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**Author:** Staveren, M.C. van  
**Title:** DPD screening to prevent toxicity in fluoropyrimidine treated patients  
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Chapter 7

Evaluation of clinical implementation of prospective DPYD genotyping in 5-fluorouracil or capecitabine treated patients

Maurice van Staveren
Carin Lunenburg
Hans Gelderblom
Henk-Jan Guchelaar
Jesse Swen

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ABSTRACT

Introduction: Prospective *DPYD* screening reduces severe fluoropyrimidine-induced toxicity. This study evaluated the routinely application of prospective *DPYD* screening at the Leiden University Medical Center.

Methods: Prospective *DPYD* screening as part of routine patient care was evaluated by retrospectively screening databases and patient files to determine genotype, treatment, dose recommendations and dose adjustments.

Results: 86.9% of all patients with a first fluoropyrimidine prescription were screened. 14 out of 275 patients (5.1%) carried a *DPYD* variant and received a 25–50% dose reduction recommendation. None of the *DPYD* carriers treated with an initial dose reduction developed toxicities.

Conclusions: Prospective *DPYD* screening can be implemented successfully in a real world clinical setting is well accepted by physicians and results in low toxicity.
INTRODUCTION

Fluoropyrimidines like 5-fluorouracil (5FU) and its oral pro-drug capecitabine (CAP) are the cornerstone anti-cancer drugs for several types of cancer such as colorectal cancer, head-neck cancer and breast cancer. Approximately 10–30% of the patients receiving 5FU or CAP experience severe (grade ≥ 3) toxicity, such as diarrhoea, mucositis and hand-foot syndrome [1]. 5FU is extensively metabolized (> 80%) by the liver enzyme dihydropyrimidine dehydrogenase (DPD). DPD is encoded by the gene DPYD for which more than 160 genetic variants are known, some of them being pathogenic by reducing enzyme function [2, 3]. There is a strong correlation between reduced DPD activity and increased risk for severe and potentially lethal toxicity following treatment with a normal dose of 5FU [4-7]. Toxicity occurred in 73% of DPYD*2A carriers, compared to 23% of wild-types [8]. Several meta-analyses have consistently shown that DPYD*2A, c.2846A>T, DPYD*13 and c.1236C>G/HapB3 are associated with toxicity [1, 6, 9]. Although the sensitivity of DPYD genotyping is low (< 14.5% for DPYD*2A and c.2846A>T combined), prospective screening for genetic variants in DPYD is a well-known strategy to detect patients who have reduced DPD enzyme activity (DPD deficient) [8, 10, 11]. Patients with no or reduced DPD enzyme activity can be treated more safely when applying a 25–50% dose reduction of 5FU or CAP, or using an alternative drug [10, 12, 13]. Recently it was shown that prospective screening for DPYD*2A followed by a 50% dose reduction significantly reduces the number of severe toxicities and is cost-effective [8]. Several pharmacogenetic guidelines are available that provide dose recommendations when a reduced function DPYD variant is present. The pharmacogenetic guidelines of the Dutch Pharmacogenetic Working Group (DPWG), recommend a 25–50% dose reduction of 5FU or CAP for the first treatment cycle followed by dose titration guided upon toxicity during subsequent cycles for patients with a variant in DPYD (DPYD*2A, DPYD*13, c.2846A>T or c.1236G>A). A minimum of 50% reduction or alternative therapy is advised for homozygous patients, depending on the variant [14]. The Clinical Pharmacogenetics Implementation Consortium (CPIC) [15, 16] recommends a 50% dose reduction of 5FU or CAP for patients with DPYD*2A, DPYD*13 and c.2846A>T and alternative therapy for patients who are homozygous for these variants. While these guidelines are very useful for dose adjustments in patients with a genetic variant, they do not advocate prospective DPYD testing prior to initiation of therapy.

At Leiden University Medical Center (LUMC), a routine DPYD screening programme prior to prescribing 5FU or CAP was initiated in April 2013. In this retrospective study we evaluated
the physician’s acceptance of prospective $DPYD$ screening for patients who were prescribed 5FU or CAP in LUMC and the adherence of the recommended dose reduction.

**METHODS**

**Setting**

At LUMC all patients with an indication for a fluoropyrimidine containing therapy were routinely screened for $DPYD$ variants by the laboratory of the department of Clinical Pharmacy and Toxicology (CPT) using two independent techniques (Taqman assay and pyrosequencing (PSQ), described previously) [17]. Within LUMC the Electronic Medication Record (EMR) system EZIS (version 5.2, Chipsoft) is used, which can be consulted electronically by the responsible pharmacist into the EMR and are visible for other users of the EMR.

The prospective screening programme was initiated on April 15th 2013. During a kick-off meeting attended by medical oncologists and fellows, the staff was informed and agreed on the prospective programme. New medical oncologists and fellows were informed about the prospective screening programme during the regular introduction programme for new staff members. Genotyping was performed 3 times per week (Monday, Wednesday, Friday) in order to minimize the lag time between sampling and test. This resulted in a turnaround time of 2 days, allowing rapid start of treatment if needed. Ethical approval by the Institutional Review Board of LUMC was not required for the current study as it evaluates standard care. Patient data from the EMR was handled following the Codes of Proper Use and Proper Conduct in the Self-Regulatory Codes of Conduct (www.federa.org).

**Study endpoints**

Three study endpoints were evaluated to determine the successfulness of the screening programme that was introduced at LUMC. We evaluated:

1. The ‘implementation’, i.e. requests of the $DPYD$ tests as standard care in daily practice;
2. The proportion of test results with a dose recommendation provided by the pharmacist;
3. The follow up of the dose recommendations by oncologists, calculated as the number of follow-ups of dose recommendations by prescribers, excluding the patients in which a follow-up was not possible (e.g. no therapy).

**Study procedures**

The implementation, or routinely application of the prospective (pre-treatment) *DPYD* screening in daily practice was evaluated by determining the proportion of patients who were screened for *DPYD* variants when an incident prescription for 5FU or CAP was given. The data was extracted from two electronic databases. The first database contains data of all patients who are genotyped for *DPYD* variants. The second database (EMR EZIS) contains individual patient medical records. This system is also used by oncologists to electronically prescribe 5FU and CAP. Prescription data prior to the start of the study was studied as well, to ascertain that 5FU or CAP prescription was indeed the first prescription for the patient. The patient identification number was used to connect data from both databases. Discrepancies between information in the queried databases were resolved by manually checking the individual electronic patient records to identify the reason of their absence in one of the two searches. After connecting the data from both databases, all patient data was anonymized. All manual changes (additional information, removal of duplicates, etc.) to the queries were double checked by the two first authors (CL and MS).

To evaluate the follow up of the recommended dose reductions by the oncologists, medical records of patients carrying a variant in *DPYD* were inventoried as to determine if the oncologist followed the dose advice. The genotyping data of the laboratory of CPT was used to determine the patients carrying a *DPYD* variant. Prospective execution of the genotyping could be determined by comparing the genotyping date and start date of the therapy. Regular drug regimens and notations of dose reductions in the medical records were searched to check applied dose reductions.

After completion of the study, an explorative analysis was executed in order to describe the course of toxicity in relation to the provided dose recommendations. In order to perform this analysis, toxicity information regarding the 5FU or CAP therapy was retrieved from the EMR for patients with a *DPYD* variant. Toxicity was scored by the oncologists using the Common Toxicity Criteria (CTC), version 4.
RESULTS

The implementation of the prospective screening programme for *DPYD*

The prospective *DPYD* screening programme was implemented on April 15th 2013 (start date study) at LUMC. From this date until December 31st 2014 (end date study) 540 patients were genotyped for *DPYD* variants at LUMC. Initially, patients were screened only for the presence of the *DPYD* *2A* variant. Later on *DPYD* *13*, c.2846A>T and c.1236G>A were added to the *DPYD* screening. An overview is shown in Table 7.1. After removal of duplicate or invalid records, 529 evaluable genotyped patients remained. Of these 529 patients, 275 patients were patients treated at the LUMC and 254 patients were treated at other hospitals, but genotyped as a service provided by the department of CPT of the LUMC. The dose reductions that were advised for each individual *DPYD* variant are displayed in Table 7.1.

<table>
<thead>
<tr>
<th>DPYD variant</th>
<th>Initial dose reduction (%)</th>
<th>Inclusion in screening programme</th>
<th>Patients screened</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>DPYD</em>2A (c.1905+1G&gt;A)</td>
<td>50</td>
<td>April 15th, 2013</td>
<td>529</td>
</tr>
<tr>
<td><em>DPYD</em>13 (c.1679T&gt;G)</td>
<td>50</td>
<td>October 10th, 2013</td>
<td>440</td>
</tr>
<tr>
<td>c.2846A&gt;T</td>
<td>50 → 25*</td>
<td>October 10th, 2013</td>
<td>440</td>
</tr>
<tr>
<td>c.1236G&gt;A</td>
<td>25</td>
<td>May 28th, 2014</td>
<td>254</td>
</tr>
</tbody>
</table>

Advice given by CPIC and DPWG guidelines at the time the variant was added to the routine screening. *The dose reduction advice for c.2846A>T has been updated to 25% in February 2015.

2,498 records of 5FU or CAP prescriptions prior to December 31st 2014 were found. After removal of duplicates, invalid records (e.g. incomplete data) or patients not meeting eligibility criteria (e.g. prescription prior to April 2013), 337 patients remained who were prescribed 5FU (16%) or CAP (84%) for the first time at LUMC within the study period.

Genotyped patients were compared with patients who were prescribed 5FU or CAP, resulting in 236 matching patients. 39 patients were genotyped for *DPYD*, but were not prescribed 5FU or CAP. Also, 101 patients were prescribed 5FU or CAP, but were not genotyped for *DPYD* variants (Figure 7.1).

Two patients, who received 5FU or CAP and were genotyped, were excluded because their medical records revealed they had received 5FU or CAP prior to April 15th 2013. Of
the 39 patients who were genotyped without receiving 5FU or CAP therapy, 33 patients eventually did not start their therapy, although there was an intention to treat at the time of requesting the screening test. Six patients started their therapy after December 31st 2014 and were therefore not identified by the search. Of the 101 patients with a 5FU or CAP prescription and no DYPD-genotyping record, the medical records were screened resulting in a legitimate reason not to genotype in 60 cases (Table 7.2). Legitimate reasons included; any notes on prior treatment with 5FU or CAP (e.g. outside LUMC) or invalid patient files (e.g. no medical dossier found for the oncology department). For 41 patients who had a

Table 7.2  Excluded patients

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Reason not to perform DYPD genotyping</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>SFU or CAP therapy started just prior to the start date of April 15th, 2013</td>
</tr>
<tr>
<td>30</td>
<td>SFU or CAP was used before April 2013 without problems and would start again after April 15th</td>
</tr>
<tr>
<td>20</td>
<td>No medical dossier at the Medical Oncology department was found, therefore the patient was not treated at the LUMC</td>
</tr>
<tr>
<td>2</td>
<td>These dossiers were fake patients used for education purposes</td>
</tr>
</tbody>
</table>

Patients (n = 60) with legitimate reasons not to screen were excluded from analysis.
Figure 7.2  Proportion of eligible patients that were genotyped.
The figure shows the eligible patients for evaluation per month in actual patient numbers. If the intention to treat with SFU or CAP was present, patients were eligible. Also the actual patient numbers of the genotyped patients per month are shown and the calculated percentage which represents the clinical acceptance, or how well implemented the prospective DPYD screening is.
prescription for newly 5FU or CAP no reason was found to neglect genotyping. After data cleaning, 314 patients with a newly 5FU or CAP prescription remained in the dataset and 273 of these patients were genotyped as depicted in Figure 7.1. The clinical acceptance of the prospective DPYD screening programme is displayed as percentage per month in Figure 7.2. The average clinical acceptance was 86.9%.

The clinical acceptance of the prospective DPYD screening programme is displayed as percentage per month in Figure 7.2. The average clinical acceptance was 86.9%.

**Proportion of test results with a dose recommendation**

During the study period 275 patients were screened for DPYD variants. Of these 275 patients, 14 patients (5.1%) were found to carry one or more variants. Shown in Table 7.3 are the variants that were screened for, and of each variant the frequency in comparison to the literature.

<table>
<thead>
<tr>
<th><strong>DPYD variant</strong></th>
<th><strong>#</strong></th>
<th><strong># of tested patients</strong></th>
<th><strong>% LUMC</strong></th>
<th><strong>% literature</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DPYD*2A / c.1905+1G&gt;A</strong></td>
<td>6</td>
<td>275</td>
<td>2.2</td>
<td>~1.0–1.8 [10, 18]</td>
</tr>
<tr>
<td><strong>DPYD*13 / c.1679T&gt;G</strong></td>
<td>0</td>
<td>214</td>
<td>0</td>
<td>~0.1 [12]</td>
</tr>
<tr>
<td>c.2846A&gt;T</td>
<td>1</td>
<td>214</td>
<td>0.5</td>
<td>~1.0–1.4 [10, 12]</td>
</tr>
<tr>
<td>c.1236G&gt;A</td>
<td>8</td>
<td>109</td>
<td>7.3</td>
<td>~2.6–4.9 [10, 19]</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>15 (n = 14)</td>
<td>275</td>
<td>5.1</td>
<td>4.7–8.2</td>
</tr>
</tbody>
</table>

DPYD variants found in LUMC patients and these numbers compared to frequencies in the literature.

For eight patients with a c.1236G>A variant a dose reduction of 25% was recommended. Five patients with a DPYD*2A variant received a recommendation to reduce the dose by 50%. One patient carried both DPYD*2A and c.2846A>T (Table 7.4, patient 9). For this patient no dose reduction was recommended. Instead it was advised to determine the DPD enzyme activity in PBMCs as applied Taqman and PSQ assays were not able to identify if the found mutations were in cis or trans configuration. Turnaround time of the DPD enzyme activity test is approximately 1–2 weeks, which could not be awaited for. The treating physician decided to treat this patient with a 50% dose reduction, taking into account the results of the genotyping and the fact that this patient had tolerated 5FU-containing regimens before.
Table 7.4  

<table>
<thead>
<tr>
<th>Pt #</th>
<th>Cancer type</th>
<th>Therapy</th>
<th>DPYD variant</th>
<th>Prospective screening?</th>
<th>Initial dose adjustment?</th>
<th>Toxicity (gr 3–4)?</th>
<th>Toxicity specifications</th>
<th>Hospital admissions?</th>
<th>Second dose adjustment?</th>
<th>Toxicity (gr 3–4)?</th>
<th>Toxicity specifications</th>
<th>Hospital admissions?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Colorectal</td>
<td>CAPOX</td>
<td>c.1236G&gt;A</td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
<td>N/A</td>
<td>N/A</td>
<td>YES (to 100%)</td>
<td>NO</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>2</td>
<td>Mouth</td>
<td>TPF + RT</td>
<td>c.1236G&gt;A</td>
<td>NO</td>
<td>NO</td>
<td>YES</td>
<td>Diarrhoea IV + Neutropenia/Thrombocytopenia III</td>
<td>YES (6 + 16 days)</td>
<td>N/A (Quit after 2nd cycle)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>3</td>
<td>Colon (met.)</td>
<td>OXACAPBEV</td>
<td>c.1236G&gt;A</td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
<td>N/A</td>
<td>N/A</td>
<td>NO</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>4</td>
<td>Anus</td>
<td>5FU + RT</td>
<td>c.1236G&gt;A</td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
<td>N/A</td>
<td>N/A</td>
<td>NO</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>5</td>
<td>Colon</td>
<td>N/A</td>
<td>c.1236G&gt;A</td>
<td>YES</td>
<td>DNS*1</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>6</td>
<td>Pharynx</td>
<td>5FU + RT</td>
<td>c.1236G&gt;A</td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
<td>N/A</td>
<td>N/A</td>
<td>NO</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>7</td>
<td>Pancreas</td>
<td>CAP</td>
<td>c.1236G&gt;A</td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A (Quit)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>8</td>
<td>Rectal</td>
<td>CAP + RT</td>
<td>DPYD*2A</td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
<td>N/A</td>
<td>N/A</td>
<td>YES (to ± 80%)</td>
<td>YES</td>
<td>Diarrhoea III + Enteritis</td>
<td>YES (31 days)</td>
</tr>
</tbody>
</table>
### Table 7.4  
**Continued**

<table>
<thead>
<tr>
<th>Pt #</th>
<th>Cancer type</th>
<th>Therapy</th>
<th><em>DPYD</em> variant</th>
<th>Prospective screening?</th>
<th>Initial dose adjustment?</th>
<th>Toxicity (gr 3–4)?</th>
<th>Toxicity specifications</th>
<th>Hospital admissions?</th>
<th>Second dose adjustment?</th>
<th>Toxicity (gr 3–4)?</th>
<th>Toxicity specifications</th>
<th>Hospital admissions?</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>Mamma (met.)</td>
<td>CAPOX</td>
<td><em>DPYD</em>2A + c.2846A&gt;T</td>
<td>YES</td>
<td>No dose recomm.*4</td>
<td>NO</td>
<td>N/A</td>
<td>N/A (Quit)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>10</td>
<td>Mamma (met.)</td>
<td>CAPOX</td>
<td><em>DPYD</em>2A</td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
<td>N/A</td>
<td>YES (to 100%)</td>
<td>YES (not in first cycles)</td>
<td>HFS II–III</td>
<td>NO (switch to Paxclitaxel, after 8 cycles)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Rectal</td>
<td>CAPOX</td>
<td><em>DPYD</em>2A</td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
<td>N/A</td>
<td>YES (to 60%)</td>
<td>NO</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>12</td>
<td>Gastric (met.)</td>
<td>EOX</td>
<td><em>DPYD</em>2A</td>
<td>NO*2</td>
<td>NO</td>
<td>YES</td>
<td>Diarrhoea III</td>
<td>NO</td>
<td>YES (to 50%)</td>
<td>NO</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>13</td>
<td>Rectal</td>
<td>CAP + RT</td>
<td><em>DPYD</em>2A</td>
<td>YES</td>
<td>NO</td>
<td>YES</td>
<td>Diarrhoea IV + Enteritis + Leukopenia</td>
<td>YES (18 days)</td>
<td>N/A (Quit after TOX first cycle)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>14</td>
<td>Rectum</td>
<td>N/A</td>
<td>c.1236G&gt;A</td>
<td>DNS*5</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

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*1 Genotyping was performed on November 7th 2014, while therapy started on November 5th, 2014.

*2 Both genotyping and start of therapy where on January 24th, 2014. Therefore the result of the genotyping was not awaited.

*3 Patient did not start therapy on its own wish.

*4 For this patient no dose reduction advice was given because this patient was compound heterozygous (carrying two variants), and it was not possible to predict the remaining DPD enzyme activity with the current information. The advice given was to test the actual DPD enzyme activity with another method.

*5 Patient did not start therapy due to renal failure and presence of the *DPYD* variant.

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* Initial dose adjustment is the dose adjustment made prior to the first dose of SFU or CAP.

RT = radiotherapy, CAPOX = Capecitabine + Oxaliplatin, TPF = Docetaxel + Cisplatin + 5-fluorouracil, OXACAPBEV = Oxaliplatin + Capecitabine + Bevacizumab, SFU = 5-fluorouracil, CAP = Capecitabine, EOX = Epirubicin + Oxaliplatin + Capecitabine, DNS = Did not start, dose recomm. = dose recommendation.
Fluoropyrimidine therapy was stopped in this patient after the first cycle due to toxicity (≤ grade 3).

The follow up of the dose recommendations by oncologists

Dose reduction was advised after the first administration of 5FU or CAP (post-dose) for 2 patients. The medical record of the first patient showed that the initial screening result became available after the start of therapy. Dose adjustments could not be applied, toxicity occurred and the advised dose reduction was applied in the second cycle (Table 7.4, patient 12). The other patient was screened after start of therapy, but stopped therapy completely due to toxicity, thus applying a dose reduction was not applicable. For this patient the reason not to screen prospectively was absent in the medical record (Table 7.4, patient 2).

For eleven patients a dose reduction was recommended prior to the start of therapy (prospective). This resulted in an initial dose reduction in 8 out of 11 patients. For one patient the recommend dose reduction was not applied and full dose was given (Table 7.4, patient 13). In two patients the recommended dose reduction could not be applied since they did not start therapy. One patient did not start therapy due to renal failure and the presence of a DPYD variant (Table 7.4, patient 14), and one patient refused to start therapy (Table 7.4, patient 5). Also one patient was genotyped prospectively, but received a recommendation for phenotyping due to compound heterozygosity (Table 7.4, patient 9). This patient started treatment with a 50% reduced dose at the oncologists discretion. An overview of the above mentioned data is displayed in Table 7.4. The adherence to the dose recommendations (pre- and post-dose) is 90% (9 out of 10).

Analysis of results on clinical outcomes

The explorative analysis showed that the prospective dose recommendations given, resulted in initial dose reductions in eight patients. None of these eight patients developed severe toxicity (grade ≥ 3) during the first cycle. After the first or second cycle it was possible to increase the dosages, guided by toxicity. Dosages were increased in four patients (from 50% up to 60, 80 and 100%, and from 75% to 100%, respectively, all receiving CAP). However, this led to the development of severe toxicity in two DPYD*2A carrying patients (80% CAP led to diarrhoea grade 3 followed by 31 days of hospitalisation and 100% CAP led to hand-foot syndrome grade 3). Toxicity data can be found in Table 7.4.
In one patient with a *DPYD*\(^*2A\) variant who received CAP in combination with radiotherapy, the dose recommendation was not followed by the physician and this patient experienced diarrhoea (grade 4), enteritis and leukopenia, for which hospitalisation of 18 days was required and CAP therapy was permanently terminated (Table 7.4, patient 13).

**DISCUSSION**

In this study, the successffulness of routine application of a prospective *DPYD* screening programme followed by pharmacogenetically guided dose recommendations was studied. The percentage of patients in which screening was performed was relatively high: 86.9% of all eligible (newly prescribed 5FU or CAP) patients. In the study period, 13.1% of the patients were not screened prior to receiving 5FU or CAP therapy, which on average comes down to one patient per month. Follow-up of dose recommendations given by the pharmacist were applied in all cases except one, resulting in a high acceptance.

Our study has several limitations. Due to the retrospective design of our study, available data may not always have been fully complete. For example for some patients, it was not possible to retrieve why *DPYD* screening was not requested or whether a patient actually started fluoropyrimidine therapy. In addition, the study was performed with data obtained in a real world clinical setting instead of a regulated and controlled case report form. We had to manually check patient files to obtain specific information and not all physicians may have systematically annotated CTC grading continuously to describe toxicity. Due to the low number of *DPYD* variant carriers our study was not powered to formally test the effect of *DPYD* screening on fluoropyrimidine-induced toxicity and only explorative analyses could be performed.

In this study we determined the level of routine application of *DPYD* screening in daily practice, which increased at the end of the study period to 90–100%. This might indicate that prescribers were undergoing a learning or acceptance curve following the initial start, and were getting used to apply *DPYD* genotyping increasingly in their daily routine.

We believe patients do not need to be genotyped if previous 5FU or CAP usage without toxicity is known or if patients were genotyped (*DPYD*) or phenotyped (DPD) previously. However, within the 41 (13.1%) remaining patients legitimate reasons can still exist (e.g. well-tolerated treatment before 2013 with 5FU or CAP), but might not have been filed in the medical record. Therefore we can conclude the 90–100% (≤ 1 patient not tested per
month) rate was an effective prospective DPYD screening implementation. Disputable is, if this clinical acceptance can become 100% continuously. In order to support the clinical implementation, the use of a clinical decision support (CDS) system might be suitable. In LUMC a CDS entitled adverse drug event alerting system (ADEAS) is used in daily practice in the hospital pharmacy of LUMC [20]. This system is used by hospital pharmacists to systematically select patients at risk of possible adverse drug events. It retrieves data from several information systems, and uses clinical rules to select the patient at risk of adverse drug events.

As mentioned before, sensitivity of genotyping is relatively low (< 14.5% for DPYD*2A and c.2846A>T combined) [11]. Even if all patients with a DPYD variant are identified and treated with an appropriately reduced dose, not all fluoropyrimidine-related toxicity can be prevented. Adding a DPD phenotyping test may increase sensitivity, but is expensive and logistically challenging to implement in clinical practice [13]. SNPs located in other genes than DPYD (e.g. TYMS) have been associated with fluoropyrimidine-induced toxicity with conflicting results. However, testing for these SNPs holds the potential to increase sensitivity [21]. Even though DPYD screening cannot prevent all fluoropyrimidine-related toxicity, we feel that the available evidence strongly supports implementation in clinical practice and can prevent fluoropyrimidine-induced deaths [8, 11, 22].

The presence of one of the four DPYD variants that were pre-emptively tested resulted in a recommendation to the oncologist to reduce the initial dose of 5FU or CAP by 25–50% depending on the identified variant. In February 2015 the recommended dose reduction for c.2846A>T was changed from 50% to 25%, following the updated guidelines of the DPWG [23, 24].

One patient (Table 7.4, patient 13) received full CAP dose, since the treating oncologist argued that she was afraid of under dosing the patient as the dosage of CAP in chemoradiation schemes is already lower compared to other treatments and there is less opportunity to increase the dose in subsequent treatment cycles. The patient developed severe toxicity illustrating that the recommended dose reductions should also be applied to lower CAP doses used in chemoradiation, despite lack of published data about CAP toxicity during chemoradiation therapy.

In conclusion, this study for the first time shows that systematic prospective DPYD screening can be implemented successfully in real world daily clinical practice. The applied 25–50%
dose reduction for patients with a DPYD variant resulted in absence of toxicity. However, a more active follow-up of adherence to provided dose recommendations might improve patient safety even further.

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