Standard fluoropyrimidine dosages in chemoradiation therapy result in an increased risk of severe toxicity in *DPYD* variant allele carriers

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Abstract

Prospective *DPYD* genotyping prevents severe fluoropyrimidine-induced toxicity by decreasing dosages in *DPYD* variant allele carriers. Fluoropyrimidine dosages in chemoradiation therapy (CRT) are lower compared to other fluoropyrimidine-containing regimens. Pharmacogenetic guidelines do not distinguish between regimens, leaving physicians in doubt to apply dose reductions. Our aim was to investigate severe toxicity in *DPYD* variant allele carriers receiving CRT.

Medical records of 828 patients who received fluoropyrimidine-based CRT were reviewed from three centers. Severe (grade ≥3) toxicity in *DPYD* variant allele carriers receiving upfront fluoropyrimidine dose reductions according to pharmacogenetic dosing guidelines and *DPYD* variant allele carriers not receiving fluoropyrimidine dose reductions was compared with *DPYD* wild-type patients receiving standard dose of fluoropyrimidines in CRT.

DPYD variant allele carriers treated with standard dosages (*N*=34) showed an increased risk of severe gastrointestinal (adjusted OR: 2.58, 95% confidence interval [95%CI]: 1.02–6.53, p=0.045) or severe haematological (adjusted OR: 4.19, 95%CI: 1.32–13.25, p=0.015) toxicity compared with wild-type patients (*N*=771). *DPYD* variant allele carriers who received dose reductions (*N*=22) showed a comparable frequency of severe gastrointestinal toxicity compared with wild-type patients, but more (not statistically significant) severe haematological toxicity. Hospitalisations for all *DPYD* variant allele carriers were comparable, independent of dose adjustments; however, the mean duration of hospitalisation was significantly shorter in the dose reduction group (p=0.010).

Standard fluoropyrimidine dosages in CRT resulted in an increased risk of severe toxicity in *DPYD* variant allele carriers. We advise to apply fluoropyrimidine dose reductions according to current guidelines in *DPYD* variant allele carriers starting CRT.

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Introduction

Fluoropyrimidines, such as 5-fluorouracil (5-FU) and capecitabine, are the backbone of chemotherapy regimens for solid tumours such as colorectal and breast cancer.[1-3] Since the 90's, 5-FU has been in use in neoadjuvant chemoradiation therapy (CRT) for patients with stages 2-3 rectal cancer.[4,5] Fluoropyrimidines affect nucleotide metabolism and inhibit the repair of radiation-induced DNA damage in patients and act as a radiation sensitiser.[6] Fluoropyrimidines in combination with radiotherapy are used at lower dosages than those in other treatment regimens. An example; for patients with advanced colorectal cancer capecitabine, dosages are usually 1,250 mg/m^2 bid (twice daily) for two weeks followed by one week rest, repeated every three weeks.[7] In combination with radiotherapy, a continuous regimen is preferred to optimise radio-sensitisation. The maximum tolerated dose of capecitabine was 825 mg/m^2 bid for patients with rectal cancer.[8,9]

Adverse events are well known in fluoropyrimidine treatment and differ between treatment regimens. Severe (grade ≥3) side-effects in stage 3 or 4 colorectal cancer patients treated with capecitabine monotherapy dosed 1,250 mg/m^2 bid in three-week cycles, were hand-foot syndrome (~18%), diarrhoea (~14%), stomatitis (~3%), vomiting (~3%) and neutropenia (~3%).[10-12] Severe side-effects in locally advanced rectal cancer patients treated with CRT, including 825 mg/m^2 capecitabine continuously for five weeks, were grade ≥3 radiation dermatitis (~9%), diarrhoea (~2-7%), fatigue (~2%), neutropenia (~2%) and anaemia (~2%).[13,14]

Over 80% of 5-FU is degraded into inactive metabolites by the key enzyme dihydropyrimidine dehydrogenase (DPD).[15] DPD is encoded by the gene *DPYD*. DPD and variants in *DPYD* are associated with the onset of severe fluoropyrimidine-induced toxicity. To prevent severe fluoropyrimidine-induced toxicity prospective *DPYD* genotyping is increasingly used in clinical practice, followed by dose reductions in patients who carry a *DPYD* variant. For four variants (*DPYD**2A, c.1905+1G>A, rs3918290; *DPYD**13, c.1679T>G, rs55886062; c2846A>T, rs67376798; c.1236G>A/HapB3, rs56038477) individual dosing guidelines are currently given by the Dutch Pharmacogenetics Working Group and Clinical Pharmacogenetics Implementation Consortium.[16,17] Dosing guidelines advise that *DPYD* variant allele carriers should receive a percentage of the standard dose, for example 50 or 75%, depending on the specific variant.[18] These guidelines do not distinguish between treatment regimens in which different fluoropyrimidine dosages are given. Because fluoropyrimidine dosages in CRT regimens are lower than those in other treatment regimens, it is questioned if dose adjustments in dosing guidelines should be applied in patients receiving fluoropyrimidines in CRT. The objective of this study was to investigate the frequency of severe treatment-related toxicity in *DPYD* variant allele carriers receiving reduced or standard fluoropyrimidine dosages in CRT, to determine whether dose reductions are required.

Methods

Study population

The study population consisted of three combined databases. All patients were treated with fluoropyrimidine-based CRT according to the various tumour types and were genotyped for the aforementioned four variants in *DPYD.*

At the Netherlands Cancer Institute (NKI), Amsterdam, the Netherlands, a prospective clinical trial was executed in which patients were prospectively genotyped for *DPYD**2A followed by dose

reductions of ≥50% in *DPYD**2A carriers (NCT00838370).[19] The trial was approved by the institutional review board (IRB) of all participating institutes, and all *DPYD**2A carriers provided written informed consent before study registration. The patients were retrospectively genotyped for the three other variants (*DPYD**13, c.2846A>T, c.1236G>A). A total of 497 patients received CRT and were selected for the present study. Two patients had missing genotypes and were excluded. Radiation dose in Gray (Gy) and fractions (Fr) given to the patient could be collected retrospectively for 425 patients.

At Leiden University Medical Center (LUMC), Leiden, the Netherlands, a retrospective database was created for the purpose of this study. The study was reviewed and approved by the IRB. All patients scheduled to start fluoropyrimidine-based CRT between April 2013 and September 2017 were evaluated. In total, 253 patients started therapy. In April 2013, only *DPYD**2A was genotyped; *DPYD**13 and c.2846A>T were added to the genotyping panel in October, and c.1236G>A was added in May 2014. Some patients were prospectively genotyped for *DPYD**2A alone (*N*=20) or *DPYD**2A, *DPYD**13 and c.2846A>T (*N*=35). Missing genotypes were determined retrospectively. Thirteen patients could not be genotyped and were excluded. Data were collected from the electronic patient files. Ten percent of the data was checked by an independent data manager. Ten percent of toxicity data was checked by an oncologist and radiation oncologist. Limited discrepancies were discussed and similar errors were searched and corrected.

At CRO-Aviano National Cancer Institute, Northern Italy, 207 patients were enrolled in a study from December 1993 to April 2016. All procedures were reviewed and approved by the IRB and patients signed written informed consent for research purposes. Ninety-five patients were included in the present study of whom additional chemotherapy treatment details could be collected. Sixteen patients were prospectively tested for *DPYD**2A, *DPYD**13 and c.2846A>T, and 79 patients were tested after start of treatment. Missing genotypes of c.1236G>A were determined retrospectively. Two patients had incomplete genotype data and were excluded.

Groups

All included patients in the combined database were grouped into wild-types receiving standard fluoropyrimidine dosages in CRT, *DPYD* variant allele carriers receiving standard fluoropyrimidine dosages in CRT or *DPYD* variant allele carriers receiving upfront reduced fluoropyrimidine dosages in CRT. *DPYD* variant allele carriers are heterozygous or homozygous for a *DPYD* variant (*DPYD**2A, *DPYD**13, c.2846A>T or c.1236G>A). Initial dose reductions (25 or 50%) were applied corresponding to pharmacogenetic guidelines.[16,17]

Toxicity

Treatment-related toxicity data were scored prospectively according to the National Cancer Institute common terminology criteria for adverse events (CTC-AE) v3.0[20] for the NKI and CRO databases, and retrospectively using CTC-AE v4.03[21] for the LUMC database. It was not possible to determine missing toxicities retrospectively. In CRT a continuous regimen is used, and there are no cycles; therefore, the highest toxicity grade over the entire treatment period was used. Gastrointestinal toxicity included diarrhoea, mucositis, nausea and vomiting (nausea or vomiting were not scored by all databases). Haematological toxicity included leukopenia, thrombocytopenia and neutropenia.

Statistics

To study the association between study groups and severe gastrointestinal or haematological

toxicity multivariable logistic regression models with grouped diagnosis as covariate were estimated. Gastrointestinal and haematological toxicity outcomes were dichotomised (grades 0–2 versus grades 3–5). Diagnoses were grouped according to tumour location, either pelvic or non-pelvic region (grouped diagnosis). Differences in baseline characteristics between study groups were tested using Pearson Chi-square or Kruskal Wallis tests. Owing to the retrospective character of this study, there was no protocol on how to deal with additional dose adjustments during treatment in the analysis. A Mann–Whitney U test was applied to compare duration of hospitalisation between *DPYD* variant allele carriers who received dose reductions or standard dosages. P-values of <0.05 were considered statistically significant. Statistical analyses were performed using SPSS (v23, Chicago, IL, USA).

Results

Study population

The combined database of 828 patients was divided into three study groups. Seven hundred seventy-one patients were wild-types, 34 patients were *DPYD* variant allele carriers who received standard fluoropyrimidine dosages in CRT and 23 patients were *DPYD* variant allele carriers who received upfront reduced (50 or 75%) fluoropyrimidine dosages in CRT. Baseline characteristics per database and study group are shown in Tables 1 and 2. Each original database included patients in each study group, described in Table 2. Cancer of the rectum was the most present in 71.7% of the patients. 86.6% of the patients received capecitabine. Baseline characteristics between study groups showed no significant differences.

In one *DPYD* *2A carrier, dose reductions were applied during treatment but not at the first drug administration. In three *DPYD**2A carriers initial reduced dosages were increased during treatment. Three out of four patients had a total dose intensity of approximately 50% (according to current dosing guidelines). The fourth patient was excluded from statistical analyses. These four patients were described in Table 2.

Toxicity

Toxicity of patients from this study treated with comparable treatment schedules was similar to toxicity of rectal cancer patients described in literature (Supplementary Table 1). Differences in toxicity between databases were observed. Grade 2 radiation dermatitis and grade 2 'other toxicity' were very high in the LUMC and CRO database, respectively, resulting in a high overall toxicity percentage in these databases (Supplementary Table 2). Toxicity separated per study group is shown in Table 3.

Percentages of severe gastrointestinal and haematological toxicity were 8 and 2.9% for wild-types, 17.6 and 11.8% for *DPYD* variant allele carriers treated with a standard dose, and 9.1 and 9.1% for *DPYD* variant allele carriers who received a reduced dose, respectively (Figure 1, Table 3). *DPYD* variant allele carriers treated with a standard dose had a significantly increased risk to develop severe gastrointestinal toxicity (adjusted OR: 2.58, 95% confidence interval [95%CI]: 1.023–6.534, p=0.045) and severe haematological toxicity (adjusted OR:4.19, 95%CI: 1.323–13.253, p=0.015) compared with *DPYD* wild-type patients treated with standard dose. No statistical significant difference was found for the risk of developing severe gastrointestinal toxicity (adjusted OR: 1.10, 95%CI: 0.250–4.804, p=0.904) or severe haematological toxicity (adjusted OR: 3.88, 95%CI: 0.837–18.016, p=0.083) in *DPYD* variant allele carriers who received an initially reduced dose

compared with wild-types. Grouped diagnosis was not significantly associated with the development of severe gastrointestinal toxicity (adjusted OR: 0.26, 95%CI: 0.061–1.069), while it was for severe haematological toxicity (adjusted OR: 4.21, 95%CI: 1.760–10.053, p=0.001), with more toxicity in pelvic malignancies.

Table 1. Baseline characteristics of patients from three original databases and of the combined database (total)

Characteristics	**DB#1:NKI (*N*=495)** *N* **(%)**	**DB#2:LUMC (*N*=240)** *N* **(%)**	**DB#3:CRO (*N*=93)** *N* **(%)**	**TOTAL (*N*=828)** *N* **(%)**
Sex, male	283 (57.2)	122 (50.8)	60 (64.5)	465 (56.2)
Age, median [range]	62 [32–86]	65 [23–86]	63 [33–88]	63 [23–88]
BSA, median [range]	1.9 [1.38–2.71]	1.89 [1.39–2.54]	1.85 [1.4–2.2]	1.9 [1.38–2.71]
Diagnosis				
Rectum cancer	344 (69.5)	157 (65.4)	93 (100)	594 (71.7)
Anus cancer	80 (16.2)	36 (15.0)	-	116 (14)
Vulva/vagina cancer	1 (0.2)	17 (7.1)	-	18 (2.2)
Pancreas cancer	-	5 (2.1)	-	5 (0.6)
Upper GI cancer	54 (10.9)	10 (4.2)	-	64 (7.7)
Other cancers	16 (3.2)	15 (6.3)	-	31 (3.7)
Grouped diagnosis				
Pelvic region cancer[a]	432 (87.3)	223 (92.9)	93 (100)	748 (90.7)
Non-pelvic region cancer[b]	60 (12.1)	17 (7.1)	-	77 (9.3)
Other cancers	3 (0.6)	-	-	3 (0.4)
Treatment type				
Capecitabine	442 (89.3)	183 (76.3)	92 (98.9)	717 (86.6)
5-FU	53 (10.7)	57 (23.8)	1 (1.1)	111 (13.4)
Treatment date [range]	[01/2007–02/2012]	[12/2012–09/2017]	[04/2006–04/2016]	[05/2006–09/2017]
Radiotherapy				
Gy: median [range]	50 [20–78][c]	50 [7.2–69.4]	55 [31.5–55.2]	50 [7.2–78][c]
Fr: median [range]	25 [5–39][c]	25 [4–38]	25 [15–28][d]	25 [4–39][e]
DPYD carriers total	36 (7.3)	18 (7.5)	3 (3.2)	57 (6.9)
*DPYD*2A*	7 (1.4)	6 (2.5)	-	13 (1.6)
*DPYD*13*	1 (0.2)	-	-	1 (0.1)
c.2846A>T	9 (1.8)	-	1 (1.1)	10 (1.2)
c.1236G>A	17 (3.4)	12 (5)	2 (2.2)	31 (3.7)
c.1236G>A homozygote	2 (0.4)	-	-	2 (0.2)

[a] Included are cancers of the colon sigmoidal, rectum, anus, vulva, vagina, cervix, uterus, endometrium, bladder, urethra, prostate and double tumours with one tumour in the pelvic area;

[b] Included are cancers of the breast, stomach, oesophagus, pancreas, skin, tongue;

[c] Seventy-one patients have missing data;

[d] One patient has missing data;

[e] Seventy-two patients have missing data.

Abbreviations: BSA: body surface area; CRO: Aviano National Cancer Institute; DB: database; *DPYD*: gene encoding dihydropyrimidine dehydrogenase; 5-FU: 5-fluorouracil; Fr: fractions; GI: gastro-intestinal tract; Gy: gray; LUMC: Leiden University Medical Center; NKI: Netherlands Cancer Institute.

Table 2. Baseline characteristics per study group

No significant differences between study groups in baseline characteristics were found. Differences in median dose intensity, treatment date and *DPYD* carriers were not tested. All original databases were able to include patients in each study group. Of the 34 *DPYD* variant allele carriers who received standard fluoropyrimidine dosages in CRT, 29 patients were included from NKI, three patients from LUMC (2x *DPYD**2A, 1x c.1236G>A) and two c.1236G>A carriers from the CRO database. Of the 23 *DPYD* variant allele carriers who received upfront dose reductions in CRT, 15 patients were included from LUMC, seven *DPYD**2A carriers from NKI and one c.2846A>T carrier from the CRO database.

Characteristics	**WT + standard[a] (*N*=771) *N* (%)**	***DPYD* + standard[b] (*N*=34) *N* (%)**	***DPYD* + reduced[c] (*N*=23) *N* (%)**
Sex, male	432 (56)	20 (58.8)	13 (56.5)
Age, median [range]	63 [23–88]	64 [45–79]	66 [50–78]
BSA, median [range]	1.89 [1.38–2.71]	1.93 [1.51–2.34]	2 [1.50–2.44]
Diagnosis			
Rectum cancer	554 (71.9)	22 (64.7)	18 (78.3)
Anus cancer	106 (13.7)	7 (20.6)	3 (13.0)
Vulva/vagina cancer	18 (2.3)	-	-
Pancreas cancer	5 (0.6)	-	-
Upper GI cancer	58 (7.5)	5 (14.7)	1 (4.3)
Other cancers	30 (3.9)	-	1 (4.3)
Grouped diagnosis			
Pelvic region cancer[d]	697 (90.8)	29 (85.3)	22 (95.7)
Non-pelvic region cancer[e]	71 (9.2)	5 (14.7)	1 (4.3)
Other cancers	3 (0.4)	-	-
Treatment type			
Capecitabine	668 (86.6)	29 (85.3)	20 (87)
5-FU	103 (13.4)	5 (14.7)	3 (13)
Median dose intensity[f]	97%	91%	61%
Treatment date [range]	[05/2006–09/2017]	[02/2008–10/2014]	[12/2007–08/2017]
Radiotherapy			
Gy: median [range]	50 [7.2–73.6]	50 [36–64.8]	50 [45–78]
Fr: median [range]	25 [4–39]	25 [18–36]	25 [23–39]
DPYD carriers			
*DPYD*2A*	-	2 (5.9)	11 (47.8)
*DPYD*13*	-	1 (2.9)	-
c.2846A>T	-	9 (26.5)	1 (4.3)
c.1236G>A	-	20 (58.8)	11 (47.8)
c.1236G>A homozygote	-	2 (5.9)	-

[a] Wild-type patients receiving standard fluoropyrimidine dosages in chemoradiation therapy;

[b] *DPYD* variant allele carriers receiving standard fluoropyrimidine dosages in chemoradiation therapy;

[c] *DPYD* variant allele carriers receiving initially reduced fluoropyrimidine dosages according to current guidelines compared with standard fluoropyrimidine dosages used in chemoradiation therapy. One *DPYD**2A

variant carrier started intravenous 5-FU therapy at a 100% dose before the genotype result became available. When the genotype was known, the administration of 5-FU was prematurely stopped after 2 instead of 4 days. In the second cycle a 50% dose reduction over 4 days was applied. The overall dose intensity of this patient was 49%. In three *DPYD**2A carriers initial reduced dosages were increased during treatment. One patient was included in the clinical trial (NCT00838370) before existence of dosing guidelines and started with 30% of the standard total dose, which was increased to 46%. One patient went from 50 to 60% of the standard total dose and for another patient the dose was increased from 50 to 83%. The latter patient was excluded from statistical analyses, due to the substantial dose increase. The c.2846A>T variant carrier who received a dose reduction, was treated with a 60% dose;

[d] Included are cancers of the colon sigmoidal, rectum, anus, vulva, vagina, cervix, uterus, endometrium, bladder, urethra, prostate and double tumours with one tumour in the pelvic area;

[e] Included are cancers of the breast, stomach, oesophagus, pancreas, skin and tongue;

[f] Dose intensity was calculated by dividing the received amount of mg of chemotherapy by the initial scheduled amount of mg of chemotherapy.

Abbreviations: BSA: body surface area; *DPYD:* gene encoding dihydropyrimidine dehydrogenase; 5-FU: 5-fluorouracil; Fr: fractions; GI: gastro-intestinal tract; Gy: gray; WT: wild-type patients.

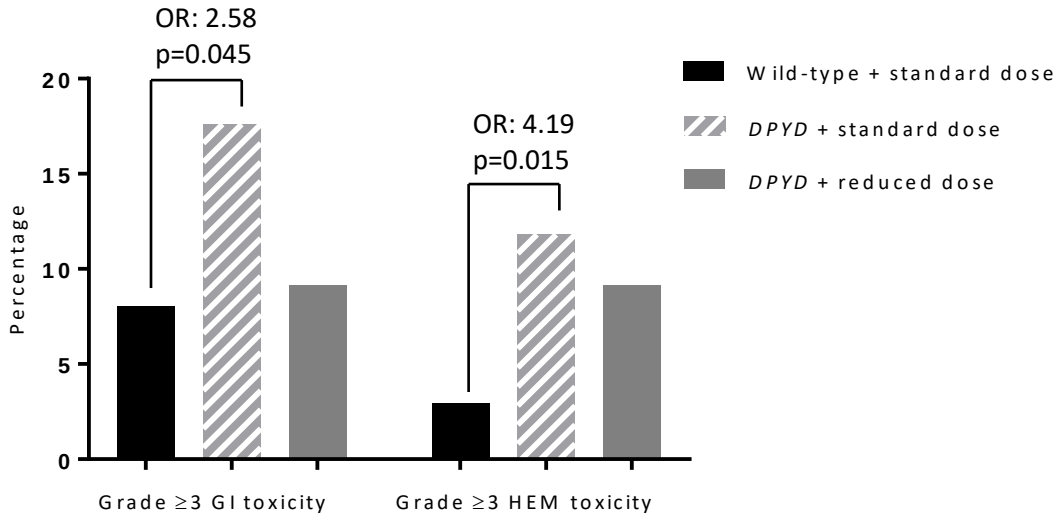

Figure 1. Percentages of severe toxicity

Shown are the percentages of severe gastrointestinal and severe haematological toxicity of *DPYD* variant allele carriers with and without fluoropyrimidine dose reductions and wild-type patients in chemoradiation treatment.

Abbreviations: OR: adjusted odds ratio; *DPYD*: gene encoding dihydropyrimidine dehydrogenase; GI= gastrointestinal; HEM: haematological.

Included in Table 3 are any changes applied in chemotherapy during treatment due to adverse events, such as dose interruptions. Compared with wild-type patients, *DPYD* variant allele carriers had more dose reductions during treatment, stopped treatment prematurely and were hospitalised more often, regardless of any received dose reductions. However, the mean duration of hospitalisation of *DPYD* variant allele carriers who received a dose reduction was notably shorter (4 days) compared with the *DPYD* variant allele carriers treated with a standard dose (23 days, p=0.010).

Table 3. Toxicity of patients per study group

Shown per study group are percentages of several types of (grouped) toxicity after chemoradiation therapy and actions following toxicity. *P*-values are shown for executed statistical tests.

Type of event	**WT + standard[a] (*N*=771) *N* (%)**	***DPYD* + standard[b] (*N*=34) *N* (%)**		***DPYD* + reduced[c] (*N*=22) *N* (%)**	
Grade 2 Diarrhoea	122 (15.8)	5 (14.7)		3 (13.6)	
Grade ≥3 Diarrhoea	58 (7.5)	6 (17.6)		2 (9.1)	
Grade 2 Mucositis	51 (6.6)	2 (5.9)		2 (9.1)	
Grade ≥3 Mucositis	13 (1.7)	-		-	
Grade 2 Nausea[d]	13 (4.2)	2 (40)		1 (6.7)	
Grade ≥3 Nausea[d]	2 (0.6)	1 (20)		-	
Grade 2 Vomiting[e]	12 (5.4)	2 (66.7)		1 (7.1)	
Grade ≥3 Vomiting[e]	1 (0.5)	1 (33.3)		-	
Grade 2 Neutropenia	8 (1)	1 (2.9)		1 (4.5)	
Grade ≥3 Neutropenia	12 (1.6)	2 (5.9)		2 (9.1)	
Grade 2 Leukocytopenia	60 (7.8)	7 (20.6)		2 (9.1)	
Grade ≥3 Leukocytopenia	17 (2.2)	4 (11.8)		2 (9.1)	
Grade 2 Thrombocytopenia	6 (0.8)	-		1 (4.5)	
Grade ≥3 Thrombocytopenia	5 (0.6)	-		-	
Grade 2 Anaemia[d]	25 (8)	1 (20)		2 (13.3)	
Grade ≥3 Anaemia[d]	1 (0.3)	1 (20)		-	
Grade 2 Radiation dermatitis[d]	77 (24.7)	1 (20)		5 (33.3)	
Grade ≥3 Radiation dermatitis[d]	13 (4.2)	-		-	
Grade 2 HFS	19 (2.5)	-		1 (4.5)	
Grade 3 HFS	5 (0.6)	-		-	
Grade 2 Cardio toxicity	21 (2.7)	-		-	
Grade ≥3 Cardio toxicity	11 (1.4)	-		-	
Grade 2 Fatigue[e]	28 (12.6)	1 (33.3)		4 (28.6)	
Grade ≥3 Fatigue[e]	2 (0.9)	2 (66.7)		-	
Grouped type of events					
Grade 2 GI toxicity[f]	138 (17.9)	5(14.7)		6 (27.3)	
Grade ≥3 GI toxicity[f]	62 (8)	6 (17.6)	p=0.045[g]	2 (9.1)	p=0.904[g]
Grade 2 HEM toxicity[h]	62 (8)	7 (20.6)		2 (9.1)	
Grade ≥3 HEM toxicity[h]	22 (2.9)	4 (11.8)	p=0.015[g]	2 (9.1)	p=0.083[g]
Grade 2 Overall toxicity[i]	252 (32.7)	7 (20.6)		7 (31.8)	
Grade ≥3 Overall toxicity[i]	105 (13.6)	8 (23.5)		5 (22.7)	
Actions					
Chemotherapy changes					
Dose reductions	34 (4.4)	4 (11.8)[j]		2 (9.1)[k]	
Dose increases	4 (0.5)	-		2 (9.1)[l]	
Interruptions	38 (4.9)	-		1 (4.5)	
Prematurely stopped	76 (9.9)	6 (17.6)		4 (18.2)	
Treatment-related hospitalization	60 (7.8)	6 (17.6)		4 (18.2)	
Days of hospitalization, mean [range]	13 [1–76]	23 [6–36]		4 [2–5]	p=0.010[m]

[a] Wild-type patients receiving standard fluoropyrimidine dosages in chemoradiation therapy;

[b] *DPYD* variant allele carriers receiving standard fluoropyrimidine dosages in chemoradiation therapy;

[c] *DPYD* variant allele carriers receiving initially reduced fluoropyrimidine dosages according to current guidelines compared with standard fluoropyrimidine dosages used in chemoradiation therapy;

[d] Data of 332 patients in total, data of 5 patients in the group of *DPYD* variant allele carriers treated with a standard dose and data of 15 patients in the group of *DPYD* variant allele carriers who received dose reductions;

[e] Data of 239 patients in total, data of 3 patients in the group of *DPYD* variant allele carriers treated with a standard dose and data of 14 patients in the group of *DPYD* variant allele carriers who received dose reductions;

[f] GI toxicity includes diarrhoea, mucositis, nausea, vomiting;

[g] *P*-values shown are compared with wild-type patients;

[h] HEM toxicity includes neutropenia, thrombocytopenia, leukocytopenia;

[i] Overall toxicity includes diarrhoea, mucositis, nausea, vomiting, neutropenia, thrombocytopenia, leukocytopenia, anaemia, radiation dermatitis, HFS, cardio toxicity, fatigue and other toxicity;

[j] Dosages were reduced from 100 to 60-77%;

[k] Dosages were reduced from 70 to 45% and 100 to 50% (applying dosing guidelines 2 days after start of therapy);

[l] Dosages were increased from 30 to 46% and from 50 to 60%;

[m] *P*-values shown are compared with *DPYD* variant allele carriers who received a standard dose.

Abbreviations: DPYD: gene encoding dihydropyrimidine dehydrogenase; GI: gastrointestinal; HFS: hand-foot syndrome; HEM: haematological; WT: wild-type patients.

Case description

To illustrate the importance of dose reductions in *DPYD* variant allele carriers, we have shown the course of one *DPYD**2A carrier in Figure 2. This patient was excluded from the statistical analyses due to a substantially increased dose during treatment. Being one of the first *DPYD* variant allele carriers who received 50% dosed CRT, it was decided that the fluoropyrimidine dose would be titrated up to 100% if the patient would have no side-effects after two weeks. However, diarrhoea grade 1–2 was present, and the dose was increased to 83%. After four weeks, severe toxicity (diarrhoea, vomiting, nausea grade 3 and dermatitis grade 2) occurred and chemotherapy, and later radiotherapy, was stopped prematurely. The patient was hospitalised for 31 days, of which three days at the intensive care unit. After hospitalisation, the patient had to recover completely from toxicity for 39 days in a nursing home (rehabilitation). Although it cannot be excluded that toxicity would have evolved in the severity as was now shown at an 83% dose level when treated entirely with a 50% dose level, it is clear that the dose increase was most likely a reason for the development of severe toxicity.

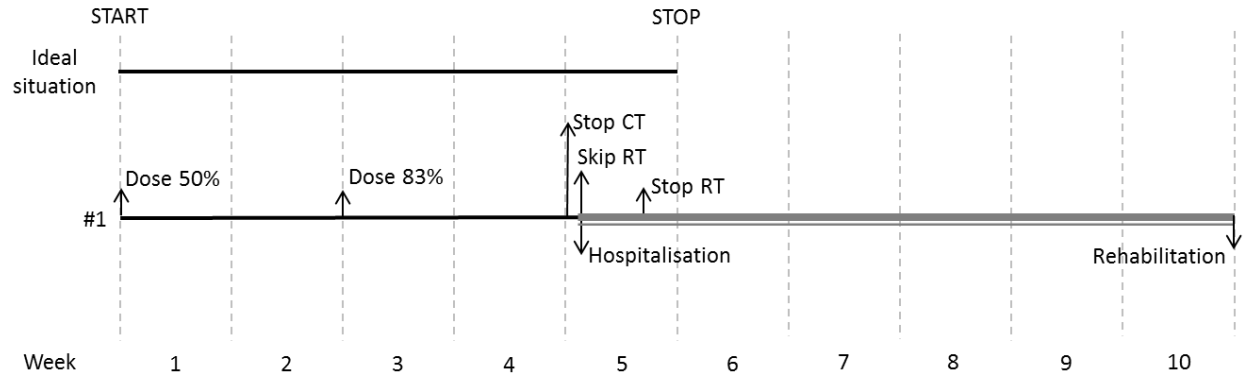

Figure 2. Course of treatment and toxicity

Shown is the course of an ideal treatment, and the treatment and toxicity for one patient (#1). The patient is a carrier of the *DPYD**2A variant and started therapy on a 50% dose. After two weeks, the dose was increased to 83%. Thereafter, the patient developed severe toxicity, and therapy was discontinued. The patient was hospitalised for 31 days (including three days at the intensive care unit) and had to recover completely from toxicity for 39 days in a nursing home (rehabilitation).

Abbreviations: CT: chemotherapy; RT: radiotherapy.

Discussion

Fluoropyrimidine dosages are lower in CRT compared with other fluoropyrimidine treatment regimens, and it is unclear if pharmacogenetic dose adjustments should be made for *DPYD* variant allele carriers receiving CRT. Dose titration in CRT is more difficult compared with other treatment regimens where the schedule contains so-called stop weeks. To our knowledge, this is the first study specifically investigating *DPYD* pharmacogenetics of fluoropyrimidines in CRT. *DPYD* variant allele carriers treated with standard fluoropyrimidine dosages in CRT showed a significantly increased risk to develop severe toxicity compared with wild-type patients. This indicates the need for pharmacogenetic dose reductions in CRT, despite the lower standard dosages.

Although over 800 patients are considered, the number of patients with a *DPYD* variant remains limited due to the low prevalence of *DPYD* variants. We were unable to show that the risk of toxicity in *DPYD* variant allele carriers who received dose reductions was equivalent to the risk of wild-type patients. Also, 85% of the *DPYD* variant allele carriers treated with a standard dose were carriers of the c.1236G>A and c.2846A>T variants. *DPYD**2A and *DPYD**13 carriers have a higher risk of toxicity when treated with standard dosages compared with c.1236G>A and c.2846A>T carriers. Therefore, it is possible that more toxicity could have occurred in this group if *DPYD* variants would have been equally distributed, increasing the difference in toxicity compared with the other study groups. Moreover, in the *DPYD* group with initial dose reductions, *DPYD* variants and corresponding dose reductions (25 versus 50%) were equally distributed.

Noteworthy, the number of hospitalisations due to toxicity was similar in both groups of *DPYD* variant allele carriers, yet the duration of hospitalisation was significantly shorter in *DPYD* variant allele carriers treated with a reduced fluoropyrimidine dose. A possible explanation for this could be that treating physicians are alarmed of a potentially increased risk of toxicity because of DPD deficiency and more rapidly decide to hospitalise a patient in response to signs of potential toxicity. A second explanation is that *DPYD* variant allele carriers who received dose reductions recovered faster of toxicity.

In two *DPYD* variant allele carriers who received initially reduced dosages and did not experience (severe) toxicity, the dose was increased during treatment. This shows that physicians might still have fear of underdosing patients and reducing efficacy of the treatment.

Grouped diagnosis was significantly associated to severe haematological toxicity, with more severe toxicity in pelvic malignancies. A possible explanation may be that more bone marrow is exposed to radiation in the pelvic area compared with other areas, increasing the chance of myelosuppression.

With over 800 patients included, this study provides a large amount of toxicity data of wild-type patients and *DPYD* variant allele carriers receiving CRT. However, our study has several limitations. First, three databases were combined and were partly retrospective, possibly introducing bias. However, each database included patients in each study group, limiting bias. General differences in scoring toxicity per database could exist; however, criteria for toxicity grades are well marked and should therefore be limited. One database used the new version of CTC-AE; however, updates did not influence the grading of toxicity of interest for this study.

Second, not all databases contained the full toxicity spectrum of interest in this study (e.g. nausea, vomiting, radiation dermatitis, fatigue); therefore, overall toxicity consisted of different toxicities per original database and was not used as a primary end-point.
And third, pharmacokinetic sampling was not executed in this study, which could have shown that dose reductions in *DPYD* variant allele carriers result in equivalent fluoropyrimidine metabolite plasma levels compared with wild-types treated with standard dosages, as was done previously for *DPYD**2A variant allele carriers.[19]

Conclusions

Our study is the first to show that *DPYD* variant allele carriers have an increased risk of severe toxicity when treated with standard dosages in CRT, indicating that dose reductions are necessary in these patients as well. The present study provides the only evidence at this time, and based on these data we advise that fluoropyrimidine dose reductions should also be applied in *DPYD* variant allele carriers who will start CRT to prevent severe fluoropyrimidine-induced toxicity.

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Supplement

Standard fluoropyrimidine dosages in chemoradiation therapy result in an increased risk of severe toxicity in *DPYD* variant allele carriers

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Supplementary table 1. Toxicity of study patients compared to patients from literature

Selected studies from literature had relatively large patient cohorts and reported toxicity extensively.

Type of event	Study[a] (*N*=674) %	Literature[1-3] %
Grade 2 Diarrhoea	17.4	3–20
Grade ≥3 Diarrhoea	8.6	2–7
Grade 2 Nausea[b]	3.8	2–10
Grade ≥3 Nausea[b]	1.1	-
Grade 2 Mucositis	2.1	7
Grade ≥3 Mucositis	0.7	-
Grade 2 Radiation dermatitis[b]	15.8	15–25
Grade ≥3 Radiation dermatitis[b]	2.3	0–9
Grade 2 Fatigue[c]	9.3	11
Grade ≥3 Fatigue[c]	1.7	2
Grade 2 HFS	3	3–4
Grade 3 HFS	0.6	-
Grade 2 Neutropenia	0.9	5–8
Grade ≥3 Neutropenia	0.7	1–2
Grade 2 Leukocytopenia	8.5	19–20
Grade ≥3 Leukocytopenia	1.6	1–2
Grade 2 Thrombocytopenia	0.3	-
Grade ≥3 Thrombocytopenia	0.3	-
Grade 2 Anaemia[b]	5.6	7–20
Grade ≥3 Anaemia[b]	0.4	-

[a] Selected from this study are all patients with a similar capecitabine schedule to referenced locally advanced rectal patients from literature (825 mg/m2 twice daily, for 5 weeks with or without weekends);

[b] Data of 266 patients;

[c] Data of 172 patients.

Abbreviations: HFS: hand-foot syndrome.

Supplementary table 2. Toxicity of patients from three original databases and of the combined database (total)

Type of event	DB#1:NKI (*N*=495) *N* (%)	DB#2:LUMC (*N*=239) *N* (%)	DB#3:CRO (*N*=93) *N* (%)	TOTAL (*N*=827) *N* (%)
Grade 2 Diarrhoea	56 (11.3)	50 (20.9)	24 (25.8)	130 (15.7)
Grade ≥3 Diarrhoea	43 (8.7)	19 (7.9)	4 (4.3)	66 (8)
Grade 2 Mucositis	15 (3)	5 (2.1)	1 (1.1)	21 (2.5)
Grade ≥3 Mucositis	3 (0.6)	4 (1.7)	-	7 (0.8)
Grade 2 Nausea	*ND*	14 (5.9)	2 (2.2)	16 (4.8)[a]
Grade ≥3 Nausea	*ND*	3 (1.3)	-	3 (0.9)[a]
Grade 2 Vomiting	*ND*	15 (6.3)	*ND*	15 (6.3)[b]
Grade ≥3 Vomiting	*ND*	2 (0.8)	*ND*	2 (0.8)[b]
Grade 2 Neutropenia	5 (1)	5 (2.1)	-	10 (1.2)
Grade ≥3 Neutropenia	10 (2)	5 (2.1)	1 (1.1)	16 (1.9)
Grade 2 Leukocytopenia	27 (5.5)	24 (10)	18 (19.4)	69 (8.3)
Grade ≥3 Leukocytopenia	10 (2)	7 (2.9)	6 (6.5)	23 (2.8)
Grade 2 Thrombocytopenia	3 (0.6)	3 (1.3)	1 (1.1)	7 (0.8)
Grade ≥3 Thrombocytopenia	2 (0.4)	3 (1.3)	-	5 (0.6)
Grade 2 Anaemia	*ND*	26 (10.9)	2 (2.2)	28 (8.4)[a]
Grade ≥3 Anaemia	*ND*	2 (0.8)	-	2 (0.6)[a]
Grade 2 Radiation dermatitis	*ND*	80 (33.5)	3 (3.2)	83 (25)[a]
Grade ≥3 Radiation dermatitis	*ND*	9 (3.8)	4 (4.3)	13 (3.9)[a]
Grade 2 HFS	12 (2.4)	7 (2.9)	1 (1.1)	20 (2.4)
Grade 3 HFS	4 (0.8)	1 (0.4)	-	5 (0.6)
Grade 2 Cardio toxicity	10 (2)	7 (2.9)	4 (4.3)	21 (2.5)
Grade ≥3 Cardio toxicity	5 (1)	1 (0.4)	5 (5.4)	11 (1.3)
Grade 2 Fatigue	*ND*	33 (13.8)	*ND*	33 (13.8)[b]
Grade ≥3 Fatigue	*ND*	4 (1.7)	*ND*	4 (1.7)[b]
Grade 2 Other toxicity[c]	*ND*	13 (5.4)	38 (40.9)	51 (15.4)[a]
Grade ≥3 Other toxicity[c]	*ND*	7 (2.9)	6 (6.5)	13 (3.9)[a]
Grouped type of events				
Grade 2 GI toxicity[d]	67 (13.5)	56 (23.4)	26 (28)	149 (18)
Grade ≥3 GI toxicity[d]	44 (8.9)	22 (9.2)	4 (4.3)	70 (8.5)
Grade 2 HEM toxicity[e]	29 (5.9)	24 (10)	18 (19.4)	71 (8.6)
Grade ≥3 HEM toxicity[e]	12 (2.4)	10 (4.2)	6 (6.5)	28 (3.4)
Grade 2 Overall toxicity[f]	93 (18.8)	118 (49.4)	55 (59.1)	266 (32.2)
Grade ≥3 Overall toxicity[f]	61 (12.3)	38 (15.9)	19 (20.4)	118 (14.3)

[a] Data of 332 patients;

[b] Data of 239 patients;

[c] Other toxicity includes anal pain, chronic enteritis, cystitis (genitourinary tract), dizziness, dysgeusia, enterocolitis, fever, headache, multi-organ failure, papulopustular rash, proctitis, radio-enteritis, skin hyperpigmentation, stomatitis, thromboembolic event, ulcer of the small intestine;

[d] GI toxicity includes diarrhoea, mucositis, nausea, vomiting;

[e] HEM toxicity includes neutropenia, thrombocytopenia, leukocytopenia;

[f] Overall toxicity includes diarrhoea, mucositis, nausea, vomiting, neutropenia, thrombocytopenia, leukocytopenia, anaemia, radiation dermatitis, HFS, cardio toxicity, fatigue and other toxicity.

Abbreviations: CRO: Aviano National Cancer Institute; DB: database; GI: gastrointestinal; HFS: hand-foot syndrome; HEM: haematological; LUMC: Leiden University Medical Center; ND: not defined in database; NKI: Netherlands Cancer Institute.

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