Evolution of Dihydropyrimidine Dehydrogenase Diagnostic Testing in a Single Center during an 8-Year Period of Time

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Objective: Fluoropyrimidine treatment can be optimized based on dihydropyrimidine dehydrogenase (DPD) activity. DPD dysfunction leads to increased exposure to active metabolites, which can result in severe or even fatal toxicity.

Methods: We provide an overview of 8 years of DPD diagnostic testing (n = 1194).

Results: Within the study period, our diagnostic test evolved from a single-enzyme measurement using first a radiochemical and then a nonradiochemical assay by ultra HPLC-MS in peripheral blood mononuclear cells with uracil, to a combined enzymatic and genetic test (ie, polymerase chain reaction) followed by Sanger sequence analysis of 4 variants of the DPD gene (ie, DPD2A, DPD13, c.2846A->T, and 1129-5923C>G; allele frequencies 0.58%, 0.03%, 0.29%, and 1.35%, respectively). Patients who have 1 of the 4 variants tested (n = 614) have lower enzyme activity than the overall patient group. The majority of patients with the DPD2A variant (83%) consistently showed decreased enzyme activity. Only 24 (25.3%) of 95 patients (tested for 4 variants) with low enzyme activity carried a variant. Complete DPDY sequenc- ing in a subgroup with low enzyme activity and without DPD2A variant (n = 47) revealed 10 genetic variants, of which 4 have not been described previously. We did not observe a strong link between DYPD genotype and enzyme activity.

Conclusions: Previous studies have shown that DPD status should be determined before treatment with fluoropyrimidines to prevent unnecessary side effects with possible fatal consequences. Our study in combination with literature shows that there is a discrepancy between the DPD enzyme activity and the presence of clinically relevant single nucleotide polymorphisms. At this moment, a combination of a genetic and enzyme test is preferable for diagnostic testing. (Curr Ther Res Clin Exp. 2018; 79:XXX-XXX.)

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Introduction

The main chemotherapeutic agents used in many types of cancer, such as colorectal, gastrointestinal, and breast cancer, are the fluoropyrimidines 5-fluorouracil (5-FU), capecitabine, and tegafur. Treatment with these agents is not well tolerated in a subgroup of patients. In 20% to 40%, moderate to severe (fatal) toxicity occurs, including nausea and vomiting, diarrhea, mucositis/stomatitis, myelosuppression, and hand-foot syndrome.* The enzyme responsible for degradation of fluoropyrimidines is dihydropyrimidine dehydrogenase (DPD), the first and rate-limiting enzyme of the pyrimidine degradation pathway. DPD dysfunction leads to an increased exposure to active metabolites, which can result in severe or even fatal toxicity. From the literature, the estimated percentage of individuals who are DPD deficient is 3% to 5% in the Caucasian populations. This deficiency can in most cases be related to genetic variants in the dihydropyrimidine dehydrogenase gene (DPYD).* The DPYD gene on chromosome 1p22 has 23 exons and more than 100 variants have been reported in DPYD, of which only few have been studied in relation to decreased DPD enzyme activity and/or toxicity. Of the variants studied, only 3 variants have been reported that were consistently associated with toxicity and decreased DPD enzyme activity in patients treated with
Figure 1. Evolution of dihydroprymidine dehydrogenase (DPD) diagnostic testing over a period of time lasting 8 years.

a fluoropyrimidine agent: DYPD*2A (c.1905+1G>A; rs3918290), DYPD*13 (c.1679T>G p.[Ile560Ser]; rs55886062), and c.2846A>T p.(Asp949Val); rs67376798, 13,14 Allele frequencies for these variants are, respectively, 0.58%, 0.03%, and 0.29%. More recently, a meta-analysis indicated that also the 1129-5923C>G (rs75017182) variant (with an allele frequency of 1.35%) is a clinically relevant predictor for fluoropyrimidine toxicity.5

Several methods have been described to directly or indirectly determine whether a patient is DPD deficient.5 Each of these methods has advantages and disadvantages and currently, no assay is stated to be the most optimal in terms of predicting toxicity, sensitivity and specificity, and cost-effectiveness. In this article, we provide an overview of 8 years of DPD diagnostic testing, in which our method changed from only DPD enzyme activity measurement to a combination of DPD enzyme activity assessment in peripheral blood mononuclear cells and DYPD genotyping for 4 variants (i.e., DYPD*2A, DYPD*13, rs67376798, and rs75017182).

Materials and Methods

Patients

A total of 1194 patients were diagnostically tested (enzyme and DNA level) for the presence of complete or partial DPD deficiency in 8 years. Based on the type of screening, these were divided into 3 groups (Figure 1). Patient group 1 consisted of 256 patients, who were phenotypically (DPD enzyme activity) analyzed between 2009 and 2013. Patients with decreased enzyme activity were subsequently genotyped for the DYPD*2A variant. Patient group 2 consisted of 132 patients who were simultaneously phenotypically analyzed and genotyped for the DYPD*2A variant. Patient group 3 consisted of 814 patients who were simultaneously phenotypically analyzed and genotyped for DYPD*2A, DYPD*13, rs67376798, and rs75017182. The procedures were in accordance with the ethical standards of the Helsinki Declaration of 1975 (as revised in 1983). The study was performed alongside standard diagnostic procedures, and thus waived from institutional review board approval.

Phenotypic DPD analysis

In the presence of reduced nicotinamide adenine dinucleotide phosphate, DPD converts thymine and uracil into 5,6-dihydrothymine and 5,6-dihydrouracil, respectively. Until December 2014, DPD activity was measured according to the method of van Kuilenburg et al.7 All subsequent samples were measured using a nonradiochemical method with uracil as substrate instead of 14C-labelled thymine. Peripheral blood mononuclear cells were isolated from 10 mL EDTA-anticoagulated blood within 24 hours after blood draw. Pellets were suspended in 200 mL milli-Q Merck, (Darmstadt, Germany) and cells were lysed by sonication for 2 minutes (microtip MS2 (Hilscher GmbH, Teltow, Germany), cycle 0.5, amplitude 80%). Cell debris was removed by centrifugation at 11,500 g for 20 minutes at 4 °C. The supernatant was used for the enzymatic analysis. Protein concentration was determined spectrophotometrically using the PierceTM BCA Protein Assay Kit: Thermo (Fisher Scientific, Waltham Massachusetts). The assay mixture contained 35 mM potassium dihydrogen phosphate pH 7.4, 2.5 mM magnesium chloride, 1 mM dihistiothreitol (DTT), 250 μM NADPH, 25 μM uracil, 5 μM internal standard, and 50 μg total protein in a final volume of 100 μL. The reaction tubes were incubated at 37°C for 60 minutes using a dry heating block. The reaction was terminated by the addition of 25 μL ice-cold 10% perchloric acid. The samples were placed on crushed ice for 10 minutes to obtain complete precipitation. After centrifugation at 11,000 g for 5 minutes at 4°C, subsequently 10 μL supernatant and 500 μL mobile phase ammonium formate buffer was transferred into a Costar spin-X (Corning, New York) centrifuge tube filter, 0.2 μm nylon membrane, and centrifuged at 11,000 g for 1 minutes at 4°C. The flow-through was transferred into a 96-well collection plate. Components were separated using a Waters Acuity ultra-high performance liquid chromatography (Waters, Etten-Leur, the Netherlands) and analyzed on the Water Xevo TQ-S tandem mass spectrometer. DPD activity was expressed as the amount of 5,6-dihydrouracil formed per milligram total protein per hour. All samples were run in duplicate. Data acquisition and chromatographic analysis was performed by using MassLynx software (Waters, Milford, Massachusetts). The threshold for decreased DPD enzyme activity was determined by testing the enzyme activity of controls and set at a cut-off of 30% of the lower end of the spectrum. For more analytical information regarding the measurement of DPD enzyme activity we refer to the supplementary information.

Genetic DPDY analysis

Total DNA was extracted from blood using the Blood L Kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) and the Hamilton Microlab STAR Line (Hamilton, Bonaduz, Switzerland) according to the protocol of the manufacturer. Specific primers with an additional M13-tag were used to amplify the protein coding exons and immediate flanking intrinsic regions of the fragments containing the DYPD c.1905+1G>A (rs3918290), c.1679T>G (rs55886062), c.2846A>T (rs67376798), and the 1129-5923C>G (rs75017182) variants for standard diagnostic analysis. A subset of the samples was screened for the entire coding region of the DYPD gene, including intron/exon boundaries. Primer sequences for this screening are provided in Supplementary Table S1. Amplification was performed in a 10 μL reaction volume using AmpliTaq Gold 360 Master Mix with 5% 360 GC Enhancer (ThermoFisher, Nieuwerkerk a/d IJssel, the Netherlands), 2 pmol of each primer and 10 ng DNA. The cycle conditions were 96°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, 60°C for 45 seconds, and 72°C for 45 seconds with a final elongation step of 72°C for 10 minutes. The resulting polymerase chain reaction products were bidirectionally sequenced using the ABI Big Dye Terminator Cycle Sequencing Ready Reaction Kit (ThermoFisher) and the ABI3730XL genetic analyzer (ThermoFisher). The DPDY-relevant variants were determined using Mutation Surveyor DNA variant analysis software (SoftGenetics, State College, Pennsylvania) with genomic National Center for Biotechnology Information, reference sequence NM_000110.3. All sequences were evaluated by 2 independent laboratory experts.

Prediction of variants of unknown significance

For in silico prediction we used similar methods as described in Offer et al21 but in more detail and using other additional fea-
Statistical Analysis

Enzyme activities were measured with 2 different methods: a radiochemical enzyme activity method (n = 380) and a nonradiochemical method using ultra-HPLC–MS (n = 814). The methods gave slightly different values, the cut-off point for decreased enzyme activity for the radiochemical enzyme activity method is 7.57 nmol/mg protein/h and for the mass spectrometry method 8.69 nmol/mg protein/h. Differences between groups were assessed using Pearson χ² test or ANOVA. Pearson correlation and χ² tests were performed to identify relationships between variables. Analyses were performed using SPSS version 22.0.0.1 (IBM-SPSS Inc, Armonk, New York).

Results

General characteristics of the patient population

In a period of 8 years, 1311 DPD diagnostics were performed in our center. For 117 samples, only DNA analysis was performed. Therefore, only 1194 patients were included in the comparisons shown below. Data on the development of toxicities were not available. An increase in the number of tests requests is observed in the last years (Supplementary Table S2). Within this period, the diagnostic approach evolved from a focus on enzyme activity measurement to a combined analysis of DPD enzyme activity and DPYD genetic testing of 4 genetic variants (Figure 1). The mean age of the patients analyzed for DPD in the diagnostic setting was 64.9 years and 50.1% were men (Table 1).

Enzyme activities

Enzyme activity was measured with 2 different methods. The radiochemical method was replaced by a nonradiochemical method in December 2014. Accuracy, precision, and reproducibility of this method was ascertained (ISO 15189 accreditation). In group 3, only the nonradiochemical method was used. The reference value for low enzyme activity appeared to be slightly different for the radiochemical and nonradiochemical method (7.57 vs 8.69 nmol/mg protein/h, respectively). Therefore, we analyzed both groups separately. The percentage of patients with decreased enzyme activity is lower in the patients analyzed in group 3. This is in line with the transition of only screening after evidence of toxicity in a patient to pretreatment screening of all patients. In total, DPD enzyme activity was measured in 1194 patients (380 patients measured with the radiochemical method [groups 1 and 2] and 814 patients measured with the nonradiochemical method [group 3]). We observed a small but significant correlation between age and DPD enzyme activity in groups 1 and 2 (Pearson correlation = 0.127; P = 0.043 and Pearson correlation = 0.218; P = 0.014, respectively). In group 3, no significant correlation was observed (P = 0.423). Sex did not influence enzyme activity.

Enzyme activity distribution is shown for group 3, the largest group tested for 4 variants (measured by the nonradiochemical method), in Figure 2 with 1 patient (0.1%) displaying 0 activity, 94 (11.5%) with intermediate activity (between 0 and 8.69 nmol/mg protein/h), and 719 (88.3%) with normal activity (>8.69 nmol/mg protein/h).

Genetic DPYD analyses

Standard DPYD analysis of the 4 clinically relevant variants (group 3) showed that only 18% of patients with a genetic variant had decreased enzyme activity (ie, below the threshold) (Figure 2). Their mean (SD) enzyme activity was significantly lower (P < 0.001) than the patients without a genetic variant (11.0 [5.0] and 15.7 [5.6], respectively). The correlation between the enzyme activity and the presence of the c.2A variant was most clear, with 83.3% of patients showing decreased enzyme activity (Table 2). In group 3, we observed 95 patients with decreased enzyme activity and of these only 24 (25.3%) carried at least 1 of the 4 tested variants.

In a subgroup of patients (n = 47) derived from patient groups 1 and 2, the entire coding region of the DPYD gene was analyzed. The subgroup consisted of patients with a decreased enzyme activity without the DPYD c.2A variant (n = 46) who were routinely screened at that time and 1 patient of whom only DNA was available. A genetic variant was identified in 11 (23.4%) patients. In 3 patients, DPYD c.13 was identified. One of these patients showed an absence of enzyme activity (0 activity). In 4 patients, rs75017182 was identified and in 1 patient rs67376798 was identified. All patients were heterozygous for the detected variants. In 3 other patients, a variant of unknown significance was identified: c.601A>G p.(Ser201Arg) (rs72549308), c.2279C>T p.(Thr760Ile), and c.2843T>C p.(Ile948Thr).

Discussion

This is to our knowledge the first article presenting 8 years of routine DPD testing in a clinical setting. Within this period, our diagnostic approach has evolved from a focus solely on enzyme activity measurement to a combined analysis of DPD enzyme activity

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Overview of the characteristics of the patient population.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (n = 1194)</td>
<td>Group 1 (n = 254)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>64.9 (10.3)</td>
</tr>
<tr>
<td>Male sex</td>
<td>598 (50.1)</td>
</tr>
<tr>
<td>DPD enzyme activity (nmol/mg protein/h)</td>
<td>n.d.</td>
</tr>
<tr>
<td>Patients with decreased enzyme activity*</td>
<td>213 (17.8)</td>
</tr>
</tbody>
</table>

DPD = dihydropyrimidine dehydrogenase; n.d. = not determined because the enzyme activity measurements are slightly different for group 1 and 2 in comparison to group 3 due to different methods used.

* Values are presented as mean (SD).
† Values are presented as n (%).
‡ Reference value for low enzyme activity 7.57 nmol/mg protein/h (group 1 and 2) and 8.69 nmol/mg protein/h (group 3).
and DYPD genetic testing of 4 clinically relevant genetic variants. We observed a strong interindividual variability in DPD enzyme activity within different DYPD genotypes. Patients with the DYPD*2A variant showed most consistently enzyme activity below the defined threshold. The genotype–phenotype correlation for the other variants tested was less clear.

In line with previous publications,\textsuperscript{5,8} we show that there is a weak correlation between DPD enzyme activity and the presence of the variants tested. With respect to this correlation, we observed a sensitivity of 0.25 (24 / [24 + 71]) and a specificity of 0.93 (667 / [667 + 52]); that is, most patients do not carry a variant as expected and all these patients have normal DPD enzyme activity (ie, high specificity). However, only 25% of patients with reduced activity carry a variant. The majority of patients with the DYPD*13, rs67376798, or rs75017182 variants showed enzyme activity within the normal range. Similar results are reported for the rs67376798 variant in functional assays, in which this variant still showed ~50% activity compared with controls, whereas the DYPD*2A variant showed a complete absence of activity when homozygously expressed.\textsuperscript{5} These observations are also in line with previous studies in which dose reductions based on the presence of a certain variant showed safe treatment with capecitabine\textsuperscript{10} and are reflected in existing pharmacogenetic dosing guidelines. The Dutch Pharmacogenetics Working Group recommends for DYPD*2A, DYPD*13, and rs67376798 variant carriers a starting dose of 50%, 50%, and 75%, respectively. Guidelines from the Clinical Pharmacogenetics Implementation Consortium (both DPWG and CPIC PGx guidelines available at www.pharmgkb.org) recommend starting with 50% in general (independent of the variant) followed by a monitored dose increase. Patients homozygous for the rs75017182 variant were found to still have a remaining DPD enzyme activity of 30%,\textsuperscript{11} indicating that also this variant does not result in a completely dysfunctional enzyme. These differences are likely caused by the different effects of the variant on enzyme function. For DYPD*2A, exon 14 skipping due to the induced splicing defect leads to a completely nonfunctional enzyme. DYPD*13 is believed to lead to destabilization of a sensitive region of the DPD protein,\textsuperscript{12} although the exact functional consequences are not clear. The rs67376798 variant is believed to interfere with cofactor binding or electron transport due to a structural change in the DPD protein. The rs75017182 variant leads to

Figure 2. Dihydropyrimidine dehydrogenase (DPD) genotype/phenotype correlation in the diagnostic patient population screened for 4 variants.

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might (data rs75017182).

burg creased of also shown). This parable by DPYD in

ing DPYD∗2A variant (from group 1), 3 variants of unknown sig-
nificance were identified. The c.601A>G p.(Ser201Arg) variant (rs72549308) was iden-
tified homozygously in a patient without DPD activity. The variant was not reported in the Exome Sequenc-

ing Project or the 1000 Genomes Project, whereas the Genome Aggregation Database browser (277,264 alleles from unrelated in-

dividuals) showed an allele frequency of 0.0029%. In silico prediction

indicated that the variant was probably damaging and expres-

sion of the genetic variant in HEK293T/C17 cells performed by Offer et al13 showed a clear decrease in enzyme activity com-

parable to cell lines with a DPYD∗2A variant (<12.5% activity). This explains the absence of DPD activity in the homozygous

patient (the presence of a deletion/depletion was excluded; data not shown). The c.2279C>T p.(Thr760Ile) variant (ie, rs112766203) was al-

so classified as probably damaging by us and others and is only present in Genome Aggregation Database with an allele frequency of

0.061%. Offer et al13 showed a decrease in DPD enzyme activity after functional analysis comparable to the clinically relevant

rs67376798 variant (~50% activity). These results indicate that the presence of the variant in heterozygous state can explain the de-

creased DPD enzyme activity found in the patient. The c.2843T>C p.(Ile948Thr) variant was classified as probably damaging by Ala-
mut Visual and was not present in the Exome Sequencing Project and 1000 Genomes Project and showed an allele frequency of

0.00041% in the Genome Aggregation Database. Recently, Kuien-
burg et al15 showed residual DPD activity of 30% after functional analysis of this variant. Based on this information, the variant

identified in our patient may explain the observed decreased en-

zyme activity. One patient (heterozygous DPYD∗13) showed 0 en-

zyme activity, whereas no other DPD variants were detected by

sequencing the entire coding region of the DPD gene (including

rs75017182). Moreover, no deletions or duplications were observed

(data not shown) to explain this absence of activity. The patient

might have a genetic variant in a regulatory region of the DPD

gene located outside the sequenced coding region or other non-

genetic factors might play a role.

Genotyping of 4 genetic variants can only explain the genetic

background of a small part of the patients with decreased enzyme

activity. Presence of other variants in regions of the DPD gene that

have not been sequenced and genetic variants in other (ie, modi-
fier) gene regions (eg, miR27a and miR27b)5,8 that determine DPD

enzyme activity might be the reason that we cannot explain all

cases of a decreased enzyme activity. In addition, other (eg, envi-

ronmental) factors determine part of DPD enzyme activity. In our

population, we observed a weak but statistically significant corre-

lation with age, which is in line with a previous publication.16 We

could not confirm a relationship between sex and DPD enzyme

activity,17–19 although this has been published before.16 Several re-

ports indicated that DPD enzyme activity is partly determined by
circadian rhythm,20 although this circadian expression seems to be

abolished in patients with gastrointestinal carcinomas.21 In addi-

tion, it is not unlikely that comedication can influence the expres-

sion of DPD enzyme. Studies have indicated that oxaliplatin (an of-
	en used anticancer agent) results in a reduced DPD activity.22,23 This observation could not be confirmed by Boidron-Celle et al,24

although they did show an effect of oxaliplatin on 5-FU plasma

clearance. In addition, the time from blood draw to processing of

the samples is known to influence enzyme activity. Therefore,

our laboratory flow is set up in such a way that the samples are

processed within 18 hours. Within this time frame it is expected

that enzyme activity is stable.25,26 In practice, our turnaround time from when we receive patient blood to finishing the report is max-

imal 7 working days. Usually we have the results around 4 to 5

working days. The referring oncologists have agreed with our

turnaround-times because this aligns well with other procedures

required for the treatment of these patients and does not affect the

start of treatment or outcome compared with not testing for

DPD deficiency.

When using both the enzymatic and genetic test results to opti-

mize fluoropyrimidine treatment advice, patients who show no
detectable DPD activity should not receive treatment with fluo-

ropyrimidine agents, according to the existing guidelines, because

of the high risk of fatal toxicity (regardless of single nucleotide polymorphism [SNP] status).3 Alternatives should be considered in

Table 2

<table>
<thead>
<tr>
<th>Genetic test (n [%])</th>
<th>Variant (n)</th>
<th>Mean enzyme activity (min-max)</th>
<th>Patients with a genetic variant and decreased enzyme activity (n [%])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (n = 254)</td>
<td>DPYD∗2A (n = 6)</td>
<td>4.9 (1.8–7.4)</td>
<td>6 (100)</td>
</tr>
<tr>
<td>Group 2 (n = 126)</td>
<td>DPYD∗2A (n = 2)</td>
<td>7.5 (7.5–7.5)</td>
<td>2 (100)</td>
</tr>
<tr>
<td>Group 3 (n = 814)</td>
<td>DPYD∗12/2A (n = 12)</td>
<td>5.4 (1.2–10.5)</td>
<td>10 (83.3)</td>
</tr>
<tr>
<td></td>
<td>DPYD∗12/13 (n = 3)</td>
<td>12.8 (9.6–18.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Heterozygous rs67376798 (n = 12)</td>
<td>10.8 (4.9–18.7)</td>
<td>25 (3.8)</td>
</tr>
<tr>
<td></td>
<td>heterozygous rs75017182 (n = 46)</td>
<td>12.8 (4.5–23.3)</td>
<td>8 (17.4)</td>
</tr>
<tr>
<td></td>
<td>rs67376798/ rs75017182 (n = 2)</td>
<td>4.3 (3.3–5.3)</td>
<td>2 (100)</td>
</tr>
<tr>
<td></td>
<td>homozygous rs75017182 (n = 1)</td>
<td>8.06</td>
<td>1 (100)</td>
</tr>
<tr>
<td>Subgroup entire screen (n = 47)</td>
<td>DPYD∗13 (n = 3)</td>
<td>3.8 (0–7.5)</td>
<td>1 (25)</td>
</tr>
<tr>
<td></td>
<td>rs75017182 (n = 4)</td>
<td>6.1 (5.0–7.0)</td>
<td>4 (17.4)</td>
</tr>
<tr>
<td></td>
<td>rs67376798 (n = 1)</td>
<td>7.9</td>
<td>2 (100)</td>
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<tr>
<td></td>
<td>c.601A&gt;G-C (n = 1)</td>
<td>0</td>
<td>2 (100)</td>
</tr>
<tr>
<td></td>
<td>c.2279C&gt;T-T (n = 1)</td>
<td>4.6</td>
<td>2 (100)</td>
</tr>
<tr>
<td></td>
<td>c.2843T&gt;C-C (n = 1)</td>
<td>3.2</td>
<td>2 (100)</td>
</tr>
</tbody>
</table>

DPYD = dihydroprimidine dehydrogenase.

Table only includes patients for which the enzyme activity was measured.

†All patients were heterozygous for the variant; only c.601A>G-C was homozygous.

Enzyme measurements were performed for 3 samples.
this situation. Patients either partially defective (ie, decreased DPD enzyme activity) or carrying 1 of the 4 relevant SNPs are at risk according to the existing guidelines and may receive an adjustment of the dose accordingly. In our opinion, only focusing on the enzyme test would ignore patients with low-normal activity who could still carry another DPDV variant and thus may be at risk for developing toxicity. On the other hand, focusing only on the genotype results is not ideal because currently we only test 4 DPDV variants, which are certainly not the only variants that may cause DPDV deficiency.27 Starting all patients on a reduced dose and monitoring them afterward is not a realistic option because most patients will not have a (partial or complete) DPDV deficiency and will experience a period of undertreatment. Because each test has its own advantages and disadvantages, we prefer the combination of both the enzyme and genotype tests as the best predictor for the development of side effects.

A limitation of our study is that we did not systematically collect information concerning the development of side effects related to fluoropyrimidine treatment. Therefore it is unclear whether the genetic variants or the enzyme activity predicts treatment outcome best. Currently, a study is being performed in the Netherlands addressing this issue (ClinicalTrials.gov identifier: NCT02324452). The literature indicates that genotype-guided dosing of fluorouracil based on DPDV2A results in a reduction of grade ≥ 3 toxicity of 45% (from 73% in DPDV2A carriers with standard dose to 28% in DPDV2A carriers with genotype-guided dosing).26 In addition, for DPDV enzyme activity measurements, 56% of patients with decreased enzyme activity develop severe complications (eg, grade 4 neutropenia) versus 29% of patients with normal enzyme activity.26 Based on our data, 76 patients (in group 3) carried a genetic variant, of whom 24 showed decreased activity, whereas 95 patients showed decreased enzyme activity in total. This means that severe toxicity might have been prevented in ~18% of patients. The evidence that DPDV testing is beneficial is also reflected in the increase of the number of diagnostic DPDV requests. In the initial years of clinical DPDV testing, it was expected that most patients who had been tested were screened after the development of side effects. During the last year of clinical testing for this study, we observed an increase in requests for DPDV testing before 5-FU treatment, which includes a shift to pretreatment screening in centers that only performed testing after toxicity, but also hospitals that never requested this type of test before. It is expected that more and more patients will be tested before treatment because it also has become evident that pharmacogenetic screening is likely cost-effective28 and more extended studies are underway (eg, ClinicalTrials.gov identifiers: NCT01547923 and NCT02324452).

Conclusions

DPDV status should be determined before treatment with fluoropyrimidines to prevent patients from developing possible fatal toxicity and to prevent unnecessary side effects. Our study in combination with the literature shows that there is a discrepancy between DPDV enzyme activity and the presence of clinically relevant SNPs. Therefore, in our view, a combination of a genetic and enzyme test is preferable for diagnostic testing until it is clear which method most reliably predicts the observed side effects.

Conflicts of Interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

Author Contributions

All authors listed were involved in the study design and interpretation of data for the work. Aimée Paulussen, Marc Breuer, Martijn Lindhout, Demis Tserpelis, Jörgen Bierau, Anja Steyls, Bianca van den Bosch collected data. Martijn Lindhout was responsible for enzyme activity measurements, genotyping was performed by Anja Steyls and Demis Tserpelis. Data analysis was performed by Marijke Coenen and Bianca van den Bosch, with input from the other investigators. The manuscript was written by Marijke Coenen and Bianca van den Bosch, all other authors edited the manuscript and gave final approval for publication. Marijke Coenen and Bianca van den Bosch had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.curtheres.2018.10.001.

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