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Review

Dihydropyrimidine dehydrogenase and the efficacy and toxicity of 5-fluorouracil

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Abstract

The identification of genetic factors associated with either responsiveness or resistance to 5-fluorouracil (5-FU) chemotherapy, as well as genetic factors predisposing patients to the development of severe 5-FU-associated toxicity, is increasingly being recognised as an important field of study. Dihydropyrimidine dehydrogenase (DPD) is the initial and rate-limiting enzyme in the catabolism of 5-fluorouracil (5-FU). Although the role of tumoral levels as a prognostic factor for clinical responsiviness has not been firmly established, there is ample evidence that a deficiency of DPD is associated with severe toxicity after the administration of 5-FU. Patients with a partial DPD deficiency have an increased risk of developing grade IV neutropenia. In addition, the onset of toxicity occurred twice as fast compared with patients with a normal DPD activity. To date, 39 different mutations and polymorphisms have been identified in *DPYD*. The IVS14 + 1G > A mutation proved to be the most common one and was detected in 24–28% of all patients suffering from severe 5-FU toxicity. Thus, a deficiency of DPD appears to be an important pharmacogenetic syndrome.

Keywords: Chemotherapy; Dihydropyrimidine dehydrogenase; DPYD; Fluorouracil; Mutations; Pharmacokinetics; Pharmacogenetics; Pharmacogenomics; Polymorphisms

1. Introduction

Colorectal cancer is one of the most common fatal cancer types in elderly men and women [1]. The standard treatment for locally advanced colon cancer is surgical resection followed by adjuvant treatment with 5-fluorouracil (5-FU) and leucovorin. For patients suffering from Dukes' C stage colon cancer, this post-operative treatment has increased the 3-year overall survival rate by 12% [2]. Nevertheless, approximately 50% of these patients will die from metastatic disease, despite surgery and adjuvant chemotherapy. 5-FU, with or without folinic acid, has also been the favoured chemotherapy for the treatment of metastatic colorectal cancer. Although the palliative treatment of advanced colorectal cancer prolongs the medium survival, when compared with patients receiving best supportive care only, long-term survival is rare [3–5]. In metastatic disease, response rates with fluoropyrimidine-based regimens are approximately 22% with a medium survival of 11 months [6].

A challenging field is the identification of patients with tumours, before the commencement of treatment, that are likely to be responsive or resistant to first-line chemotherapy with 5-FU [7,8]. Patients with an unfavourable clinical or genetic make-up would be candidates for alternative treatment modalities using new agents with novel mechanisms of action [7]. Similarly, the identification of patients with an increased risk of development of severe 5-FU-associated toxicity would allow either dose-adaptation or the application of new non-fluoropyrimidine-based chemotherapeutic drugs [9].

Adverse drug reactions are a major clinical problem and it has been estimated that, in 1994, they accounted for over 100 000 deaths in the United States [10]. As such, adverse drug reactions were the fourth largest cause of death in the United States after heart diseases,

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cancer and stroke [10]. A meta-analysis involving 1219 patients with colorectal cancer receiving 5-FU, showed that grade 3 to 4 toxicity was encountered in 31–34% of the patients, with 0.5% of the patients experiencing lethal toxicity [11]. It is likely that a significant proportion of these adverse drug reactions are due to genetically-based differences, between individuals, in the response to 5-FU. Recent advances in our understanding of the metabolism of 5-FU and the key-enzymes involved in the activation and degradation of 5-FU have led to an increased awareness that the catabolic route of 5-FU plays an important role in the determination of toxicity as well as the efficacy towards 5-FU [12–14].

In this paper, the pivotal role of dihydropyrimidine dehydrogenase (DPD) in the metabolism of 5-FU is described and the pharmacogenetic consequences associated with a deficiency of this enzyme.

2. Metabolism of 5-FU

In order to exert its cytotoxic effect against cancer, 5-FU must first be anabolised to the nucleotide level (Fig. 1). Fluoropyrimidine nucleotides exert their cytotoxic effects through different mechanisms [15,16]. 5-FU is extensively incorporated into both nuclear and cytoplasmic RNA species. The incorporation of 5-fluorouridine 5′-triphosphate (FUTP) into RNA is accompanied by profound effects on the synthesis, stability, processing and methylation of RNA [15,16]. To some extent, 5-fluoro-2′-deoxyuridine 5′-triphosphate (FdUTP) can be incorporated into DNA leading to the inhibition of DNA elongation and to DNA fragmentation [15–18]. In addition to the incorporation of 5-FU into RNA and DNA, it has also been demonstrated that metabolites of 5-FU can produce alterations of the cellular membrane [19,20]. This phenomenon is probably attributable to FUDP-hexoses which may alter the structure of membranes by impairing the biosynthesis of glycoproteins. The relative importance of these membrane effects has not been studied thoroughly and their contribution to toxicity remains unclear. However, the most important anti-tumour effect of 5-FU can be ascribed to the inhibition of thymidylate synthase (TS) by FdUMP [21–24]. TS is a crucial enzyme for the *de novo* synthesis of thymidylate (dTMP) which is needed for the synthesis of DNA. This enzyme is responsible for the methylation of 2′-deoxyuridine 5′-monophosphate (dUMP) into thymidine 5′-monophosphate (dTMP) with the concomitant oxidation of N^5,N^{10}-methylenetetrahydrofolate to dihydrofolate. The relative contribution of RNA-directed and DNA-directed mechanisms of cytotoxicity of 5-FU depends on both the concentration of 5-FU and the duration of exposure. It has been suggested that short-term exposure to high concentrations of 5-FU induce RNA-directed 5-FU toxicity, whereas longer exposures to lower concentrations favour DNA-directed effects [25].

Today, a number of prodrugs have been developed from 5-FU such as capecitabine. Capecitabine is an

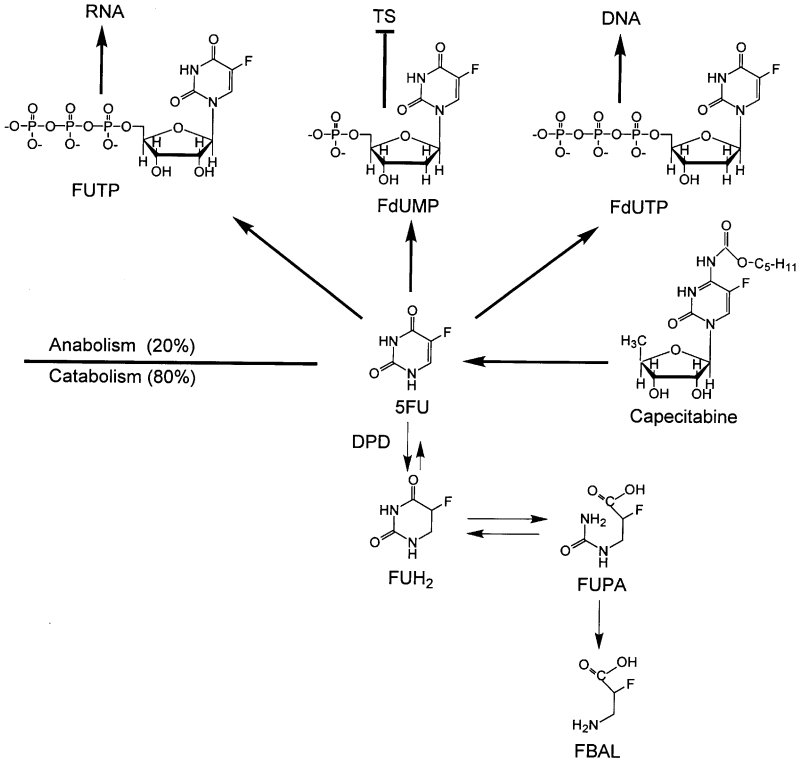

Fig. 1. The anabolism and catabolism of 5-fluorouracil (5-FU). TS, thymidylate synthase.

orally-administered fluoropyrimidine carbamate which is preferentially converted to 5-FU within the tumour site [26]. Capecitabine and its intermediate metabolites are not cytotoxic themselves, but instead become effective after the conversion to 5-FU.

Opposing the activation of 5-FU to the level of fluoropyrimidine nucleotides are the enzymes of the pyrimidine degradation pathway. DPD catalyses the conversion of 5-FU to fluoro-5,6-dihydrouracil (FUH_2) which is the initial and rate-limiting step in the catabolism of 5-FU. FUH_2 can be further degraded to fluoro-β-ureidopropionate (FUPA) and subsequently to fluoro-β-alanine (FBAL) by dihydropyrimidinase and β-ureidopropionase, respectively.

3. DPD activity and the levels of 5-FU

Although the cytotoxic effects of 5-FU are probably directly mediated via the anabolic pathways, the catabolic route plays a significant role as more than 80% of the administered 5-FU is catabolised by DPD [27] (Fig. 1). The activity of DPD can be detected in a variety of tissues, although the liver is the main organ responsible for the catabolism of 5-FU [28,29]. Patients with a severe impairment of the liver function might be prone to the development of severe toxicity due to a decreased capacity to catabolise 5-FU [30,31]. Since the activity of DPD in normal liver correlates well with that of peripheral blood mononuclear cells (PBM cells), the latter have been used as a surrogate for total body DPD activity [32]. The role of DPD, as an important factor in regulating the availability of 5-FU, was demonstrated in initial studies by Harris and colleagues, who observed a profound circadian rhythm in the activity of DPD [33]. The DPD activity was shown to be inversely related to the plasma concentrations of 5-FU in patients treated with continuous infusion of 5-FU [33]. Although there is ample evidence that the activity and mRNA levels of DPD in rodents follow a circadian rhythm [34], the presence of such a circadian rhythm in man is much debated as subsequent studies in both healthy volunteers and cancer patients failed to demonstrate the presence of a clear circadian rhythm in the DPD activity [35,36], mRNA [37] and 5-FU levels [38].

For DPD activities within the normal range, conflicting results have been published as to whether a correlation exists between the DPD activity and the clearance of 5-FU [30,39–41]. However, compelling results have recently shown that patients with a partial or complete DPD deficiency have a reduced capacity to degrade 5-FU and are at risk of developing severe 5-FU-associated toxicity. In a patient with a partial deficiency of DPD, due to heterozygosity for the IVS14 + 1G > A mutation, the clearance of 5-FU was 2.5 times lower and the area under the curve (AUC) of 5-FU (24.1 $mg.h.l^{-1}$) was 2.5 times higher, compared with controls [42]. Furthermore, a clinical pharmacological study of a patient with a complete deficiency of DPD demonstrated minimal catabolism of 5-FU, with a 10-fold longer half-life of 5-FU compared with patients with a normal DPD activity [43]. The important role of DPD in the metabolism of 5-FU was also highlighted by the observation of a lethal drug interaction between 5-FU and the antiviral agent sorivudine (1-β-D-arabinofuranosyl-(*E*)-5-(2-bromovinyl) uracil [44,45]. Sorivudine is catabolised in the gut flora to (*E*)-5-(2-bromovinyl) uracil which is a powerful inhibitor of DPD, resulting in a prolonged systemic exposure to 5-FU [44,45].

4. DPD and the efficacy of 5-FU

It was shown that a modest correlation exists between the DPD activity in PBM cells and colorectal tumours [46]. However, the use of PBM cells as a surrogate for monitoring the DPD activity in tumour cells is hampered by the fact that the DPD activity in colorectal tumours proved to be highly variable, the variability of which could not be explained by the DPD activity in PBM cells [46]. In line with this observation is the fact that no correlation was observed between the DPD activity in PBM cells and the response to 5-FU-based chemotherapy [47,48].

The analysis of more than 60 human cancer cell lines demonstrated that a highly significant inverse correlation exists between both the DPD mRNA expression and DPD activity with the 5-FU response [49,50]. In addition, it was shown that human tumour xenografts expressing low levels of DPD mRNA and DPD activity showed a significantly better response to 5-FU than tumours with a high DPD mRNA level and DPD activity [51]. Furthermore, the overexpression of DPD in a number of different cell types conferred resistance towards 5-FU [52]. These studies suggested that the intra-tumoral levels of DPD may be an important prognostic factor for the response to 5-FU. However, conflicting results have been reported in clinical studies evaluating the prognostic value of the mRNA, protein and activity levels of DPD with respect to the response and outcome of patients to 5-FU-based chemotherapy (Table 1).

The identification of DPD as a prognostic factor for response to 5-FU may have been hampered by the large variation which has been reported in the mRNA [53–57], protein [58] and activity levels [46,53,55,56,59,60] of DPD in tumour biopsies. Furthermore, a number of studies have indicated that there is no strong link between the mRNA levels of DPD and the protein or activity of DPD [34,53,55,56,61–63]. These studies suggest, therefore, that the activity of DPD is not only regulated at the transcriptional and translational levels,

Table 1
DPD expression in tumours and efficacy of 5-FU-based chemotherapy

Cancer	Patients	DPD parameter	Prognostic factor	[References]
Colorectal	37	mRNA	Yes	[67]
	33	mRNA	Yes	[23]
	142	mRNA	No	[111]
	309	mRNA	No	[57]
	100	Protein	No	[58]
	103	Activity	No	[112]
Head and neck	46	Activity	Yes	[59]
	72	Activity	No	[60]
Gastric	42	Activity	Yes	[113]
	14	Activity	Yes	[114]
	41	Activity	No	[115]
	55	Protein	No	[116]
	41	Protein	No	[117]
	93	Protein	No	[118]
	14	mRNA	No	[114]
	41	mRNA	No	[115]
Non-small cell lung	66	Protein	Yes	[119]
Ovarian	85	mRNA	No	[120]
Breast	119	Protein	Yes	[121]
Oral squamous cell carcinoma	22	mRNA	Yes	[122]
	27	Protein	Yes	[123]
Bladder	53	Activity	Yes	[124]

DPD, dihydropyrimidine dehydrogenase; 5-FU, 5-fluorouracil.

but also at the post-transcriptional level. Previously, it has been shown that the activity of DPD decreased with an increase in the growth rate of hepatomas and that the decreased DPD activity in hepatoma cells and regenerating liver appears to be specific for neoplastic cell growth [64,65]. In addition, it has been shown that both the activity of DPD, as well as its mRNA expression, is reduced in colorectal tumour cells when compared with normal mucosa cells [46,53,56,66]. The reduced levels of DPD in colorectal tumours might thus provide an explanation for the lack of a clear correlation between the levels of DPD in tumours and the clinical response of patients treated with 5-FU. Although the role of tumoral DPD levels as a prognostic factor for the efficacy of 5-FU-based chemotherapy has not been demonstrated unambiguously, the combination of the expression of DPD with that of TS, thymidine phosphorylase and orotate phosphoribosyltransferase, which are three other crucial enzymes in the metabolism of 5-FU, appears to be a promising approach [23,67,68].

5. DPD deficiency and 5-FU toxicity

In contrast to the questionable role of tumoral DPD levels as a prognostic factor of the efficacy of 5-FU-based chemotherapy, there is ample evidence to suggest that a systemic low DPD activity is associated with an increased risk of development of severe 5-FU-associated toxicity. 5-FU has a relatively narrow therapeutic index and a strong correlation has been described between exposure to 5-FU and both haematological and gastrointestinal toxicity [69]. Despite the many different treatment schedules that exist for 5-FU, comparable AUC thresholds (24–30 $mg.h.l^{-1}$) have been observed for the onset of severe toxicity [69]. Thus, in the case of a deficiency of DPD, profound alterations in the metabolism of 5-FU can be expected with an increased likelihood of developing severe toxicity [42,43]. In two small prospective studies, it was shown that 5-FU-related side-effects occurred more frequently in patients with a low DPD activity in PBM cells compared with patients with a normal DPD activity [48,70].

To date, a number of patients with a (partial) DPD deficiency have been reported as suffering from severe (lethal) toxicity after the administration of 5-FU [71–84]. Based on these experiences, a threshold limit for the DPD activity (70% of the mean DPD activity of a control population) has been proposed for patients at risk [83]. The importance of a DPD deficiency in the aetiology of unexpected severe 5-FU toxicity has been demonstrated by the fact that in 39–61% of the cases, a decreased DPD activity could be detected in PBM cells [76,80,83,84]. The DPD activity in controls and obligate heterozygotes follows a Normal or Gaussian distribution, with the mean DPD activity in individuals heterozygous for a mutation in *DPYD* being 48% of that observed in controls [39,80,85]. Using a threshold level of less then 70% of the mean of a control population, 14% of the population would be at risk of developing severe 5-FU-related toxicity [80]. Furthermore, a cut-off value for the DPD activity of 70% allows the identification of heterozygotes with a probability of 90% [80].

With respect to toxicity, it has been reported that the overall toxicity was twice as high in patients with a profound DPD deficiency (<45% of the mean DPD activity of a control population) when compared with patients with a moderate DPD deficiency (between 45% and 70% of the mean DPD activity of a control population) [83]. However, in another study, no differences were observed between patients with a low DPD activity and patients with a normal DPD activity in haematological, gastrointestinal, flu-like symptoms or other types of toxicities, with the exception of grade IV neutropenia (Table 2). Patients with a partial DPD deficiency proved to have a 3.4-fold higher risk of developing grade IV neutropenia than patients with a normal DPD activity [80]. Furthermore, in patients with a low DPD activity, the onset of toxicity occurred, on average, twice as fast compared with patients with a normal DPD activity [76]. To date, conflicting results exist as to whether the activity of DPD might be influenced by gender

Table 2
Clinical aspects of patients suffering from severe 5-FU toxicity

	n[a]	Total group	DPD ⩽ 70%	DPD > 70%	[Ref.]
Grade IV	54	46%	58%	29%	[80]
Neutropenia	25	72%	n.d.	n.d.	[78]
	53	n.d.	58%	n.d.	[83]
Time to onset of toxicity (days, mean ± SD)	37	13.9 ± 12.3	10.0 ± 7.6	19.1 ± 15.3	[76]
Females	54	65%	61%	71%	[80]
	53	57%	79%	44%	[83]
	103	45%	n.d.	n.d.	[84]

n.d., not determined.
[a] Number of patients investigated. The percentages concern the number of individuals suffering from grade IV neutropenia in each group or being female.

[39,66,85,86]. It has been reported that the DPD activity, as well as the clearance of 5-FU is, on average, 15% lower in women than in men [39,86]. This would be in line with the fact that women experience greater toxicity than men do, after receiving 5-FU-based chemotherapy [87]. However, the suggestion that women are particularly prone to a DPD deficiency in addition is questionable [80,83,84].

6. Structural organisation of DPD

Mammalian DPD appears to be relatively conserved throughout evolution and the high sequence identities (>92%) between human DPD and that of other mammals, such as bovine and pig, suggest that very similar reaction mechanisms and three-dimensional structures exist in these species [88]. The crystal structure of pig DPD revealed that the native enzyme is a homodimer of 2×111 kDa and each subunit of 1025 amino acids carries one flavin-adenine dinucleotide (FAD), one flavin mononucleotide (FMN) and four [4Fe-4S] clusters [88]. Each subunit appears to be arranged in a highly modular way with five distinctive domains, each carrying a subset of the prosthetic groups. Domain I (residues 27–172) contains two [4Fe-4S] clusters. Domain II (residues 173–286, 442–524) and domain III (residues 287–441) bind FAD and reduced nicotinamide-adenine dinucleotide (NADPH), respectively. Domain IV (525–847) contains FMN and the uracil/thymine binding site. Finally, the remaining two [4Fe-4S] clusters are bound to the core of the C-terminal domain V (residues 1–26 and 848–1025) [88]. The availability of the three-dimensional structure of DPD now permits the fascinating possibility to study the structural consequences of mutations in the DPD gene of patients with a DPD deficiency [88,89].

7. DPD mutations and polymorphisms

The human DPD gene (*DPYD*) is present as a single copy gene on chromosome 1p22 and consists of 23 exons [90,91]. A physical map indicates that *DPYD* is at least 950 kb in length with 3 kb of coding sequence and an average intron size of approximately 43 kb [91]. To date, 39 different mutations and polymorphisms have been identified in *DPYD* including 1 splice-site mutation, 5 frameshift mutations, 2 nonsense mutations, 23 mutations/polymorphisms and 8 intronic mutations [73,74,76,81,89,92–94]. The vast majority of these mutations have been detected in patients with a complete deficiency of DPD, which was accompanied by a wide variety in clinical presentation [89,92,93]. Fig. 2 shows that the mutations in the coding region of *DPYD* are not evenly distributed over the 23 exons of *DPYD* and 81% of all mutations were confined to exons 2–14, representing only 61% of the coding sequence. Typical hotspots proved to be exon 2, 6 and 13 in which 4 mutations/polymorphisms have been identified. The distribution of the mutations along the five distinctive domains that can be identified in DPD shows a slightly higher frequency of mutations in domain I (1.1%) and V (1.1%) compared with domain II (0.8%), domain III (0.9%) and domain IV (0.8%).

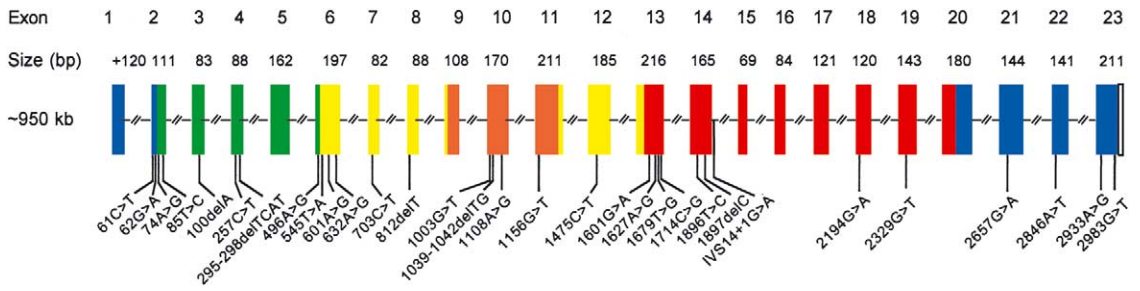

Fig. 2. Organisation of the DPD gene (*DPYD*). *DPYD* consists of 23 exons with an open reading frame of 3075 bp. The various colours represent the five different domains: green for domain I (N-terminal Fe-S clusters), yellow for domain II (FAD binding), orange for domain III (NADPH binding), red for domain IV (FMN/pyrimidine binding) and blue for domain V (C-terminal Fe-S clusters). The different mutations and polymorphisms identified in patients with a partial or complete deficiency of DPD are indicated, numbers correspond to the cDNA position.

8. DPD genotype in patients with severe 5-FU toxicity

A number of studies have been reported, demonstrating the presence of a mutant *DPYD* allele in patients suffering from severe toxicity after the administration of 5-FU (71–82). In these patients, 14 mutations have been identified including (a) 1 splice site mutation (IVS14 + 1G > A); (b) 2 nonsense mutations (R21X, E386X); (c) 6 missense mutations (M166V, M182K, V335L, I560S, A777S and D949V) and 5 polymorphisms (C29R, R21Q, S534N, I543V, V732I) (Fig. 3).

The G to A mutation in the invariant GT splice donor site [IVS14 + 1G > A] leads to the skipping of exon 14 immediately upstream of the mutated splice donor site in the process of DPD pre-mRNA splicing. As a result, the mature DPD mRNA lacks a 165 nucleotide (nt) segment encoding the amino acids 581–635 [95]. The nonsense mutations 61C > T (R21X) and 1156G > T (E386X) create a translation stop codon leading to premature termination of translation before the FAD and uracil/thymine binding sites and thus to a non-functional protein without any residual activity. The analysis of the crystal structure of pig DPD suggested that the point mutation I560S would most likely destabilise the DPD protein, whereas the amino acid exchanges D949V and V335L interfered directly with cofactor binding or electron transport [88,89]. To date, it is not yet known whether the M166V, M182K and A777S mutations affect the protein conformation and/or the binding of various cofactors.

It has been suggested that the C29R mutation might be a common polymorphism as homozygosity for this mutation was noted in two individuals with almost normal DPD activity [74]. This observation appears to be supported by analysis of the DPD protein structure, indicating that the C29R mutation would not introduce major changes in protein configuration or the binding of the various cofactors and substrates. Expression of the R21Q mutation in *Escherichia coli* resulted in the formation of a mutant protein with normal activity [73]. Previously, it has been shown that the mutations observed at codons 543 (allele frequency 28%), 732 (allele frequency 5.8%) and 534 (allele frequency 0.8%) are common polymorphisms and are not associated with a low DPD activity [96].

Analysis of the prevalence of the various mutations reported in cancer patients suffering from severe 5-FU-associated toxicity showed that the G to A point mutation in the invariant splice donor site is the most common one (Fig. 3). Homozygosity for the IVS14 + 1G > A mutation was observed in 3 patients and 26 patients were heterozygous for the IVS14 + 1G > A mutation, with a lethal toxicity occurring in 4 patients [77,78].

9. Prevalence of the IVS14 + 1G > A mutation

To date, a number of assays have been described for the detection of the IVS14 + 1G > A mutation [71,77,78, 95,97,98]. Recently, a restriction fragment length polymorphism (RFLP) procedure has been developed for the detection of the IVS14 + 1G > A mutation which allows large numbers of individuals to be screened on a routine basis [77]. Using this genotyping test, it has been shown that there is a relatively high frequency of the mutated allele in the normal Dutch population, with a frequency of 1.8% of heterozygotes [77]. Using the Hardy-Weinberg equilibrium and a frequency of

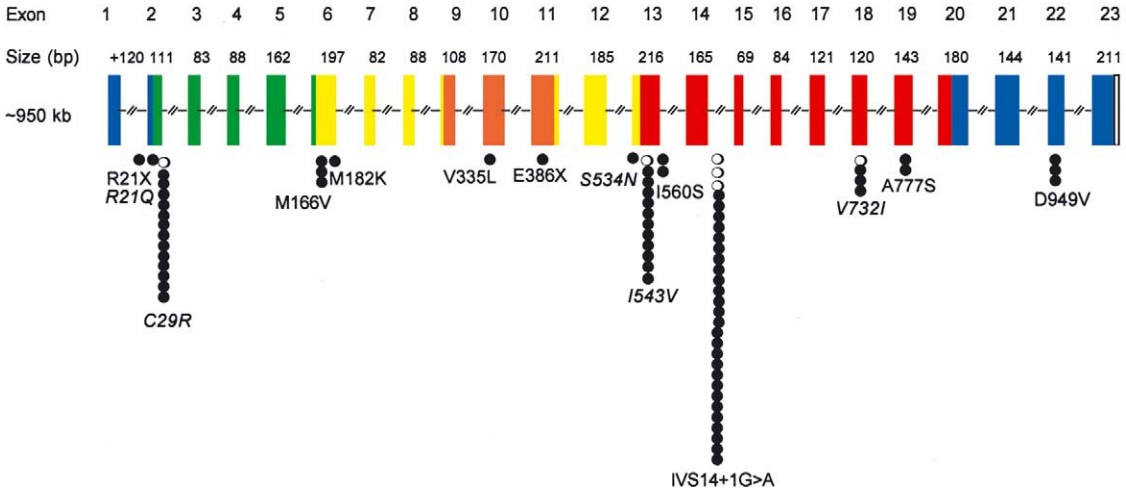

Fig. 3. Mutations and polymorphisms in *DPYD* of patients suffering from severe 5-FU-associated toxicity. The various colours represent the five different domains: green for domain I (N-terminal Fe-S clusters), yellow for domain II (FAD binding), orange for domain III (NADPH binding), red for domain IV (FMN/pyrimidine binding) and blue for domain V (C-terminal Fe-S clusters). Each symbol represents a patient homozygous (○) or heterozygous (●) for the indicated mutation. Known polymorphisms are written in italic.

heterozygotes of 1.8%, one can estimate the number of individuals, homozygous for the G→A mutation, to be 1.2 in 10 000. Screening of individuals of six other nationalities has demonstrated the presence of the IVS14 + 1G > A mutation in the German, Finnish, Turkish and Taiwanese populations whereas, this mutation has not been detected in Japanese and African-American populations (Table 3). Furthermore, the IVS14 + 1G > A mutation has also been detected in paediatric patients with a complete DPD deficiency from Denmark and Sweden [92]. Thus, there appears to be some kind of homogeneity for the IVS14 + 1G > A mutation in Northern Europe [92].

Screening for the presence of the IVS14 + 1G > A mutation in *DPYD* of patients suffering from grade 3 or 4 5-FU-associated toxicity showed that 24–28% of all patients were heterozygous or homozygous for the IVS14 + 1G > A mutation (Table 4) [78,82]. Surprisingly, Collie-Duguid and colleagues could not identify the IVS14 + 1G > A mutation in 23 patients with a reduced DPD activity [74]. In contrast, we demonstrated that the common splice site mutation was almost completely confined to the group of patients with a decreased DPD activity and 44% of these patients were carriers for this mutation. The IVS14 + 1G > A mutation could be detected in only 1 out of 24 (4%) patients with a normal DPD activity. The DPD activity of this patient proved to be just above the upper threshold level indicative of patients at risk [82]. Thus, these results clearly demonstrate that carriers of the IVS14 + 1G > A mutation are at risk of developing severe toxicity after the administration of 5-FU.

Table 3
Prevalence of IVS14 + 1G > A in different populations

Population	n[a]	Allele frequency (%)	[Ref.]
Dutch	2714	0.91	[77]
German	1702	0.47	[78]
	500	1.2	[98]
Turkish	400	0.75	[125]
Finnish	180	1.1	[91]
	90	2.2	[71]
Taiwanese	262	0.0	[91]
	72	2.7	[71]
Japanese	100	0.0	[91]
	214	0.0	[94]
African-Americans	210	0.0	[91]

[a] Number of alleles analysed.

Table 4
5-FU toxicity and the prevalence of the IVS14 + 1G > A mutation

IVS14 + 1G > A	Total group	DPD ⩽ 70%	DPD > 70%	[Ref.]
Patients ($n = 60$)				[82]
Heterozygotes	16 (27%)	15 (42%)	1 (4%)	
Homozygotes	1 (2%)	1 (3%)	0	
Patients ($n = 25$)				[78]
Heterozygotes	5 (20%)	n.d.	n.d.	
Homozygotes	1 (4%)	n.d.	n.d.	

n.d., not determined.

10. Screening for a DPD deficiency

In this review, it has been shown that a (partial) DPD deficiency plays an important role in the aetiology of severe 5-FU toxicity. Therefore, should the analysis of the DPD activity or screening for the presence of mutations be routinely carried out prior to the commencement of the 5-FU treatment? To date, a number of high-throughput procedures such as pyrosequencing [99] and denaturing high-performance liquid chromatography [100–102] have been developed to detect clinically relevant polymorphisms and mutations in *DPYD*. However, through sequencing the coding region of the DPD gene it appeared that at least 57% (8 of 14) of the patients with a reduced phenotype have a molecular basis for their deficient phenotype [76]. Thus, screening for coding mutations alone cannot unambiguously identify patients at risk. Currently, sensitive and highly accurate assays have been developed to measure the activity of DPD [103]. Although measuring the activity of DPD may be the 'method of choice', this type of analysis is less suitable for the screening of large samples on a routine basis. It has also been suggested that the 5,6-dihydrouracil/uracil ratio in the plasma of tumour patients reflects the activity of DPD and that this ratio is a prognostic factor for the toxicity of 5-FU-based chemotherapy [104]. It remains to be established whether the indicated threshold level of the 5,6-dihydrouracil/uracil ratio, indicative of an increased risk of toxicity, is also valid for other 5-FU regimens. Controlling the AUC of 5-FU seems an attractive approach, as a clear relationship has been demonstrated between the plasma levels of 5-FU and toxicity, in addition to response [40,42,69]. However, high inter- and intrapatient variations have been observed in the 5-FU plasma levels during continuous infusion [38]. In contrast, screening for the common splice-site mutation IVS14 + 1G > A is relatively easy and significantly cheaper.

To date, no large prospective studies have been performed to evaluate the role of a DPD deficiency and the onset of severe 5-FU toxicity. Nevertheless, one can estimate the probability that the presence of the IVS14 + 1G > A mutation would be accompanied by severe grade 3–4 toxicity by applying Bayes' theorem [105,106]. According to this theorem, the probability of toxicity given the presence of the IVS14 + 1G > A mutation can be calculated according to Eq. 1:

$$\Pr(\text{Tox, Mut}) = \frac{\Pr(\text{Mut, Tox}) \times \Pr(\text{Tox})}{\Pr(\text{Mut, Tox}) \times \Pr(\text{Tox}) + \Pr(\text{Mut, no Tox}) \times \Pr(\text{no Tox})} \quad (1)$$

The estimated probability Pr(Mut, Tox) of observing the IVS14 + 1G > A mutation in patients with severe grade 3–4 toxicity has been shown to be 28% [82]. A recent meta-analysis showed that the overall probability Pr(Tox) of observing severe 5-FU-associated toxicity was 31% [11]. Assuming that the probability of detecting the IVS14 + 1G > A mutation in patients without toxicity is identical to the prevalence of this mutation in the normal population (1.8%, Ref. [77]), the probability Pr(Tox, Mut) of severe 5-FU toxicity in case the IVS14 + 1G > A mutation is detected is 87%. In the event that all patients with the IVS14 + 1G > A mutation would be excluded from therapy with 5-FU, the frequency of toxicity would be reduced from 31% to 25% if screening for this mutation was carried out. Since patients suffering from severe toxicity require extensive and expensive medical care, a reduction in the number of patients with toxicity by 6% would have profoud implications.

11. Alternative treatment modalities

Patients with a deficiency of DPD might be selected for alternative treatment modalities containing novel non-fluoropyrimidine compounds. Irinotecan, oxaliplatin and raltitrexed have been shown to possess anti-neoplastic activity in colorectal cancer and these agents have been safely applied in the treatment of a patient suffering from a partial DPD deficiency [9]. An interesting option to explore might be the application of UFT (tegafur, uracil in a molar ratio of 1:4) in patients with a (partial) deficiency of DPD. After oral administration, 5-FU is slowly released from tegafur in the liver. A DPD-deficient state is achieved by the inclusion of uracil which is a reversible inhibitor of DPD and thus prevents the rapid degradation of 5-FU. A favourable efficacy and toxicity pattern has been observed for UFT treatment in colorectal cancer patients [107].

12. Concluding remarks

The identification of genetic factors leading to an increased risk of developing severe 5-FU-associated toxicity in patients is increasingly being recognised as an important field of study. Recently, it has been shown that the C677T polymorphism in the methylenetetrahydrofolate reductase gene might be responsible for the severe toxicity observed in some breast cancer patients receiving adjuvant treatment with cyclophosphamide, methotrexate and 5-FU [108]. In addition, it has been suggested that a polymorphism in the enhancer region of the TS gene promoter and a partial deficiency of dihydropyrimidinase are associated with toxicity towards 5-FU [109,110]. Although the precise role of DPD as a prognostic factor in the efficacy of 5-FU still has to be fully established, there is now ample evidence to suggest that patients with a (partial) DPD deficiency are prone to the development of severe 5-FU-associated toxicity. The common use of 5-FU in the treatment of cancer patients, the severe 5-FU-related toxicities in patients with a low activity of DPD in addition to the apparently high prevalence of the IVS14 + 1G > A mutation, thus warrants the analysis of the DPD activity in PBM cells and screening for the IVS14 + 1G > A mutation in cancer patients prior to the administration of 5-FU.

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References

1. Ries LAG, Wingo PA, Miller DS, *et al*. The annual report to the nation on the status of cancer, 1973–1997, with a special section on colorectal cancer. *Cancer* 2000, **88**, 2398–2424.
2. Efficacy of adjuvant fluorouracil and folinic acid in colon cancer. International Multicentre Pooled Analysis of Colon Cancer Trials (IMPACT) investigators. *Lancet* 1995, **345**, 939–944.
3. Simmonds PC. Palliative chemotherapy for advanced colorectal cancer: systematic review and meta-analysis. Colorectal Cancer Collaborative Group. *Br Med J* 2000, **321**, 531–535.
4. Jonker DJ, Maroun JA, Kocha W. Survival benefit of chemotherapy in metastatic colorectal cancer: a meta-analysis of randomized controlled trials. *Br J Cancer* 2000, **82**, 1789–1794.
5. Magné N, François E, Broisin L, *et al*. Palliative 5-fluorouracil-based chemotherapy for advanced colorectal cancer in the elderly: results of a 10-year experience. *Am J Clin Oncol* 2002, **25**, 126–130.
6. Meta-analysis Group In Cancer. Efficacy of intravenous continuous infusion of fluorouracil compared with bolus administration in advanced colorectal cancer. *J Clin Oncol* 1998, **16**, 301–308.
7. Adlard JW, Richman SD, Seymour MT, Quirke P. Prediction of the response of colorectal cancer to systemic therapy. *Lancet Oncol* 2002, **3**, 75–82.
8. Köhne CH, Cunningham D, Di Costanzo F, *et al*. Clinical determinants of survival in patients with 5-fluorouracil-based treatment for metastatic colorectal cancer: results of a multivariate analysis of 3825 patients. *Ann Oncol* 2002, **13**, 308–317.
9. Volk J, Reinke F, van Kuilenburg ABP, *et al*. Safe administration of irinotecan, oxaliplatin and raltitrexed in a DPD-deficient patient with metastatic colon cancer. *Ann Oncol* 2001, **12**, 569–571.

10. Lazarou J, Pomeranz BH, Corey PN. Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies. *JAMA* 1998, **279**, 1200–1205.
11. Meta-Analysis Group In Cancer. Toxicity of fluorouracil in patients with advanced colorectal cancer: effect of administration schedule and prognostic factors. *J Clin Oncol* 1998, **16**, 3537–3541.
12. Mattison LK, Soong R, Diasio RB. Implications of dihydropyrimidine dehydrogenase on 5-fluorouracil pharmacogenetics and pharmacogenomics. *Pharmacogenomics* 2002, **3**, 485–492.
13. Gardiner SJ, Begg EJ, Robinson BA. The effect of dihydropyrimidine dehydrogenase deficiency on outcomes with fluorouracil. *Adverse Drug React Toxicol Rev* 2002, **21**, 1–16.
14. van Kuilenburg ABP, De Abreu RA, van Gennip AH. Pharmacogenetic and clinical aspects of dihydropyrimidine dehydrogenase deficiency. *Ann Clin Biochem* 2003, **40**, 41–45.
15. Parker WB, Cheng YC. Metabolism and mechanism of action of 5-fluorouracil. *Pharmacol Ther* 1990, **48**, 381–395.
16. Grem JL. 5-Fluorouracil: forty-plus and still ticking. A review of its preclinical and clinical development. *Invest New Drugs* 2000, **18**, 299–313.
17. Schuetz JD, Diasio RB. The effect of 5-fluorouracil on DNA chain elongation in intact bone marrow cells. *Biochem Biophys Res Commun* 1985, **133**, 361–367.
18. Schuetz JD, Collins JM, Wallace HJ, Diasio RB. Alteration of the secondary structure of newly synthesized DNA from murine bone marrow cells by 5-fluorouracil. *Cancer Res* 1986, **46**, 119–123.
19. Kessel D. Cell surface alterations associated with exposure of leukemia L1210 cells to fluorouracil. *Cancer Res* 1980, **40**, 322–324.
20. Peters GJ, Pinedo HM, Ferwerda W, de Graaf TW, van Dijk W. Do antimetabolites interfere with the glycosylation of cellular glycoconjugates? *Eur J Cancer* 1990, **26**, 516–523.
21. 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.
22. Aschele C, Debernardis D, Casazza S, *et al*. Immunohistochemical quantitation of thymidylate synthase expression in colorectal cancer metastases predicts for clinical outcome to fluorouracil-based chemotherapy. *J Clin Oncol* 1999, **17**, 1760–1770.
23. Salonga D, Danenberg KD, Johnson MR, *et al*. Colorectal tumors responding to 5-fluorouracil have low gene expression levels of dihydropyrimidine dehydrogenase, thymidylate synthase, and thymidine phosphorylase. *Clin Cancer Res* 2000, **6**, 1322–1327.
24. Iacopetta B, Grieu F, Joseph D, Elsaleh H. A polymorphism in the enhancer region of the thymidylate synthase promoter influences the survival of colorectal cancer patients treated with 5-fluorouracil. *Br J Cancer* 2001, **85**, 827–830.
25. Sobrero AF, Aschele C, Bertino JR. Fluorouracil in colorectal cancer—a tale of two drugs: implications for biochemical modulation. *J Clin Oncol* 1997, **15**, 368–381.
26. Schüller J, Cassidy J, Dumont E, *et al*. Preferential activation of capecitabine in tumor following oral administration to colorectal cancer patients. *Cancer Chemother Pharmacol* 2000, **45**, 291–297.
27. Heggie GD, Sommadossi JP, Cross DS, Huster WJ, Diasio RB. Clinical pharmacokinetics of 5-fluorouracil and its metabolites in plasma, urine, and bile. *Cancer Res* 1987, **47**, 2203–2206.
28. Naguib FNM, el Kouni MH. Enzymes of uracil catabolism in normal and neoplastic human tissues. *Cancer Res* 1985, **45**, 5405–5412.
29. van Kuilenburg ABP, van Lenthe H, Blom MJ, Mul EPJ, van Gennip AH. Profound variation in dihydropyrimidine dehydrogenase activity in human blood cells: major implications for the detection of partly deficient patients. *Br J Cancer* 1999, **79**, 620–626.
30. Etienne MC, Chatelut E, Pivot X, *et al*. Co-variables influencing 5-fluorouracil clearance during continuous venous infusion. A NONMEM analysis. *Eur J Cancer* 1998, **34**, 92–97.
31. Stéphan F, Etienne MC, Wallays C, Milano GA, Clergue F. Depressed hepatic dihydropyrimidine dehydrogenase activity and fluorouracil-related toxicities. *Am J Med* 1995, **99**, 685–688.
32. Chazal M, Etienne MC, Renée N, Bourgeon A, Richelme H, Milano GA. Link between dihydropyrimidine dehydrogenase activity in peripheral blood mononuclear cells and liver. *Clin Cancer Res* 1996, **2**, 507–510.
33. Harris BE, Song R, Soong SJ, Diasio RB. Relationship between dihydropyrimidine dehydrogenase activity and plasma 5-fluorouracil levels with evidence for circadian variation of enzyme activity and plasma drug levels in cancer patients receiving 5-fluorouracil by protracted continuous infusion. *Cancer Res* 1990, **50**, 197–201.
34. Porsin B, Formento JL, Filipski E, *et al*. Dihydropyrimidine dehydrogenase circadian rhythm in mouse liver: comparison between enzyme activity and gene expression. *Eur J Cancer* 2003, **39**, 822–828.
35. Grem JL, Yee LK, Venzon DJ, Takimoto CH, Allegra CJ. Inter- and intraindividual variation in dihydropyrimidine dehydrogenase activity in peripheral blood mononuclear cells. *Cancer Chemother Pharmacol* 1997, **40**, 117–125.
36. van Kuilenburg ABP, Poorter RL, Peters GJ, *et al*. No circadian variation of dihydropyrimidine dehydrogenase, uridine phosphorylase, beta-alanine, and 5-fluorouracil during continuous infusion of 5-fluorouracil. *Advances in Experimental Medicine & Biology* 1998, **431**, 811–816.
37. Raida M, Kliche KO, Schwabe W, *et al*. Circadian variation of dihydropyrimidine dehydrogenase mRNA expression in leukocytes and serum cortisol levels in patients with advanced gastrointestinal carcinomas compared to healthy controls. *J Cancer Res Clin Oncol* 2002, **128**, 96–102.
38. Takimoto CH, Yee LK, Venzon DJ, *et al*. High inter- and intrapatient variantion in 5-Florouracil plama concentrations during a prolonged drug infusion. *Clin Cancer Res* 1999, **5**, 1347–1352.
39. Etienne MC, Lagrange JL, Dassonville O, *et al*. Population study of dihydropyrimidine dehydrogenase in cancer patients. *J Clin Oncol* 1994, **12**, 2248–2253.
40. Di Paolo A, Danesi R, Falcone A, *et al*. Relationship between 5-fluorouracil disposition, toxicity and dihydropyrimidine dehydrogenase activity in cancer patients. *Ann Oncol* 2001, **12**, 1301–1306.
41. Terashima M, Irinoda T, Kawamura H, *et al*. Intermittent FLDP: 24-h infusion of 5-FU on days 1, 3 and 5 combined with low-dose cisplatin on days 1–5 for gastric cancer, and its pharmacologic and kinetic rationale. *Cancer Chemother Pharmacol* 2003, **51**, 240–246.
42. Maring JG, van Kuilenburg ABP, Haasjes J, *et al*. Reduced 5-FU clearance in a patient with low DPD activity due to heterozygosity for a mutant allele of the DPYD gene. *Br J Cancer* 2002, **86**, 1028–1033.
43. 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.
44. Okuda H, Nishiyama T, Ogura K, *et al*. Lethal drug interactions of sorivudine, a new antiviral drug, with oral 5-fluorouracil prodrugs. *Drug Metab Dispos* 1997, **25**, 270–273.

45. Diasio RB. Sorivudine and 5-fluorouracil; a clinically significant drug-drug interaction due to inhibition of dihydropyrimidine dehydrogenase. *Br J Clin Pharmacol* 1998, **46**, 1–4.
46. McLeod HL, Sludden J, Murray GI, *et al*. Characterization of dihydropyrimidine dehydrogenase in human colorectal tumours. *Br J Cancer* 1998, **77**, 461–465.
47. Vokes EE, Mick R, Kies MS, *et al*. Pharmacodynamics of fluorouracil-based induction chemotherapy in advanced head and neck cancer. *J Clin Oncol* 1996, **14**, 1663–1671.
48. Saeki H, Ito S, Futatsugi M, Kimura Y, Ohga T, Sugimachi K. Role of dihydropyrimidine dehydrogenase activity in patients with esophageal cancer. *Anticancer Res* 2002, **22**, 3789–3792.
49. Beck A, Etienne MC, Chéradame S, *et al*. A role for dihydropyrimidine dehydrogenase and thymidylate synthase in tumour sensitivity to fluorouracil. *Eur J Cancer* 1994, **30A**, 1517–1522.
50. Scherf U, Ross DT, Waltham M, *et al*. A gene expression database for the molecular pharmacology of cancer. *Nat Genet* 2000, **24**, 236–244.
51. Ishikawa Y, Kubota T, Otani Y, *et al*. Dihydropyrimidine dehydrogenase activity and messenger RNA level may be related to the antitumor effect of 5-fluorouracil on human tumor xenografts in nude mice. *Clin Cancer Res* 1999, **5**, 883–889.
52. Takebe N, Zhao SC, Ural AU, *et al*. Retroviral transduction of human dihydropyrimidine dehydrogenase cDNA confers resistance to 5-fluorouracil in murine hematopoietic progenitor cells and human CD34+-enriched peripheral blood progenitor cells. *Cancer Gene Ther* 2001, **8**, 966–973.
53. Johnston SJ, Ridge SA, Cassidy J, McLeod HL. Regulation of dihydropyrimidine dehydrogenase in colorectal cancer. *Clin Cancer Res* 1999, **5**, 2566–2570.
54. Grem JL, Danenberg KD, Behan K, *et al*. Thymidine kinase, thymidylate synthase, and dihydropyrimidine dehydrogenase profiles of cell lines of the National Cancer Institute's Anticancer Drug Screen. *Clin Cancer Res* 2001, **7**, 999–1009.
55. Miyamoto S, Ochiai A, Boku N, *et al*. Discrepancies between the gene expression, protein expression, and enzymatic activity of thymidylate synthase and dihydropyrimidine dehydrogenase in human gastrointestinal cancers and adjacent normal mucosa. *Int J Oncol* 2001, **18**, 705–713.
56. Uetake H, Ichikawa W, Takechi T, Fukushima M, Nihei Z, Sugihara K. Relationship between intratumoral dihydropyrimidine dehydrogenase activity and gene expression in human colorectal cancer. *Clin Cancer Res* 1999, **5**, 2836–2839.
57. Kornmann M, Schwabe W, Sander S, *et al*. Thymidylate synthase and dihydropyrimidine dehydrogenase mRNA expression levels: predictors for survival in colorectal cancer patients receiving adjuvant 5-Fluorouracil. *Clin Cancer Res* 2003, **9**, 4116–4124.
58. Ikeguchi M, Makino M, Kaibara N. Thymidine phosphorylase and dihydropyrimidine dehydrogenase activity in colorectal carcinoma and patients prognosis. *Langenbecks Arch Surg* 2002, **387**, 240–245.
59. Etienne MC, Chéradame S, Fischel JL, *et al*. Response to fluorouracil therapy in cancer patients: the role of tumoral dihydropyrimidine dehydrogenase activity. *J Clin Oncol* 1995, **13**, 1663–1670.
60. Etienne MC, Pivot X, Formento JL, *et al*. A multifactorial approach including tumoural epidermal growth factor receptor, p53, thymidylate synthase and dihydropyrimidine dehydrogenase to predict treatment outcome in head and neck cancer patients receiving 5-fluorouracil. *Br J Cancer* 1999, **79**, 1864–1869.
61. 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**, 1144–1153.
62. Tanaka-Nozaki M, Onda M, Tanaka N, Kato S. Variations in 5-fluorouracil concentrations of colorectal tissues as compared with dihydropyrimidine dehydrogenase (DPD) enzyme activities and DPD messenger RNA levels. *Clin Cancer Res* 2001, **7**, 2783–2787.
63. Tamada H, Fukushima M, Koizumi K, *et al*. Regulation of dihydropyrimidine dehydrogenase gene expression in regenerating mouse liver. *Int J Oncol* 2003, **22**, 359–364.
64. Queener SF, Morris HP, Weber G. Dihydrouracil dehydrogenase activity in normal, differentiating and regnerating liver and in hepatomas. *Cancer Res* 1971, **31**, 1004–1009.
65. Jiang W, Lu Z, He Y, Diasio RB. Dihydropyrimidine dehydrogenase activity in hepatocellular carcinoma: implication in 5-fluorouracil-based chemotherapy. *Clin Cancer Res* 1997, **3**, 395–399.
66. Yamashita K, Mikami Y, Ikeda M, *et al*. Gender differences in the dihydropyrimidine dehydrogenase expression of colorectal cancers. *Cancer Lett* 2002, **188**, 231–236.
67. Ichikawa W, Uetake H, Shirota Y, *et al*. Combination of dihydropyrimidine dehydrogenase and thymidylate synthase gene expressions in primary tumors as predictive parameters for the efficacy of fluoropyrimidine-based chemotherapy for metastatic colorectal cancer. *Clin Cancer Res* 2003, **9**, 786–791.
68. Ichikawa W, Uetake H, Shirota Y, *et al*. Both gene expression for orotate phosphoribosyltransferase and its ratio to dihydropyrimidine dehydrogenase influence outcome following fluoropyrimidine-based chemotherapy for metastatic colorectal cancer. *Br J Cancer* 2003, **89**, 1486–1492.
69. Gamelin E, Boisdron-Celle M. Dose monitoring of 5-fluorouracil in patients with colorectal or head and neck cancer — status of the art. *Crit Rev Oncol Hematol* 1999, **30**, 71–79.
70. Katona C, Kralovászky J, Rosta A, *et al*. Putative role of dihydropyrimidine dehydrogenase in the toxic side effect of 5-fluorouracil in colorectal cancer patients. *Oncology* 1998, **55**, 468–474.
71. Wei X, McLeod HL, McMurrough J, Gonzalez FJ. Molecular basis of the human dihydropyrimidine dehydrogenase deficiency and 5-fluorouracil toxicity. *J Clin Invest* 1996, **98**, 610–615.
72. van Kuilenburg ABP, Vreken P, Beex LVAM, *et al*. Heterozygosity for a point mutation in an invariant splice donor site of dihydropyrimidine dehydrogenase and severe 5-fluorouracil related toxicity. *Eur J Cancer* 1997, **33**, 2258–2264.
73. 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.
74. Collie-Duguid ESR, Etienne MC, Milano GA, McLeod HL. Known variant DPYD alleles do not explain DPD deficiency in cancer patients. *Pharmacogenetics* 2000, **10**, 217–223.
75. Johnson MR, Hageboutros A, Wang K, High L, Smith JB, Diasio RB. Life-threatening toxicity in a dihydropyrimidine dehydrogenase-deficient patient after treatment with topical 5-fluorouracil. *Clin Cancer Res* 1999, **5**, 2006–2011.
76. 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**, 4705–4712.
77. 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**, 1149–1153.
78. Raida M, Schwabe W, Häusler 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.

79. Johnson MR, Wang K, Diasio RB. Profound dihydropyrimidine dehydrogenase deficiency resulting from a novel compound heterozygote genotype. *Clin Cancer Res* 2002, **8**, 768–774.
80. van Kuilenburg ABP, Meinsma JR, 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**, 253–258.
81. van Kuilenburg ABP, Baars JW, Meinsma JR, van Gennip AH. Lethal 5-fluorouracil toxicity associated with a novel mutation in the dihydropyrimidine dehydrogenase gene. *Ann Oncol* 2003, **14**, 341–342.
82. van Kuilenburg ABP, Meinsma JR, 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**, 555–558.
83. Milano GA, Etienne MC, Pierrefite V, Barberi-Heyob M, Deporte-Fety R, Renée N. Dihydropyrimidine dehydrogenase deficiency and fluorouracil-related toxicity. *Br J Cancer* 1999, **79**, 627–630.
84. Johnson MR, Diasio RB. Importance of dihydropyrimidine dehydrogenase (DPD) deficiency in patients exhibiting toxicity following treatment with 5-fluorouracil. *Adv Enzyme Regul* 2001, **41**, 151–157.
85. Lu ZH, Zhang RW, 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.
86. Milano GA, Etienne MC, Cassuto-Viguier E, *et al*. Influence of sex and age on fluorouracil clearance. *J Clin Oncol* 1992, **10**, 1171–1175.
87. Sloan JA, Goldberg RM, Sargent DJ, *et al*. Women experience greater toxicity with fluorouracil-based chemotherapy for colorectal cancer. *J Clin Oncol* 2002, **20**, 1491–1498.
88. Dobritzsch D, Schneider G, Schnackerz KD, Lindqvist Y. Crystal structure of dihydropyrimidine dehydrogenase, a major determinant of the pharmacokinetics of the anti-cancer drug 5-fluorouracil. *EMBO J* 2001, **20**, 650–660.
89. van Kuilenburg ABP, Dobritzsch D, Meinsma JR, *et al*. Novel disease-causing mutations in the dihydropyrimidine dehydrogenase gene interpreted by analysis of the three-dimensional protein structure. *Biochem J* 2002, **364**, 157–163.
90. Takai S, Kimura S, Gonzalez FJ, Yamada K. Assignment of the human dihydropyrimidine dehydrogenase gene (DPYD) to chromosome region 1p22 by fluorescence in situ hybridisation. *Genomics* 1994, **24**, 613–614.
91. Wei X, Elizondo G, Sapone A, McLeod HL, Raunio H, Gonzalez FJ. Characterization of the human dihydropyrimidine dehydrogenase gene. *Genomics* 1998, **51**, 391–400.
92. van Kuilenburg ABP, Vreken P, Abeling NGGM, *et al*. Genotype and phenotype in patients with dihydropyrimidine dehydrogenase deficiency. *Human Genetics* 1999, **104**, 1–9.
93. van Kuilenburg ABP, Meinsma JR, Poll-The BT, Zoetekouw L, van Gennip AH. Identification of a novel disease-causing mutation (100delA) in the dihydropyrimidine dehydrogenase gene. *Int Arch Biocsi* 2002, **1**, 1096–1100.
94. Yamaguchi K, Arai Y, Kanda Y, Akagi K. Germline mutation of dihydropyrimidine dehydrogenese gene among a Japanese population in relation to toxicity to 5-Fluorouracil. *Jpn J Cancer Res* 2001, **92**, 337–342.
95. Vreken P, van Kuilenburg ABP, Meinsma JR, *et al*. A point mutation in an invariant splice donor site leads to exon skipping in two unrelated Dutch patients with dihydropyrimidine dehydrogenase deficiency. *J Inherit Metab Dis* 1996, **19**, 645–654.
96. Ridge SA, Sludden J, Brown O, *et al*. Dihydropyrimidine dehydrogenase pharmacogenetics in Caucasian subjects. *Br J Clin Pharmacol* 1998, **46**, 151–156.
97. Stott MK, Fellowes AP, Upton JD, Burt MJ, George PM. Common DPYD mutation associated with 5-fluorouracil toxicity detected by PCR-mediated site-directed mutagenisis. *Clin Chem* 2000, **46**, 309–310.
98. Nauck M, Gierens H, März W, Wieland H. Rapid detection of a common dihydropyrimidine dehydrogenase mutation associated with 5-fluorouracil toxicity and congenital thymine uraciluria using fluorogenic hybridization probes. *Clin Biochem* 2001, **34**, 103–105.
99. Ahluwalia R, Freimuth R, McLeod HL, Marsh S. Use of pyrosequencing to detect clinically relevant polymorphisms in dihydropyrimidine dehydrogenase. *Clin Chem* 2003, **49**, 1661–1664.
100. 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 dihydropyrimidine dehydrogenase deficiency. *Anal Biochem* 2002, **306**, 63–73.
101. 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.
102. Gross E, Seck K, Neubauer S, *et al*. High-throughput genotyping by DHPLC of the dihydropyrimidine dehydrogenase gene implicated in (fluoro)pyrimidine catabolism. *Int J Oncol* 2003, **22**, 325–332.
103. van Kuilenburg ABP, van Lenthe H, Tromp A, Veltman PCJ, van Gennip AH. Pitfalls in the diagnosis of patients with a partial dihydropyrimidine dehydrogenase deficiency. *Clin Chem* 2000, **46**, 9–17.
104. Gamelin E, Boisdron-Celle M, Guérin-Meyer V, *et al*. Correlation between uracil and dihydrouracil plasma ratio, fluorouracil (5-FU) pharmacokinetic parameters, and tolerance in patients with advanced colorectal cancer: a potential interest for predicting 5-FU toxicity and determining optimal 5-FU dosage. *J Clin Oncol* 1999, **17**, 1105–1110.
105. Behnke D, Raida M, Kliche K-O, Pichlmeier U. Reply. *Clin Cancer Res* 2002, **8**, 1315–1316.
106. Altman DG. *Calculations. Practical Statistics for Medical Research*. London, Chapman and Hall, 1991, 414–416.
107. Malet-Martino M, Martino R. Clinical studies of three oral prodrugs of 5-fluorouracil (capecitabine, UFT, S-1): a review. *Oncologist* 2002, **7**, 288–323.
108. 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**, 373–374.
109. 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.
110. van Kuilenburg ABP, Meinsma JR, Zonnenberg BA, *et al*. Dihydropyrimidinase deficiency and severe 5-Fluorouracil toxicity. *Clin Cancer Res* 2003, **9**, 4363–4367.
111. Kornmann M, Link KH, Galuba I, *et al*. Association of time to recurrence with thymidylate synthase and dihydropyrimidine dehydrogenase mRNA expression in stage II and III colorectal cancer. *J Gastrointest Surg* 2002, **6**, 331–337.
112. Etienne MC, Chazal M, Laurent-Puig P, *et al*. Prognostic value of tumoral thymidylate synthase and p53 in metastatic colorectal cancer patients receiving fluorouracil-based chemotherapy: phenotypic and genotypic analyses. *J Clin Oncol* 2002, **20**, 2832–2843.
113. Terashima M, Irinoda T, Fujiwara H, *et al*. Roles of thymidylate synthase and dihydropyrimidine dehydrogenase in tumor

progression and sensitivity to 5-fluorouracil in human gastric cancer. *Anticancer Res* 2002, **22**, 761–768.
114. Takabayashi A, Iwata S, Kawai Y, *et al*. Dihydropyrimidine dehydrogenase activity and mRNA expression in advanced gastric cancer analyzed in relation to effectiveness of preoperative 5-fluorouracil-based chemotherapy. *Int J Oncol* 2000, **17**, 889–895.
115. Ishikawa Y, Kubota T, Otani Y, *et al*. Thymidylate synthetase and dihydropyrimidine dehydrogenase levels in gastric cancer. *Anticancer Res* 1999, **19**, 5635–5640.
116. Nozawa H, Tsukui H, Nishida K, Yakumaru K, Nagawa H, Sekikawa T. Dihydropyrimidine dehydrogenase expression in preoperative biopsy and surgically resected specimens of gastric carcinoma. *Cancer Chemother Pharmacol* 2002, **49**, 267–273.
117. Miyamoto S, Boku N, Ohtsu A, *et al*. Clinical implications of immunoreactivity of thymidylate synthase and dihydropyrimidine dehydrogenase in gastric cancer treated with oral fluoropyrimidine (S-1). Study Group of S-1 for Gastric Cancer. *Int J Oncol* 2000, **17**, 653–658.
118. Terashima M, Fujiwara H, Takagane A, *et al*. Role of thymidine phosphorylase and dihydropyrimidine dehydrogenase in tumour progression and sensitivity to doxifluridine in gastric cancer patients. *Eur J Cancer* 2002, **38**, 2375–2381.
119. Nakagawa T, Tanaka F, Takata T, *et al*. Predictive value of dihydropyrimidine dehydrogenase expression in tumor tissue, regarding the efficacy of postoperatively administered UFT (Tegafur + Uracil) in patients with p-stage I nonsmall-cell lung cancer. *J Surg Oncol* 2002, **81**, 87–92.
120. Fujiwaki R, Hata K, Nakayama K, Fukumoto M, Miyazaki K. Gene expression for dihydropyrimidine dehydrogenase and thymidine phosphorylase influences outcome in epithelial ovarian cancer. *J Clin Oncol* 2000, **18**, 3946–3951.
121. Horiguchi J, Takei H, Koibuchi Y, *et al*. Prognostic significance of dihydropyrimidine dehydrogenase expression in breast cancer. *Br J Cancer* 2002, **86**, 222–225.
122. Hoque MO, Kawamata H, Nakashiro KI, *et al*. Dihydropyrimidine dehydrogenase mRNA level correlates with the response to 5-fluorouracil-based chemo-immuno-radiation therapy in human oral squamous cell cancer. *Int J Oncol* 2001, **19**, 953–958.
123. Kawasaki G, Yoshitomi I, Yanamoto S, Mizuno A. Thymidylate synthase and dihydropyrimidine dehydrogenase expression in oral squamous cell carcinoma: an immunohistochemical and clinicopathologic study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002, **94**, 717–723.
124. Mizutani Y, Wada H, Fukushima M, *et al*. The significance of dihydropyrimidine dehydrogenase (DPD) activity in bladder cancer. *Eur J Cancer* 2001, **37**, 569–575.
125. Çelik L, Kars A, Guc D, Tekuzman G, Ruacan S. Dihydropyrimidine dehydrogenase enzyme deficiency: clinical and genetic assessment of prevalence in Turkish cancer patients. *Cancer Invest* 2002, **20**, 333–339.