PERGAMON

European Journal of Cancer 38 (2002) 639–644

European Journal of Cancer

www.ejconline.com

Review

Update on pharmacogenetics in cancer chemotherapy

F. Innocenti, M.J. Ratain*

Department of Medicine, Committee on Clinical Pharmacology and Pharmacogenomics, Cancer Research Center, The University of Chicago, Chicago, IL, USA

Received 3 December 2001; accepted 12 December 2001

Abstract

This review describes how genetic differences among patients may change the therapeutic outcome in cancer chemotherapy. Severe toxicity in genetically predisposed patients is predominantly associated with mutations in drug metabolism enzyme genes, and an update on genetic intolerance to 6-mercaptopurine, 5-fluorouracil, and irinotecan is provided. Moreover, recent findings pointed out that the methylenetetrahydrofolate reductase (*MTHFR*) C677T mutation might change patient susceptibility to the toxic effects of the cyclophosphamide, methotrexate, 5-fluorouracil (CMF) regimen and raltitrexed. Finally, it is emerging that not only toxicity, but also response to chemotherapy could be influenced by pharmacogenetic determinants, and the clinical relevance of polymorphisms in thymidylate synthase (*TS*) and glutathione-S-transferase (*GST*) genes is discussed.

Keywords: Cancer; Chemotherapy; Pharmacogenetics; Toxicity; Response

1. Introduction

The role of pharmacogenetics in cancer chemotherapy is progressively changing. Besides the "classical" examples of reduced tolerance to chemotherapy, genetic determinants might also affect patients' response and survival. Genotypic stratification of patients might identify subgroups with a better prognostic profile. The presence of alleles associated with reduced responsiveness to a certain drug might guide the selection of alternative therapies.

The need for optimisation in cancer chemotherapy is urgent. Adjusting the dose by body surface area does not correct for inter-individual differences in drug disposition. The intrinsic potency of cytotoxic agents and their use at maximally tolerated doses render chemotherapeutic treatment a high risk procedure for those patients that deviate from the average population. Identifying the genetic reasons behind either the occurrence of toxicity or lack of tumour response will reduce the unpredictability of cancer treatment. This review highlights the most recent findings on pharmacogenetic correlations between toxicity/response and mutated genetic traits.

2. Pharmacogenetics of drug toxicity. The examples of 6-mercaptopurine, 5-fluorouracil, irinotecan,[1] cyclophosphamide, methotrexate, 5-fluorouracil (CMF) regimen and raltitrexed

2.1. 6-Mercaptopurine and thiopurine methyltransferase pharmacogenetics

Mutations in the thiopurine methyltransferase (*TPMT*) gene have profound effects on 6-mercaptopurine (6-MP) tolerance and dose intensity in maintenance treatment of acute lymphoblastic leukaemia (ALL) in children [3,4]. A recent trial estimated that 71% of patients with bone marrow intolerance to 6-MP were phenotypically-TPMT deficient [5]. When 14 of these patients were typed for open reading frame (ORF) mutations associated with TPMT deficiency (*TPMT*2*, *TPMT*3A*, *TPMT*3C* (Table 1), 9 of them were found to be positive, with 100% concordance in the homozygous-mutant patients. However, less concordance was found in patients with intermediate TPMT activity. 6-MP dose adjustment strategy in TPMT-deficient patients was applied, and appropriate dose reductions (91% of the reduction in homozygous mutants (from

* Corresponding author at: The University of Chicago, 5841 South Maryland Avenue, MC 2115, Chicago, IL, USA. Tel.: +1-773-702-4400; fax: +1-773-702-3969.

E-mail address: mratain@medicine.bsd.uchicago.edu (M.J. Ratain).

[1] Concerning 6-mercaptopurine, 5-fluorouracil (5-FU), and irinotecan pharmacogenetics, details on related metabolic pathways and candidate genes can be obtained from two previously published articles [1,2].

0959-8049/02/$ - see front matter
PII: S0959-8049(01)00434-8

Table 1
Summary of gene mutations associated with changes in clinical and pharmacological phenotypes in cancer patients[a]

Drugs	*Clinical* phenotype	*Pharmacological* phenotype	Genes	Mutated alleles
Amonafide	Toxicity	Caffeine used as a probe	NAT2	*NAT2*5* *NAT2*6A* *NAT2*7* *NAT2*13* *NAT2*14*[b]
5-FU	Toxicity	DPD activity	*DPYD*	*DPYD*2A*
	Downstaging Response Toxicity	n.d.	*TS*	*TSER*3*
6-MP	Toxicity	TPMT activity	*TPMT*	*TPMT*2*, *TPMT*3A*, *TPMT*3C*[c]
CPT-11	Toxicity	Plasma metabolic ratios	*UGT1A1*	*UGT1A1*28*
CMF regimen Raltitrexed	Toxicity	n.d.	*MTHFR*	C677T
Epirubicin[d]	–	Plasma metabolic ratios	*UGT2B7*[e]	n.d.
Flavopiridol	Toxicity	Plasma metabolic ratios	*UGT1A9*[e]	n.d.

CMF, cyclophosphamide, methotrexate, 5-fluorouracil; 5-FU, 5-fluorouracil; DPD, dihydropyrimidine dehydrogenase; *DPYD*, dihydropyrimidine dehydrogenase gene; 6-MP, 6-mercaptopurine; CPT-11, irinotecan; TPMT, thiopurine methyltransferase; NAT, *N*-acetyltransferase; n.d., not determined.

[a] *Clinical* phenotypes include response, downstaging, survival and toxicity. *Pharmacological* phenotypes include *in vivo* and *ex vivo* indexes like blood cell enzyme activity, plasma metabolic ratios and use of phenotypic probes.

[b] These mutated *NAT2* alleles account for the slow acetylator phenotype [2].

[c] *TPMT*2, TPMT*3A, TPMT*3C* are associated with up to 95% of the TPMT deficiency [1].

[d] Concerning polymorphic epirubicin glucuronidation, no clear pharmacodynamic correlation was found in high and low glucuronidators [57], and no clinical phenotype is reported.

[e] We are currently investigating the functional role of *UGT2B7* and *UGT1A9* polymorphisms for epirubicin and flavopiridol glucuronidation, respectively.

50% to 94%)) allowed patients to tolerate full doses of all other chemotherapy treatments. In 8 wild-type patients who did not tolerate standard 6-MP dosage, median 6-MP dose reduction was 8% (from −20% to 91%), suggesting that toxicity was not caused by genetic differences in 6-MP disposition. Although 6-MP dose intensity was reduced in homozygous mutants compared with either heterozygous or wild-type patients, no differences in survival were observed among the genotypes. After 6-MP dose individualisation in TPMT-deficient patients, survival outcomes are similar between deficient and wild-type patients [4,6].

Although the genetic basis of TPMT deficiency has been elucidated, TPMT activity among patients without ORF mutations (approximately 90% of individuals) is very variable and showed familial correlation [7]. The variable number tandem repeat (*VNTR*) region of the *TPMT* promoter contains several potential binding sites for transcription factors. *In vitro* evidence of an inverse correlation between the number of repeats and TPMT activity was found [8–10]. When not only the number of repeats, but also their different structure was related to TPMT activity among individuals without ORF mutations, individuals with one *VNTR*6* allele were located at the low end of the frequency distribution curve [11]. Although statistically significant, the reduction in enzyme activity associated with *VNTR*6* was approximately 15% when compared with the maximal activity, suggesting minimal phenotypic changes induced by *VNTR* polymorphisms, as previously reported in Ref. [12].

Finally, sequencing of the entire *TPMT* genomic structure in Japanese individuals evidenced one promoter, three 3′-untranslated region (3′-UTR) and 26 intronic mutations [13], and their effect on mRNA turnover and splicing mechanisms should be assessed in functional studies.

2.2. 5-Fluorouracil and dihydropyrimidine dehydrogenase pharmacogenetics

Dihydropyrimidine dehydrogenase (DPD) activity is completely or partially deficient in approximately 0.1 and 3–5% of individuals, respectively [14]. At least 350 documented cases of DPD deficiency and seven toxic deaths have been reported so far (Diasio, 2001[2]). The

[2] 5th International Workshop on Pharmacodynamics of Anticancer Agents, Sea Island, GA, USA, October 2001.

complex molecular basis of genetic DPD deficiency and the multifactorial nature of 5-FU toxicity hamper the application of DPD pharmacogenetics to clinical practice. In two studies, normal DPD phenotype was found in approximately 33 and 66% of the patients who suffered from severe toxicity after 5-FU [15,16], suggesting the presence of other determinants of toxicity in addition to DPD genetics. Moreover, at least 20 functional mutations in the *DPYD* gene have been reported. *DPYD*2A* is a splice site mutation (intron 14 G1A) resulting in the production of a truncated mRNA (frequency of 0.094) [17]. So far, *DPYD*2A* has been associated with lethal outcomes in at least four cancer patients [17,18]. When 25 patients with grade 3–4 toxicity were genotyped for *DPYD*2A*, six of them (24%) were *DPYD*2A* carriers. Another study failed to demonstrate any association, with no *DPYD*2A* carrier among 14 cases of severe toxicity, rather showing the presence of other variants in these patients [19]. Due to the *DPYD*2A* prevalence among patients with 5-FU severe toxicity compared with normal individuals, it has been proposed that patients should be screened for this mutation [18]. The clinical utility of this genetic test is minimal due to its low sensitivity and unknown specificity [20]. DPD deficiency is probably a genetic disorder resulting from multiple mutations at a single gene locus. Genotyping for one single *DPYD* mutation does not have the sensitivity to identify high-risk patients.

2.3. Irinotecan and UDP-glucuronosyltransferase 1A1 pharmacogenetics

The clinical pharmacogenetics of irinotecan (CPT-11) is mainly focused on polymorphic glucuronidation of SN-38, the active metabolite of CPT-11. Gilbert's syndrome (mild unconjugated hyperbilirubinaemia) is mainly due to a reduced UDP-glucuronosyltransferase 1A1 (UGT1A1) expression caused by a TA insertion in the *UGT1A1* promoter region resulting in the variant allele $(TA)_7TAA$ (*UGT1A1*28*) [21–23]. A recent prospective trial of *UGT1A1* pharmacogenetics in cancer patients receiving 300 mg/m^2 CPT-11 found that the presence of the *UGT1A1*28* allele markedly altered SN-38 disposition. Significantly lower SN-38G/SN-38 AUC ratios and a trend towards more severe neutropenia and diarrhoea were found in *UGT1A1*28* carriers compared with wild-type [24]. This trial is now including patients receiving 350 mg/m^2 CPT-11 in order to evaluate the impact of *UGT1A1*28* for CPT-11 pharmacodynamics, and the role of other possible genetic determinants of CPT-11 and SN-38 disposition (CYP3A4/5, carboxylesterase, p-glycoprotein, multidrug resistance protein (MRP).

A minority of the Gilbert's syndrome patients do not have promoter mutations and are heterozygotes for coding missense mutations [25]. A retrospective pharmacogenetic trial evaluated the role of *UGT1A1* coding mutations for irinotecan toxicity in Japanese patients. All three C686A (P229Q, *UGT1A1*27*) carriers experienced severe toxicity. Surprisingly, no association was found with G211A (*UGT1A1*6*), an allele highly prevalent among Asians [26,27], and associated with approximately 60% of the Gilbert's syndrome cases among Japanese individuals [25,28].

2.4. Methylenetetrahydrofolate reductase pharmacogenetics in oncology

The occurrence of severe myelotoxicity after CMF in a very small series of breast cancer patients was associated with a C677T change in exon 4 in the methylenetetrahydrofolate reductase (*MTHFR*) gene [29]. This polymorphism is common, with approximately 10% of homozygous individuals (TT) in Caucasian populations (frequency of 0.05–0.54 in different populations) [30].

MTHFR regulates the pool of intracellular folates for nucleic acid and protein synthesis. MTHFR converts 5,10-methylenetetrahydrofolate (CH_2THF) to 5-methyltetrahydrofolate (CH_3THF), a methyl donor in the conversion of homocysteine to methionine during protein synthesis [31]. When alanine is substituted by valine due to C677T, this MTHFR variant has *in vitro* thermolability and 35% residual activity [32]. The C677T polymorphism alters the distribution of intracellular folates, creating retention of folates committed for purine and pyrimidine synthesis (i.e. CH_2THF) [33]. CH_2THF is required as a donor of monocarbon groups when thymidylate synthase converts deoxyuridine monophosphate (dUMP) into deoxythymidine monophosphate (dTMP). Qualitatively altered distribution of intracellular folates in breast cancer patients with a TT genotype could have increased the bone marrow sensitivity to CMF. Genetic MTHFR deficiency increases the availability of CH_2THF, probably potentiating 5-fluoro (5-F)dUMP inhibition of thymidylate synthase and leading to severe myelosuppression.

The clinical relevance of the C677T mutation was also highlighted in a phase I trial of the CPT-11/raltitrexed combination [34]. Patients with a TT genotype seemed to be protected from raltitrexed-associated toxicities compared with CC/CT patients. Raltitrexed is a potent thymidylate synthase (TS) inhibitor and folate analogue. High intracellular levels of CH_2THF in TT patients might have competed with raltitrexed for its binding to TS and folylpolyglutamate synthase, resulting in overall reduced cytotoxic effects.

Larger trials should investigate the impact of *MTHFR* C677T genotypes on response rates in patients receiving antifolates and fluoropyrimidines.

3. Pharmacogenetics of the drug response. The examples of polymorphic *thymidylate synthase* and *glutathione-S-transferase* genes

3.1. Thymidylate synthase pharmacogenetics in colorectal cancer patients

Acute induction of TS has been associated with resistance to fluoropyrimidine derivatives [35,36], and tumour TS expression is inversely related to clinical response [37–39]. In the *TS* enhancer region, two, three, four and nine copies of 28-bp tandem repeated sequences (*TSER*2*, *TSER*3*, *TSER*4*, *TSER*9*) have been described [40–42]. TS expression was increased by 2.6-fold when the triple repeat was compared with the double repeat in transient expression assays [40], and a similar trend was observed in tumour specimens [43].

In colorectal cancer patients, the relative probability of achieving downstaging after radiation and 5-FU treatment was different depending upon the *TSER* genotype. *TSER*2* carriers had 3.7-fold higher probability compared with *TSER*3* homozygotes [44]. In a larger study, *TSER* genotypes correlated with *TS* tumour expression levels, response and toxicity after 5-FU in colorectal cancer patients [45]. In liver metastases, higher (3.6-fold) mRNA levels were present in *TSER*3* homozygotes compared with *TSER*2* homozygotes. This difference probably affected the responsiveness of the tumour to 5-FU, as response rates were 9% in *TSER*3* homozygotes and 50% in *TSER*2* homozygotes. Concerning survival rates, a trend towards longer survival in *TSER*2* homozygotes was reported, in agreement with another study [45]. Grade 3 toxicities were 3-fold less frequent in *TSER*3* homozygotes compared with *TSER*2* homozygotes [46], suggesting that better prediction of 5-FU-related toxicity might derive from combined genotyping of dihydropyrimidine dehydrogenase gene (*DPYD*) and *TSER* functional variants.

Allelic imbalance leading to loss of heterozygosity (LOH) should be taken into account when tumour samples are genotyped. The *TS* gene is located on the short arm of chromosome 18, which is deleted in approximately 40% of colorectal cancers [47]. Out of 30 *TSER*2*/*TSER*3* rectal cancer patients, either one of the two alleles was lost [48]. Such a high LOH rate was not reported in another study, where only one of 23 *TSER*2*/*TSER*3* tumour biopsies showed LOH [45].

The search for matching expressed sequence tags by using the BLAST database (http://www.ncbi.nlm.nih.gov/BLAST) led to the discovery of a 6-bp deletion in the 3′UTR of *TS* mRNA in a Caucasian population (frequency of 0.29) [49]. The 3′ end of a mRNA can contain sequences that are important for maintaining adequate mRNA stability. Two 3′UTR polymorphisms were detected in normal colonic mucosa, primary tumours and colorectal metastases. In four metastatic tumour samples with high TS expression, no correlation was found with 3′UTR mutations [50].

3.2. Glutathione-S-transferase pharmacogenetics in oncology

Detoxification of xenobiotics is efficiently mediated by conjugation of their nucleophilic centre with reduced glutathione (GSH) by glutathione-*S*-transferase (GST) [51,52]. High phenotypic variability might derive from *GST* gene duplication (ultrarapid phenotype), deletions ('null' alleles, like *GSTM1*0* and *GSTT1*0*), and promoter/coding mutations that might increase or reduce GST function.

The role of GST pharmacogenetics in oncology is not fully established, and different studies showed contradictory results. For instance, in breast cancer patients receiving cyclophosphamide, 5-fluorouracil and doxorubicin, patients homozygous for both *GSTM1*0* and *GSTT1*0* had a reduced risk of recurrence and hazard of death compared with patients carrying wild-type alleles [53]. Reduced inactivation of doxorubicin- and cyclophosphamide-generated reactive oxygen species might have led to increased antitumour activity and better survival rates. On the contrary, when ovarian cancer patients received platinum and alkylating agents, *GSTM1*0* and *GSTT1*0* patients had poorer survival rates when compared with wild-type patients [54]. In the absence of differences in toxicity, reduced systemic drug detoxification in null genotype patients should have led to better survival rates compared with wild-type, but the opposite results were observed.

Similarly, conflicting results were shown in two studies in ALL children. Patients having at least two of the 'low risk' genotypes (i.e. *GSTM1*0*, *GSTT1*0*, *GSTP1 Val105*) had a 3.5-fold reduced risk of relapse compared with patients with no 'low risk' genotypes [55]. However, a previous study did not demonstrate any impact of *GSTM1*0* and *GSTT1*0* alleles on survival [56]. Although different patient selection criteria were applied, the patient population in the study by Chen and colleagues [56] is more representative of all childhood ALL patients.

The clinical studies on GST pharmacogenetics are not supported by pharmacological evidence (*ex vivo* or *in vivo*) of altered detoxification rates in patients carrying *GST* mutations. Correlative studies that might clarify the interpretation of pharmacogenetic data on GSTs include: (1) investigation of plasma pharmacokinetics of GST substrates and their metabolites, (2) investigation of GST activity in blood cells, for DPD and TPMT enzymes.

4. Conclusions

The availability of potent and reliable genetic techniques can change the way patients will receive chemotherapy in

the near future. With this perspective in mind, oncologists and clinical pharmacologists should prompt the inclusion of pharmacogenetic investigation and DNA collection into early phases of clinical drug development.

How to recognise the possible presence of a pharmacogenetic issue in cancer patients under chemotherapy treatment? If highly variable or bimodal pharmacokinetics are observed, underlying functional mutations should be investigated. For instance, bimodality in plasma metabolic ratios of epirubicin and flavopiridol are suggestive of the presence of functional mutations in drug metabolising enzymes [57,58] (Table 1). Moreover, recurrent (even after dose reduction) or unexplainable toxicity can be induced by genetically reduced drug inactivation/elimination. When polymorphic genes involved in the systemic disposition of a new agent are identified, prospective phenotype/genotype correlation analysis should be performed in phase I-II clinical trials, following the example of two recent phase I and pharmacogenetic studies [34,59].

Acknowledgements

This Pharmacogenetics of Anticancer Agents Research Group (www.pharmacogenetics.org) article was supported by a grant GM61393 from the National Institute of Health, Bethesda, MD, USA.

References

1. Innocenti F, Iyer L, Ratain MJ. Pharmacogenetics: a tool for individualizing antineoplastic therapy. *Clin Pharmacokinet* 2000, **39**, 315–325.
2. Innocenti F, Iyer L, Ratain MJ. Pharmacogenetics of anticancer agents: lessons from amonafide and irinotecan. *Drug Metab Dispos* 2001, **29**, 596–600.
3. McLeod HL, Coulthard S, Thomas AE, *et al*. Analysis of thiopurine methyltransferase variant alleles in childhood acute lymphoblastic leukaemia. *Br J Haematol* 1999, **105**, 96–700.
4. 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**, 2001–2008.
5. 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**, 2293–2301.
6. Relling MV, Hancock ML, Boyett JM, *et al*. Prognostic importance of 6-mercaptopurine dose intensity in acute lymphoblastic leukemia. *Blood* 1999, **93**, 2817–2823.
7. Vuchetich JP, Weinshilboum RM, Price RA. Segregation analysis of human red blood cell thiopurine methyltransferase activity. *Genet Epidemiol* 1995, **12**, 1–11.
8. Krynetski EY, Fessing MY, Yates CR, *et al*. Promoter and intronic sequences of the human thiopurine S-methyltransferase (TPMT) gene isolated from a human PAC1 genomic library. *Pharm Res* 1997, **14**, 1672–1678.
9. Spire-Vayron de la Moureyre C, Debuysere H, Mastain B, *et al*. Genotypic and phenotypic analysis of the polymorphic thiopurine S-methyltransferase gene (TPMT) in a European population. *Br J Pharmacol* 1998, **125**, 879–887.
10. Spire-Vayron de la Moureyre C, Debuysere H, Fazio F, *et al*. Characterization of a variable number tandem repeat region in the thiopurine S-methyltransferase gene promoter. *Pharmacogenetics* 1999, **9**, 189–198.
11. Alves S, Amorim A, Ferreira F, *et al*. Influence of the variable number of tandem repeats located in the promoter region of the thiopurine methyltransferase gene on enzymatic activity. *Clin Pharmacol Ther* 2001, **70**, 165–174.
12. Yan L, Zhang S, Eiff B, *et al*. Thiopurine methyltransferase polymorphic tandem repeat: genotype-phenotype correlation analysis. *Clin Pharmacol Ther* 2000, **68**, 210–219.
13. Seki T, Tanaka T, Nakamura Y. Genomic structure and multiple single-nucleotide polymorphisms (SNPs) of the thiopurine S-methyltransferase (TPMT) gene. *J Hum Genet* 2000, **45**, 299–302.
14. Lu Z, Zhang R, Diasio RB. Dihydropyrimidine dehydrogenase activity in human peripheral blood mononuclear cells and liver: population characteristics, newly identified deficient patients, and clinical implication in 5-fluorouracil chemotherapy. *Cancer Res* 1993, **53**, 5433–5438.
15. Milano G, Etienne MC, Pierrefite V, *et al*. Dihydropyrimidine dehydrogenase deficiency and fluorouracil-related toxicity. *Br J Cancer* 1999, **79**, 627–630.
16. 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**, 4705–4712.
17. 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**, 1149–1153.
18. 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**, 2832–2839.
19. Collie-Duguid ES, Etienne MC, Milano G, McLeod HL. Known variant DPYD alleles do not explain DPD deficiency in cancer patients. *Pharmacogenetics* 2000, **10**, 217–223.
20. Innocenti F, Ratain MJ. Correspondence re: Raida M. *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, published in *Clin Cancer Res* 2001, **7**, 2832–2839. *Clin Cancer Res* (in press).
21. Bosma PJ, Chowdhury JR, Bakker C, *et al*. The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. *N Engl J Med* 1995, **333**, 1171–1175.
22. Monaghan G, Ryan M, Seddon R, *et al*. Genetic variation in bilirubin UDP-glycuronyltransferase gene promoter and Gilbert's syndrome. *Lancet* 1996, **347**, 578–581.
23. 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 USA* 1998, **95**, 8170–8174.
24. Iyer L, Das S, Janish L, *et al*. *UGT1A1*28* polymorphism as a determinant of irinotecan disposition and toxicity. *Pharmacogenomics J* (in press).
25. Sato H, Adachi Y, Koiwai O. The genetic basis of Gilbert's syndrome. *Lancet* 1996, **347**, 557–558.
26. Akaba K, Kimura T, Sasaki A, *et al*. Neonatal hyperbilirubinemia and mutation of the bilirubin uridine diphosphate-glucuronosyltransferase gene: a common missense mutation among Japanese, Koreans and Chinese. *Biochem Mol Biol Int* 1998, **46**, 21–26.

27. Akaba K, Kimura T, Sasaki A, *et al*. Neonatal hyperbilirubinemia and a common mutation of the bilirubin uridine diphosphate-glucuronosyltransferase gene in Japanese. *J Hum Genet* 1999, **44**, 22–25.
28. Ando Y, Saka H, Ando M, *et al*. Polymorphisms of UDP-glucuronosyltransferase gene and irinotecan toxicity: a pharmacogenetic analysis. *Cancer Res* 2000, **60**, 6921–6926.
29. Toffoli G, Veronesi A, Boiocchi M, *et al*. MTHFR gene polymorphism and severe toxicity during adjuvant treatment of early breast cancer with cyclophosphamide, methotrexate, and fluorouracil (CMF). *Ann Oncol* 2000, **11**, 373–374.
30. Brattstrom L, Zhang Y, Hurtig M, *et al*. A common methylenetetrahydrofolate reductase gene mutation and longevity. *Atherosclerosis* 1998, **141**, 315–319.
31. Ueland PM, Hustad S, Schneede J, *et al*. Biological and clinical implications of the MTHFR C677T polymorphism. *Trends Pharmacol Sci* 2001, **22**, 195–201.
32. 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**, 111–113.
33. Bagley PJ, Selhub J. A common mutation in the methylenetetrahydrofolate reductase gene is associated with an accumulation of formylated tetrahydrofolates in red blood cells. *Proc Natl Acad Sci USA* 1998, **95**, 13217–13220.
34. 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**, 4081–4087.
35. Berger SH, Jenh CH, Johnson LF, *et al*. Thymidylate synthase overproduction and gene amplification in fluorodeoxyuridine-resistant human cells. *Mol Pharmacol* 1985, **28**, 461–467.
36. Johnston PG, Drake JC, Trepel J, *et al*. Immunological quantitation of thymidylate synthase using the monoclonal antibody TS 106 in 5-fluorouracil-sensitive and-resistant human cancer cell lines. *Cancer Res* 1992, **52**, 4306–4312.
37. Aschele C, Debernardis D, Tunesi G, *et al*. Thymidylate synthase protein expression in primary colorectal cancer compared with the corresponding distant metastases and relationship with the clinical response to 5-fluorouracil. *Clin Cancer Res* 2000, **6**, 4797–4802.
38. Johnston PG, Lenz HJ, Leichman CG, *et al*. Thymidylate synthase gene and protein expression correlate and are associated with response to 5-fluorouracil in human colorectal and gastric tumors. *Cancer Res* 1995, **55**, 1407–1412.
39. Leichman CG, Lenz HJ, Leichman L, *et al*. Quantitation of intratumoral thymidylate synthase expression predicts for disseminated colorectal cancer response and resistance to protracted-infusion fluorouracil and weekly leucovorin. *J Clin Oncol* 1997, **15**, 3223–3229.
40. Kaneda S, Takeishi K, Ayusawa D, *et al*. Role in translation of a triple tandemly repeated sequence in the 5′-untranslated region of human thymidylate synthase mRNA. *Nucleic Acids Res* 1987, **15**, 1259–1270.
41. Horie N, Aiba H, Oguro K, *et al*. 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**, 191–197.
42. Marsh S, Ameyaw MM, Githang'a J, *et al*. Novel thymidylate synthase enhancer region alleles in African populations. *Hum Mutat* 2000, **16**, 528.
43. Kawakami K, Omura K, Kanehira E, *et al*. Polymorphic tandem repeats in the thymidylate synthase gene is associated with its protein expression in human gastrointestinal cancers. *Anticancer Res* 1999, **19**, 3249–3252.
44. Villafranca E, Okruzhnov Y, Dominguez MA, *et al*. Polymorphisms of the repeated sequences in the enhancer region of the thymidylate synthase gene promoter may predict downstaging after preoperative chemoradiation in rectal cancer. *J Clin Oncol* 2001, **19**, 1779–1786.
45. Marsh S, McKay JA, Cassidy J, *et al*. Polymorphism in the thymidylate synthase promoter enhancer region in colorectal cancer. *Int J Oncol* 2001, **19**, 383–386.
46. 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.
47. Vogelstein B, Fearon ER, Kern SE, *et al*. Allelotype of colorectal carcinomas. *Science* 1989, **244**, 207–211.
48. Zinzindohoue F, Ferraz JM, Laurent-Puig P. Thymidylate synthase promoter polymorphism. *J Clin Oncol* 2001, **19**, 3442.
49. Ulrich CM, Bigler J, Velicer CM, *et al*. Searching expressed sequence tag databases: discovery and confirmation of a common polymorphism in the thymidylate synthase gene. *Cancer Epidemiol Biomarkers Prev* 2000, **9**, 1381–1385.
50. Yousef AM, Davis RA, Sticca RP, *et al*. Structural analysis of cDNA encoding thymidylate synthase in hepatic metastases of human colorectal tumors. *Int J Colorectal Dis* 2001, **16**, 318–325.
51. Hayes JD, Strange RC. Glutathione S-transferase polymorphisms and their biological consequences. *Pharmacology* 2000, **61**, 154–166.
52. Strange RC, Spiteri MA, Ramachandran S, *et al*. Glutathione-S-transferase family of enzymes. *Mutat Res* 2001, **482**, 21–26.
53. 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**, 7130–7135.
54. Howells RE, Redman CW, Dhar KK, *et al*. Association of glutathione S-transferase GSTM1 and GSTT1 null genotypes with clinical outcome in epithelial ovarian cancer. *Clin Cancer Res* 1998, **4**, 2439–2445.
55. Stanulla M, Schrappe M, Brechlin AM, *et al*. Polymorphisms within glutathione S-transferase genes (GSTM1, GSTT1, GSTP1) and risk of relapse in childhood B-cell precursor acute lymphoblastic leukemia: a case-control study. *Blood* 2000, **95**, 1222–1228.
56. Chen CL, Liu Q, Pui CH, *et al*. Higher frequency of glutathione S-transferase deletions in black children with acute lymphoblastic leukemia. *Blood* 1997, **89**, 1701–1707.
57. Robert J, David M, Granger C. Metabolism of epirubicin to glucuronides: relationship to the pharmacodynamics of the drug. *Cancer Chemother Pharmacol* 1990, **27**, 147–150.
58. Innocenti F, Stadler WM, Iyer L, *et al*. Flavopiridol metabolism in cancer patients is associated with the occurrence of diarrhea. *Clin Cancer Res* 2000, **6**, 3400–3405.
59. Ewesuedo RB, Iyer L, Das S, *et al*. Phase I clinical and pharmacogenetic study of weekly TAS-103 in patients with advanced cancer. *J Clin Oncol* 2001, **19**, 2084–2090.