available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Review

Should DPD analysis be required prior to prescribing fluoropyrimidines?

*Jane L. Yen, Howard L. McLeod**

Department of Pharmacotherapy and Experimental Therapeutics, University of North Carolina School of Pharmacy and the UNC Institute for Pharmacogenomics and Individualized Therapy, Chapel Hill, NC 27599-7360, USA

ARTICLE INFO

Article history:
Received 15 January 2007
Accepted 23 January 2007
Available online 12 March 2007

Keywords:
Dihydropyrimidine dehydrogenase
DPYD
5-Fluorouracil
Pharmacogenetics
Pharmacogenomics
Capecitabine
Chemotherapy
Toxicity

ABSTRACT

Dihydropyrimidine dehydrogenase (DPD) is a key enzyme in the metabolic catabolism of chemotherapeutic agent 5-fluorouracil (5FU) and its derivatives, including capecitabine. Numerous genetic mutations have been identified in the DPD gene locus (*DPYD*), with a few key variants having functional consequences on enzymatic activity. Deficiencies in DPD activity have been shown to cause 5FU-treated cancer patients to experience severe drug-related toxicities, often requiring extensive medical intervention. We review the performance of assays that assess DPD and *DPYD* status, with an emphasis on the robustness for routine clinical applications. None of the current strategies are adequate to mandate routine DPD testing prior to starting a fluoropyrimidine-based therapy. However, further research and technological improvements will hopefully allow prospective identification of potentially toxic patients, in order to reduce the number of patients with severe, life-threatening side effects to 5FU treatment.

1. Introduction

5-Fluorouracil (5FU) and its prodrug, capecitabine, are amongst the most commonly used drug therapies for cancers in the United States. These agents are administered either as single agent or combination therapy for cancers of the head and neck, cervix, breast, and gastrointestinal tract. Numerous serious adverse side effects have been reported with fluoropyrimidine treatment, including myelosuppression, cardiac toxicity, mucositis, hand-foot syndrome (HFS), and diarrhoea.[1,2] More recently, capecitabine has been favoured because of the convenience of its oral administration. In addition, capecitabine is better tolerated by patients, who reported fewer cases of stomatitis, alopecia, neutropenia, diarrhoea, and nausea, but more cases of HFS, with capecitabine compared to 5FU.[3]

Dihydropyrimidine dehydrogenase (DPD) is the initial and rate-limiting enzyme in the pathway that catabolises the pyrimidines such as uracil and thymine. The degradation of uracil by this process is solely responsible for the endogenous biosynthesis of β-alanine, a structural homologue of two inhibitory neurotransmitters, glycine and γ-aminobutyric acid (GABA). Complete DPD deficiency has been reported in paediatric patients presenting with high levels of thymine and uracil in the urine, blood, and cerebrospinal fluid, and can be accompanied by varying levels of neurological abnormalities.[4]

DPD plays a key role in the catabolic breakdown of fluorinated pyrimidines, like 5FU. It has been reported to catabolise

* *Corresponding author*: Tel.: +1 919 966 0512; fax: +1 919 962 0644.
E-mail address: hmcleod@unc.edu (H.L. McLeod).
0959-8049/$ - see front matter
doi:10.1016/j.ejca.2007.01.030

>80% of 5FU administered to cancer patients into fluorinated β-alanine.[5] Incorporation of the remaining 5FU into anabolic pathways induces anti-tumour and cytotoxic effects, primarily, by inhibition of thymidylate synthase (TS), a necessary enzyme for the *de novo* synthesis of dTMP. 5FU is converted into 5FdUMP and forms a stable complex with TS, limiting further enzymatic activity and impairing RNA and DNA synthesis and stability.[6,7] Capecitabine is an orally administered inactive prodrug that is converted into 5FU by a three-step process *in situ*.[8] The key step is catalysed by thymidine phosphorylase (TP), which is expressed at elevated levels by the liver and many tumours, allowing capecitabine to be specifically targeted to the site of the cancer, leading to relatively high local concentrations of 5FU in tumour cells.[3,7]

For the past 20 years, a causative link between deficiency in DPD activity and severe toxicity in response to 5FU treatment, including grade 4 symptoms and death, has been extensively studied. *DPYD*, the gene encoding DPD, is located within human chromosomal region 1p22 and is composed of 23 exons encompassing approximately 950 kb.[9,10] Over 30 single nucleotide polymorphisms (SNPs) and deletion mutations have been identified within *DPYD*, although most of these variants have no functional consequences on enzymatic activity. Of particular interest is the IVS14 + 1 G > A variant (DPYD*2A), which has been found in up to 40–50% of people with partial or complete DPD deficiency.[4,11,12] Recent studies have suggested that epigenetic factors may also influence DPD activity. Aberrant methylation of the *DPYD* promoter was found to cause a partially DPD deficient phenotype.[13–15]

The issue of DPD deficiency and severe toxicity from 5FU is of significant importance due to the widespread usage of 5FU. Meta-analysis of over 1200 patients suggested that more than 30% of patients treated with 5FU experience severe drug-related toxicity.[16] The frequency of low DPD enzymatic activity, indicating partial DPD deficiency, in the general population was initially estimated at between 3% and 5%,[17,18] although additional studies have shown significant variability among different ethnic subpopulations. Phenotypic and genotypic analyses of Asian (Japanese, Taiwanese, Korean),[10,11,19–21] Southwest Asian (Indian, Pakistani, Sri Lankan),[22,23] African (Egyptian, Kenyan, Ghanian),[22,24] European Caucasian (British, Dutch, French, German, Portuguese)[25–27] and American (Caucasian and African-American)[10,28,29] populations found partial DPD deficiency to occur at varying rates. For instance, a cohort of 114 Korean subjects was found to have a higher mean value for DPD activity than previously published Caucasian populations.[19] In contrast, a study population of African-Americans, especially female African-Americans, had significantly lower mean DPD activity and higher prevalence of partial DPD deficiency than a control Caucasian group.[29] Interpretation of these studies has been complicated by the fact that there is not a current consensus definition of deficiency. Initial studies used the lower 95th percentile as an arbitrary cut-off point. Other groups have suggested using the lower 70th percentile of DPD activity from a normal population as a threshold level, which would put nearly 14% of the general population at risk for developing 5FU-related toxicity.[12] Most patients have no symptoms of partial DPD deficiency and are unaware of their condition prior to 5FU treatment and the subsequent development of adverse side effects. This raises the question: What is the clinical utility of screening all cancer patients for DPD deficiency before starting on a 5FU or capecitabine treatment regimen?

2. The clinical assays

A number of screening methods have been developed for assessing DPD activity. The most common test, used as a benchmark to compare the quality of newer assays, involves the *ex vivo* incubation of a patient's peripheral blood mononuclear cells (PBMCs) with radiolabelled 5FU and measuring the resulting rate of catabolite formation by high-performance liquid chromatography (HPLC).[30,31] Although levels are highest in the liver, activity in PBMCs was found to be strongly correlated to liver DPD activity, and PBMCs are often used because they are easier to access.[32] Still, this assay is limited by a cumbersome sample preparation process and is complicated to perform. Thus, the number of patients who can be tested by this method is limited.

Real-time quantitative PCR of DPD mRNA has been suggested as an alternative method of measuring DPD enzymatic activity. Quantitative PCR has the advantage of being less complicated than the radiolabelling assay and relatively high-throughput. Yet, it remains unclear whether the quantification of cellular DPYD mRNA expression accurately represents the level of DPD enzyme activity, with several reports offering conflicting data.[28,33–36] Of particular concern is the detection of patients with DPD deficiency, where the molecular basis may result from RNA splicing, non-synonymous variants that do not change expression level, or post-genome variables (e.g. drug interactions or dietary intake).

A recent report, using a small number of patients, suggested measuring plasma levels of fluoro-β-alanine (FBAL), the final metabolite of 5FU in the catabolic pathway, by HPLC to assess DPD enzyme activity.[37] Decreased levels of FBAL were significantly correlated with reduced DPD activity, as measured by radio-assay. However, this assay requires the administration of at least a test dose of a fluoropyrimidine. Further research needs to be performed to confirm the clinical utility of this technique.

More promising alternate assays involve measuring uracil levels to determine DPD activity, prior to starting on a 5FU or capecitabine therapeutic regimen. Partial DPD deficiency impairs the metabolic breakdown of uracil, causing a build up of uracil that can be detected in plasma or urine samples by HPLC. Several recent studies found that elevated levels of uracil in plasma were significantly associated with impaired clearance of 5FU and development of 5FU-related toxicity.[36,38] Similarly, a ratio comparison of 5,6-dihydrouracil to uracil (UH_2/U), measured prior to 5FU treatment, could also be significantly correlated with later development of adverse side effects.[38,39] Additionally, for particularly low values, the UH_2/U ratio was associated with decreased 5FU clearance rates.[36,40] This method has been further streamlined, cutting the assay time in half and reducing the cost, in an effort to make it available for routine clinical use.[41]

Recently, a new test for uracil catabolism was developed.[42] Subjects ingested an aqueous solution of 2-^{13}C-uracil, and exhaled levels of $^{13}CO_2$ and $^{12}CO_2$ were measured

using infrared spectrophotometry. This study, as well as several follow up studies, demonstrated that reduced levels of exhaled $^{13}CO_2$ were strongly correlated to partial or complete DPD deficiency, as measured by the radiolabelling enzyme assay.[23,29] The breath test was non-invasive and rapid, giving accurate results of DPD activity within an hour of consuming the 2-^{13}C-uracil solution. The $^{13}CO_2$ exhaled breath samples that were collected into sealed bags were reported to be stable for up to 210 days, so the time needed for transport from the clinic to a laboratory testing facility should not be limiting. The analysis method used for measuring 2-^{13}C-uracil is the same as is used for several commonly performed tests, including the *H. pylori* breath test. However, the general usage of this method is restricted by the limited availability of the substrate, since 2-^{13}C-uracil is not available worldwide, yet. Other problems with screening for DPD deficiency utilising uracil-based methods exist. Inter-assay variability remains a problem, particularly if the tests are done by different laboratory facilities, a necessary consequence of widespread patient testing. Likewise, it has been difficult to define standardised threshold values for separating normal patients from those with DPD deficiency.

Other techniques for predicting partial DPD deficiency utilise genomics-based approaches. Several groups have reported using the WAVE™ DNA Fragment Analysis System, developed by Transgenomic, connected to a denaturing HPLC (DHPLC) for the detection of DNA mutations in *DPYD*.[43–45] PCR amplified exons of a patient's *DPYD* gene were allowed to duplex with wild-type DNA fragments, and heteroduplexes were separated from homoduplexes using DHPLC. Using optimised conditions in pilot studies, two groups reported no false positives or false negatives, which would give both sensitivity and specificity of 1.00 for the assay.[43,45] With this system whole exons can be screened, without the need to have previously identified the mutation, at reasonably low cost. Standard reference peaks can be established using PCR-generated homoduplexes, minimising the inter-assay variability. However, the method is relatively slow, taking up to 12.5 h per patient to screen the entire *DPYD* gene, which could limit the utility of this technique for clinical laboratory applications. Also, the method will identify any genetic change in the evaluated sequence, with the majority being of unclear clinical consequence.

Pyrosequencing, a procedure that sequences targeted micro-domains containing putative mutations, has been used in multiple studies to investigate SNPs and nucleotide deletions in *DPYD*.[36,38,46,47] This method is able to rapidly screen a large number of individual samples. As with the other tests, there are drawbacks to pyrosequencing. The assay can only be used to analyse previously identified mutations, and most mutations have to be sequenced separately. Screening of each of the more than 30 known *DPYD* mutations would be a lengthy process for every patient. However, because some of the mutations have no effect on DPD enzyme activity, and others are found at very low frequencies, pyrosequencing could be made efficient enough for widespread clinical use by concentrating on screening patients for the most commonly found functional SNPs.

3. The clinical case for DPD screening

Importantly, genetic mutation in *DPYD* does not always translate into severe 5FU toxicity. Seven patients heterozygous for the 2846A > T SNP were recently described.[36] Five of the seven patients developed severe, grade III or IV toxicities shortly after beginning the 5FU regimen. One patient had only mild, grade I toxicity after 5FU administration, leading to a reduction in dosage and no further toxicity. The other patient reported no toxicity to 5FU treatment. The possibility of getting false positive genetic testing results, causing an unnecessary reduction in chemotherapy dosage for a patient, is a risk that must be balanced with the benefit of identifying patients who will be harmed by the treatment regimen.

Many reports have studied the correlation between specific *DPYD* mutations or DPD activity measures and the development of grade 3 or 4 adverse side effects (see Table 1). Within a group of 25 German cancer patients experiencing severe toxicity, six patients (24%) were found to carry the DPYD*2A mutation, with one individual being homozygous.[26] Three of the patients with mutations died as a result of treatment. A similar study of 60 Dutch cancer patients with grade 3 or 4 toxicity found that 17 patients (28%) were carriers of the IVS14 + 1 G > A variant, one of whom was homozygous.[48] In a separate report from the Netherlands, 59% (22/37) of cancer patients with severe toxicity on a 5FU regimen were found to have partial DPD deficiency, as determined by the radioisotope assay.[49] Fourteen of these patients with reduced DPD activity were genotyped, and eight (57%) were found to have *DPYD* mutations, including six with the IVS14 + 1 G > A SNP and one with the 2846 A > T SNP. Similar results were found

Table 1 – Analytical performance of DPYD genetic testing to predict fluoropyrimidine toxicity

Study	*n*	Type	Sensitivity	Specificity	PPV	NPV
Raida et al.[26]	25 Patients with severe toxicity	Case only	24%	N.D.	N.D.	N.D.
van Kuilenburg et al.[48]	60 Patients with severe toxicity	Case only	28%	N.D.	N.D.	N.D.
van Kuilenburg et al.[49]	37 Patients with severe toxicity	Case only	57.1%	N.D.	N.D.	N.D.
Salguiero et al.[27]	73 Consecutive patients, 8 with severe toxicity	Cohort	25%	100%	100%	92%
Morel et al.[47]	44 Patients with severe toxicity	Case only	31%	98%	62%	94%
Largillier et al.[50]	105 Consecutive patients, 16 with severe toxicity	Cohort	6.3%	100%	100%	85.6%
Ciccolini et al.[39]	80 Patients with severe toxicity	Case only	71.3%	N.D.	N.D.	N.D.
Boisdron-Celle et al.[36]	252 Patients, 15 with severe toxicity	Cohort	83%	82%	70%	N.D.

PPV, positive predictive value; NPV, negative predictive value. N.D., not determinable.

in a small study of three patients who died after treatment with 5FU in the United States.[44] Genomic sequencing determined that two of the individuals were heterozygous for both the IVS14 + 1 G > A and 2846 A > T SNPs, while the third also had a distinct complex heterozygous genotype for *DPYD*. In Portugal, a cohort of 73 cancer patients being treated with 5FU was analysed.[27] Of the eight patients who developed early grade 3 or 4 toxicity, two had mutations in the DPD gene – one IVS14 + 1 G > A mutation and one 1845 G > T mutation. More recently, a large prospective study was performed in France with 487 patients, 44 of whom suffered a severely toxic reaction to 5FU.[47] A combination of 3 SNPs (IVS14 + 1 G > A, 2846 A > T and 1679 T > G) was examined, and it was determined that the presence of any of these SNPs significantly increased the patient's risk of developing severe 5FU toxicity by about 4-fold. Six of ten patients with the IVS14 + 1 G > A SNP developed grade 3 or 4 toxicity, but two of the four non-toxic individuals received a lower initial dosage of 5FU. Likewise, 6/10 individuals carrying the 2846 A > T mutation and 1/1 with the 1679 T > G SNP experienced grade 3 or 4 adverse side effects. In a cohort of 105 breast cancer patients being treated with capecitabine, a single individual was found to carry the IVS14 + 1 G > A mutation; she experienced lethal toxicity in response to treatment.[50]

Fewer reports have been published using the alternative methods to predict toxicity caused by partial DPD deficiency. A patient of Spanish descent who experienced lethal toxicity in response to 5FU treatment was found to have an abnormally high baseline level of plasma uracil and a low plasma UH_2/U ratio.[38] Additional genetic testing demonstrated that the patient carried a novel *DPYD* 464 T > A SNP. From a cohort of 615 French patients treated with 5FU or capecitabine, 80 were identified who developed grade 3 or 4 toxicity.[39] Fifty-seven of the 80 patients (71%) demonstrated a UH_2/U ratio lower than the cut-off value determined from the reference population. However, none of the 80 patients experiencing toxicity carried the IVS14 + 1 G > A SNP. Similarly, a patient treated with capecitabine and oxaliplatin, who developed severe grade 4 toxicities, did not carry the IVS14 + 1 G > A mutation but demonstrated a depressed UH_2/U ratio.[51] A combination of methods – starting with pyrosequencing and plasma uracil levels measurement, followed by determination of the UH_2/U ratio to verify the results – in a retrospective study of a cohort of 252 French Caucasian patients was shown to achieve 0.83 sensitivity and 0.84 specificity.[36] Using this combination of assays, the authors reported that 98.8% of patients who experienced grade 3 or 4 toxicities could have been identified prior to beginning 5FU treatment.

Overall, the analysis of DPYD*2A, alone or in combination with other common variants 2846 A > T and 1679 T > G, gave a sensitivity of 6.3–83% (median 30%) and a specificity of 82–100% (median 99%). Only 2 studies provided enough data to assess DPD enzyme activity; both had a sensitivity of 60%. More valuable indicators of the utility of a particular assay are the positive predictive value (PPV) and the negative predictive value (NPV), which ranged from 62% to 100% (median 85%) and 85.6% to 94% (median 92%), respectively, for the genetic analyses.

While the genomics and plasma uracil quantitation approaches to screening for 5FU-induced toxicity are promising, the uracil breath test appears to be fast, effective, and non-invasive. As an indirect, but functional, measure of DPD activity it holds great potential as a viable clinical tool. Currently, there have been no published reports on the efficacy of this assay for identifying patients experiencing severe toxicity, conducted either prospectively or retrospectively. Its current usage is limited by reagent availability, and the cost for performing the assay is unclear. However, the demonstrated long-term stability of $^{13}CO_2$ and consistency of assay results indicates that this assay could have great benefits for future cancer patients by identifying those individuals who would be at risk for developing severe 5FU-related toxicities prior to starting the treatment regimen.

Clearly, the goal of clinical testing for DPD genetic mutation or enzymatic deficiency is to identify patients who will develop severe adverse reactions to 5FU or capecitabine prior to drug administration, while minimising the number of patients who are mischaracterised and, thus, receive an unnecessarily altered dosage. However, the detection of 'at risk' patients must be the priority, from both a humanitarian and an economic viewpoint. Thus, developing assays with moderately good PPV (i.e. >70%), but very high NPV (i.e. >90%) is critical. Currently, several recently published reports approach these standards.[27,36,47] It is imperative to comprehensively recognise all patients who are potentially toxic, so they can receive a lower initial dosage of chemotherapeutic drug, and thus, avoid the toxic side effects. The improper dosing of patients who are misidentified as potentially toxic can be corrected during follow-up rounds of chemotherapy by escalation of dose in patients not experiencing toxicity. Oncologists and patients must be able to have confidence that the patients will not experience severe toxicity to 5FU or capecitabine given test results that indicate that the patient is not DPD deficient.

4. Conclusions

Fluoropyrimidines remain a staple of chemotherapeutic cancer medicine, nearly 50 years after the discovery of 5FU. Although advancements in treatment have been made, most notably the development of rationally designed 5FU derivatives, like capecitabine or uracil and tegafur, many patients still experience severe adverse side effects from the drugs. In the absence of new, better alternative chemotherapies, screening patients for conditions that would predispose them to being unable to tolerate 5FU, such as DPD deficiency, remains the best solution for improving patient outcomes. Still, with the currently available clinical laboratory assays, it is not yet possible to screen cancer patients with a high level of predictive accuracy. However, as an understanding of the molecular basis of 5FU-related toxicity continues to improve, and the techniques for assessing DPD deficiency are further refined, there is hope that screening will become practicable in the future.

Conflict of interest statement

None declared.

Acknowledgement

This work was supported in part by the Pharmacogenetics Research Network (U01 GM63340). The study sponsors had no involvement in the preparation or submission of this manuscript.

REFERENCES

1. Das P, Lin EH, Bhatia S, et al. Preoperative chemoradiotherapy with capecitabine versus protracted infusion 5-fluorouracil for rectal cancer: a matched-pair analysis. *Int J Radiat Oncol Biol Phys* 2006;**66**:1378–83.
2. Schmoll H-J, Cartwright T, Tabernero J, et al. Phase III trial of capecitabine plus oxaliplatin as adjuvant therapy for stage III colon cancer: a planned safety analysis in 1864 patients. *J Clin Oncol* 2007;**25**:102–9.
3. Walko CM, Lindley C. Capecitabine: a review. *Clin Ther* 2005;**27**:23–44.
4. van Kuilenburg ABP, Vreken P, Abeling NGGM, et al. Genotype and phenotype in patients with dihydropyrimidine dehydrogenase deficiency. *Hum Genet* 1999;**104**:1–9.
5. Heggie GD, Sommadossi J-P, Cross DS, Huster WJ, Diasio RB. Clinical pharmacokinetics of 5-fluorouracil and its metabolites in plasma, urine, and bile. *Cancer Res* 1987;**47**:2203–6.
6. van Kuilenburg ABP. Dihydropyrimidine dehydrogenase and the efficacy and toxicity of 5-fluorouracil. *Eur J Cancer* 2004;**40**:939–50.
7. de Bono JS, Twelves CJ. The oral fluorinated pyrimidines. *Invest New Drugs* 2001;**19**:41–59.
8. Ishikawa T, Utoh M, Sawada N, et al. Tumor selective delivery of 5-fluorouracil by capecitabine, a new oral fluoropyrimidine carbamate, in human cancer Xenografts. *Biochem Pharmacol* 1998;**55**:1091–7.
9. Takai S, Fernandez-Salguero P, Kimura S, Gonzalez FJ, Yamada K. Assignment of the human dihydropyrimidine dehydrogenase gene (DPYD) to chromosome region 1p22 by fluorescence in situ hybridization. *Genomics* 1994;**24**:613–4.
10. Wei X, Elizondo G, Sapone A, et al. Characterization of the human dihydropyrimidine dehyrdogenase gene. *Genomics* 1998;**51**:391–400.
11. Wei X, McLeod HL, McMurrough J, Gonzalez FJ, Fernandez-Salguero P. Molecular basis of the human dihydropyrimidine dehydrogenase deficiency and 5-fluorouracil toxicity. *J Clin Invest* 1996;**98**:610–5.
12. Van Kuilenburg ABP, 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 + 1 G > A mutation. *Int J Cancer* 2002;**101**:253–8.
13. Noguchi T, Tanimoto K, Shimokuni T, et al. Aberrant methylation of DPYD promoter, DPYD expression, and cellular sensitivity to 5-fluorouracil in cancer cells. *Clin Cancer Res* 2004;**10**:7100–7.
14. 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.
15. Yu J, McLeod HL. Methylation of the DPYD promoter and dihydropyrimidine dehydrogenase deficiency. *Clin Cancer Res* 2006;**12**:3864.
16. Cancer M-AGI. Toxicity of fluorouracil in patients with advanced colorectal cancer: effect of administration schedule and prognostic factors. *J Clin Oncol* 1998; **16**: 3537–41.
17. Etienne MC, Lagrange JL, Dassonville O, et al. Population study of dihydropyrimidine dehydrogenase in cancer patients. *J Clin Oncol* 1994;**12**:2248–53.
18. Lu Z, Zhang R, Carpenter JT, Diasio RB. Decreased dihydropyrimidine dehydrogenase activity in a population of patients with breast cancer: implication for 5-fluorouracil-based chemotherapy. *Clin Cancer Res* 1998;**4**:325–9.
19. Sohn D-R, Cho MS, Chung P-J. Dihydropyrimidine dehydrogenase activity in a Korean population. *Ther Drug Monit* 1999;**21**:152–4.
20. Yamaguchi K, Arai Y, Kanda Y, Akagi K. Germline mutation of dihydropyrimidine dehydrogenase gene among a Japanese population in relation to toxicity to 5-fluorouracil. *Jpn J Cancer Res* 2001;**92**:337–42.
21. Ogura K, Ohnuma T, Minamide Y, et al. Dihydropyrimidine dehydrogenase activity in 150 healthy Japanese volunteers and identification of novel mutations. *Clin Cancer Res* 2005;**11**:5104–11.
22. Morsman JM, Sludden J, Ameyaw M-M, et al. Evaluation of dihydropyrimidine dehydrogenase activity in South-west Asian, Kenyan and Ghanaian populations. *Br J Clin Pharmacol* 2000;**50**:269–72.
23. Saif MW, Mattison L, Carollo T, Ezzeldin H, Diasio RB. Dihydropyrimidine dehydrogenase deficiency in an Indian population. *Cancer Chemother Pharmacol* 2006;**58**:396–401.
24. Hamdy SI, Hiratsuka M, Narahara K, et al. Allele and genotype frequencies of polymorphic cytochromes P450 (CYP2C9, CYP2C19, CYP2E1) and dihydropyrimidine dehydrogenase (DPYD) in the Egyptian population. *Br J Clin Pharmacol* 2002;**53**:596–603.
25. van Kuilenburg ABP, 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.
26. 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.
27. Salgueiro N, Veiga I, Fragoso M, et al. Mutations in exon 14 of dihydropyrimidine dehydrogenase and 5-fluoruracil toxicity in Portuguese colorectal cancer patients. *Genet Med* 2004;**6**:102–7.
28. Seck K, Riemer S, Kates R, et al. Analysis of the DPYD gene implicated in 5-fluorouracil catabolism in a cohort of caucasian individuals. *Clin Cancer Res* 2005;**11**:5886–92.
29. Mattison LK, Fourie J, Desmond RA, Modak A, Saif MW, Diasio RB. Increased prevalence of dihydropyrimidine dehydrogenase deficiency in African-Americans compared with caucasians. *Clin Cancer Res* 2006;**12**:5491–5.
30. Johnson MR, Yan J, Shao L, Albin N, Diasio RB. Semi-automated radioassay for determination of dihydropyrimidine dehydrogenase (DPD) activity. Screening cancer patients for DPD deficiency, a condition associated with 5-fluorouracil toxicity. *J Chromatogr* 1997;**696**:183–91.
31. van Kuilenburg ABP, Van Lenthe H, Van Gennip AH. Radiochemical assay for determination of dihydropyrimidinase activity using reversed-phase high-performance liquid chromatography. *J Chromatogr B* 1999;**729**:307–14.
32. van Kuilenburg A, van Lenthe H, van Gennip A. Activity of pyrimidine degradation enzymes in normal tissues. *Nucleosides Nucleotides Nucleic Acids* 2006;**25**:1211–4.
33. Takechi T, Okabe H, Fujioka A, Murakami Y, Fukushima M. Relationship between protein levels and gene expression of dihydropyrimidine dehydrogenase in human tumor cells

during growth in culture and in nude mice. *Jpn J Cancer Res* 1998;**89**:1144–53.
34. Johnson MR, Wang K, Smith JB, Heslin MJ, Diasio RB. Quantitation of dihydropyrimidine dehydrogenase expression by real-time reverse transcription polymerase chain reaction. *Anal Biochem* 2000;**278**:175–84.
35. Johnson MR, Wang K, Diasio RB. Profound dihydropyrimidine dehydrogenase deficiency resulting from a novel compound heterozygote genotype. *Clin Cancer Res* 2002;**8**:768–74.
36. Boisdron-Celle M, Remaud G, Traore S, et al. 5-Fluorouracil-related severe toxicity: a comparison of different methods for the pretherapeutic detection of dihydropyrimidine dehydrogenase deficiency. *Cancer Lett* 2006 [in press, corrected proof].
37. Furuhata T, Kawakami M, Okita K, et al. Plasma level of a 5-fluorouracil metabolite, fluoro-beta-alanine correlates with dihydropyrimidine dehydrogenase activity of peripheral blood mononuclear cells in 5-fluorouracil treated patients. *J Exp Clin Cancer Res* 2006;**25**:79–82.
38. Morel A, Boisdron-Celle M, Fey L, Laine-Cessac P, Gamelin E. Identification of a novel mutation in the dihydropyrimidine dehydrogenase gene in a patient with a lethal outcome following 5-fluorouracil administration and the determination of its frequency in a population of 500 patients with colorectal carcinoma. *Clin Biochem* 2007;**40**:11–7.
39. Ciccolini J, Mercier C, Evrard A, et al. A rapid and inexpensive method for anticipating severe toxicity to fluorouracil and fluorouracil-based chemotherapy. *Ther Drug Monit* 2006;**28**:678–85.
40. Jiang H, Lu J, Jiang J, Hu P. Important role of the dihydrouracil/uracil ratio in marked interpatient variations of fluoropyrimidine pharmacokinetics and pharmacodynamics. *J Clin Pharmacol* 2004;**44**:1260–72.
41. Garg M, Sevester JC, Sakoff JA, Ackland SP. Simple liquid chromatographic method for the determination of uracil and dihydrouracil plasma levels: a potential pretreatment predictor of 5-fluorouracil toxicity. *J Chromatogr B* 2002;**774**:223–30.
42. Mattison LK, Ezzeldin H, Carpenter M, Modak A, Johnson MR, Diasio RB. Rapid identification of dihydropyrimidine dehydrogenase deficiency by using a novel 2-13C-uracil breath test. *Clin Cancer Res* 2004;**10**:2652–8.
43. Ezzeldin H, Okamoto Y, Johnson MR, Diasio RB. A high-throughput denaturing high-performance liquid chromatography method for the identification of variant alleles associated with dihydropyrimidne dehydrogenase deficiency. *Anal Biochem* 2002;**306**:63–73.
44. Ezzeldin H, Johnson MR, Okamoto Y, Diasio R. Denaturing high performance liquid chromatography analysis of the DPYD gene in patients with lethal 5-fluorouracil toxicity. *Clin Cancer Res* 2003;**9**:3021–8.
45. Fischer J, Schwab M, Eichelbaum M, Zanger UM. Mutational analysis of the human dihydropyrimidine dehydrogenase gene by denaturing high-performance liquid chromatography. *Genet Test* 2003;**7**:97–105.
46. Ahluwalia R, Freimuth R, McLeod HL, Marsh S. Use of pyrosequencing to detect clinically relevant polymorphisms in dihydropyrimidine dehydrogenase. *Clin Chem* 2003;**49**:1661–4.
47. Morel A, Boisdron-Celle M, Fey L, et al. Clinical relevance of different dihydropyrimidine dehydrogenase gene single nucleotide polymorphisms on 5-fluorouracil tolerance. *Mol Cancer Ther* 2006;**5**:2895–904.
48. van Kuilenburg ABP, 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.
49. van Kuilenburg ABP, 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.
50. Largillier R, Etienne-Grimaldi M-C, Formento J-L, et al. Pharmacogenetics of capecitabine in advanced breast cancer patients. *Clin Cancer Res* 2006;**12**:5496–502.
51. Ciccolini J, Mercier C, Dahan L, et al. Toxic death-case after capecitabine + oxaliplatin (XELOX) administration: probable implication of dihydropyrimidine deshydrogenase deficiency. *Cancer Chemother Pharmacol* 2006;**58**:272–5.