>OS↑</td>
<td>GSTM1/T1 both null HR = 0.3 (0.11–0.70)</td>
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<td>CAF</td>
<td>DFS↑</td>
<td>GSTM1/T1 both null P = 0.01</td>
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<td>GSTT1 null</td>
<td>79</td>
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<td>OS↑</td>
<td>GSTT1 null HR = 0.2 (0.0–0.9)</td>
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<td>GSTP1</td>
<td>GSTP1 Ile&lt;sup&gt;105&lt;/sup&gt;Val</td>
<td>1034</td>
<td>CP-based</td>
<td>OS↑</td>
<td>GSTP1 Val&lt;sup&gt;105&lt;/sup&gt;/Val&lt;sup&gt;105&lt;/sup&gt; HR = 0.4 (0.2–0.8)</td>
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<td>GSTP1</td>
<td>GSTP1 Ile&lt;sup&gt;105&lt;/sup&gt;Val</td>
<td>240</td>
<td>CP-based</td>
<td>OS↑</td>
<td>GSTP1 Val&lt;sup&gt;105&lt;/sup&gt;/Val&lt;sup&gt;105&lt;/sup&gt; HR = 0.3 (0.1–1.0)</td>
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<tr>
<td>GSTA1</td>
<td>GSTA1&lt;sup&gt;*&lt;sub&gt;B&lt;/sub&gt;&lt;/sup&gt;</td>
<td>245</td>
<td>CP-based</td>
<td>OS↑</td>
<td>GSTA1&lt;sup&gt;<em>&lt;sub&gt;B&lt;/sub&gt;/</em>&lt;sub&gt;B&lt;/sub&gt; HR = 0.3 (0.1–0.8)</td>
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<tr>
<td><strong>Ovarian cancer</strong></td>
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<td>GSTM1/T1</td>
<td>GSTM1/T1 null</td>
<td>148</td>
<td>Platinum-based</td>
<td>OS↓</td>
<td>GSTM1/T1 both null HR = 3.44 (1.67–7.09)</td>
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<tr>
<td>GSTM1</td>
<td>GSTM1 null</td>
<td>24</td>
<td>Platinum-based</td>
<td>OS↑</td>
<td>GSTM1 null P = 0.013</td>
<td>49</td>
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<tr>
<td>GSTM1/P1</td>
<td>GSTM1 null/GSTP1 Ile&lt;sup&gt;105&lt;/sup&gt;Val</td>
<td>215</td>
<td>Platinum-based</td>
<td>DFS↑</td>
<td>GSTM1 null and GSTP1 Val&lt;sup&gt;105&lt;/sup&gt; HR = 0.42 (0.24–0.75)</td>
<td>47</td>
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<tr>
<td>GSTP1</td>
<td>GSTP1 Ile&lt;sup&gt;105&lt;/sup&gt;Val</td>
<td>266</td>
<td>Platinum-based</td>
<td>OS↑</td>
<td>GSTP1 Val&lt;sup&gt;105&lt;/sup&gt; HR = 0.77 (0.61–0.99)</td>
<td>50</td>
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<tr>
<td>GSTP1</td>
<td>GSTP1 Ile&lt;sup&gt;105&lt;/sup&gt;Val</td>
<td>81</td>
<td>Platinum-based</td>
<td>OS↑</td>
<td>GSTP1 heterozygous Ile&lt;sup&gt;105&lt;/sup&gt;Val HR = 0.34 (0.12–0.98)</td>
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<tr>
<td><strong>Colorectal cancer</strong></td>
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<td>GSTP1 Ile&lt;sup&gt;105&lt;/sup&gt;Val</td>
<td>107</td>
<td>Platinum-based</td>
<td>OS↑</td>
<td>GSTP1 Val&lt;sup&gt;105&lt;/sup&gt; P = 0.042</td>
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<td>GSTP1</td>
<td>GSTP1 Ile&lt;sup&gt;105&lt;/sup&gt;Val</td>
<td>125</td>
<td>Platinum-based</td>
<td>OS↑</td>
<td>GSTP1 Val&lt;sup&gt;105&lt;/sup&gt;/Val&lt;sup&gt;105&lt;/sup&gt; and Ile&lt;sup&gt;105&lt;/sup&gt; / Val&lt;sup&gt;105&lt;/sup&gt; HR = 5.3 (1.26–19.53)</td>
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<td><strong>Lung cancer</strong></td>
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<td>GSTP1</td>
<td>GSTP1 Ala&lt;sup&gt;114&lt;/sup&gt;Val</td>
<td>425</td>
<td>Platinum-based</td>
<td>OS↑</td>
<td>GSTP1 Ala&lt;sup&gt;114&lt;/sup&gt;/Val&lt;sup&gt;114&lt;/sup&gt; or Val&lt;sup&gt;114&lt;/sup&gt;/Val&lt;sup&gt;114&lt;/sup&gt; HR = 0.75 (0.54–1.05)</td>
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<td>GSTM1 null</td>
<td>274</td>
<td>Chemotherapy/radiotherapy</td>
<td>OS↓</td>
<td>GSTM1null RR = 1.36 (1.04–1.80)</td>
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<td>Other malignancies</td>
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<td>Subjects</td>
<td>Treatment</td>
<td>Effect on end point</td>
<td>Risk measure (95% CI)</td>
<td>Reference</td>
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<td>Gastric cancer</td>
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<td>52</td>
<td>Platinum-based</td>
<td>OS↑</td>
<td>GSTP1 Val&lt;sup&gt;105&lt;/sup&gt; / Val&lt;sup&gt;105&lt;/sup&gt; P = 0.037</td>
<td>59</td>
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<td>Pancreatic cancer</td>
<td>GSTP1 Ile&lt;sup&gt;105&lt;/sup&gt;/Val&lt;sup&gt;105&lt;/sup&gt;</td>
<td>52</td>
<td>5-FU</td>
<td>OS↑</td>
<td>GSTP1 Val&lt;sup&gt;105&lt;/sup&gt; / Val&lt;sup&gt;105&lt;/sup&gt; P = 0.037</td>
<td>59</td>
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<tr>
<td>Hodgkin’s Lymphoma</td>
<td>GSTM1/T1 null</td>
<td>90</td>
<td>Alkylating agents and anthracyclines</td>
<td>DFS↑</td>
<td>GSTM1 null or GSTT1 null HR = 0.29 (0.10–0.86)</td>
<td>64</td>
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<td>GSTP1 Ile&lt;sup&gt;105&lt;/sup&gt;/Val&lt;sup&gt;105&lt;/sup&gt;</td>
<td>97</td>
<td>Alkylating agents and anthracyclines</td>
<td>Failure-free survival↑</td>
<td>GSTP1 Val&lt;sup&gt;105&lt;/sup&gt; HR = 0.42 (0.21–0.85)</td>
<td>60</td>
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<tr>
<td>Multiple Myeloma</td>
<td>GSTP1 Ile&lt;sup&gt;105&lt;/sup&gt;/Val&lt;sup&gt;105&lt;/sup&gt;</td>
<td>101</td>
<td>Alkylating agents and anthracyclines</td>
<td>PFS↑</td>
<td>GSTP1 Val&lt;sup&gt;105&lt;/sup&gt; / Val&lt;sup&gt;105&lt;/sup&gt; P = 0.037</td>
<td>59</td>
</tr>
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<td></td>
<td>GSTP1 Ile&lt;sup&gt;105&lt;/sup&gt;/Val&lt;sup&gt;105&lt;/sup&gt;</td>
<td>233</td>
<td>Platinum-based</td>
<td>OS↓</td>
<td>GSTP1 Val&lt;sup&gt;105&lt;/sup&gt; HR = 1.36 (1.01–1.84)</td>
<td>63</td>
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<td>GSTP1 Ala&lt;sup&gt;114&lt;/sup&gt;/Val&lt;sup&gt;114&lt;/sup&gt;</td>
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<td>OS↓</td>
<td>GSTP1 Ala&lt;sup&gt;114&lt;/sup&gt; / Val&lt;sup&gt;114&lt;/sup&gt; HR = 2.10 (1.14–3.89)</td>
<td>80</td>
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<td>Oesophageal cancer</td>
<td>GSTP1 Ile&lt;sup&gt;105&lt;/sup&gt;/Val&lt;sup&gt;105&lt;/sup&gt;, Ala&lt;sup&gt;114&lt;/sup&gt;/Val&lt;sup&gt;114&lt;/sup&gt;</td>
<td>278</td>
<td>Alkylating agent-based</td>
<td>OS↑</td>
<td>GSTP1 Val&lt;sup&gt;105&lt;/sup&gt; / Ala&lt;sup&gt;114&lt;/sup&gt; + GSTM1 null P = 0.06</td>
<td>65</td>
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</table>

CP, cyclophosphamide; CAF, cyclophosphamide, doxorubicin, 5-fluorouracil; 5-FU, 5-fluorouracil; DFS, disease-free survival; PFS, progression-free survival; OS, overall survival; HR, hazard ratio; RR, relative risk.
gut, skin, placenta, breast and prostate gland. Most of the UGTs are expressed in the liver as well as other extrahepatic tissues; however, some are exclusively extrahepatic.

Thus far, 17 functional UGTs encoded by the UGT gene family have been identified in humans, with some substrate specificity but also significant overlap in their ability to detoxify substrates. Human UGT isoforms have been classified into two families of proteins, UGT1 and UGT2. UGT has several functional genetic polymorphisms. The UGT1A1*6 variant is associated with defective glucuronidating function while the UGT2B15*2 variant is located within the putative substrate recognition site of the enzyme and is associated with increased catalytic activity.

UGT1A1 is responsible for the inactivation of SN-38 (the active metabolite of irinotecan) to SN-38 glucuronide. UGT2B15 catalyzes the biotransformation of a number of steroid substrates, including 4-hydroxytamoxifen, thereby facilitating their excretion. Table 4 gives an overview of studies describing a relation between polymorphisms in UGT genes and survival.

Han et al. studied a group of 81 patients with non-small cell lung cancer, who were treated with irinotecan. The patients who had the UGT1A1*6/6 genotype had significantly shorter overall survival compared to patients with other genotypes (Table 4). This finding is difficult to explain, since decreased inactivation of SN-38 in tumour cells, as a result of the presence of the defective glucuronidation, should be associated with better survival. These data, however, were not controlled for other genetic and environmental factors, patient demographics and tumour histology, which can result in the identification of false positive results. UGT1A1*6 homozygous patients also had more toxicity. Therefore, another explanation for the negative effect of UGT1A1*6 on efficacy is that irinotecan dose-intensity/density or cycle number might have been lower in *6 carriers because of toxicity observed during the first cycle of chemotherapy. However, dose delays and number of cycles were not reported.

The UGT2B15*2 variant appears to be a risk factor for the recurrence and poorer survival of breast cancer patients treated with tamoxifen who also have the variant SULT1A1*2 allele. A study by Nowell et al. in breast cancer patients treated with tamoxifen showed, that patients with at least one UGT2B15*2 allele and also the SULT1A1*2 allele had significantly reduced 5-year survival rates (P = 0.003).

### Table 4: Association studies of polymorphisms in other phase II enzyme genes and survival

<table>
<thead>
<tr>
<th>Genes</th>
<th>SNP</th>
<th>Subjects</th>
<th>Disease</th>
<th>Treatment</th>
<th>Effect on end point</th>
<th>Risk measure (95% CI)</th>
<th>Reference</th>
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<td>UGT1A1</td>
<td>UGT1A1*6</td>
<td>81</td>
<td>NSCLC</td>
<td>Irinotecan</td>
<td>OS</td>
<td>UGT1A1*6/*6 P = 0.017</td>
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<td>UGT2B15</td>
<td>UGT2B15*2</td>
<td>160</td>
<td>Breast cancer</td>
<td>TAM</td>
<td>OS</td>
<td>UGT2B15<em>2 and SULT1A1</em>2</td>
<td>68</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HR = 4.40 (1.17–16.55)</td>
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</tr>
<tr>
<td>SULT1A1</td>
<td>SULT1A1*2</td>
<td>160</td>
<td>Breast cancer</td>
<td>TAM</td>
<td>OS</td>
<td>SULT1A1*2/*2 HR = 2.9 (1.1–7.6)</td>
<td>75</td>
</tr>
<tr>
<td>SULT1A1</td>
<td>SULT1A1*1</td>
<td>112</td>
<td>Breast cancer</td>
<td>TAM</td>
<td>RFS†</td>
<td>SULT1A1*1/<em>1 and/or CYP2D6</em>4</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RR = 0.38 (0.19–0.74)</td>
<td>13</td>
</tr>
</tbody>
</table>

CP, cyclophosphamide; TAM, tamoxifen; 5-FU, 5-fluorouracil; DFS, disease-free survival; EFS, event-free survival; RFS, recurrence-free survival; OS, overall survival; HR, hazard ratio; RR, relative risk.

### Sulotransferase

Cytosolic sulotransferases (SULTs) transfer sulphate-moieties to nucleophilic groups of xenobiotics and small endogenous compounds, thereby increasing water solubility and decreasing passive penetration of cell membranes. This enhances the urinary and biliary excretion.

All known cytosolic sulotransferases are members of a single superfamily, named SULT. In humans, 10 SULT genes are known. Genetic polymorphisms have been described for three human SULTs, namely SULT1A1, SULT1A2 and SULT2A1. SULT1A1 is present in various human tissues such as the liver, brain and platelets. A wide racial variation in SULT1A1 polymorphisms is reported. The allele frequency for SULT1A1*2 in Caucasian subjects is 33.2%, while Chinese subjects have an allele frequency of 8.0% and African-American subjects have an allele frequency of 29.4%.

The SULT1A1*2 variant allele is associated with decreased catalytic activity. 4-Hydroxytamoxifen and endoxifen are subject to conjugation leading to excretion by sulfation, via SULT1A1.

Table 4 gives an overview of studies describing a relation between polymorphisms in the SULT1A1 gene and survival.

Two studies have demonstrated an association between the presence of SULT1A1*2 and shorter survival (Table 4). In 160 breast cancer patients who were treated with tamoxifen (dose not specified), those patients homozygous for the low-activity SULT1A1*2 allele had a threefold increase in risk of death as those with one or more common alleles. This association persisted even when adjustments were made for age, race, clinical stage of tumour at diagnosis and presence or absence of progesterone receptor. Similar associations were reported by Wegman et al. They showed that in 112 breast cancer patients who received tamoxifen (40 mg daily for 2 years), SULT1A1*2 was associated with an increased risk of recurrence.

Although the results of these studies are in contrast to what was expected (that lower SULT1A1 activity would theoretically result in reduced elimination of active metabolites), sulfation of 4-hydroxytamoxifen may modify the pharmacokinetics of tamoxifen therapy or beneficially
alter the receptor-binding properties of 4-hydroxy-tamoxifen.

**Drug transporters**

**Multidrug resistance 1**

The multidrug resistance 1 (MDR1) gene (ABCB1) in humans is responsible for multidrug resistance. The protein product of this gene is a 170-kDa transmembrane glycoprotein referred to as P-glycoprotein (P-gp), which is a membrane protein. P-gp is a member of the adenosine triphosphate-binding cassette (ABC) superfamily of membrane transporters and is involved in the active transport of a great number of amphipathic molecules through lipid membranes. P-gp is expressed in tumour cells, but also in normal tissues with an excretory function (intestine, liver, kidney).\(^7\)

The most important physiological role of P-gp is the protection against toxic xenobiotics. Moreover, a wide range of anti-cancer agents can be actively extruded by P-gp, which can lead to chemoresistance.

To date, at least 50 SNPs and 3 insertion/deletion polymorphisms have been reported in the MDR1 gene, some of which appear to be associated with altered transporter function and expression, thereby affecting the metabolism and disposition of drugs.\(^7\)\(^7\) The G2677T SNP at exon 26 leads to a change in the amino acid sequence from Ala to Ser (G2677T) or Thr (G2677A) in the second transmembrane domain of P-gp. The functional role for this SNP still remains controversial. A SNP in exon 26 position 3435 (C3435T) has been found to be associated with the expression and function of P-gp.\(^7\)\(^9\) The variant TT genotype of the C3435T SNP is associated with lower MDR1 expression and higher plasma drug concentration.\(^8\)\(^0\) Table 5 gives an overview of studies describing a relation between polymorphisms in the MDR1 gene and survival.

Anthracyclines, vinca alkaloids and epipodophyllotoxins, which are drugs commonly used in the treatment of AML and ALL, belong to the P-gp substrates.\(^8\)\(^1\) Functional polymorphisms of MDR1 may, therefore, be involved in the outcome of AML and ALL by two mechanisms. The first involves the multidrug resistance related to P-gp expression in AML/ALL blasts and the other involves the influence of P-gp on the pharmacokinetics of anti-cancer drugs. Carriers of low P-gp expression-coding genotype may show higher exposure to chemotherapy considering the role of P-gp in multidrug resistance and in the pharmacokinetics of anti-cancer agents, resulting in a better survival.

A study by Illmer et al.\(^8\)\(^2\) that investigated three MDR1 SNPs at exons 12, 21 and 26 revealed that the genotype at exon 26 (position 3435) was associated with overall survival and the probability of relapse in the Caucasian AML population (Table 5). They found that AML patients who were wild-type for the polymorphism of the MDR1 gene at position 3435 were likely to have decreased survival because of an increased relapse risk. They were not able to determine whether this phenomenon was attributable to characteristics of the AML blast population or attributable to altered P-gp-mediated drug pharmacokinetics. This finding is in accordance with the finding of Jamroziak et al.\(^7\)\(^6\) who found a worse prognosis in children with ALL with the wild-type genotype.

In contrast, Kim et al.\(^8\)\(^3\) reported a better event-free survival in AML patients with the wild-type genotype at exon 26. This could be due to ethnic differences in the patient groups in those studies (Caucasians and Koreans). The C3435T mutation is a silent mutation that does not cause amino acid substitution and is suggested to be linked with

<table>
<thead>
<tr>
<th>Genes</th>
<th>SNP</th>
<th>Subjects</th>
<th>Disease</th>
<th>Treatment</th>
<th>Effect on end point</th>
<th>Risk measure (95% CI)</th>
<th>Ref.</th>
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<tr>
<td>MDR1</td>
<td>MDR1 G2677A</td>
<td>99</td>
<td>Hepatocellular carcinoma</td>
<td>Prednisone, tacrolimus, mycophenolate, cyclosporine</td>
<td>RFS↑</td>
<td>MDR1 GA/AA P = 0.015</td>
<td>77</td>
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<tr>
<td>MDR1</td>
<td>MDR1 G2677A</td>
<td>82</td>
<td>AML, CML, ALL</td>
<td>Etoposide, mitoxantrone, idarubicin, idarubicin, cytarabine</td>
<td>OS↓</td>
<td>MDR1 GG HR = 2.651 (1.386–5.070)</td>
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<tr>
<td>MDR1</td>
<td>MDR1 G2677T</td>
<td>30</td>
<td>AML</td>
<td>Etoposide, mitoxantrone, idarubicin</td>
<td>OS↓</td>
<td>MDR1 GG or TT P = 0.02</td>
<td>85</td>
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<tr>
<td>MDR1</td>
<td>MDR1 G2677T/A + C3435T</td>
<td>81</td>
<td>AML</td>
<td>Etoposide, mitoxantrone, idarubicin, cytarabine</td>
<td>EFS↓</td>
<td>MDR1 without homozygous GC haplotype HR = 2.455 (1.088–5.539)</td>
<td>83</td>
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<tr>
<td>MDR1</td>
<td>MDR1 C3435T</td>
<td>405</td>
<td>AML</td>
<td>Etoposide, mitoxantrone, daunorubicin</td>
<td>OS↓</td>
<td>MDR1 CC P &lt; 0.01</td>
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<td>MDR1 C3435T</td>
<td>111</td>
<td>ALL</td>
<td>Etoposide, daunorubicin, vincristine</td>
<td>OS↓</td>
<td>MDR1 CC HR = 3.3 (P = 0.02)</td>
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<tr>
<td>MDR1</td>
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<td>OS↑</td>
<td>MDR1 CT/TT HR = 0.44 (0.23–0.85)</td>
<td>80</td>
</tr>
<tr>
<td>ABCG2</td>
<td>ABCG2 C421A</td>
<td>51</td>
<td>Prostate cancer</td>
<td>Docetaxel</td>
<td>OS↑</td>
<td>ABCG2 CA/AA P = 0.05</td>
<td>89</td>
</tr>
</tbody>
</table>

EFS, event-free survival; RFS, recurrence-free survival; OS, overall survival; HR, hazard ratio.
Table 5. The effect of therapy in the presence of the patients survived beyond 15 months with docetaxel-based zymes, the clearest relation is found for in relation to cancer treatment response. A number of polymorphisms in genes encoding drug meta-

Discussion

The ATP-binding cassette transporter G2 gene (ABCG2) is a member of the G subfamily of ABC transporters and encodes breast cancer resistance protein (BCR), which is also called mitoxantrone resistant protein (MXR) or placenta-specific ATP binding cassette transporter (ABCP). The protein with six transmembrane segments and one ATP-binding cassette is located in the plasma membrane. BCRP protects tissues by actively transporting toxic substances and xenobiotics out of the cells. SNPs have been reported in the ABCG2 gene.86 The C421A polymorphism is associated with decreased protein expression.85 Hahn et al.89 showed that a greater proportion of patients survived beyond 15 months with docetaxel-based therapy in the presence of the ABCG2 C421A polymorphism (Table 5). The effect of ABCG2 polymorphisms on docetaxel disposition is unknown. The increased survival observed in individuals with an ABCG2 C421A polymorphism may suggest a less functional drug efflux pump, leading to increased intracellular docetaxel concentrations and improved cytotoxic activity. However, this hypothesis should be interpreted cautiously due to the small patient sample size and potential confounding variables. No multivariate analysis and no adjustment for multiple testing were performed.

A number of polymorphisms in genes encoding drug metabolising enzymes and drug transporters have been described in relation to cancer treatment response. Concerning polymorphisms in genes encoding CYP enzymes, the clearest relation is found for CYP2D6 and tamoxifen treatment. Several studies have found that patients with breast cancer and CYP2D6 non-functional alleles treated with tamoxifen have shorter relapse-free periods, worse event-free survival and lower overall survival. Even though the studies differed in number of patients and patient groups with different ethnicity were included, these studies have identified consistent results. Based on these results it can, however, not be concluded yet that genotyping for the treatment of tamoxifen is needed. The data available at present represent retrospective analyses of banked samples. Therefore, the association between polymorphisms in the CYP2D6 gene and survival needs further evaluation in prospective studies in a large population of breast cancer patients treated with tamoxifen.

The GST genes are the most extensively studied genes of the phase II enzymes. Several associations have been found between polymorphisms in the various GST genes and cancer treatment response. Polymorphisms in GSTP1 and GSTA1, leading to a less active enzyme, have been associated with improved survival. Deletions in GSTM1 and GSTT1, leading to reduced or no GST activity, resulted in a significantly better prognosis in most malignancies due to a better response to chemotherapy. However, in AML patients with GSTM1 null and/or GSTT1 null genotypes, a worse prognosis was seen due to an increase in toxic deaths. Therefore, the significance of GST enzymes may be different for each malignancy and therapy.

Pharmacogenetic studies in cancer patients have several limitations. Firstly, the small number of patients and the lack of control for other genetic and environmental factors, patient demographics and tumour histology, can result in the identification of false positive results. Secondly, the use of different genotyping techniques and patients of different ethnic origin, that often show different frequencies for mutant alleles, make it difficult to compare the different studies.

Most pharmacogenetic studies investigate the effect of only one or a few single nucleotide polymorphisms in a specific gene at a time, the candidate gene approach. Obviously, this mechanistic approach appears logical. This approach is hypothesis driven, uses a priori knowledge of single nucleotide polymorphisms and gene functions and has produced informative data. The disadvantage of this approach is that it is limited by the present knowledge of pathophysiology and the mechanism of action of a drug. Therefore, future research will also use hypothesis-free whole genome approaches such as SNP arrays. The genome wide approach can discover previously unknown associations of factors as well as identify potential multigenic associations. A drawback is that the results can be affected by false positives, associated with unimportant genes identified by chance.

It is evident that large studies based upon combined analysis of multiple genes within the metabolic pathway are needed for unravelling prognostically important individual polymorphism profiles. Furthermore, prospective trials are required to establish clinical value and cost-effectiveness of pharmacogenetic testing in oncology.

Conclusions

A single nucleotide polymorphism is not likely to afford a straightforward risk factor for analysis of survival. Population diversities and pharmacokineti/pharmacodynamic polymorphisms must also be taken into account. The studies presented here suggest that genetic polymorphisms may provide useful prognostic markers in some situations. More insight into the mechanism of action of these markers, the biological determinants of response to treatment and prognosis in cancer will ultimately lead to individualised cancer
treatment based on a combination of genotype and tumour characteristics of a patient.

**Conflict of interest statement**

None of the authors report a conflict of interest.

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