Personalised medicine of fluoropyrimidines using DPYD pharmacogenetics

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CHAPTER 8

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

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Abstract
Fluoropyrimidines are commonly used anti-cancer drugs, but lead to severe toxicity in 10—30% of patients. Prospective *DPYD* screening identifies patients at risk for toxicity and leads to a safer treatment with fluoropyrimidines. This study evaluated the routinely application of prospective *DPYD* screening at the Leiden University Medical Center.

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.

86,9% of all patients with a first fluoropyrimidine prescription were screened. Fourteen out of 275 patients (5.1%) carried a *DPYD* variant and received a 25—50% dose reduction recommendation. None of the patients with a *DPYD* variant treated with a reduced dose developed toxicities.

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

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Introduction

Fluoropyrimidines such as 5-fluorouracil (5-FU) and its oral prodrug capecitabine are the cornerstone anticancer drugs for several types of cancer such as colorectal cancer, head–neck cancer and breast cancer. Approximately 10–30% of the patients receiving 5-FU or capecitabine experience severe (grade ≥3) toxicity, such as diarrhea, mucositis and hand-foot syndrome.[1] 5-FU 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 5-FU.[4-7] Toxicity occurred in 73% of *DPYD**2A carriers, compared with 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 5-FU or capecitabine, 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 5-FU or capecitabine 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 5-FU or capecitabine 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; Leiden, The Netherlands), a routine *DPYD* screening program prior to prescribing 5-FU or capecitabine was initiated in April 2013. In this retrospective study we evaluated the physician's acceptance of prospective *DPYD* screening for patients who were prescribed 5-FU or capecitabine 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® Genotyping SNP assay from Thermo Fisher Scientific [MA USA], and a home-brew pyrosequencing (PSQ),

described previously).[17] Within LUMC the Electronic Medication Record (EMR) system EZIS (version 5.2, Chipsoft) is used, which can be consulted by physicians, pharmacists and nurses. *DPYD* genotyping results are communicated electronically by the responsible pharmacist into the EMR and are visible for other users of the EMR.

The prospective screening program was initiated on 15 April 2013. During a kick-off meeting attended by medical oncologists and fellows, the staff was informed and agreed on the prospective program. New medical oncologists and fellows were informed about the prospective screening program during the regular introduction program for new staff members. Genotyping was performed three times per week (Monday, Wednesday and 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.[18]

Study end points

Three study end points were evaluated to determine the successfulness of the screening program that was introduced at LUMC. We evaluated:

- The 'implementation', in other words, requests of the *DPYD* tests as standard care in daily practice;
- The proportion of test results with a dose recommendation provided by the pharmacist;
- 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 (pretreatment) *DPYD* screening in daily practice was evaluated by determining the proportion of patients who were screened for *DPYD* variants when an incident prescription for 5-FU or capecitabine was given. The data were 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 5-FU and capecitabine. Prescription data prior to the start of the study was studied as well, to ascertain that 5-FU or capecitabine 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 were anonymized. All manual changes (additional information, removal of duplicates, among others) to the queries were double checked by the two first authors (CL and MvS).

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 5-FU or capecitabine therapy was retrieved from the EMR for patients with a *DPYD* variant. Toxicity was scored by the oncologists using the National Cancer Institute common terminology criteria for adverse events (CTC-AE), version 4.03.[19]

Results

The implementation of the prospective screening program for DPYD

The prospective *DPYD* screening program was implemented on 15 April 2013 (study start date) at LUMC. From this date until 13 December 2014 (study end date) 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 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 1.

Table 1. Recommended reductions of initial 5-fluorouracil or capecitabine dose

Advice given by CPIC and DPWG guidelines at the time the variant was added to the routine screening.

***DPYD* variant**	**Initial dose reduction (%)**	**Inclusion in screening program**	**Patients screened**
*DPYD**2A (c.1905+1G>A)	50	April 15th, 2013	529
*DPYD**13 (c.1679T>G)	50	October 10th, 2013	440
c.2846A>T	50 → 25[a]	October 10th, 2013	440
c.1236G>A	25	May 28th, 2014	254

[a] The dose reduction advice for c.2846A>T has been updated to 25% in February 2015.

A total of 2,498 records of 5-FU or capecitabine prescriptions prior to 31 December 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 5-FU (16%) or capecitabine (84%) for the first time at LUMC within the study period.

Genotyped patients were compared with patients who were prescribed 5-FU or capecitabine, resulting in 236 matching patients. Thirty-nine patients were genotyped for *DPYD*, but were not prescribed 5-FU or capecitabine. Also, 101 patients were prescribed

5-FU or capecitabine, but were not genotyped for *DPYD* variants (Figure 1).

Two patients, who received 5-FU or capecitabine and were genotyped, were excluded because their medical records revealed they had received 5-FU or capecitabine prior to 15 April 2013. Of the 39 patients who were genotyped without receiving 5-FU or capecitabine 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 31 December 2014 and were therefore not identified by the search. Of the 101 patients with a 5-FU or capecitabine prescription and no *DPYD*-genotyping record, the medical records were screened resulting in a legitimate reason not to genotype in 60 cases (Table 2). Legitimate reasons included; any notes on prior treatment with 5-FU or capecitabine (e.g., outside LUMC) or invalid patient files (e.g., no medical dossier found for the oncology department). For 41 patients who had a prescription for newly 5-FU or capecitabine no reason was found to neglect genotyping. After data cleaning, 314 patients with a newly 5-FU or capecitabine prescription remained in the dataset and 273 of these patients were genotyped as depicted in Figure 1. The clinical acceptance of the prospective *DPYD* screening program is displayed as percentage per month in Figure 2. The average clinical acceptance was 86.9%.

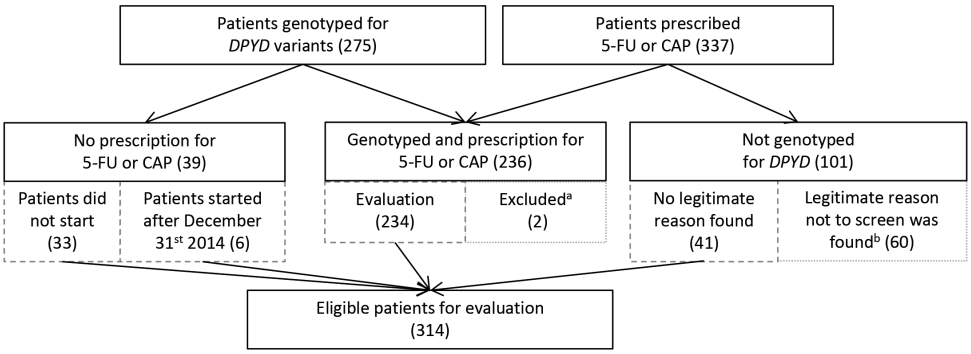

Figure 1. Patient selection

Flowchart following the results from the two searches. Patients could be both genotyped and prescribed 5-FU or capecitabine, or only genotyped, or only prescribed 5-FU or capecitabine. If the intention to treat was present, patients should have been genotyped and these patients are 'eligible for evaluation'.

[a] These two patients were excluded because their medical records revealed they had received 5-FU or capecitabine prior to April 15th 2013;

[b] Legitimate reasons were: e.g., any notes on prior treatment with 5-FU or capecitabine (e.g., outside LUMC) or invalid patient files (e.g., no medical dossier found for the oncology department).

Abbreviations: 5-FU: 5-fluorouracil, CAP: capecitabine.

Table 2. Excluded patients

Patients (*N*=60) with legitimate reasons not to screen were excluded from analysis.

Patients (*N*)	Reason not to perform *DPYD* genotyping
8	5-FU or CAP therapy started just prior to the start date of 15 April 2013
30	5-FU or CAP was used before April 2013 without problems and would start again after 15 April 2013
20	No medical dossier at the Medical Oncology department was found, therefore the patient was not treated at the LUMC
2	These dossiers were fake patients used for education purposes

Abbreviations: 5-FU: 5-fluorouracil; CAP: capecitabine.

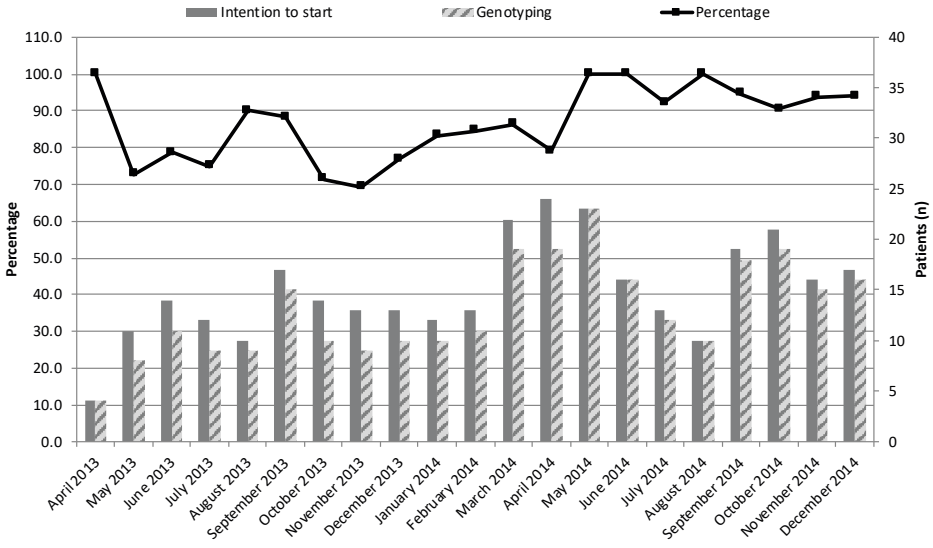

Figure 2. Proportion of eligible patients that were genotyped

The figure shows the eligible patients for evaluation per month in actual patient numbers. If the intention to treat with 5-FU or capecitabine 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.

Table 3. *DPYD* variants

***DPYD* variant**	**SNPs (*N*)**	**Tested patients (*N*)**	**LUMC (%)**	**Literature (%)**	**Ref.**
*DPYD**2A (c.1905+1G>A)	6	275	2.2	~1.0–1.8	[10,20]
*DPYD**13 (c.1679T>G)	0	214	0	~0.1	[12]
c.2846A>T	1	214	0.5	~1.0–1.4	[10,12]
c.1236G>A	8	109	7.3	~2.6–4.9	[10,21]
Total	15 (*N*=14)	275	5.1	4.7–8.2	

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

The follow-up of the dose recommendations by oncologists

Dose reduction was advised after the first administration of 5-FU or capecitabine (post-dose) for two 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 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 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 eight of 11 patients. For one patient the recommend dose reduction was not applied and full dose was given (Table 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 4, patient 14), and one patient refused to start therapy (Table 4, patient 5). Also one patient was genotyped prospectively, but received a recommendation for phenotyping due to compound heterozygosity (Table 4, patient 9). This patient started treatment with a 50% reduced dose at the oncologists discretion. An overview of the above mentioned data are displayed in Table 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 capecitabine). However, this led to the development of severe toxicity in two *DPYD**2A carrying patients (80% capecitabine led to diarrhea grade 3 followed by 31 days of hospitalization and 100% capecitabine led to hand-foot syndrome grade 3). Toxicity data can be found in Table 4.

In one patient with a *DPYD**2A variant who received capecitabine in combination with radiotherapy, the dose recommendation was not followed by the physician and this patient experienced diarrhea (grade 4), enteritis and leukopenia, for which hospitalization of 18

days was required and capecitabine therapy was permanently terminated (Table 4, patient 13).

Discussion

In this study, the successfulness of routine application of a prospective *DPYD* screening program 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 5-FU or capecitabine) patients. In the study period, 13.1% of the patients were not screened prior to receiving 5-FU or capecitabine 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-AE 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 5-FU or capecitabine 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 5-FU or capecitabine), 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 system might be suitable. In LUMC a clinical decision support system entitled adverse drug event alerting system (ADEAS) is used in daily practice in the hospital pharmacy of LUMC.[22] 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.[23] 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.[8,11,24]

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 5-FU or capecitabine 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.[25,26]

One patient (Table 4, patient 13) received full capecitabine dose, since the treating oncologist argued that she was afraid of under dosing the patient as the dosage of capecitabine in chemoradiation schemes is already lower compared with 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 capecitabine doses used in chemoradiation, despite lack of published data about capecitabine toxicity during chemoradiation therapy.

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.

Table 4. *DPYD* variant carrying patients
Listed for LUMC patients who carry a *DPYD* variant are genotyping data, therapy details and toxicity data.

pt#	Cancer type	Therapy	*DPYD* variant	Pro-spective screen-ing?	Initial dose adjust-ment?[a]	Toxicity (grade 3–4)?	Toxicity specifications	Hospital admissions?	Second dose adjustment?	Toxicity (grade 3–4)?	Toxicity specifica-tions	Hospital admissions?
1	Colo-rectal	CAPOX	c.1236G>A	YES	YES	NO	N/A	N/A	YES (to 100%)	NO	N/A	N/A
2	Mouth	TPF + RT	c.1236G>A	NO[b]	NO	YES	Diarrhoea IV + Neutropenia/ Thrombocyto-penia III	YES (6+16 days)	N/A (Quit af-ter 2nd cycle)	N/A	N/A	N/A
3	Colon (met.)	CAPOX + BEV	c.1236G>A	YES	YES	NO	N/A	N/A	NO	N/A	N/A	N/A
4	Anus	5-FU + RT	c.1236G>A	YES	YES	NO	N/A	N/A	NO	N/A	N/A	N/A
5	Colon	N/A	c.1236G>A	YES	DNS[c]	N/A	N/A	N/A	N/A	N/A	N/A	N/A
6	Pharynx	5-FU + RT	c.1236G>A	YES	YES	NO	N/A	N/A	NO	N/A	N/A	N/A
7	Pancreas	CAP	c.1236G>A	YES	YES	NO	N/A	N/A	N/A (Quit)	N/A	N/A	N/A
8	Rectal	CAP + RT	*DPYD**2A	YES	YES	NO	N/A	N/A	YES (to ±80%)	YES	Diarrhoea III + Enteritis	YES (31 days)
9	Mamma (met.)	CAPOX	*DPYD**2A + c.2846A>T	YES	No dose recomm.[d]	NO	N/A	N/A	N/A (Quit)	N/A	N/A	N/A
10	Mamma (met.)	CAPOX	*DPYD**2A	YES	YES	NO	N/A	N/A	YES (to 100%)	YES (not in first cycles)	HFS II–III	NO (switch to Paxclitaxel, after 8 cycles)

table continues

pt#	Cancer type	Therapy	*DPYD* variant	Pro-spective screen-ing?	Initial dose adjust-ment?[a]	Toxicity (grade 3–4)?	Toxicity specifications	Hospital admissions?	Second dose adjustment?	Toxicity (grade 3–4)?	Toxicity specifica-tions	Hospital admissions?
11	Rectal	CAPOX	*DPYD**2A	YES	YES	NO	N/A	N/A	YES (to 60%)	NO	N/A	N/A
12	Gastric (met.)	EOX	*DPYD**2A	NO[e]	NO	YES	Diarrhoea III	NO	YES (to 50%)	NO	N/A	N/A
13	Rectal	CAP + RT	*DPYD**2A	YES	NO	YES	Diarrhoea IV + Enteritis + Leukopenia	YES (18 days)	N/A (Quit after TOX in first cycle)	N/A	N/A	N/A
14	Rectum	N/A	c.1236G>A	YES	DNS [f]	N/A	N/A	N/A	N/A	N/A	N/A	N/A

[a] Initial dose adjustment is the dose adjustment made prior to the first dose of 5-FU or CAP;
[b] Genotyping was performed on November 7th 2014, while therapy started on November 5th, 2014;
[c] Patient did not start therapy on its own wish;
[d] 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;
[e] Both genotyping and start of therapy where on January 24th, 2014. Therefore the result of the genotyping was not awaited;
[f] Patient did not start therapy due to renal failure and presence of the *DPYD* variant.
Abbreviations: RT: radiotherapy; CAPOX: Capecitabine + Oxaliplatin; TPF: Docetaxel + Cisplatin + 5-fluorouracil; CAPOX + BEV: Capecitabine + Oxaliplatin + Bevacizumab; 5-FU: 5-fluorouracil; CAP: Capecitabine; EOX: Epirubicin + Oxaliplatin + Capecitabine; DNS: Did not start; dose recomm.: dose recommendation.

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