Universiteit
Leiden
The Netherlands

Pharmacogenetics of advanced colorectal cancer treatment

Pander, J.

Citation
Pander, J. (2011, June 29). *Pharmacogenetics of advanced colorectal cancer treatment*. Retrieved from https://hdl.handle.net/1887/17746

Version:	Corrected Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/17746

Note: To cite this publication please use the final published version (if applicable).

5

Current insights in the role of heritable genetic variation in the pharmacokinetics and pharmacodynamics of anti-cancer drugs

Jan Pander • Hans Gelderblom • Henk-Jan Guchelaar

Expert Opinion on Pharmacotherapy 2007 Jun;8(9):1197-210

Abstract

Pharmacogenetics in oncology ideally will allow oncologists to individualise therapy based upon a genetic test result. Severe toxicity and clinically significant under-dosing may be avoided, whereas predicted non-responders can be offered alternative therapy.
This manuscript gives an overview of heritable variants in the genes of nine enzymes or pathways that have been studied most extensively in anti-cancer chemotherapy.
Even though many pharmacogenetic association studies have been published, there is need for more research. In particular, there is need for replication of data and development of predictive models. Prospective trials are required to establish clinical value and cost-effectiveness of pharmacogenetic testing in oncology.

Introduction

Pharmacogenetics studies the association between heritable functional variants in DNA (genotype) with outcome of therapy (phenotype). In the recent years, pharmacogenetics in oncology has become an increasing field of research. Ideally, pharmacogenetic testing will allow oncologists to individualise therapy, with respect to the choice of a drug and the dose of the drug administered, based upon a genetic test result. Severe toxicity may be avoided, whereas predicted non-responders can be offered alternative therapy.

A polymorphism is an inheritable variant that occurs within at least 1% of the population. Moreover, a polymorphism is a neutral variant: the variant may have functional consequences on the protein level, without influencing existence of the individual.
Variants in DNA can be single nucleotide polymorphisms (SNPs), deletions or insertions of a number of base pairs (bp) or variable number of tandem repeats resulting in changes in exons, introns or in untranslated regions (UTR), such as the promoter region of the gene. When DNA is transcripted into mRNA, some of these variants may result in altered mRNA stability. Some variants result in different amino acid composition of proteins or truncated proteins which may lead to altered enzyme activity and thus functionality (non-synonymous variants), whereas other variants do not result in amino acid change (synonymous or silent variants). Finally, variants in a UTR of a gene can alter the transcriptional activity of a gene and thus change the expression of an enzyme.
As specific regions in DNA are conserved though generations, variants are often inherited as so called haplotypes, which can be measured by assessing linkage disequilibrium (LD).

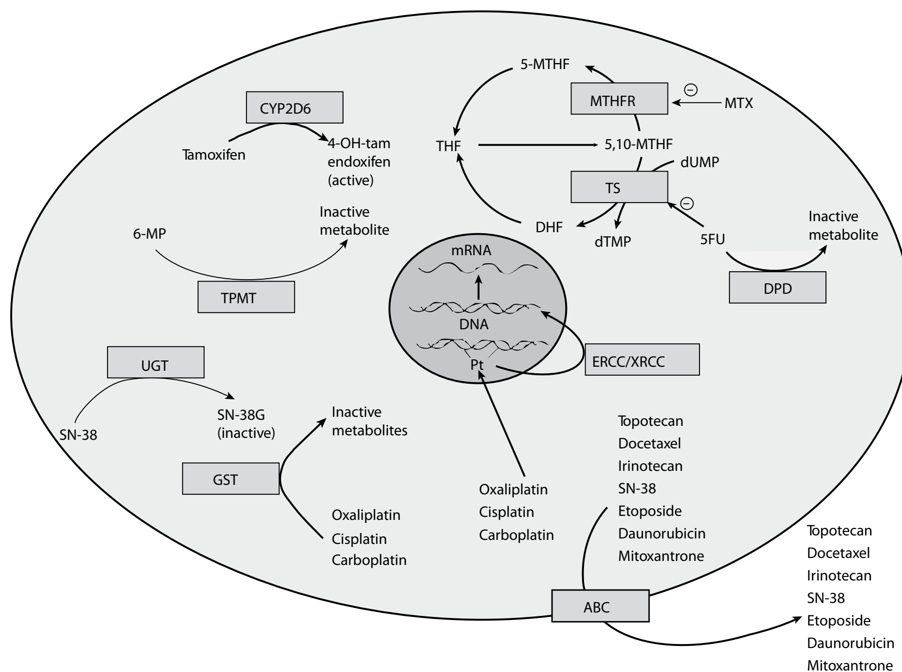
In contrast to somatic variants, heritable (germ-line) variants in DNA are inherited from parents, and the presence of a variant can be either heterozygous (carrier of one normal and one variant allele) or homozygous (carrier of two variant alleles) as compared to wild-type (two normal alleles). The determination of germ-line variants in, for example DNA isolated from peripheral blood is much more feasible than determination of variants in DNA isolated from tumour samples. Interestingly, in a recent paper it was shown that there is a high degree of concordance between germ-line and somatic variants for a number of SNPs.[1] However, genetic mutations related to the origin of the malignant phenotype are by definition in discordance to the germ-line phenotype.

In this manuscript, we give an overview of heritable variants in the genes encoding nine enzymes or pathways that have been studied most extensively in patients

treated with anti-cancer drugs (Figure 1). The variants in these genes are summarised in Table 1.

Medline was systematically searched (from July 1st to September 30th 2006) with the following set of keywords: pharmacogenetics, pharmacogenomics, polymorphism, SNP, genotype, phenotype, antineoplastic (protocols), chemotherapy, combined with the names of genes and enzymes, limiting results to human research published in English.

Figure 1 Schematic overview of enzymes involved in cellular response and metabolism to anti-cancer drugs



Abbreviations: CYP2D6: cytochrome P450 2D6; 4-OH-tam: 4-hydroxy-tamoxifen; DPD: dihydropyrimidine dehydrogenase; 5FU: 5-fluorouracil; MTHF: methylene tetrahydrofolate; MTHFR: methylene tetrahydrofolate reductase; MTX: methotrexate; THF: tetrahydrofolate; 6-MP: 6-mercaptopurine; dUMP: deoxyuridine monophosphate; TPMT: thiopurine S-methyltransferase; TS: thymidylate synthase; DHF: dihydrofolate; dTMP: deoxythymidine monophosphate; UGT: uridine diphosphate glucuronosyl transferase; ERCC: excision repair cross complementing; XRCC: X-ray repair cross complementing; SN-38: active metabolite of irinotecan; SN-38G: SN-38 glucuronide; GST: glutathione S-transferase; ABC: ATP-binding cassette

Table 5 Enzymes involved in response to anti-cancer drugs and their common polymorphisms

enzyme	variant allele	polymorphism	phenotype
TPMT	*TPMT*2*	238G>C	Ala80Pro
	*TPMT*3A*	460G>A + 719A>G	Ala154Thr + Tyr240Cys
DPD	*DPYD*2A*	IVS14+1G>A	Δ exon 14
UGT	*UGT1A1*28*	$(TA)_7TAA$	reduced enzyme activity
GST π	*GSTP1-105*	313A>G	Ile105Val
GST μ	*GSTM1-null*		deletion of gene
GST θ	*GSTT1-null*		deletion of gene
P-glycoprotein	*ABCB1 = MDR1*	1236C>T	silent
		3435C>T	silent
		2677G>T/A	Ala893Ser/Thr
BCRP	*ABCG2-421*	421C>A	Gln141Lys
ERCC1	*ERCC1-118*	496C>T	Asn118Asn
XPD	*ERCC2-321*	965G>A	Asp321Asn
	ERCC2-751	2251A>C	Lys751Gln
XRCC1	*XRCC1-399*	1301G>A	Arg399Gln
CYP2D6	*CYP2D6*4*	1846G>A	null enzyme activity
MTHFR	*MTHFR-677*	677C>T	Ala222Val
TS	*TYMS TSER*	28 bp insert in TSER	increased expression
	TYMS	G>C at bp 12 in TSER-3	restored enzyme activity
	TYMS 3' UTR	1494 6bp indel	decreased expression

TPMT: thiopurine S-methyltransferase; DPD/DPYD: dihydropyrimidine dehydrogenase; UGT: uridine diphosphate glucuronosyl transferase; GST: glutathione S-transferase; ABCB1: ATP-binding cassette B1; MDR1: multi drug resistance 1; BCRP: breast cancer resistance protein; ABCG2: ATP-binding cassette G2; ERCC1: excision repair cross complementing group 1; XPD: xeroderma pigmentosum group 1; ERCC2: excision repair cross complementing group 2; XRCC1: X-ray repair cross complementing group 1; CYP2D6: cytochrome P450 2D6; MTHFR: methylene tetrahydrofolate dehydrogenase; TS/TYMS: thymidylate synthase; TSER: thymidylate synthase enhancer region; UTR: untranslated region; bp: base pair; indel: insertion/deletion

Pharmacogenetic association studies

Thiopurine S-methyltransferase

An alkylating agent commonly used in maintenance treatment of acute lymphoblastic leukaemia (ALL) 6-mercaptopurine (6MP), which is deactivated by the enzyme thiopurine S-methyltransferase (TPMT). Approximately 0.3% and 10% of the population has undetectable and intermediate TPMT enzyme activity respectively.[2,3] TPMT activity is inversely associated with exposure to the cytotoxic metabolite of 6MP, 6-thioguanine (6TGN), in red blood cells[4] and in ALL blasts.[5] Because of severe haematological

toxicity, 6MP dose must be reduced[6], with as much as 90% and 50-66% for the respective phenotypes.[7] Because dose intensity proved to be a prognostic marker for outcome in ALL patients treated with 6MP, it is important to administer the right dose with regard to toxicity[8] and efficacy.[4]

Therefore, TPMT activity is a determinant for predicting the occurrence of toxicity. However, it must be noticed that TPMT activity is influenced by several common factors in ALL, such as methotrexate (MTX)/trimethoprim treatment[9] or administration of red blood cells transfusions.[10]
The molecular basis of decreased TPMT activity was found in 1995. A 238G>C SNP resulting in amino acid change of alanine to proline in codon 80 (Ala80Pro), and in 100 fold decrease in enzyme activity was found in a patient who experienced severe toxicity to 6MP.[11] This allele is referred to as *TPMT*2*.
The variant allele *TPMT*3A* was found a year later (460G>A; Ala154Thr + 719A>G; Tyr240Cys) in a patient with almost absent TPMT activity.[12] To date, at least 25 variant alleles have been found, and their functional significance has been described.[13,14] However, approximately 85-95% of all variant alleles in Caucasians is *TPMT*2*, *TPMT*3A* and *TPMT*3C*.[15,16] The *TPMT*3A* has an allele frequency of 4%[17] but is absent in African and Asian populations.[16,18-21] In these populations, the *TPMT*3C* allele (719A>G; Tyr240Cys) is the most frequent variant allele[16,18-21] and its functional impact has been demonstrated in Japanese children with ALL.[22]
A strong relationship between genotype and phenotype has been demonstrated, resulting in 90% sensitivity and 99% specificity (variants *TPMT*2-*18*).[3] However, in another report no *TPMT*2*, *TPMT*3A* and *TPMT*3C* allele was detected in 5 of 9 patients with intermediate TPMT activity.[7]
Even though cost effectiveness models of TPMT genotyping have been reported recently[23], and the Food and Drug Administration (FDA) has included more information on inherited TPMT deficiency in the 6MP label [201], only few institutions commonly genotype patients prior to 6MP treatment.[24]

Interestingly, exposure to the cytotoxic metabolite 6TGN is not only related to toxicity, but also to efficacy of 6MP therapy as shown by Stanulla *et al.* They found a 2.9 fold lower occurrence of residual disease in ALL patients who were heterozygous for any *TPMT*2*, *TPMT*3A*, *TPMT*3C* or *TPMT*9* (356A>C; Lys119Thr) allele and treated with a similar 6MP dose. They did not find a difference in the occurrence of toxicity.[25]

Dihydropyrimidine dehydrogenase
An important anti-metabolite used for a vast number of different types of solid tumours is 5-Fluorouracil (5FU). Over 80% of 5FU is inactivated in the liver by the enzyme dihydropyrimidine dehydrogenase (DPD), encoded by the gene *DPYD*. Since

a DPD deficient patient experiencing severe haematological toxicity to 5FU was described in 1988[26] many mutations in the *DPYD* gene that result in decreased DPD activity have been identified. Apart from association studies between DPD enzyme activity and 5FU toxicity, genetic associations have also been described. It must be noted that not all 5FU related toxicity can be attributed to decreased DPD activity though.
Despite having an allele frequency of <1% in Caucasians[27,28], a SNP in the 5' invariant splice donor sequence in intron 14 of the *DPYD* gene (IVS14+1G>A; deletion of exon 14; *DPYD*2A*) seems to be one of the key mutations resulting in low DPD activity and increased incidence of 5FU toxicity.[29] Two studies have shown considerable effect of this polymorphism on the incidence of 5FU toxicity. In 60 cancer patients who experienced grade 3-4 toxicity (according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) [202]) to 5FU containing chemotherapy, the frequency of the *DPYD*2A* allele was 15%, which was significantly higher than 0.91% in the control population (P=0.001).[30] Also, 50% of patients who experienced NCI-CTC grade 4 neutropenia carried the *DPYD*2A* allele.[31] The *DPYD*2A* allele has not been detected in Asian populations.[32,33]
Other variants of the *DPYD* gene that have been linked to 5FU toxicity include the *DPYD*4* (1601G>A; Ser534Asn), *DPYD*11* (1003G>T; Val335Leu), *DPYD*12* (62G>A; Arg21Gln + 1156G>T; Glu386Ter) and *DPYD*13* (1679T>G; Ile560Ser) alleles.[34,35] On the other hand, methylation of the promoter region of the *DPYD* gene also seems to reduce DPD activity due to a decrease of transcription.[36]
Homozygous patients with two low DPD activity alleles are rare, but multiple cases have been described of lethal outcome to 5FU treatment in these patients.[27,28]

Uridine diphosphate glucuronosyl transferase
Uridine diphosphate glucuronosyl transferase (UGT) is a phase II metabolic enzyme responsible for glucuronidation of several endogenous (such as bilirubin) and exogenous compounds. SN-38, the active metabolite of the topo-isomerase I inhibitor irinotecan, is predominantly inactivated by the isoforms UGT1A1 and UGT1A9 in the liver and by UGT1A7 in the upper gastro-intestinal tract.[37]
A TA insert polymorphism in the TATA box of the promoter region of the *UGT1A1* gene has been studied extensively. The *UGT1A1*28* allele has 7 TA repeats, whereas wild-type has 6 TA repeats. This polymorphism is associated with Gilbert's syndrome, a condition of reduced bilirubin glucuronidation[38] and has also been associated with decreased SN-38 glucuronidation *in vitro*[39] and *in vivo*.[40,41] The allele frequency of *UGT1A1*28* is higher in Caucasians (22-39%)[41-44] than in Asians (7-17%)[44-49], and even higher in Blacks (~45%).[45,46]
Patients with metastatic colorectal cancer (mCRC) or other solid tumours who were treated with irinotecan, and who were homozygous for the *UGT1A1*28* allele had

5

significant higher occurrence of grade 3 or 4 diarrhoea (according to criteria of the World Health Organization (WHO)[50]) [51] and NCI-CTC grade 3 or 4 neutropenia[40,43] compared with patients who carried at least one wild-type allele. Other studies have shown higher incidence of NCI-CTC grade 3 or 4 diarrhoea in mCRC patients[52] and higher incidence of grade 3 or 4 diarrhoea and/or grade 4 leukopenia (according to criteria of the Japan Society for Cancer Therapy[53]) in patients with solid tumours[47] when carriers of the variant allele were compared with homozygote wild-type patients. In a recent report of 250 mCRC patients, the odds ratio for the incidence of NCI-CTC grade 3 or 4 haematological toxicity was 8.63 for patients homozygous for the variant allele compared with patients who were homozygote wild-type. However, this was only significant after the first cycle of treatment.[54] Interestingly, response to irinotecan therapy was also improved for homozygote individuals for the variant allele compared with homozygote individuals for the wild-type allele because of increased SN-38 exposure.[54]

From these studies it is clear that the *UGT1A1*28* allele is associated with increased risk for neutropenia in patients receiving irinotecan. Due to increased exposure to SN-38, the active metabolite of irinotecan, it may also be expected that carriers of this allele experience increased efficacy but this has not yet been proven.

A SNP in the phenobarbital responsive enhancer module (PBREM) of the *UGT1A1* gene (-3279T>G; *UGT1A1*60*) has been associated with severe toxicity (grade 4 leukopenia and grade 3 or 4 diarrhoea; Japanese criteria) in Japanese cancer patients treated with irinotecan.[55] However, the *UGT1A1**60 variant allele was linked to the *UGT1A1*28* variant.[39]

Several SNPs in various regions of the *UGT1A1*, *UGT1A7* and *UGT1A9* genes have been found to be in linkage disequilibrium (LD).[44,48,49,56] The functional and clinical relevance of these haplotypes has not yet been established.

In 2005, the FDA approved the Invader® UGT1A1 molecular assay, a test for the *UGT1A1*28* variant allele [203]. Also, the package insert of irinotecan was modified in 2005 by the FDA, to include information on UGT1A1 variability [204]. Unfortunately, because no studies have determined the optimal dose per genotype, no advice for dose adjustment is made.

Glutathione S-transferase

Glutathione S-transferases (GSTs) make up a family of phase II enzymes that catalyze the conjugation of reduced glutathione to toxic substances. Members of this family are GST π, GST μ and GST θ, which are products of distinct loci in the genome. Among substrates for GSTs are cyclophosphamide, etoposide, doxorubicin, cisplatin, carboplatin and oxaliplatin and their metabolites. Theoretically, reduced activity of these enzymes would result in increased exposure to these drugs, possibly resulting in increased efficacy and toxicity.

The gene for GST π, *GSTP1*, is known to be polymorphic. One polymorphism resulting in a non-synonymous SNP at codon 105 (313A>G; Ile105Val) in exon 5 causes decreased GST *π* activity.[57] The allele frequency is approximately 20%, 30% and 40% in Asian, Caucasian and African American populations respectively.[57-60]

A significant association toward better survival after cyclophosphamide containing chemotherapy was found for breast cancer patients carrying the variant 105Val allele.[58,59] The variant allele was also associated with increased survival in 107 mCRC patients who were treated with a combination of 5FU and oxaliplatin.[61] Survival was 24.9, 13.3 and 7.9 months for Val/Val, Val/Ile and Ile/Ile genotypes respectively (P=0.001).[61] The variant allele was also associated with better response and longer survival in gastric cancer patients who were treated with 5FU and cisplatin.[62] Colorectal, gastric and pancreatic cancer patients carrying at least one *GSTP1*-105Val allele experienced less toxicity to oxaliplatin containing chemotherapy.[63]

The genes for subclasses GST μ (*GSTM1*) and GST θ (*GSTT1*) both have 'null' polymorphisms, where the total gene is deleted on both alleles. Both null genotypes were associated with increased survival among breast cancer patients, irrespective of treatment (either chemotherapy or radiation).[64] However, this association was not found in another cohort of breast cancer patients[59], nor in a cohort of colorectal cancer (CRC) patients.[61] Survival of ovarian cancer patients treated with platinum containing chemotherapy was also better for *GSTM1* null patients.[65,66]

These studies demonstrate that, because of decreased inactivation of the respective anti-cancer agents, carriers of the less active variant GST alleles have increased response and survival to chemotherapy.

Drug transporters

ABCB1

The ATP-binding cassette (ABC) B1 gene (*ABCB1*), formerly known as multi-drug resistance (*MDR1*) gene, encodes the P-glycoprotein (PGP), an ATP-dependent efflux pump that exports exogenous substances across the cell membrane. Through this mechanism, substances such as cytostatics are unable to retain sufficient intracellular concentrations to exert their anti-tumour activity. Two synonymous SNPs in exons 12 and 26 (1236C>T and 3435C>T respectively) and a non-synonymous SNP in exon 21 (2677G>T/A; Ala893Ser/Thr) have been studied extensively. These variant alleles occur together in a common haplotype (*MDR1*2*), with a frequency of 27%, 31-49% and 6.5% in Caucasians, Asians and Blacks respectively.[67-69] The *MDR1*2* haplotype was associated with lower irinotecan and SN-38 clearance[69] and with lower C_{max} for glucuronidated SN-38 (SN-38G).[70]

The individual polymorphisms have been associated with decreased PGP function *in vivo*.[71,72] Cancer patients homozygous for the 1236C>T variant allele had higher

5

exposure to both irinotecan and SN-38.[73] In 58 patients with solid tumours, all patients homozygous for the 3435T variant allele experienced grade 3-4 neutropenia (specified as neutrophil count between 0.5 and 1.0 x 10^9/L and less than 0.5 x 10^9/L respectively) to docetaxel, compared with 77% and 54% for heterozygote and wild-type individuals respectively.[74] As exposure to docetaxel is increased in carriers of the variant allele, this finding would be expected.

ABCG2

Another ATP binding cassette, formerly known as the breast cancer resistance protein (BCRP) is coded by the gene *ABCG2*. Overexpression of this enzyme is related to the occurrence of resistance to several anticancer agents such as SN-38, mitoxantrone, topotecan, daunorubicin and etoposide.[75] A SNP in exon 5 (421C>A; Gln141Lys) has an allele frequency of 34%, 12% and 1-5% in Han Chinese, Caucasians and Blacks respectively[76], and results in lower BCRP expression and higher SN-38 and topotecan sensitivity *in vitro*.[77] However, this SNP has found not to be associated with pharmacokinetic parameters of irinotecan and its metabolites in a cohort of cancer patients.[76]

DNA repair

Excision repair cross complementing group 1

As part of the nucleotide excision (NER) pathway, excision repair cross complementing group 1 (ERCC1) is involved in DNA damage repair caused by platinum containing compounds such as cisplatin, carboplatin and oxaliplatin.[78,79] Increased ERCC1 expression has been shown to lead to cisplatin resistance *in vitro*[80] and to lower response in cisplatin treated bladder cancer patients *in vivo*.[81] A prospective ERCC1 mRNA expression guided phase III study is ongoing.[82] A silent SNP has been identified in exon 4 at codon 118 in the *ERCC1* gene (496C>T; Asn118Asn). The allele frequency of the T allele is 0.58 in Caucasians, 0.24-0.36 in Asians and 0 in Blacks.[83,84]

Even though encoding the same amino acid, the variant codon is believed to occur less commonly, therefore resulting in reduced ERCC1 expression.[85,86]

Homozygote *ERCC1* wild-type patients with stage IIIb-IV non-small cell lung cancer (NSCLC) who were treated with cisplatin containing chemotherapy had longer survival than patients carrying the variant allele.[87] This same association was found in another cohort of NSCLC stage IIIb-IV patients treated with cisplatin and docetaxel.[88]

Homozygote wild-type mCRC patients were also found to have longer survival compared to carriers of the variant allele when treated with oxaliplatin and 5FU[83], whereas in another cohort of mCRC patients, the variant genotype was associated with better response to oxaliplatin and 5FU.[89]

In melanoma patients (stage IV) treated with cisplatin containing chemotherapy, the wild-type genotype was associated with worse response and shorter overall survival.[90]

Ovarian cancer patients who carried the variant allele had reduced risk of platinum resistance, but survival was not affected by genotype.[84]

One would expect that the SNP leading to reduced ERCC1 expression and hence to decreased DNA repair of DNA-platinum adducts, would result in increased platinum sensitivity and consequently to increased response and survival. However, most studies that are presented show the opposite result. The occurrence of linkage disequilibrium of the evaluated variant with other variants with opposing effects on enzyme function as well as that of other enzymes and pathways with a role in the drug's pharmacokinetics and clinical variables could be of importance and explain this discrepancy.

Excision repair cross complementing group 2

The enzyme xeroderma pigmentosum group D (XPD) is coded by the excision repair cross complementing group 2 (*ERCC2*) gene, and is also involved in the NER pathway. Two SNPs in this gene (965G>A, Asp321Asn and 2251A>C, Lys751Gln) are associated with reduced DNA repair capacity.[91] The allele frequency of the XPD-321 variant allele was 0.32 in a general Western population.[92] The allele frequency of the XPD-751 variant allele is 0.44 in Caucasians, 0.16 in Blacks and 0.09 in Asians.[83]

Patients with mCRC who were homozygous for the variant *ERCC2-751* allele and who were treated with oxaliplatin based chemotherapy had higher mortality compared with patients carrying the wild-type allele.[83,93] The two SNPs in the *ERCC2* gene were not associated with response and survival in cisplatin treated NSCLC patients[87,88], but the wild-type *ERCC2-751* allele was associated with increased incidence of WHO grade 2 or higher neutropenia.[88] In stage III-IV NSCLC patients treated with platinum containing chemotherapy, homozygote individuals for the *ERCC2-321* variant had worse survival compared to carriers of a wild-type allele.[92]

X-ray repair cross complementing group 1

The X-ray repair cross complementing group 1 (XRCC1) enzyme is involved in repair of single-strand breaks in DNA. A SNP in the *XRCC1* gene (1301G>A, Arg399Gln, allele frequency of 0.35 in Caucasians, 0.36 in Blacks and 0.22 in Asians[83]) has been associated with worse response to oxaliplatin and 5FU in mCRC patients[94], but there was no difference in time to progression or survival.[83] In stage III NSCLC patients, survival was shorter for individuals homozygote for the variant allele compared with carriers of the wild-type allele.[92]

An increased number of variant alleles of the *ERCC2* and *XRCC1* genes have been associated with worse survival in NSCLC patients who were treated with platinum containing chemotherapy.[92] In contrast, in patients with squamous cell cancer of the head and neck (SCCHN) treated with cisplatin containing therapy, an increased number of variant alleles of *ERCC2-321*, *ERCC2-751*, *XRCC1-399* and *ERCC1* (8092C>A in

the 3'UTR) was associated with increased survival.[95] Moreover, cisplatin treated patients with muscle invasive bladder cancer who carried one or more variant *ERCC2-751* or *XRCC1-399* allele had better survival compared to wild-type patients.[96]
These inconsistent and non-intuitive findings could in part be explained by the reasons that are given for conflicting results for ERCC1.

CYP2D6
Tamoxifen is a widely used agent in treatment of breast cancer and is hydroxylated into the 100 times more active metabolites 4-hydroxy-tamoxifen and endoxifen by the cytochrome P450 iso-enzyme CYP2D6.[97-99] The *CYP2D6*4* (1846G>A) allele results in gene deletion and thus in absent CYP2D6 activity and has a frequency of 15-20% in the general population in Western countries.[100-105] Other alleles resulting in lower CYP2D6 activity are *CYP2D6*3* (Δ2549A), *CYP2D6*5* (deletion of entire *CYP2D6* gene) and *CYP2D6*6* (Δ1707T). The *CYP2D6*4* allele has been linked to reduced conversion of tamoxifen into 4-hydroxy-tamoxifen[106] and the *CYP2D6*3-6* genotypes have been related to lower endoxifen formation.[103,104]
As expected, breast cancer patients treated with adjuvant tamoxifen who were homozygous for the CYP2D6*4 allele had significant worse relapse-free time and shorter disease free survival compared with carriers of the wild-type allele. However, significance was not retained in multivariate analysis. Homozygote CYP2D6*4 patients did not experience moderate to severe flashes, which is a side effect of (the active metabolite of) tamoxifen.[102] A similar finding was reported in a case control study for prevention of breast cancer with tamoxifen in hysterectomised women. The frequency of the CYP2D6*4/*4 genotype was higher in women who developed breast cancer during follow up than in women free of cancer.[107]
Contradictory to this, another study found that oestrogen receptor positive (ER+) breast cancer patients who carried the CYP2D6*4 allele had significant longer recurrence free survival when treated with adjuvant tamoxifen compared with CYP2D6*4 carriers who were not treated with tamoxifen. This difference was not observed for wild-type patients.[100] Selection bias in this study may have influenced the outcome of this study.[108]

Methylene tetrahydrofolate reductase (MTHFR)
The enzyme MTHFR is one of the key enzymes in the folate pathway (see figure 1). Reduced MTHFR expression results in reduced sensitivity to the MTHFR inhibitor MTX. On the other hand, abundance of the MTHFR substrate 5,10-methylene tetrahydrofolate (5,10-MTHF) facilitates the inhibition of thymidylate synthase (TS) by 5FU, therefore increasing sensitivity to this agent.
A SNP in the *MTHFR* gene, 677C>T results in an amino-acid change of alanine to valine at codon 222 (Ala222Val). The allele frequency in all populations is 0.27-0.57.[62,109-114]

MTHFR activity is 70% and 35% for heterozygote and homozygote individuals respectively.[109] *In vitro* assays showed that cell lines with the variant allele are more sensitive to 5FU and less sensitive to MTX.[115]
Of six patients who experienced severe toxicity to adjuvant CMF (cyclophosphamide, MTX, 5FU) for breast cancer, five were homozygous for the 677T allele.[116] In a cohort of cancer patients who were treated with the 5FU analogue raltitrexed, patients with 677TT genotype had significant more therapy related toxicity.[117] Leukaemia patients homozygous for the 677T allele experienced more MTX related toxicity compared with patients who carried at least one wild-type allele.[118] Also, ovarian cancer patients who were treated with MTX and were homozygous for the 677T allele experienced significant more WHO grade 3/4 side effects.[112]
In mCRC patients, the 677T allele has been associated with improved response to 5FU based chemotherapy in several studies[119-121], whereas another study did not find a significant association.[122] No association was found in a cohort of advanced gastric cancer patients treated with 5FU and cisplatin.[62]

Thymidylate synthase (TS)
TS is the central enzyme in the de-novo thymidine synthesis. *In vitro* resistance to 5FU is associated with increased TS activity, which is also induced by 5FU itself.[123] The TS promoter enhancer region (TSER) of the gene encoding TS (*TYMS*) has been shown to contain either two or three tandem repeats designated as TSER*2 and TSER*3 respectively. The TSER*3 genotype results in increased TS expression, either through higher mRNA levels or increase in efficiency of mRNA translation.[124,125] The allele frequency of the TSER*2 allele is 0.40-0.46 in Caucasians and Blacks[126,127], compared to 0.18-0.21 in Asian populations.[126,128-130]

The TSER*3 allele was associated with increased response in CRC patients treated with 5FU.[121] On the other hand, the TSER*2 allele was associated with improved response to capecitabine in CRC patients.[131]

These conflicting results could in part be explained by a G>C SNP in the 12th base pair of the TSER*3 allele[132] that results in TS activity similar to that of the TSER*2 allele.[133] This TSER*3C allele is found in 29%-57% of all TSER*3 alleles.[132,134,135]
Carriers of the TSER*3G allele had significantly worse response, disease free survival and overall survival in a cohort of mCRC patients treated with 5FU.[135] The TSER*3G allele was also associated with worse survival in advanced gastric cancer patients who were treated with 5FU.[62]
As expected, most studies show that the TSER*3 allele, and especially the TSER*3G allele, is associated with lower response and survival to fluoropyrimidine therapy. Conflicting results could be explained by the G>C SNP in the TSER*3 allele.

A six base pair deletion (-6bp) in the 3' UTR of the *TYMS* gene results in decreased mRNA stability and lower TS expression.[136] The -6bp mutation is in linkage disequilibrium (LD) with the TSER*3 allele, and the +6bp allele is in LD with TSER*2.[137] The -6bp variant allele is associated with decreased survival in mCRC patients treated with 5FU and oxaliplatin[83] and with decreased response to 5FU based chemotherapy in advanced gastric cancer patients.[138]

CRC patients treated with 5FU who were either homozygous for the TSER*3 allele (regardless of 3' UTR genotype) or heterozygous for the TSER allele combined with homozygous for the +6bp genotype had significant better disease free survival (DFS) and overall survival (OS) compared with the other genotypes.[139] In a cohort of gastric cancer patients, non-carriers of the TSER*3G allele together with one or two -6bp alleles had significant better DFS and OS compared to carriers of TSER*3G and two copies of +6bp.[140] The haplotype TSER*3C and -6bp was associated with significant better OS compared with the haplotype TSER*2 and +6bp in CRC patients treated with 5FU.[141]

Multiple gene studies

Drug response is a complex phenotype, especially in anti-cancer therapy, where multiple drug regimens are often applied. Only few studies have explored the influence of polymorphisms of multiple genes that are involved in the pathway of the drug.

The role of polymorphisms in genes involved in response to 5FU (*TYMS*) and metabolism of cisplatin (*GSTP1*) was investigated by Ruzzo *et al.* Advanced gastric cancer patients treated with 5FU and cisplatin who were both homozygous for the *GSTP1*-105Ile allele and carrier of the TSER*3G allele had significant shorter progression free survival and over all survival compared with patients who were carriers of the *GSTP1*-105Val allele or patients who did not carry the TSER*3G allele.[62]

Stoehlmacher *et al.* looked at genes involved in response and metabolism of oxaliplatin (*ERCC1*, *ERCC2* and *GSTP1*) and 5FU (*TYMS*). Favourable genotypes in mCRC patients were *ERCC2-751* Lys/Lys, *ERCC1-496* C/C, *GSTP1*-105Val/Val and *TYMS-3'UTR* +6bp/+6bp. Patients who carried none of these genotypes had median survival of 5.4 months, compared with 10.2 and 17.4 months for patients with one or ≥ two favourable genotypes ($P<0.001$).[83]

When multiple variants in genes or variants in multiple genes are surveyed for example in combination chemotherapy regimens, both sample size and power are of great importance since opposite effects of different genetic variants can obliterate each other in small samples, whereas multiple testing may reveal false-positive associations.

Conclusion

There is ample evidence that pharmacogenetic traits are able to predict pharmacodynamics of several anti-cancer drugs. Polymorphisms that result in decreased metabolic enzyme levels or activity have shown to result in either increased toxicity or increased efficacy or both. Other polymorphisms lead to increased exposure to chemotherapy through decreased expression of membrane efflux pumps, whereas others lead to decreased capability to repair DNA damage caused by chemotherapy. Variants in genes that code for enzymes involved in the mode of action of anti-cancer drugs give altered response to chemotherapy. Despite emerging evidence, pharmacogenetic testing has not yet found its way to routine patient care.

Expert Opinion

Many pharmacogenetic studies that point towards association of heritable genetic variants and cytotoxic drug response have been presented in this paper. These genes and variations have been studied most extensively until now. This does not necessarily imply that these genes hold most promise for implementation in the standard of oncology care in the near future. Possibly, other genes and variations may emerge as potential predictors of response or toxicity.

Consequently, there is a need for additional, but also for other types of research in pharmacogenetics to find its way to routine patient care. Obviously, there is need for replication of apparent conflicting findings, such as for the TSER polymorphism in the *TYMS* gene, or polymorphisms in the DNA repair genes, in larger cohorts in routine patient care environment.[142]

Also, cost-effectiveness of testing needs to be determined for pharmacogenetic tests. In this light, it is important to develop tests that are sensitive and specific, as well as simple and cheap.

Genetic variability is only one of the determinants of drug response. Therefore, another type of research that holds promise for the future is the development of prediction models that not only include pharmacogenetic data, but also non-genetic traits such as WHO performance status and organ function. Such models are only starting being developed, for instance regarding MTX response in rheumatoid arthritis.[143]

Until now, genes are selected mainly through the candidate pathway gene approach. Obviously, this mechanistic approach seems logical. However, the disadvantage of this approach is that it is limited by current knowledge of pathophysiology and the mechanism of action of a drug. Therefore, future research will use hypothesis-free whole genome approach such as SNP arrays.[144]

Finally, it is important to perform prospective studies on applying pharmacogenetics in patient care and to assess optimal dose and drug per genotype upon a predictive model including a pharmacogenetic test result. After all it is not possible to predict necessary dose adjustment based upon current knowledge. This is illustrated by the phrase in the irinotecan label that has recently been modified by the FDA: "A reduced initial dose should be considered for patients known to be homozygous for the *UGT1A1*28* allele" [204]. Therefore, studies are necessary that prospectively investigate an adjusted dose for a certain genotype compared with normal dose for wild-type patients.
The above mentioned future pharmacogenetic research will enable oncologists to implement pharmacogenetics and to optimize individual cancer treatment.

References

1. Marsh S, Mallon MA, Goodfellow P, McLeod HL. Concordance of pharmacogenetic markers in germline and colorectal tumor DNA. Pharmacogenomics 2005;6:873-7.
2. Weinshilboum RM, Sladek SL. Mercaptopurine pharmacogenetics: monogenic inheritance of erythrocyte thiopurine methyltransferase activity. Am J Hum Genet 1980;32:651-62.
3. Schaeffeler E, Fischer C, Brockmeier D, *et al.* Comprehensive analysis of thiopurine S-methyltransferase phenotype-genotype correlation in a large population of German-Caucasians and identification of novel TPMT variants. Pharmacogenetics 2004;14:407-17.
4. Lennard L, Lilleyman JS, Van Loon J, Weinshilboum RM. Genetic variation in response to 6-mercaptopurine for childhood acute lymphoblastic leukaemia. Lancet 1990;336:225-9.
5. McLeod HL, Relling MV, Liu Q, Pui CH, Evans WE. Polymorphic thiopurine methyltransferase in erythrocytes is indicative of activity in leukemic blasts from children with acute lymphoblastic leukemia. Blood 1995;85:1897-902.
6. Relling MV, Hancock ML, Rivera GK, *et al.* Mercaptopurine therapy intolerance and heterozygosity at the thiopurine S-methyltransferase gene locus. J Natl Cancer Inst 1999;91:2001-8.
7. Evans WE, Hon YY, Bomgaars L, *et al.* Preponderance of thiopurine S-methyltransferase deficiency and heterozygosity among patients intolerant to mercaptopurine or azathioprine. J Clin Oncol 2001;19:2293-301.
8. Relling MV, Hancock ML, Boyett JM, Pui CH, Evans WE. Prognostic importance of 6-mercaptopurine dose intensity in acute lymphoblastic leukemia. Blood 1999;93:2817-23.
9. Brouwer C, De Abreu RA, Keizer-Garritsen JJ, *et al.* Thiopurine methyltransferase in acute lymphoblastic leukaemia: biochemical and molecular biological aspects. Eur J Cancer 2005;41:613-23.
10. Cheung ST, Allan RN. Mistaken identity: misclassification of TPMT phenotype following blood transfusion. Eur J Gastroenterol Hepatol 2003;15:1245-7.
11. Krynetski EY, Schuetz JD, Galpin AJ, *et al.* A single point mutation leading to loss of catalytic activity in human thiopurine S-methyltransferase. Proc Natl Acad Sci U S A 1995;92:949-53.
12. Tai HL, Krynetski EY, Yates CR, *et al.* Thiopurine S-methyltransferase deficiency: two nucleotide transitions define the most prevalent mutant allele associated with loss of catalytic activity in Caucasians. Am J Hum Genet 1996;58:694-702.
13. Salavaggione OE, Wang L, Wiepert M, Yee VC, Weinshilboum RM. Thiopurine S-methyltransferase pharmacogenetics: variant allele functional and comparative genomics. Pharmacogenet Genomics 2005;15:801-15.
14. Schaeffeler E, Eichelbaum M, Reinisch W, Zanger UM, Schwab M. Three novel thiopurine S-methyltransferase allelic variants (TPMT*20, *21, *22) - association with decreased enzyme function. Hum Mutat 2006;27:976.
15. Yates CR, Krynetski EY, Loennechen T, *et al.* Molecular diagnosis of thiopurine S-methyltransferase deficiency: genetic basis for azathioprine and mercaptopurine intolerance. Ann Intern Med 1997;126:608-14.
16. Otterness D, Szumlanski C, Lennard L, *et al.* Human thiopurine methyltransferase pharmacogenetics: gene sequence polymorphisms. Clin Pharmacol Ther 1997;62:60-73.
17. Brouwer C, Marinaki AM, Lambooy LH, *et al.* Pitfalls in the determination of mutant alleles of the thiopurine methyltransferase gene. Leukemia 2001;15:1792-3.
18. Ameyaw MM, Collie-Duguid ES, Powrie RH, Ofori-Adjei D, McLeod HL. Thiopurine methyltransferase alleles in British and Ghanaian populations. Hum Mol Genet 1999;8:367-70.
19. Collie-Duguid ES, Pritchard SC, Powrie RH, *et al.* The frequency and distribution of thiopurine methyltransferase alleles in Caucasian and Asian populations. Pharmacogenetics 1999;9:37-42.
20. McLeod HL, Pritchard SC, Githang'a J, *et al.* Ethnic differences in thiopurine methyltransferase pharmacogenetics: evidence for allele specificity in Caucasian and Kenyan individuals. Pharmacogenetics 1999;9:773-6.

21. Chang JG, Lee LS, Chen CM, *et al.* Molecular analysis of thiopurine S-methyltransferase alleles in South-east Asian populations. Pharmacogenetics 2002;12:191-5.
22. Ando M, Ando Y, Hasegawa Y, *et al.* Genetic polymorphisms of thiopurine S-methyltransferase and 6-mercaptopurine toxicity in Japanese children with acute lymphoblastic leukaemia. Pharmacogenetics 2001;11:269-73.
23. van den Akker-van Marle ME, Gurwitz D, Detmar SB, *et al.* Cost-effectiveness of pharmacogenomics in clinical practice: a case study of thiopurine methyltransferase genotyping in acute lymphoblastic leukemia in Europe. Pharmacogenomics 2006;7:783-92.
24. Woelderink A, Ibarreta D, Hopkins MM, Rodriguez-Cerezo E. The current clinical practice of pharmacogenetic testing in Europe: TPMT and HER2 as case studies. Pharmacogenomics J 2006;6:3-7.
25. Stanulla M, Schaeffeler E, Flohr T, *et al.* Thiopurine methyltransferase (TPMT) genotype and early treatment response to mercaptopurine in childhood acute lymphoblastic leukemia. JAMA 2005;293:1485-9.
26. Diasio RB, Beavers TL, Carpenter JT. Familial deficiency of dihydropyrimidine dehydrogenase. Biochemical basis for familial pyrimidinemia and severe 5-fluorouracil-induced toxicity. J Clin Invest 1988;81:47-51.
27. Van Kuilenburg AB, Muller EW, Haasjes J, *et al.* Lethal outcome of a patient with a complete dihydropyrimidine dehydrogenase (DPD) deficiency after administration of 5-fluorouracil: frequency of the common IVS14+1G>A mutation causing DPD deficiency. Clin Cancer Res 2001;7:1149-53.
28. Raida M, Schwabe W, Hausler P, *et al.* Prevalence of a common point mutation in the dihydropyrimidine dehydrogenase (DPD) gene within the 5'-splice donor site of intron 14 in patients with severe 5-fluorouracil (5-FU)- related toxicity compared with controls. Clin Cancer Res 2001;7:2832-9.
29. Van Kuilenburg AB, Haasjes J, Richel DJ, *et al.* Clinical implications of dihydropyrimidine dehydrogenase (DPD) deficiency in patients with severe 5-fluorouracil-associated toxicity: identification of new mutations in the DPD gene. Clin Cancer Res 2000;6:4705-12.
30. Van Kuilenburg AB, Meinsma R, Zoetekouw L, Van Gennip AH. High prevalence of the IVS14 + 1G>A mutation in the dihydropyrimidine dehydrogenase gene of patients with severe 5-fluorouracil-associated toxicity. Pharmacogenetics 2002;12:555-8.
31. Van Kuilenburg AB, Meinsma R, Zoetekouw L, Van Gennip AH. Increased risk of grade IV neutropenia after administration of 5-fluorouracil due to a dihydropyrimidine dehydrogenase deficiency: high prevalence of the IVS14+1g>a mutation. Int J Cancer 2002;101:253-8.
32. Wei X, Elizondo G, Sapone A, *et al.* Characterization of the human dihydropyrimidine dehydrogenase gene. Genomics 1998;51:391-400.
33. Hsiao HH, Yang MY, Chang JG, *et al.* Dihydropyrimidine dehydrogenase pharmacogenetics in the Taiwanese population. Cancer Chemother Pharmacol 2004;53:445-51.
34. Kouwaki M, Hamajima N, Sumi S, *et al.* Identification of novel mutations in the dihydropyrimidine dehydrogenase gene in a Japanese patient with 5-fluorouracil toxicity. Clin Cancer Res 1998;4:2999-3004.
35. Collie-Duguid ES, Etienne MC, Milano G, McLeod HL. Known variant DPYD alleles do not explain DPD deficiency in cancer patients. Pharmacogenetics 2000;10:217-23.
36. Ezzeldin HH, Lee AM, Mattison LK, Diasio RB. Methylation of the DPYD promoter: an alternative mechanism for dihydropyrimidine dehydrogenase deficiency in cancer patients. Clin Cancer Res 2005;11:8699-705.
37. Gagne JF, Montminy V, Belanger P, *et al.* Common human UGT1A polymorphisms and the altered metabolism of irinotecan active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38). Mol Pharmacol 2002;62:608-17.
38. Monaghan G, Ryan M, Seddon R, Hume R, Burchell B. Genetic variation in bilirubin UPD-glucuronosyltransferase gene promoter and Gilbert's syndrome. Lancet 1996;347:578-81.

39. Innocenti F, Grimsley C, Das S, *et al.* Haplotype structure of the UDP-glucuronosyltransferase 1A1 promoter in different ethnic groups. Pharmacogenetics 2002;12:725-33.
40. Innocenti F, Undevia SD, Iyer L, *et al.* Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. J Clin Oncol 2004;22:1382-8.
41. Mathijssen RH, de Jong FA, van Schaik RH, *et al.* Prediction of irinotecan pharmacokinetics by use of cytochrome P450 3A4 phenotyping probes. J Natl Cancer Inst 2004;96:1585-92.
42. Paoluzzi L, Singh AS, Price DK, *et al.* Influence of genetic variants in UGT1A1 and UGT1A9 on the in vivo glucuronidation of SN-38. J Clin Pharmacol 2004;44:854-60.
43. Rouits E, Boisdron-Celle M, Dumont A, *et al.* Relevance of different UGT1A1 polymorphisms in irinotecan-induced toxicity: a molecular and clinical study of 75 patients. Clin Cancer Res 2004;10:5151-9.
44. Innocenti F, Liu W, Chen P, *et al.* Haplotypes of variants in the UDP-glucuronosyltransferase1A9 and 1A1 genes. Pharmacogenet Genomics 2005;15:295-301.
45. Kaniwa N, Kurose K, Jinno H, *et al.* Racial variability in haplotype frequencies of UGT1A1 and glucuronidation activity of a novel single nucleotide polymorphism 686C> T (P229L) found in an African-American. Drug Metab Dispos 2005;33:458-65.
46. Beutler E, Gelbart T, Demina A. Racial variability in the UDP-glucuronosyltransferase 1 (UGT1A1) promoter: a balanced polymorphism for regulation of bilirubin metabolism? Proc Natl Acad Sci U S A 1998;95:8170-4.
47. Ando Y, Saka H, Ando M, *et al.* Polymorphisms of UDP-glucuronosyltransferase gene and irinotecan toxicity: a pharmacogenetic analysis. Cancer Res 2000;60:6921-6.
48. Sai K, Saeki M, Saito Y, *et al.* UGT1A1 haplotypes associated with reduced glucuronidation and increased serum bilirubin in irinotecan-administered Japanese patients with cancer. Clin Pharmacol Ther 2004;75:501-15.
49. Han JY, Lim HS, Shin ES, *et al.* Comprehensive analysis of UGT1A polymorphisms predictive for pharmacokinetics and treatment outcome in patients with non-small-cell lung cancer treated with irinotecan and cisplatin. J Clin Oncol 2006;24:2237-44.
50. World Health Organization. WHO handbook for reporting results of cancer treatment. Geneva: World Health Organization; 1979.
51. Marcuello E, Altes A, Menoyo A, *et al.* UGT1A1 gene variations and irinotecan treatment in patients with metastatic colorectal cancer. Br J Cancer 2004;91:678-82.
52. Massacesi C, Terrazzino S, Marcucci F, *et al.* Uridine diphosphate glucuronosyl transferase 1A1 promoter polymorphism predicts the risk of gastrointestinal toxicity and fatigue induced by irinotecan-based chemotherapy. Cancer 2006;106:1007-16.
53. Japan Society for Cancer Therapy. Criteria for the evaluation of the clinical effects of solid cancer chemotherapy. J Jpn Soc Cancer Ther 1993;28:101-30.
54. Toffoli G, Cecchin E, Corona G, *et al.* The role of UGT1A1*28 polymorphism in the pharmacodynamics and pharmacokinetics of irinotecan in patients with metastatic colorectal cancer. J Clin Oncol 2006;24:3061-8.
55. Kitagawa C, Ando M, Ando Y, *et al.* Genetic polymorphism in the phenobarbital-responsive enhancer module of the UDP-glucuronosyltransferase 1A1 gene and irinotecan toxicity. Pharmacogenet Genomics 2005;15:35-41.
56. Carlini LE, Meropol NJ, Bever J, *et al.* UGT1A7 and UGT1A9 polymorphisms predict response and toxicity in colorectal cancer patients treated with capecitabine/irinotecan. Clin Cancer Res 2005;11:1226-36.
57. Watson MA, Stewart RK, Smith GB, Massey TE, Bell DA. Human glutathione S-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. Carcinogenesis 1998;19:275-80.
58. Sweeney C, McClure GY, Fares MY, *et al.* Association between survival after treatment for breast cancer and glutathione S-transferase P1 Ile105Val polymorphism. Cancer Res 2000;60:5621-4.

59. Yang G, Shu XO, Ruan ZX, *et al.* Genetic polymorphisms in glutathione-S-transferase genes (GSTM1, GSTT1, GSTP1) and survival after chemotherapy for invasive breast carcinoma. Cancer 2005;103:52-8.
60. Lu C, Spitz MR, Zhao H, *et al.* Association between glutathione S-transferase pi polymorphisms and survival in patients with advanced nonsmall cell lung carcinoma. Cancer 2006;106:441-7.
61. Stoehlmacher J, Park DJ, Zhang W, *et al.* Association between glutathione S-transferase P1, T1, and M1 genetic polymorphism and survival of patients with metastatic colorectal cancer. J Natl Cancer Inst 2002;94:936-42.
62. Ruzzo A, Graziano F, Kawakami K, *et al.* Pharmacogenetic profiling and clinical outcome of patients with advanced gastric cancer treated with palliative chemotherapy. J Clin Oncol 2006;24:1883-91.
63. Lecomte T, Landi B, Beaune P, Laurent-Puig P, Loriot MA. Glutathione S-transferase P1 polymorphism (Ile105Val) predicts cumulative neuropathy in patients receiving oxaliplatin-based chemotherapy. Clin Cancer Res 2006;12:3050-6.
64. Ambrosone CB, Sweeney C, Coles BF, *et al.* Polymorphisms in glutathione S-transferases (GSTM1 and GSTT1) and survival after treatment for breast cancer. Cancer Res 2001;61:7130-5.
65. Medeiros R, Pereira D, Afonso N, *et al.* Platinum/paclitaxel-based chemotherapy in advanced ovarian carcinoma: glutathione S-transferase genetic polymorphisms as predictive biomarkers of disease outcome. Int J Clin Oncol 2003;8:156-61.
66. Beeghly A, Katsaros D, Chen H, *et al.* Glutathione S-transferase polymorphisms and ovarian cancer treatment and survival. Gynecol Oncol 2006;100:330-7.
67. Kim RB, Leake BF, Choo EF, *et al.* Identification of functionally variant MDR1 alleles among European Americans and African Americans. Clin Pharmacol Ther 2001;70:189-99.
68. Tang K, Ngoi SM, Gwee PC, *et al.* Distinct haplotype profiles and strong linkage disequilibrium at the MDR1 multidrug transporter gene locus in three ethnic Asian populations. Pharmacogenetics 2002;12:437-50.
69. Sai K, Kaniwa N, Itoda M, *et al.* Haplotype analysis of ABCB1/MDR1 blocks in a Japanese population reveals genotype-dependent renal clearance of irinotecan. Pharmacogenetics 2003;13:741-57.
70. Zhou Q, Sparreboom A, Tan EH, *et al.* Pharmacogenetic profiling across the irinotecan pathway in Asian patients with cancer. Br J Clin Pharmacol 2005;59:415-24.
71. Wong M, Evans S, Rivory LP, *et al.* Hepatic technetium Tc 99m-labeled sestamibi elimination rate and ABCB1 (MDR1) genotype as indicators of ABCB1 (P-glycoprotein) activity in patients with cancer. Clin Pharmacol Ther 2005;77:33-42.
72. Michael M, Thompson M, Hicks RJ, *et al.* Relationship of Hepatic Functional Imaging to Irinotecan Pharmacokinetics and Genetic Parameters of Drug Elimination. J Clin Oncol 2006;24:1-8.
73. Mathijssen RH, Marsh S, Karlsson MO, *et al.* Irinotecan pathway genotype analysis to predict pharmacokinetics. Clin Cancer Res 2003;9:3246-53.
74. Tran A, Jullien V, Alexandre J, *et al.* Pharmacokinetics and toxicity of docetaxel: role of CYP3A, MDR1, and GST polymorphisms. Clin Pharmacol Ther 2006;79:570-80.
75. Robey RW, Honjo Y, Morisaki K, *et al.* Mutations at amino-acid 482 in the ABCG2 gene affect substrate and antagonist specificity. Br J Cancer 2003;89:1971-8.
76. de Jong FA, Marsh S, Mathijssen RH, *et al.* ABCG2 pharmacogenetics: ethnic differences in allele frequency and assessment of influence on irinotecan disposition. Clin Cancer Res 2004;10:5889-94.
77. Imai Y, Nakane M, Kage K, *et al.* C421A polymorphism in the human breast cancer resistance protein gene is associated with low expression of Q141K protein and low-level drug resistance. Mol Cancer Ther 2002;1:611-6.
78. Larminat F, Bohr VA. Role of the human ERCC-1 gene in gene-specific repair of cisplatin-induced DNA damage. Nucleic Acids Res 1994;22:3005-10.
79. Kweekel DM, Gelderblom H, Guchelaar HJ. Pharmacology of oxaliplatin and the use of pharmacogenomics to individualize therapy. Cancer Treat Rev 2005;31:90-105.

80. Ferry KV, Hamilton TC, Johnson SW. Increased nucleotide excision repair in cisplatin-resistant ovarian cancer cells: role of ERCC1-XPF. Biochem Pharmacol 2000;60:1305-13.
81. Bellmunt J, Paz-Ares L, Cuello M, *et al.* Gene expression of ERCC1 as a novel prognostic marker in advanced bladder cancer patients receiving cisplatin-based chemotherapy. Ann Oncol 2007.
82. Rosell R, Cobo M, Isla D, *et al.* ERCC1 mRNA-based randomized phase III trial of docetaxel (doc) doublets with cisplatin (cis) or gemcitabine (gem) in stage IV non-small-cell lung cancer (NSCLC) patients (p). Proc Am Assoc Clin Oncol 2005;Abstr. 7002.
83. Stoehlmacher J, Park DJ, Zhang W, *et al.* A multivariate analysis of genomic polymorphisms: prediction of clinical outcome to 5-FU/oxaliplatin combination chemotherapy in refractory colorectal cancer. Br J Cancer 2004;91:344-54.
84. Kang S, Ju W, Kim JW, *et al.* Association between excision repair cross-complementation group 1 polymorphism and clinical outcome of platinum-based chemotherapy in patients with epithelial ovarian cancer. Exp Mol Med 2006;38:320-4.
85. Yu JJ, Mu C, Lee KB, *et al.* A nucleotide polymorphism in ERCC1 in human ovarian cancer cell lines and tumor tissues. Mutat Res 1997;382:13-20.
86. Lathe R. Synthetic oligonucleotide probes deduced from amino acid sequence data. Theoretical and practical considerations. J Mol Biol 1985;183:1-12.
87. Ryu JS, Hong YC, Han HS, *et al.* Association between polymorphisms of ERCC1 and XPD and survival in non-small-cell lung cancer patients treated with cisplatin combination chemotherapy. Lung Cancer 2004;44:311-6.
88. Isla D, Sarries C, Rosell R, *et al.* Single nucleotide polymorphisms and outcome in docetaxel-cisplatin-treated advanced non-small-cell lung cancer. Ann Oncol 2004;15:1194-203.
89. Viguier J, Boige V, Miquel C, *et al.* ERCC1 codon 118 polymorphism is a predictive factor for the tumor response to oxaliplatin/5-fluorouracil combination chemotherapy in patients with advanced colorectal cancer. Clin Cancer Res 2005;11:6212-7.
90. Liu D, O'Day SJ, Yang D, *et al.* Impact of gene polymorphisms on clinical outcome for stage IV melanoma patients treated with biochemotherapy: an exploratory study. Clin Cancer Res 2005;11:1237-46.
91. Spitz MR, Wu X, Wang Y, *et al.* Modulation of nucleotide excision repair capacity by XPD polymorphisms in lung cancer patients. Cancer Res 2001;61:1354-7.
92. Gurubhagavatula S, Liu G, Park S, *et al.* XPD and XRCC1 genetic polymorphisms are prognostic factors in advanced non-small-cell lung cancer patients treated with platinum chemotherapy. J Clin Oncol 2004;22:2594-601.
93. Park DJ, Stoehlmacher J, Zhang W, *et al.* A Xeroderma pigmentosum group D gene polymorphism predicts clinical outcome to platinum-based chemotherapy in patients with advanced colorectal cancer. Cancer Res 2001;61:8654-8.
94. Stoehlmacher J, Ghaderi V, Iobal S, *et al.* A polymorphism of the XRCC1 gene predicts for response to platinum based treatment in advanced colorectal cancer. Anticancer Res 2001;21:3075-9.
95. Quintela-Fandino M, Hitt R, Medina PP, *et al.* DNA-repair gene polymorphisms predict favorable clinical outcome among patients with advanced squamous cell carcinoma of the head and neck treated with cisplatin-based induction chemotherapy. J Clin Oncol 2006;24:4333-9.
96. Sakano S, Wada T, Matsumoto H, *et al.* Single nucleotide polymorphisms in DNA repair genes might be prognostic factors in muscle-invasive bladder cancer patients treated with chemoradiotherapy. Br J Cancer 2006;95:561-70.
97. Dehal SS, Kupfer D. CYP2D6 catalyzes tamoxifen 4-hydroxylation in human liver. Cancer Res 1997;57:3402-6.
98. Boocock DJ, Brown K, Gibbs AH, *et al.* Identification of human CYP forms involved in the activation of tamoxifen and irreversible binding to DNA. Carcinogenesis 2002;23:1897-901.

99. Stearns V, Johnson MD, Rae JM, *et al.* Active tamoxifen metabolite plasma concentrations after coadministration of tamoxifen and the selective serotonin reuptake inhibitor paroxetine. J Natl Cancer Inst 2003;95:1758-64.
100. Wegman P, Vainikka L, Stal O, *et al.* Genotype of metabolic enzymes and the benefit of tamoxifen in postmenopausal breast cancer patients. Breast Cancer Res 2005;7:R284-R290.
101. Nowell SA, Ahn J, Rae JM, *et al.* Association of genetic variation in tamoxifen-metabolizing enzymes with overall survival and recurrence of disease in breast cancer patients. Breast Cancer Res Treat 2005;91:249-58.
102. Goetz MP, Rae JM, Suman VJ, *et al.* Pharmacogenetics of tamoxifen biotransformation is associated with clinical outcomes of efficacy and hot flashes. J Clin Oncol 2005;23:9312-8.
103. Jin Y, Desta Z, Stearns V, *et al.* CYP2D6 genotype, antidepressant use, and tamoxifen metabolism during adjuvant breast cancer treatment. J Natl Cancer Inst 2005;97:30-9.
104. Borges S, Desta Z, Li L, *et al.* Quantitative effect of CYP2D6 genotype and inhibitors on tamoxifen metabolism: implication for optimization of breast cancer treatment. Clin Pharmacol Ther 2006;80:61-74.
105. Sachse C, Brockmoller J, Bauer S, Roots I. Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequences. Am J Hum Genet 1997;60:284-95.
106. Coller JK, Krebsfaenger N, Klein K, *et al.* The influence of CYP2B6, CYP2C9 and CYP2D6 genotypes on the formation of the potent antioestrogen Z-4-hydroxy-tamoxifen in human liver. Br J Clin Pharmacol 2002;54:157-67.
107. Bonanni B, Macis D, Maisonneuve P, *et al.* Polymorphism in the CYP2D6 tamoxifen-metabolizing gene influences clinical effect but not hot flashes: data from the Italian Tamoxifen Trial. J Clin Oncol 2006;24:3708-9.
108. Rae JM, Goetz MP, Hayes DF, *et al.* CYP2D6 genotype and tamoxifen response. Breast Cancer Res 2005;7:E6.
109. Frosst P, Blom HJ, Milos R, *et al.* A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995;10:111-3.
110. Haagsma CJ, Blom HJ, van Riel PL, *et al.* Influence of sulphasalazine, methotrexate, and the combination of both on plasma homocysteine concentrations in patients with rheumatoid arthritis. Ann Rheum Dis 1999;58:79-84.
111. Ulrich CM, Yasui Y, Storb R, *et al.* Pharmacogenetics of methotrexate: toxicity among marrow transplantation patients varies with the methylenetetrahydrofolate reductase C677T polymorphism. Blood 2001;98:231-4.
112. Toffoli G, Russo A, Innocenti F, *et al.* Effect of methylenetetrahydrofolate reductase 677C-->T polymorphism on toxicity and homocysteine plasma level after chronic methotrexate treatment of ovarian cancer patients. Int J Cancer 2003;103:294-9.
113. Kumagai K, Hiyama K, Oyama T, Maeda H, Kohno N. Polymorphisms in the thymidylate synthase and methylenetetrahydrofolate reductase genes and sensitivity to the low-dose methotrexate therapy in patients with rheumatoid arthritis. Int J Mol Med 2003;11:593-600.
114. Shrubsole MJ, Shu XO, Ruan ZX, *et al.* MTHFR genotypes and breast cancer survival after surgery and chemotherapy: a report from the Shanghai Breast Cancer Study. Breast Cancer Res Treat 2005;91:73-9.
115. Sohn KJ, Croxford R, Yates Z, Lucock M, Kim YI. Effect of the methylenetetrahydrofolate reductase C677T polymorphism on chemosensitivity of colon and breast cancer cells to 5-fluorouracil and methotrexate. J Natl Cancer Inst 2004;96:134-44.
116. Toffoli G, Veronesi A, Boiocchi M, Crivellari D. MTHFR gene polymorphism and severe toxicity during adjuvant treatment of early breast cancer with cyclophosphamide, methotrexate, and fluorouracil (CMF). Ann Oncol 2000;11:373-4.
117. Stevenson JP, Redlinger M, Kluijtmans LA, *et al.* Phase I clinical and pharmacogenetic trial of irinotecan and raltitrexed administered every 21 days to patients with cancer. J Clin Oncol 2001;19:4081-7.
118. Chiusolo P, Reddiconto G, Casorelli I, *et al.* Preponderance of methylenetetrahydrofolate reductase C677T homozygosity among leukemia patients intolerant to methotrexate. Ann Oncol 2002;13:1915-8.
119. Cohen V, Panet-Raymond V, Sabbaghian N, *et al.* Methylenetetrahydrofolate reductase polymorphism in advanced colorectal cancer: a novel genomic predictor of clinical response to fluoropyrimidine-based chemotherapy. Clin Cancer Res 2003;9:1611-5.
120. Etienne MC, Formento JL, Chazal M, *et al.* Methylenetetrahydrofolate reductase gene polymorphisms and response to fluorouracil-based treatment in advanced colorectal cancer patients. Pharmacogenetics 2004;14:785-92.
121. Jakobsen A, Nielsen JN, Gyldenkerne N, Lindeberg J. Thymidylate synthase and methylenetetrahydrofolate reductase gene polymorphism in normal tissue as predictors of fluorouracil sensitivity. J Clin Oncol 2005;23:1365-9.
122. Marcuello E, Altes A, Menoyo A, Rio ED, Baiget M. Methylenetetrahydrofolate reductase gene polymorphisms: genomic predictors of clinical response to fluoropyrimidine-based chemotherapy? Cancer Chemother Pharmacol 2006;57:835-40.
123. Copur S, Aiba K, Drake JC, Allegra CJ, Chu E. Thymidylate synthase gene amplification in human colon cancer cell lines resistant to 5-fluorouracil. Biochem Pharmacol 1995;49:1419-26.
124. Pullarkat ST, Stoehlmacher J, Ghaderi V, *et al.* Thymidylate synthase gene polymorphism determines response and toxicity of 5-FU chemotherapy. Pharmacogenomics J 2001;1:65-70.
125. Kawakami K, Salonga D, Park JM, *et al.* Different lengths of a polymorphic repeat sequence in the thymidylate synthase gene affect translational efficiency but not its gene expression. Clin Cancer Res 2001;7:4096-101.
126. Marsh S, Collie-Duguid ES, Li T, Liu X, McLeod HL. Ethnic variation in the thymidylate synthase enhancer region polymorphism among Caucasian and Asian populations. Genomics 1999;58:310-2.
127. Marsh S, Ameyaw MM, Githang'a J, *et al.* Novel thymidylate synthase enhancer region alleles in African populations. Hum Mutat 2000;16:528.
128. Kawakami K, Ishida Y, Danenberg KD, *et al.* Functional polymorphism of the thymidylate synthase gene in colorectal cancer accompanied by frequent loss of heterozygosity. Jpn J Cancer Res 2002;93:1221-9.
129. Tsuji T, Hidaka S, Sawai T, *et al.* Polymorphism in the thymidylate synthase promoter enhancer region is not an efficacious marker for tumor sensitivity to 5-fluorouracil-based oral adjuvant chemotherapy in colorectal cancer. Clin Cancer Res 2003;9:3700-4.
130. Horie N, Aiba H, Oguro K, Hojo H, Takeishi K. Functional analysis and DNA polymorphism of the tandemly repeated sequences in the 5'-terminal regulatory region of the human gene for thymidylate synthase. Cell Struct Funct 1995;20:191-7.
131. Park DJ, Stoehlmacher J, Zhang W, *et al.* Thymidylate synthase gene polymorphism predicts response to capecitabine in advanced colorectal cancer. Int J Colorectal Dis 2002;17:46-9.
132. Mandola MV, Stoehlmacher J, Muller-Weeks S, *et al.* A novel single nucleotide polymorphism within the 5' tandem repeat polymorphism of the thymidylate synthase gene abolishes USF-1 binding and alters transcriptional activity. Cancer Res 2003;63:2898-904.
133. Kawakami K, Watanabe G. Identification and functional analysis of single nucleotide polymorphism in the tandem repeat sequence of thymidylate synthase gene. Cancer Res 2003;63:6004-7.
134. Morganti M, Ciantelli M, Giglioni B, *et al.* Relationships between promoter polymorphisms in the thymidylate synthase gene and mRNA levels in colorectal cancers. Eur J Cancer 2005;41:2176-83.
135. Marcuello E, Altes A, del Rio E, *et al.* Single nucleotide polymorphism in the 5' tandem repeat sequences of thymidylate synthase gene predicts for response to fluorouracil-based chemotherapy in advanced colorectal cancer patients. Int J Cancer 2004;112:733-7.

136. Mandola MV, Stoehlmacher J, Zhang W, *et al.* A 6 bp polymorphism in the thymidylate synthase gene causes message instability and is associated with decreased intratumoral TS mRNA levels. Pharmacogenetics 2004;14:319-27.
137. Lecomte T, Ferraz JM, Zinzindohoue F, *et al.* Thymidylate synthase gene polymorphism predicts toxicity in colorectal cancer patients receiving 5-fluorouracil-based chemotherapy. Clin Cancer Res 2004;10:5880-8.
138. Lu JW, Gao CM, Wu JZ, *et al.* Polymorphism in the 3'-untranslated region of the thymidylate synthase gene and sensitivity of stomach cancer to fluoropyrimidine-based chemotherapy. J Hum Genet 2006;51:155-60.
139. Hitre E, Budai B, Adleff V, *et al.* Influence of thymidylate synthase gene polymorphisms on the survival of colorectal cancer patients receiving adjuvant 5-fluorouracil. Pharmacogenet Genomics 2005;15:723-30.
140. Kawakami K, Graziano F, Watanabe G, *et al.* Prognostic role of thymidylate synthase polymorphisms in gastric cancer patients treated with surgery and adjuvant chemotherapy. Clin Cancer Res 2005;11:3778-83.
141. Dotor E, Cuatrecases M, Martinez-Iniesta M, *et al.* Tumor thymidylate synthase 1494del6 genotype as a prognostic factor in colorectal cancer patients receiving fluorouracil-based adjuvant treatment. J Clin Oncol 2006;24:1603-11.
142. Kirchheiner J, Fuhr U, Brockmoller J. Pharmacogenetics-based therapeutic recommendations--ready for clinical practice? Nat Rev Drug Discov 2005;4:639-47.
143. Wessels JAM, van der Kooij SM, le Cessie S, *et al.* A clinical pharmacogenetic model to predict the efficacy of methotrexate monotherapy in recent-onset rheumatoid arthritis. Arthritis Rheum 2007;In press.
144. Hattersley AT, McCarthy MI. What makes a good genetic association study? Lancet 2005;366:1315-23.

201. http://www.fda.gov/cder/foi/label/2004/09053s024lbl.pdf
FDA package label 6-mercaptopurine. Accessed 25 October 2006.
202. http://ctep.cancer.gov/reporting/ctc_archive.html
NCI-CTC toxicity criteria. Accessed 19 February 2007.
203. http://www.fda.gov/cdrh/pdf5/K051824.pdf
Invader UGT1A1 molecular assay. Accessed 25 October 2006.
204. http://www.fda.gov/cder/foi/label/2006/020571s030lbl.pdf
FDA package label irinotecan. Accessed 25 October 2006.