# Personalised medicine of fluoropyrimidines using DPYD pharmacogenetics

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# CHAPTER 5

## *DPYD* genotype-guided dose individualisation of fluoropyrimidine therapy in patients with cancer: a prospective safety analysis

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**Abstract**

Fluoropyrimidine treatment can result in severe toxicity in up to 30% of patients and is often the result of reduced activity of the key metabolic enzyme dihydropyrimidine dehydrogenase (DPD), mostly caused by genetic *DPYD* variants. In a prospective clinical trial, we investigated whether upfront screening for four *DPYD* variants and *DPYD*-guided dose individualization can reduce fluoropyrimidine-induced toxicity.

Prospective genotyping of *DPYD**2A, c.2846A>T, c.1679T>G, and c.1236G>A was performed in adult cancer patients for which fluoropyrimidine-based chemotherapy was considered in their best interest. All patients about to start with a fluoropyrimidine regimen (capecitabine or 5-fluorouracil as single agent or in combination with other chemotherapeutic agents and/or radiotherapy) could be included in the study. Heterozygous *DPYD* variant allele carriers received an initial dose reduction of 25% (c.2846A>T, c.1236G>A) or 50% (*DPYD**2A, c.1679T>G), *DPYD* wild-type patients were treated according to standard of care. The primary endpoint of the study was the incidence of severe (CTC-AE grade≥3) overall fluoropyrimidine-related toxicity. This toxicity incidence was compared between *DPYD* variant allele carriers and *DPYD* wild-type patients in the study in an intention-to-treat analysis, and relative risks for severe toxicity were compared between the current study and a historical cohort of *DPYD* variant allele carriers treated with full dose fluoropyrimidine-based therapy (derived from a previously published meta-analysis). This trial is registered under clinicaltrials.gov identifier NCT02324452 and is completed.

In total, 1,103 evaluable patients were enrolled, of whom 85 *DPYD* variant carriers (7.7%). Overall grade≥3 toxicity was higher in *DPYD* variant carriers than in wild-type patients (39% vs 23%, p=0.0013). The relative risk (RR) for grade≥3 toxicity was 1.31 (95% confidence interval [95%CI]:0.63–2.73) for genotype-guided dosing vs 2.87(95%CI:2.14–3.86) in the historical cohort for *DPYD**2A, no toxicity vs 4.30(95%CI:2.10–8.80) in c.1679T>G, 2.00(95%CI:1.19–3.34) vs 3.11(95%CI:2.25–4.28) for c.2846A>T, and 1.69(95%CI:1.18–2.42) vs 1.72(95%CI: 1.22–2.42) for c.1236G>A.

Upfront *DPYD* genotyping was feasible in routine clinical practice, and improved patient safety of fluoropyrimidine treatment. For *DPYD**2A and c.1679T>G carriers, a 50% initial dose reduction seems adequate. For c.1236G>A and c.2846A>T carriers, a larger dose reduction of 50% (instead of 25%) needs to be investigated. As fluoropyrimidines are among the most commonly used anticancer agents, the findings of this study are of high clinical importance, as they endorse implementing *DPYD* genotype-guided dosing as the new standard of care.

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**Introduction**

Fluoropyrimidine anticancer drugs, including 5-fluorouracil (5-FU) and its oral prodrug capecitabine, have been widely used for over sixty years in the treatment of different solid tumor types, such as colorectal, breast, and gastric cancer. Although these drugs are relatively well tolerated, up to 30% of patients experience severe treatment-related toxicity, including diarrhea, mucositis, myelosuppression, and hand-foot syndrome.[1-3] In addition, severe fluoropyrimidine-related toxicity can lead to treatment-related death in up to 1% of patients.[4,5] The occurrence of these severe side-effects can lead to treatment discontinuation and toxicity-related hospitalization, which in addition puts a heavy burden on health-care costs.



Fluoropyrimidine-related toxicity is often caused by reduced activity of the enzyme dihydropyrimidine dehydrogenase (DPD), the main metabolic enzyme for fluoropyrimidine inactivation.[6,7] A partial DPD deficiency (e.g. a ~50% reduced DPD activity compared to normal) is present in 3—5% of the Western population. These DPD deficient patients have a highly increased risk of developing severe treatment-related toxicity when treated with a standard dose of fluoropyrimidines.[8-10] Complete DPD deficiency is much rarer, with an estimated prevalence of 0.01—0.1%.[8,11,12] DPD deficiency is most often caused by genetic variants in *DPYD*, the gene encoding DPD. The four *DPYD* variants currently considered most clinically relevant and with convincingly demonstrated association with severe toxicity are *DPYD**2A (rs3918290, c.1905+1G>A, IVS14+1G>A), c.2846A>T (rs67376798, D949V), c.1679T>G (rs55886062, *DPYD**13, I560S), and c.1236G>A (rs56038477, E412E, in haplotype B3).[10,13,14] For these variants, available evidence suggests that heterozygous carriers of these variants have an average reduction in DPD enzyme activity of approximately 25% (c.2846A>T, c.1236G>A) to 50% (*DPYD**2A, c.1679T>G).[14]

Prospective *DPYD* genotyping and dose reduction in heterozygous *DPYD* variant allele carriers is a promising strategy for preventing severe and potentially fatal fluoropyrimidine-related toxicity without affecting treatment efficacy. In a previous study prospective genotyping and dose-individualization for one *DPYD* variant, *DPYD**2A, in a cohort of 1,631 patients showed that severe fluoropyrimidine-related toxicity could be decreased from 73% in *DPYD**2A carriers receiving a standard fluoropyrimidine dose (*N*=48) to 28% by genotype-guided dosing, i.e. *DPYD**2A carriers receiving a 50% dose reduction (*N*=18, p<0.001).[15] This study showed that by reducing the fluoropyrimidine dose by 50% in *DPYD**2A variant allele carriers, severe toxicity was reduced to a frequency (28%) comparable to that in *DPYD**2A wild-type patients treated with a standard fluoropyrimidine dose (23%).

It is expected that patient safety can be further improved by expanding the number of prospectively tested *DPYD* variants beyond *DPYD**2A alone. The objective of the current study was to assess the impact on patient safety of prospective screening for the four most relevant *DPYD* variants and subsequent *DPYD* genotype-guided dose individualization in daily clinical care.

## Patients and methods

### *Study design and participants*

This study was a prospective multicenter clinical trial in which 17 hospitals in the Netherlands participated. The study was approved by the institutional review board of The Netherlands Cancer Institute, Amsterdam, the Netherlands, and approval from the board of directors of each individual hospital was obtained for all participating centers. All patients provided written informed consent before enrollment in the study. Additional informed consent was obtained for *DPYD* variant allele carriers who participated in pharmacokinetic and DPD enzyme activity measurements.

The study population consisted of adult cancer patients (≥18 years) intended to start with a fluoropyrimidine-based anticancer therapy, either as single agent or in combination with other chemotherapeutic agents and/or radiotherapy. Patients with all tumor types for which fluoropyrimidine-based therapy was considered in their best interest could be included. Prior chemotherapy was allowed, except for prior use of fluoropyrimidines. Patients had to have a WHO performance status of 0, 1 or 2, a life expectancy of at least 12 weeks, and acceptable safety laboratory values (Supplementary methods). There were no restrictions on comorbidities, except for diseases expected to interfere with study or the patient's safety. Full inclusion and exclusion criteria can be found in the Supplementary methods.

### *Procedures*

*Treatment*

Patients were genotyped before start of fluoropyrimidine therapy for the previously mentioned four *DPYD* variants. Heterozygous *DPYD* variant allele carriers received an initial dose reduction of either 25% (for c.2846A>T and c.1236G>A) or 50% (for *DPYD**2A and c.1679T>G), in line with current recommendations from Dutch and international pharmacogenomic guidelines.[13,16] To achieve a maximal safe exposure, dose escalation was allowed after the first two cycles provided that treatment was well tolerated, and the decision to escalate was left to the discretion of the treating physician. The dose of other anticancer agents or radiotherapy were left unchanged at start of treatment. Homozygous or compound heterozygous *DPYD* variant allele carriers were excluded from the study and could be treated with personalized regimens outside this protocol.[17] Non-carriers of the above mentioned *DPYD* variants are considered wild-type patients in this study and were treated according to existing standard of care.

*Assessments*

Toxicity was graded by participating centers according to the National Cancer Institute common terminology criteria for adverse events (CTC-AE),[18] and severe toxicity was defined as grade 3 or higher. Patients were followed for toxicity during the entire treatment period and until toxicity was resolved. Toxicity scored by the treating physician or qualified nurse practitioner as possibly, probably or definitely related to fluoropyrimidine-treatment was considered treatment-related toxicity (definitions in the Supplementary methods). Toxicity-related hospitalization and treatment discontinuation due to adverse events were also investigated. Standard laboratory assessments were performed prior to start of treatment

and each new cycle according to routine clinical care, for evaluation of treatment safety.

*DPYD genotyping*
Genotyping of the four *DPYD* variants *DPYD**2A, c.2846A>T, c.1679T>G and c.1236G>A was performed before start of treatment. Genotyping was performed in a clinical laboratory of the local hospital or in one of the other participating centers of this trial. Validated assays were used and all laboratories participated in a Dutch national proficiency testing program for all four *DPYD* variants.[19]

*Pharmacokinetics and DPD enzyme activity*
In *DPYD* variant allele carriers who provided written informed consent for additional tests, plasma levels of capecitabine, 5-FU, and their metabolites were determined at the first day of a capecitabine/5-FU cycle (preferably the first cycle) to assess the pharmacokinetic profile in these patients. A validated ultra-performance liquid chromatography tandem mass-spectrometry (UPLC-MS/MS) method was used (details in the Supplementary methods). Results of pharmacokinetic parameters, including the area under the plasma concentration-time curve (AUC) and half-life ($t_{1/2}$) were calculated using non-compartmental analysis, and compared to control values derived from literature.[20]

DPD enzyme activity in peripheral blood mononuclear cells (PBMCs) was determined in a pretreatment sample in the *DPYD* variant allele carriers and compared to DPD enzyme activity measured in wild-type patients in this study, using a validated assay.[21]

***Outcomes***
The primary endpoint of the study was the frequency of severe overall fluoropyrimidine-related toxicity across the entire treatment duration. A comparison was made between the incidence of severe toxicity in *DPYD* variant allele carriers treated with reduced dose and in wild-type patients treated with standard dose in this study. In addition to this, the relative risk for severe toxicity of these *DPYD* variant allele carriers treated with reduced dose compared to non-carriers in the study was calculated. A comparison between this calculated relative risk and a similarly calculated relative risk for *DPYD* variant allele carriers treated with full dose in a historical cohort derived from a previously published meta-analysis[10] was made. Secondary endpoints included pharmacokinetics of capecitabine and 5-FU in *DPYD* variant allele carriers and measurements of DPD enzyme activity. Another secondary endpoint was a cost analysis on individualized dosing based on upfront *DPYD* genotyping, of which results will be reported separately.

***Statistical analysis***
The sample size was based on a one stage A'Hern (phase II) design[22] and calculated under the assumption that overall fluoropyrimidine-related severe toxicity could be reduced from 60% (in *DPYD* variant allele carriers receiving standard dose)[10,15] to 20% by individualized dosing in *DPYD* variant allele carriers. This resulted in a required sample size of eleven variant carriers. To reach this number of variant carriers, we used a single *DPYD* variant (c.2846A>T, assumed variant frequency of 1%) to calculate the total sample size, resulting in

a total expected sample size of 1,100 evaluable patients. Detailed information on the sample size calculation can be found in the Supplementary methods. Patients were considered evaluable when meeting the inclusion and exclusion criteria, and if they received at least one fluoropyrimidine drug administration.

Associations between dichotomous outcomes, e.g. occurrence of severe toxicity or hospitalization, and genotype status were tested using $\chi^2$ or Fisher's exact test (Fisher's exact test was chosen when the smallest cell count was 5 or lower; for this test the double one-tailed exact probability was reported). Baseline characteristics between *DPYD* variant allele carriers and wild-type patients in the study were compared using either $\chi^2$ test, Fisher's exact test or Kruskal-Wallis rank sum test depending on the type of variable. DPD enzyme activity was compared between carriers of individual *DPYD* variants and wild-type patients using Student's *t*-tests. *P*-values <0.05 were considered statistically significant. Statistical analyses on an intention-to-treat population were performed using SPSS (version 23.0) and R (version 3.1.2). This study is registered with ClinicalTrials.gov, number NCT02324452.

**Results**

***Patient and treatment characteristics***

Between April 30th, 2015 and December 21st, 2017, a total of 1,181 patients intended to start fluoropyrimidine-based treatment were enrolled in this study. In total, 78 patients were considered non-evaluable (Figure 1), as they retrospectively were identified as not meeting the inclusion criteria (*N*=48), did not start fluoropyrimidine-based treatment (*N*=26), or were homozygous or compound heterozygous *DPYD* variant allele carriers (*N*=4). This resulted in a total of 1,103 evaluable patients, of whom 85 were heterozygous *DPYD* variant allele carriers (7.7%). Baseline characteristics of *DPYD* variant allele carriers and *DPYD* wild-type patients are described in Table 1 and in the Supplementary Table 1. The most common tumor type was colorectal cancer (64%). In total, 83% of patients were treated with a capecitabine-based regimen.

Mean relative dose intensities for each patient group are presented in Table 2. In general, dose recommendations as described in the study protocol were followed by the treating physicians, which resulted in mean dose intensities in the first cycle of 74%, 73%, 51%, and 50% for c.1236G>A, c.2846A>T, *DPYD**2A and c.1679T>G, respectively. The performed dose reductions were therefore in line with the pre-specified dose reductions of 25% (for c.1236G>A and c.2846A>T) or 50% (for *DPYD**2A and c.1679T>G). However, for four patients carrying *DPYD* variants, dose reductions were not applied at start of treatment (Supplementary results). One of these patients, (c.2846A>T carrier) was treated by mistake with a full capecitabine dose for the first two cycles, which resulted in fatal fluoropyrimidine-related toxicity. Although dosing recommendations were not followed in these four patients, all results were included in the analysis (intention-to-treat analysis).

Doses were escalated during treatment in eleven out of 85 *DPYD* variant allele carriers (13%). In five of these patients (two *DPYD**2A and three c.1236G>A carriers) the higher dose was not well tolerated, leading to a dose reduction. Also, one patient (c.2846A>T carrier) discontinued treatment after the dose escalation due to toxicity. Five patients (one c.2846A>T, one c.1236G>A, one c.1679T>G, and two *DPYD**2A carriers) were able to

continue treatment with the escalated dose.

The median follow-up period (similar to the entire treatment duration or when toxicity was resolved) was 71 days (interquartile range [IQR]: 36—161 days). For wild-type patients median follow-up was 69 days (IQR 36—161 days) and for *DPYD* variant allele carriers 90 days (IQR 35—168 days).

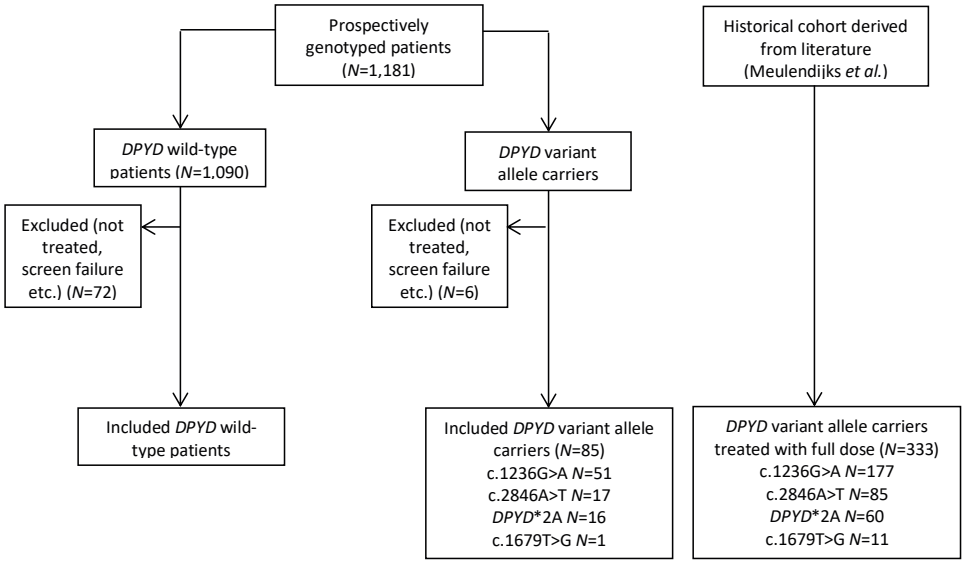


**Figure 1. Consort diagram of included patients**

***Toxicity in DPYD variant allele carriers versus wild-type patients***

Frequencies of severe toxicity for *DPYD* variant allele carriers who received genotype-guided dosing and wild-type patients who received standard dosing are depicted in Table 2. A total of 33 out of 85 (39%) *DPYD* variant allele carriers experienced severe (grade ≥3) fluoropyrimidine-related toxicity, which was significantly higher than the frequency in wild-type patients (23%, p=0.0013). The incidence of grade ≥4 toxicity was low but was comparable between both groups as well (four out of 85 (5%) for *DPYD* variant allele carriers vs 29 out of 1,018 3% for wild-type patients, p=0.49, Table 2).

The percentage of toxicity in *DPYD* variant allele carriers was mainly driven by the two most common variants, who also had higher toxicity frequencies. In total, 20 out of 51 c.1236G>A carriers experienced severe toxicity (39%) and eight out of 17 c.2846A>T carriers (47%). For *DPYD**2A carriers, five out of 16 patients (31%) experienced severe toxicity. The single c.1679T>G carrier, who did receive reduced-dose treatment, tolerated the treatment well and did not experience severe treatment-related toxicity over the course of treatment (three cycles).

For 16 out of 85 *DPYD* variant allele carriers (19%) fluoropyrimidine-related toxicity resulted in hospitalization, compared to 140 out of 1,018 wild-type patients (14%, p=0.26). Median

duration of hospitalization was five days for both *DPYD* variant allele carriers and wild-type patients (IQR 3—7 days, and 3—10 days, respectively). For 15 out of 85 *DPYD* variant allele carriers (18%) fluoropyrimidine treatment was stopped due to fluoropyrimidine-related toxicity, compared to 175 out of 1,018 wild-type patients (17%), which was comparable between both groups (p=1.0).

As described above, one c.2846A>T carrier experienced fatal fluoropyrimidine-related toxicity, but the intended dose reductions were not applied for this patient. When disregarding this patient for the critical protocol violation, no treatment-related death occurred in *DPYD* variant allele carriers. In the wild-type cohort, three patients died due to fluoropyrimidine-related toxicity (0.3%), which is comparable to literature.[4,5]

**Table 1. Demographic and clinical characteristics of patients**

| **Characteristic** | ***DPYD* variant allele carriers** | **Wild-type patients** | **Total** | **P-value**[a] |
|---|---|---|---|---|
| | ***N*=85** | ***N*=1,018** | ***N*=1,103** | |
| Sex | | | | |
| *Male* | 48 (56%) | 545 (54%) | 593 (54%) | 0.68 |
| *Female* | 37 (44%) | 473 (46%) | 510 (46%) | |
| Age | | | | |
| *Median [IQR]* | 63 [54—71] | 64 [56—71] | 64 [56—71] | 0.61 |
| Ethnic origin | | | | |
| *Caucasian* | 84 (99%) | 964 (95%) | 1,048 (95%) | 0.61 |
| *African* | 0 | 19 (2%) | 19 (2%) | |
| *Asian* | 1 (1%) | 23 (2%) | 24 (2%) | |
| *Other*[b] | 0 | 12 (1%) | 12 (1%) | |
| Tumor type | | | | |
| *Non-metastatic CRC* | 32 (38%) | 440 (43%) | 472 (43%) | 0.48 |
| *Metastatic CRC* | 24 (28%) | 208 (20%) | 232 (21%) | |
| *BC* | 10 (12%) | 131 (13%) | 141 (13%) | |
| *GC* | 6 (7%) | 57 (6%) | 63 (6%) | |
| *Other*[c] | 13 (15%) | 182 (18%) | 195 (18%) | |
| Type of treatment regimen | | | | |
| *CAP mono* | 14 (16%) | 191 (19%) | 205 (19%) | 0.40 |
| *CAP + RT* | 18 (21%) | 246 (24%) | 264 (24%) | |
| *CAPOX* | 31 (36%) | 343 (34%) | 374 (34%) | |
| *CAP other* | 5 (6%) | 67 (7%) | 72 (7%) | |
| *5-FU mono* | 1 (1%) | 1 (0%) | 2 (0%) | |
| *5-FU + RT* | 6 (7%) | 57 (6%) | 63 (6%) | |
| *FOLFOX* | 5 (6%) | 38 (4%) | 43 (4%) | 0.40 |
| *5-FU other* | 5 (6%) | 75 (7%) | 80 (7%) | |
| BSA | | | | |
| *Median [IQR]* | 1.9 [1.8—2.1] | 1.9 [1.8—2.1] | 1.9 [1.8—2.1] | 0.60 |

*table continues*

| Characteristic | *DPYD* variant allele carriers *N*=85 | Wild-type patients *N*=1,018 | Total *N*=1,103 | P-value[a] |
|---|---|---|---|---|
| WHO performance status | | | | |
| *0* | 39 (46%) | 515 (51%) | 554 (50%) | 0.68 |
| *1* | 36 (42%) | 412 (40%) | 448 (41%) | |
| *2* | 4 (5%) | 38 (4%) | 42 (4%) | |
| *NS* [d] | 6 (7%) | 53 (5%) | 59 (5%) | |
| Number of treatment cycles | | | | |
| *Median [IQR]* | 4 [1—8] | 3 [1—8] | 3 [1—8] | 0.97 |
| *DPYD* status | | | | |
| *Wild-type* | 0 | 1,018 (100%) | 1,018 (92%) | NA |
| *c.1236G>A heterozygous* | 51 (60%) | 0 | 51 (5%) | |
| *c.2846A>T heterozygous* | 17 (20%) | 0 | 17 (2%) | |
| *DPYD*2A heterozygous* | 16 (19%) | 0 | 16 (1%) | |
| *c.1679T>G heterozygous* | 1 (1%) | 0 | 1 | |

[a] P-value comparing *DPYD* variant allele carriers to *DPYD* wild-type patients. A Kruskal-Wallis rank sum test was used for age, BSA, and number of treatment cycles, a Fisher's exact test was used for ethnic origin and WHO performance status and a $\chi^2$ test for sex, tumor type, and treatment regimen;

[b] Other ethnic origins included Hispanic descent, mixed-racial parentage and unknown ethnic origin;

[c] Other tumor types included anal cancer, esophageal cancer, head and neck cancer, pancreas cancer, bladder cancer, unknown primary tumor, vulva carcinoma, and several rare tumor types;

[d] WHO performance status was not specified for these patients, but was either 0, 1, or 2, as this was required by the inclusion criteria of the study.

*Abbreviations:* 5-FU mono: 5-fluorouracil monotherapy; 5-FU other: 5-fluorouracil combined with other anticancer drugs (excluding the FOLFOX regimen); 5-FU + RT: 5-fluorouracil combined with radiotherapy (with or without mitomycin); BC: breast cancer; BSA: body surface area; CAP mono: capecitabine monotherapy (with or without bevacizumab); CAPOX: capecitabine combined with oxaliplatin (with or without bevacizumab); CAP other: capecitabine combined with other anticancer drugs; CAP + RT: capecitabine combined with radiotherapy (with or without mitomycin); CRC: colorectal cancer; *DPYD*: gene encoding dihydropyrimidine dehydrogenase; FOLFOX: 5-fluorouracil combined with oxaliplatin and leucovorin (with or without bevacizumab); GC: gastric cancer; IQR: interquartile range; NA: not applicable; NS: not specified.

**Table 2. Treatment outcome of patients included in this study**

| Type of event | *DPYD* variant allele carriers | Wild-type patients | P-value | c.1236G>A | c.2846A>T | *DPYD**2A | c.1679T>G |
|---|---|---|---|---|---|---|---|
| | *N*=85 | *N*=1,018 | | *N*=51 | *N*=17 | *N*=16 | *N*=1 |
| Relative dose intensity whole treatment *Mean [range]*[c] | 69.1% [36.7—96.6%] | 94.1% [48.8—127.6%] | NA | 73.6% [50.9—96.6%] | 71.6% [48.8—96.2%] | 52..9% [36.7—74.1%] | 54.2% |
| Relative dose intensity first cycle *Mean [range]*[c] | 69.3% [24.8—96.2%] | 96.3% [37.2—127.6%] | NA | 74.0% [50.9—87.5%] | 73.4% [55.3—96.2%] | 51.1% [24.8—81.5%] | 50.0% |
| Overall grade≥3 toxicity[d] | 33 (39%) | 231 (23%) | 0.0013[a] | 20 (39%) | 8 (47%) | 5 (31%) | 0 |
| Grade≥3 gastrointestinal toxicity | 17 (20%) | 86 (8%) | 0.00089[a] | 11 (22%) | 4 (24%) | 2 (13%) | 0 |
| Grade≥3 hematological toxicity | 13 (15%) | 65 (6%) | 0.0043[a] | 7 (14%) | 4 (24%) | 2 (13%) | 0 |
| Grade 3 hand-foot syndrome[e] | 1 (1%) | 36 (4%) | 0.41[b] | 0 | 1 (6%) | 0 | 0 |
| Grade≥3 cardiac toxicity | 1 (1%) | 9 (1%) | 1.0[b] | 1 (2%) | 0 | 0 | 0 |
| Grade≥3 other treatment-related toxicity | 9 (11%) | 78 (8%) | 0.45[a] | 7 (14%) | 1 (6%) | 1 (6%) | 0 |
| Overall grade ≥4 toxicity[d] | 4 (5%) | 29 (3%) | 0.49[b] | 3 (6%) | 1 (6%) | 0 | 0 |
| Grade≥4 gastrointestinal toxicity | 1 (1%) | 8 (1%) | 1.0[b] | 1 (2%) | 0 | 0 | 0 |
| Grade≥4 hematological toxicity | 1 (1%) | 12 (1%) | 1.0[b] | 1 (2%) | 0 | 0 | 0 |
| Grade≥4 cardiac toxicity | 0 | 1 (0%) | NA | 0 | 0 | 0 | 0 |
| Grade≥4 other treatment-related toxicity | 3 (4%) | 9 (1%) | 0.12[b] | 2 (4%) | 1 (6%) | 0 | 0 |
| Fluoropyrimidine-related hospitalization | 16 (19%) | 140 (14%) | 0.26[a] | 10 (20%) | 4 (24%) | 2 (13%) | 0 |
| Stop of FP due to adverse events | 15 (18%) | 175 (17%) | 1.0[a] | 8 (16%) | 3 (18%) | 4 (25%) | 0 |
| Fluoropyrimidine-related death | 1 (1%)[f] | 3 (0%) | 0.55[b] | 0 | 1 (6%)[f] | 0 | 0 |

[a] P-value determined with $\chi^2$ test, with Yates' continuity correction;
[b] P-value determined with Fisher's exact test with one-sided probability (with the p-value multiplied by two);
[c] The relative dose intensity is calculated as the given dose in mg/m$^2$ divided by the standard dose in mg/m$^2$ given for the indication and treatment schedule which was applicable for the patient. The relative dose intensity was calculated for the first cycle alone and for the entire treatment duration;
[d] Overall toxicity includes all toxicities evaluated as possibly, probably or definitely related to fluoropyrimidine-treatment;

[e] Defined as palmar-plantar erythrodysesthesia syndrome by the common terminology criteria for adverse events (CTC-AE) version 4.03;[18]

[f] This patient (c.2846A>T carrier) was wrongly treated with a full capecitabine dose for two cycles, which resulted in fatal fluoropyrimidine-related toxicity.

*Abbreviations: DPYD*: gene encoding dihydropyrimidine dehydrogenase; FP: fluoropyrimidines; NA: not applicable.

***Toxicity of genotype-guided dosing versus standard dosing in DPYD variant allele carriers***

As another primary comparison, the relative risk for severe toxicity of *DPYD* variant allele carriers with genotype-guided dosing was compared with the corresponding relative risk for severe toxicity of *DPYD* variant allele carriers from a historical cohort of a previously performed meta-analysis.[10] *DPYD* variant allele carriers described in the meta-analysis were not identified prior to start of treatment and were therefore treated with a full dose. Relative risks for severe toxicity for each *DPYD* variant obtained in the meta-analysis[10] are described in Table 3 (incidences of toxicity can be found in the Supplementary Table 2) and were compared to calculated relative risks in the current study. This analysis showed that genotype-guided dosing reduced the relative risk for severe toxicity in *DPYD**2A carriers from 2.87 (95% confidence interval [95%CI]: 2.14—3.86)[10] when treated with full dose to 1.31 (95%CI: 0.63—2.73) when treated with individualized dose, thus showing a clinically relevant reduction of toxicity risk.



**Table 3. Relative risk for severe toxicity of *DPYD* variant carriers compared to a historical cohort**

| ***DPYD* variant** | ***DPYD* variant carriers treated with reduced dose (this study)** | ***DPYD* variant carriers treated with full dose (meta-analysis)** |
|---|---|---|
| | **Relative risk overall grade≥3 toxicity (95%CI)[a]** | **Relative risk overall grade≥3 toxicity (95%CI)[b]** |
| c.1236G>A | 1.69 (1.18–2.42) | 1.72 (1.22–2.42) |
| c.2846A>T | 2.00 (1.19–3.34) | 3.11 (2.25–4.28) |
| *DPYD**2A | 1.31 (0.63–2.73) | 2.87 (2.14–3.86) |
| c.1679T>G | NA[c] | 4.30 (2.10–8.80) |

[a] Relative risk for overall grade ≥3 fluoropyrimidine-related toxicity compared to non-carriers of this variant as described in Table 2;

[b] Relative risk for overall grade ≥3 fluoropyrimidine-related toxicity compared to non-carriers of this variant, as determined in a random-effects meta-analysis by Meulendijks *et al*.[10] Unadjusted relative risks for the meta-analysis are depicted, as the relative risk in the current study was also calculated as an unadjusted value (as patient numbers were low);

[c] Relative risk cannot be calculated as only one patient who carried c.1679T>G was present. This patient did not experience severe toxicity.

*Abbreviations:* 95%CI: 95% confidence interval; NA: not applicable.

Interestingly, for c.1236G>A and c.2846A>T, a reduction in toxicity risk comparable to that of *DPYD* wild-type patients could not be demonstrated. The risk for c.1236G>A in the historical cohort was 1.72 (95%CI: 1.22—2.42),[10] and in our study it was 1.69 (95%CI:

1.18—2.42), showing that the toxicity risk was still increased even when applying a 25% dose reduction. For c.2846A>T, the risk of severe toxicity determined in the meta-analysis was 3.11 (95%CI: 2.25—4.28),[10] which was decreased to 2.00 (95%CI: 1.19—3.34) after 25% dose reduction. However, this risk was still higher compared to non-carriers of this variant. For the c.1679T>G variant no relative risk could be calculated, as only one patient with this variant was included.

***Pharmacokinetics of DPYD-guided dosing***

A total of 26 *DPYD* variant allele carriers (of which 16 c.1236G>A carriers, five c.2846A>T carriers, four *DPYD**2A carriers and one c.1679T>G carrier) treated with a reduced fluoropyrimidine dose gave informed consent to draw blood for pharmacokinetic analysis. Mean AUC values of the *DPYD* variant allele carriers and control values are depicted in Figure 2. Mean exposure to capecitabine and all metabolites, including 5-FU, was comparable between patients dosed based on *DPYD* genotype and control values,[20] suggesting that mean drug exposure of all combined *DPYD* variant allele carriers treated with a reduced dose was adequate. However, in line with toxicity data, AUC values for 5-FU were markedly higher for c.1236G>A carriers and especially for c.2846A>T carriers, compared to *DPYD**2A and c.1679T>G carriers as shown in the Supplementary Table 3.

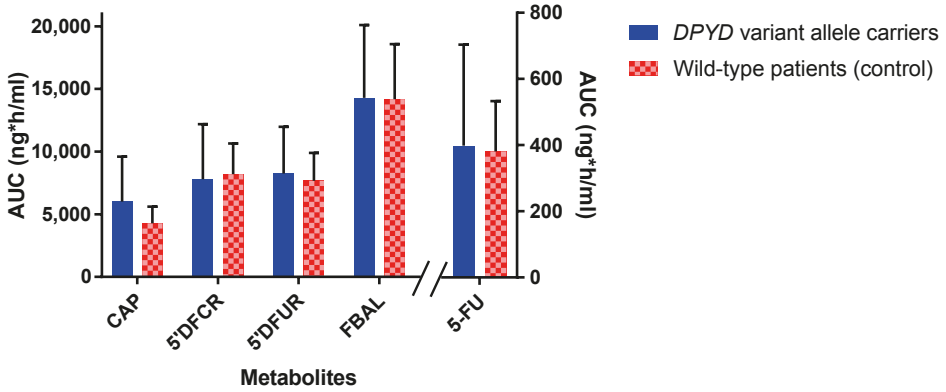


**Figure 2. Pharmacokinetics of *DPYD*-guided capecitabine dosing**

Depicted are the mean AUCs of capecitabine, and the metabolites 5'DFCR, 5'DFUR, 5-FU and FBAL of the *DPYD* variant allele carriers treated with *DPYD*-genotype guided dose (blue) and control values from wild-type patients from a published study (red).[20] Error bars represent the standard deviation.

*Abbreviations:* 5'DFCR: 5-deoxy-5-fluorocytidine; 5'DFUR: 5-deoxy-5-fluorouridine; 5-FU: 5-fluorouracil; AUC: area under the plasma concentration-time curve; CAP: capecitabine; FBAL: fluoro-β-alanine.

***DPD enzyme activity***

In 56 *DPYD* variant allele carriers and 82 wild-type patients (participating in a subgroup of the study where DPD phenotyping tests were investigated), pretreatment DPD enzyme

activity was determined (Figure 3). Mean DPD activity (with standard deviation) in *DPYD* wild-type patients was 9.4 (3.6) nmol/(mg*h), similar to as previously published.[23] For the c.1236G>A variant (*N*=35), the mean DPD activity was 7.5 (2.8) nmol/(mg*h) (i.e. a 20% reduction compared to wild-type). The mean DPD activity for c.2846A>T (*N*=12) was 6.2 (1.9) nmol/(mg*h) (34% reduction), and for *DPYD**2A (*N*=8) 5.2 (0.6) nmol/(mg*h) (45% reduction). The single patient carrying c.1679T>G had a DPD enzyme activity of 3.8 nmol/(mg*h) (60% reduction). For c.1236G>A, c.2846A>T, and *DPYD**2A, the mean DPD enzyme activity was significantly lower than the mean for wild-type patients. Statistical analysis was not possible for c.1679T>G. No correlation between DPD enzyme activity and the occurrence of severe fluoropyrimidine-related toxicity in *DPYD* variant allele carrying patients was seen (Figure 3 and Supplementary Table 4).



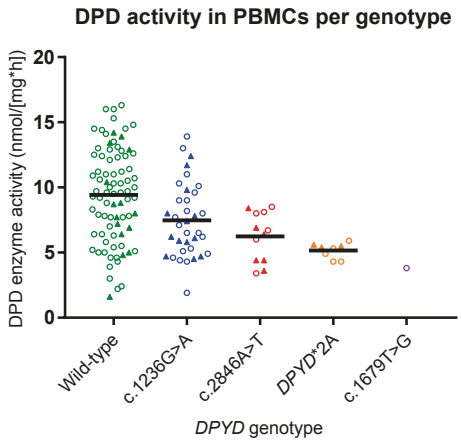


**Figure 3. DPD enzyme activity in *DPYD* variant allele carriers and wild-type patients**

Wild-type patients were wild-type for the four *DPYD* variants that were prospectively tested. Mean DPD enzyme activity was statistically significantly lower than wild-type (mean 9.4 (3.6) nmol/[mg*h]) for the *DPYD* variants as determined by a t-test: c.1236G>A (7.5 (2.8) nmol/[mg*h], p=0.0050), c.2846A>T (6.2 (1.9) nmol/[mg*h], p=0.0034), and *DPYD**2A (5.2 (0.6) nmol/[mg*h], p=0.0012). As only one patient carried c.1679T>G, no statistical test could be performed for this variant. However, the single measurement in this patient was in the range of DPD deficiency (3.8 nmol/[mg*h]). Patients with grade ≥3 fluoropyrimidine-related toxicity are depicted by closed triangles, patients without grade <3 toxicity by open circles; wild-type patients are treated with standard fluoropyrimidine doses, *DPYD* variant allele carriers with initially reduced doses according to protocol.

*Abbreviations:* DPD: dihydropyrimidine dehydrogenase; PBMCs: peripheral blood mononuclear cells.

## Discussion

This is, to our knowledge, the first prospective study to investigate the effect on fluoropyrimidine-related toxicity by dose individualization based on four *DPYD* variants. Our results demonstrate that genotype-guided dosing is feasible in clinical practice. Dose individualization markedly decreased the risk of severe toxicity for *DPYD**2A carriers, was

safe in the single c.1679T>G carrier, and moderately decreased the toxicity risk in c.2846A>T carriers. For c.1236G>A carriers, a 25% dose reduction was not enough to decrease severe treatment-related toxicity. This shows that *DPYD* genotype-guided dose-individualization is able to improve patient safety, as toxicity risk was reduced for three of the four variants in our study. Although sample sizes of variant allele carriers were modest and not all reductions in toxicity risk were statistically significant, these findings imply high clinical relevance. Also, implementation of *DPYD* genotype-guided dosing resulted in similar frequencies of toxicity-related hospitalization and discontinuation of treatment due to fluoropyrimidine-related toxicity for wild-type patients and *DPYD* variant allele carriers.

Interestingly, for *DPYD**2A carriers, the frequency of severe toxicity found in this study was 31%; drastically lower than the frequency in the historical cohort (72%). DPD enzyme activity measurements in this study showed that activity for *DPYD**2A carriers was approximately 50% reduced compared to wild-type patients, which endorses the dose recommendation of 50% for this variant.

As only one carrier of the rare c.1679T>G variant was identified in our current study, this made statistical comparisons impossible. However, while a relative risk for severe toxicity of 4.30 has been reported in literature, we showed that this patient did not experience severe toxicity in a completed treatment with 50% reduced dose. The DPD enzyme activity was about 50% decreased as well in this patient, which is in line with expectations based on previous studies.[24]

For carriers of the c.1236G>A and c.2846A>T variant, risk of severe toxicity remained relatively high despite dose individualization based on our dosing recommendations (25% reduction). In this study, 39% of the c.1236G>A carriers experienced severe toxicity and 47% of the c.2846A>T carriers. For these two variants, an initial dose reduction of 25% was applied in this study, because these variants are considered to have a less deleterious effect on DPD activity than the non-functional variants *DPYD**2A and c.1679T>G.[14,16] However, the Clinical Pharmacogenetics Implementation Consortium (CPIC) mentions that evidence is limited regarding the optimal degree of dose reduction for the decreased function variants c.1236G>A and c.2846A>T, and a 25% dosing recommendation is mainly based on one small retrospective study. Therefore, they advise a 25%—50% dose reduction in heterozygous c.1236G>A and c.2846A>T carriers.[13] Our current results suggest that applying 25% dose reduction might be insufficient for some patients, as toxicity risk was increased for carriers of c.1236G>A and c.2846A>T, compared to wild-type patients. In line with these findings, our pharmacokinetic analyses showed that exposure to 5-FU was markedly higher in c.2846A>T carriers than in *DPYD* wild-type controls. Exposure to 5-FU in the variant allele carriers was at least equal to levels observed in wild-type patients receiving standard dose, which is circumstantial evidence that the applied genotype-guided dose-reduction will not result in under-treatment. However, these pharmacokinetic results need to be interpreted with caution for some reasons. In patients with reduced DPD activity, 5-FU metabolism is affected, with 5-FU being the third metabolite derived from the parent compound capecitabine, which limits the interpretation of 5-FU exposure. Furthermore, pharmacokinetics of capecitabine and its metabolites exhibit a high inter-individual variability in exposure –even in wild-type patients– and are therefore difficult to interpret. In addition, based on the limited

number of patients with a *DPYD* variant of whom we also obtained pharmacokinetic data (Supplementary Table 3) firm conclusions on the basis of pharmacokinetic measurements alone cannot be drawn.

The mean DPD enzyme activity for c.1236G>A was approximately 20% reduced, but a large variation in DPD activity was found (Figure 3), which suggests that a proportion of patients needs a larger dose reduction, while other patients might even tolerate a full dose. This is also in line with the large variation in pharmacokinetic exposure seen in c.1236G>A carriers. Individual dose titration is important to ensure an adequate and safe dose for all patients. Therefore, we recommend a more cautious initial dose reduction of 50%, followed by close monitoring and individual dose titration.



The mean value for c.2846A>T DPD enzyme activity was approximately 35% reduced compared to normal. These DPD activity measurements show that 25% dose reduction might not be sufficient for most of the patients, and this could be an explanation for the higher toxicity risk in this patient group. A more cautious initial dose reduction of 50% should be considered in these patients as well.

In this study, initially reduced doses were escalated in eleven out of 85 (13%) *DPYD* variant allele carriers, although only five patients were able to tolerate this escalated dose. In *DPYD* wild-type patients dose escalations are uncommon in clinical practice (3% in our study, mostly patients who started with an initially reduced dose as a precaution measure).

Our study was performed in a daily clinical care setting in general regional hospitals and a few academic centers, demonstrating the feasibility of implementation of upfront *DPYD* screening. In order to make *DPYD*-guided dosing feasible in all hospitals, it is important that the turn-around time for *DPYD* genotyping is short to prevent a delay in the start of treatment. Participating laboratories in our study had a turn-around time of a few days to a maximum of a week.

A limitation of this study is that a historical cohort of *DPYD* variant allele carriers treated with full dose was used as control, and no direct comparison was made with a control cohort within the study. Inherently to this chosen design, differences between the study populations could have influenced the observed toxicity outcomes. However, this study design was chosen as a randomized clinical trial is considered unethical in this context, since it is known that *DPYD* variant allele carriers are at increased risk of severe toxicity when treated with a full dose of fluoropyrimidines.[25] A previously performed clinical study was stopped prematurely as a patient in the arm without dose individualization died due to treatment-related toxicity.[26]

This study focused on toxicity and did not evaluate survival or other effectiveness outcomes, as this was considered not feasible due to the large variation in tumor types and treatment regimens. We did, however, perform pharmacokinetic measurements, which suggest that applied dose reductions in *DPYD* variant allele carriers did not result in under-dosing.

The four *DPYD* variants investigated in this study are especially relevant to Caucasian populations. For ethnicities other than Caucasians, more research on the frequency and clinical relevance of these and other *DPYD* variants is recommended.[27] In our current study, homozygous and compound heterozygous *DPYD* variant allele carriers were not included and

were treated with individualized fluoropyrimidine dosing or alternative treatment outside this study.[17] However, for this group of patients *DPYD* genotype-guided dosing is of even greater importance than for heterozygous *DPYD* variant allele carriers, as these patients in general have less remaining DPD activity or even complete absence of DPD activity, and a full fluoropyrimidine dose, when not identified as DPD deficient patients, is therefore likely to be fatal.

Although our study revealed that the applied approach of genotype-guided adaptive dosing significantly reduced severe fluoropyrimidine-induced toxicity and prevented treatment related death, additional methods should be explored and prospectively tested to further reduce treatment related toxicity not only in poor metabolizers, but also in *DPYD* wild-type patients.

In conclusion, we showed safety of patients treated with fluoropyrimidines was improved by dose individualization based on *DPYD* genotype. Dose reduction of 50% in heterozygous *DPYD**2A and c.1679T>G carriers reduced toxicity risk markedly. The applied dose reductions of 25% in heterozygous c.1236G>A and c.2846A>T carriers appear to be insufficient to lower the risk of fluoropyrimidine-related toxicity to the background risk in wild-type patients. A larger initial dose reduction of 50% for c.2846A>T and c.1236G>A carriers with subsequent individual dose titrations should therefore be considered.

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# SUPPLEMENT CHAPTER 5

## *DPYD* genotype-guided dose individualisation of fluoropyrimidine therapy in patients with cancer: a prospective safety analysis

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**Contributed equally*

**Supplementary methods**

***Inclusion and exclusion criteria***

Patients with a pathologically confirmed malignancy for which treatment with a fluoropyrimidine drug was considered to be in the patient's best interest could be included in this study. Eligible patients were 18 years or older and were willing to undergo blood sampling for the purpose of this study (pharmacogenetic and phenotyping analysis). Patients had to have a WHO performance status of 0, 1 or 2, a life expectancy of at least 12 weeks, and acceptable safety laboratory values (neutrophil count of ≥1.5 x $10^9$/L, platelet count of ≥100 x $10^9$/L, hepatic function as defined by serum bilirubin ≤1.5 x upper limit of normal (ULN), alanine aminotransferase (ALAT), and aspartate aminotransferase (ASAT) ≤2.5 x ULN, or in case of liver metastases ALAT and ASAT≤5 x ULN, renal function as defined by serum creatinine ≤1.5 x ULN, or creatinine clearance ≥60 ml/min (by Cockcroft-Gault formula).

Exclusion criteria were prior treatment with fluoropyrimidines, patients with known substance abuse, psychotic disorders, and/or other diseases expected to interfere with study or the patient's safety, women who were pregnant or breast feeding, man and women who refused to use reliable contraceptive methods throughout the study, and patients with a homozygous polymorphic *DPYD* genotype or compound heterozygous *DPYD* genotype.

***Toxicity assessments***

For causality assessment of toxicity the following definitions were used:

- Possible: the event follows a reasonable temporal sequence from the time of drug administration, but could have been produced by other factors such as the patient's clinical state, other therapeutic interventions or concomitant drugs.
- Probable: the event follows a reasonable temporal sequence from the time of drug administration, and follows a known response pattern to the study drug. The toxicity cannot be reasonably explained by other factors such as the patient's clinical state, therapeutic interventions or concomitant drugs.
- Definite: the event follows a reasonable temporal sequence from the time of drug administration, and follows a known response pattern to the study drug, cannot be reasonably explained by other factors such as the patient's condition, therapeutic interventions or concomitant drugs; AND occurs immediately following study drug administration, improves on stopping the drug, or reappears on re-exposure.

***Sample size calculation***

A sample size calculation was made based on the primary aim of the study, which was to determine whether fluoropyrimidine-related severe toxicity can be reduced by individualized dosing in *DPYD* variant allele carriers compared to standard dosing in these patients. Using a one stage A'Hern (phase II) design and a null hypothesis of a probability of toxicity of 60% (the estimated severe treatment-related toxicity probability if *DPYD* variant allele carriers received standard dose)[1,2] and an alternative hypothesis of 20% (estimated toxicity probability of *DPYD* variant allele carriers receiving individualized dose), a sample size of eleven *DPYD* variant allele carriers would give a one-sided type I error probability α of 2.93% and power of 83.9%. It was decided that the frequency of c.2846A>T carriers (approximately

1.0%)[3] would determine the total number of patients required in the study. These patients would then arise from an expected minimum population of 1,100 treated patients. To account for a proportion of patients not evaluable for the study, the target accrual was set at 1,250 patients. Given the very low allele frequency of the c.1679T>G variant, it was considered not feasible to power this study for this particular variant. The estimated frequency of c.1236G>A is 3% and of *DPYD**2A 1%, which means that the calculated sample size would be adequate for those individual variants, or when analyzing all four variants together (estimated frequency of 5%).

***Pharmacokinetic analyses***



For pharmacokinetic analyses, peripheral blood was collected on the first day of treatment. Blood was collected in lithium heparin tubes at nine different time points up to eight hours after capecitabine intake (pre-dose, 0.25, 0.5, 1, 2, 3, 4, 6, and 8 hours after capecitabine intake). Samples were centrifuged immediately after the blood was drawn and plasma was stored at -80°C until analysis.

Capecitabine and the metabolites 5'-deoxy-5-fluorocytidine (5'DFCR), 5'-deoxy-5-fluorourdine (5'DFUR), 5-fluorouracil (5-FU), and fluoro-β-alanine (FBAL) were quantified in plasma samples using a validated ultra-performance liquid chromatography (UPLC)-tandem mass spectrometry (MS/MS) method. Lower limit of quantifications were 25 ng/ml for capecitabine, 10 ng/ml for 5'DFCR, 5'DFUR and 5-FU, and 50 ng/ml for FBAL. Stable isotopes were used as internal standard for all analytes. To a sample volume of 300 µl of plasma, 900 µl of methanol-acetonitrile (50:50 v/v) was added to precipitate the plasma proteins. Samples were vortex-mixed for 10 seconds, shaken for 10 minutes at 1,250 rpm and centrifuged at 14,000 rpm for 10 minutes. The clear supernatants were dried under a stream of nitrogen at 40°C and reconstituted in 100 µl of 0.1% formic acid in water. An Acquity UPLC® HSS T3 column (150 x 2.1 mm ID, 1.8 µlm particles) was used for chromatographic separation, at a flow rate of 300 µl/min and a gradient of 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B). The following gradient was applied: 100% A from 0—2.5 minutes, an increase from 0% to 90% B from 2.5—7.5 minutes, and 100% A from 7.5—9 minutes. For detection an API5500 triple quadruple mass spectrometer (Sciex) equipped with a turbo ionspray interphase was used, using optimized mass transitions *m/z* 360.0 → 243.9 for capecitabine, 244.9 → 128.8 for 5'DFUR, 128.9 → 42.1 for 5-FU, and 105.9 → 85.9 for FBAL.

Pharmacokinetic parameters were calculated using non-compartmental analysis and the calculated area under the plasma concentration-time curve (AUC) and half-life ($t_{1/2}$) were compared with pharmacokinetic data described in literature,[4] measured at the same laboratory as the current study.

***Data sharing statement***

Data collected in the study, including individual participant data, will not be made available to others, except to researchers involved in the study. However, upon request, data sharing for additional research is possible and will be supported. Requests will be judged on scientific and clinical rationale and may need to be reviewed by an authorized institutional review

board (IRB) prior to data sharing. The study protocol of this study is publicly available (as online supplement available with this publication).

**Supplementary results**

***Detailed information of DPYD variant allele carriers not treated according to dosing recommendations***

For four patients dosing recommendations were not followed according to protocol. One patient carrying *DPYD**2A started with a full dose as genotyping results were not awaited before start of treatment. After one week of treatment the *DPYD* genotyping result became available and the dose was reduced to 50%. The patient did not experience severe treatment-related toxicity in this course. However, from the third cycle onwards the dose was quickly titrated upwards (75% in the third cycle and 90% in the fourth cycle), hereafter treatment-related toxicity (anorexia grade 2, fatigue grade 3) occurred and the dose was reduced again. A second patient (*DPYD**2A carrier) also started with a full dose as genotyping results were not awaited before starting treatment. As results were known the following day, the patient had only taken a full dose for one day, which did not result in severe toxicity. The patient was treated with a 50% dose from the second day onwards. A third patient carrying c.2846A>T, used a full dose for four days, but continued with a 50% dose after an interruption of 5 days. The overall dose intensity of this cycle was approximately 55% and no toxicity occurred. The fourth patient (c.2846A>T carrier) was wrongly treated with a full dose for two cycles due to miscommunication with the patient. The patient experienced severe diarrhea, pancytopenia and sepsis, and passed away.

***Pharmacokinetic analyses***

A total of 26 *DPYD* variant allele carriers treated with reduced dose of capecitabine was included in the analysis. Pharmacokinetic results are shown in Supplementary Table 3. In 24 out 26 patients (92%) pharmacokinetic sampling was performed at day 1 of cycle 1. In two patients this was done at day 1 of another cycle, after a resting period of one week without capecitabine intake.

Of five patients who were treated with 5-FU, pharmacokinetic blood samplings was performed as well, but results were considered unreliable, most likely as drawing of blood was not done correctly. Results of the 5-FU treated patients are therefore not included in the analysis.

**Supplementary Table 1. Demographic and clinical characteristics of *DPYD* variant allele carriers**

| Characteristics | *DPYD* variant allele carriers | c.1236G>A | c.2846A>T | *DPYD**2A | c.1679T>G |
|---|---|---|---|---|---|
| | ***N*=85** | ***N*=51** | ***N*=17** | ***N*=16** | ***N*=1** |
| Sex | | | | | |
| *Male* | 48 (56%) | 26 (51%) | 11 (65%) | 10 (63%) | 1 (100%) |
| *Female* | 37 (44%) | 25 (49%) | 6 (35%) | 6 (38%) | 0 |
| Age | | | | | |
| *Median [IQR]* | 63 [54—71] | 62 [52—71] | 62 [53—72] | 64 [58—70] | 70 |
| Ethnic origin | | | | | |
| *Caucasian* | 84 (99%) | 51 (100%) | 17 (100%) | 15 (94%) | 1 (100%) |
| *African* | 0 | 0 | 0 | 0 | 0 |
| *Asian* | 1 (1%) | 0 | 0 | 1 (6%) | 0 |
| *Other*[a] | 0 | 0 | 0 | 0 | 0 |
| Tumor type | | | | | |
| *Non-metastatic CRC* | 32 (38%) | 15 (29%) | 7 (40%) | 9 (56%) | 1 (100%) |
| *Metastatic CRC* | 24 (28%) | 17 (33%) | 4 (24%) | 3 (19%) | 0 |
| *BC* | 10 (12%) | 5 (10%) | 3 (18%) | 2 (13%) | 0 |
| *GC* | 6 (7%) | 4 (8%) | 1 (6%) | 1 (6%) | 0 |
| *Other*[b] | 13 (15%) | 10 (20%) | 2 (12%) | 1 (6%) | 0 |
| Type of treatment regimen | | | | | |
| *CAP mono* | 14 (16%) | 8 (16%) | 4 (24%) | 2 (13%) | 0 |
| *CAP + RT* | 18 (21%) | 8 (16%) | 5 (29%) | 5 (31%) | 0 |
| *CAPOX* | 31 (36%) | 19 (37%) | 5 (29%) | 6 (38%) | 1 (100%) |
| *CAP other* | 5 (6%) | 3 (6%) | 1 (6%) | 1 (6%) | 0 |
| *5-FU mono* | 1 (1%) | 0 | 0 | 1 (6%) | 0 |
| *5-FU + RT* | 6 (7%) | 6 (12%) | 0 | 0 | 0 |
| *FOLFOX* | 5 (6%) | 2 (4%) | 2 (12%) | 1 (6%) | 0 |
| *5-FU other* | 5 (6%) | 5 (10%) | 0 | 0 | 0 |
| BSA | | | | | |
| *Median [IQR]* | 1.9 [1.8—2.1] | 1.9 [1.7—2.1] | 2.0 [1.7—2.1] | 2.0 [1.5—2.5] | 2.1 |
| WHO performance status | | | | | |
| *0* | 39 (46%) | 26 (51%) | 8 (47%) | 4 (25%) | 1 (100%) |
| *1* | 36 (42%) | 18 (35%) | 9 (53%) | 9 (56%) | 0 |
| *2* | 4 (5% | 3 (6%) | 0 | 1 (6%) | 0 |
| *NS*[c] | 6 (7%) | 4 (8%) | 0 | 2 (13%) | 0 |
| Number of treatment cycles | | | | | |
| *Median [IQR]* | 4 [1—8] | 4 [2—8] | 3 [1—7] | 3 [1—7] | 3 |

[a] Other ethnic origins included Hispanic descent, mixed-racial parentage and unknown ethnic origin;

[b] Other tumor types included anal cancer, esophageal cancer, head and neck cancer, pancreas cancer, bladder cancer, unknown primary tumor, vulva carcinoma, and several rare tumor types;

[c] WHO performance status was not specified for these patients, but was either 0, 1, or 2, as this was required by the inclusion criteria of the study.

*Abbreviations:* 5-FU mono: 5-fluorouracil monotherapy; 5-FU other: 5-fluorouracil combined with

other anticancer drugs (excluding the FOLFOX regimen); 5-FU + RT: 5-fluorouracil combined with radiotherapy (with or without mitomycin); BC: breast cancer; BSA: body surface area; CAP mono: capecitabine monotherapy (with or without bevacizumab); CAPOX: capecitabine combined with oxaliplatin (with or without bevacizumab); CAP other: capecitabine combined with other anticancer drugs; CAP + RT: capecitabine combined with radiotherapy (with or without mitomycin); CRC: colorectal cancer; *DPYD*: gene encoding dihydropyrimidine dehydrogenase; FOLFOX: 5-fluorouracil combined with oxaliplatin and leucovorin (with or without bevacizumab); GC: gastric cancer; IQR: interquartile range; NS: not specified.

**Supplementary Table 2. Incidences of severe toxicity in *DPYD* variant allele carriers in this study and the historical cohort**

| ***DPYD* variant** | ***DPYD* variant carriers treated with reduced dose (this study)** | ***DPYD* variant carriers treated with full dose (meta-analysis)** |
|---|---|---|
| | ***N* of patients with overall grade ≥3 toxicity / total *N* of patients with this variant (%)** | ***N* of patients with overall grade ≥3 toxicity / total *N* of patients with this variant (%)** |
| c.1236G>A | 20 / 51 (39%) | 65 / 177 (37%) |
| c.2846A>T | 8 / 17 (47%) | 53 / 85 (62%) |
| *DPYD**2A | 5 / 16 (31%) | 43 / 60 (72%) |
| c.1679T>G | 0 / 1 (0%) | 6 / 11 (55%) |

**Supplementary Table 3. Pharmacokinetic parameters of capecitabine and metabolites in *DPYD* variant allele carriers and controls**

| Metabolite | Mean $AUC_{0-\infty}$ (ng*h/ml) [CV%][a] *DPYD* variant allele carriers (*N*=26) | Wild-type control patients (*N*=23)[b] | Mean $T_{1/2}$ (h) [CV%] *DPYD* variant allele carriers (*N*=26) | Wild-type control patients (*N*=23)[b] |
|---|---|---|---|---|
| Capecitabine | 6007 [60%] | 4281 [31%] | 0.73 [49%] | 0.76 [55%] |
| 5'DFCR | 7792 [56%] | 8192 [30%] | 0.83 [43%] | 1.0 [45%] |
| 5'DFUR | 8243 [45%] | 7673 [29%] | 0.85 [38%] | 0.9 [34%] |
| 5-FU | 398 [77%] | 381 [40%] | 0.92 [112%] | 1.0 [57%] |
| FBAL | 14295 [41%] | 14177 [31%] | 2.2 [133%] | 2.6 [33%] |

| Metabolite | Mean $AUC_{0-\infty}$ (ng*h/ml) [CV%][a] c.1236G>A (*N*=16) | c.2846A>T (*N*=5) | *DPYD**2A (*N*=4) | c.1679T>G (*N*=1) | Mean $T_{1/2}$ (h) [CV%] c.1236G>A (*N*=16) | c.2846A>T (*N*=5) | *DPYD**2A (*N*=4) | c.1679T>G (*N*=1) |
|---|---|---|---|---|---|---|---|---|
| Capecitabine | 6579 [65%] | 5944 [26%] | 4460 [51%] | 3350 | 0.77 [48%] | 0.68 [50%] | 0.66 [61%] | 0.53 |
| 5'DFCR | 9162 [45%] | 8320 [50%] | 2552 [25%] | 4185 | 0.84 [51%] | 0.88 [28%] | 0.83 [21%] | 0.51 |
| 5'DFUR | 9319 [41%] | 8150 [46%] | 4824 [17%] | 5161 | 0.84 [38%] | 0.73 [41%] | 0.84 [19%] | 1.6 |
| 5-FU | 346 [49%] | 765 [64%] | 197 [54%] | 219 | 1.1 [120%] | 0.75 [47%] | 0.54 [20%] | 0.82 |
| FBAL | 16217 [30%] | 15627 [36%] | 6244 [18%] | 9082 | 2.9 [78%] | 2.5 [14%] | 2.2 [15%] | 2.9 |

[a] Note that for all metabolites the AUC is calculated until infinity ($AUC_{0-\infty}$, extrapolated from the last time point). Only for FBAL, the metabolite with the longest half-life, this resulted in a difference between the AUC until last time point ($AUC_{0-t}$) and $AUC_{0-\infty}$, with the $AUC_{0-\infty}$ being on average 26% higher than $AUC_{0-t}$. For the metabolites capecitabine, 5'DFCR, 5'DFUR and 5-FU the difference between $AUC_{0-t}$ and $AUC_{0-\infty}$ was respectively 0.5%, 0.8%, 1%, and 4%;

[b] Control values are derived from Deenen *et al.*[4] for patients with advanced cancer of the stomach or gastroesophageal junction after administration of capecitabine 850 mg/m2 (dose level 2 of the study).

*Abbreviations:* 5'DFCR: 5'-deoxy-5-fluorocytidine; 5'DFUR: 5'-deoxy-5-fluorouridine; 5-FU: 5-fluorouracil; AUC: area under the plasma concentration-time curve; CV%: coefficient of variation; FBAL: fluoro-β-alanine; $T_{1/2}$: half-life.

**Supplementary Table 4. DPD enzyme activity in patients with and without severe toxicity**

| ***DPYD* genotype** | **Patients without severe toxicity[a]** | | **Patients with severe toxicity[a]** | | **P-value[b]** |
|---|---|---|---|---|---|
| | **Mean activity (SD)** | ***N* of patients** | **Mean activity (SD)** | ***N* of patients** | |
| Wild-type | 9.6 (3.6) | 67 | 8.7 (3.7) | 15 | 0.36 |
| c.1236G>A | 7.6 (3.0) | 22 | 7.3 (2.6) | 13 | 0.79 |
| c.2846A>T | 6.8 (1.9) | 6 | 5.7 (1.8) | 6 | 0.33 |
| *DPYD**2A | 4.9 (0.7) | 5 | 5.5 (1.1) | 3 | 0.22 |
| c.1679T>G | NA | 1 | NA | 0 | NA |

[a] Severe toxicity is defined as CTC-AE grade 3 or higher;

[b] P-value determined with *t*-test.

*Abbreviations:* CTC-AE: common terminology criteria for adverse events; NA: not applicable.

**Supplementary Table 5. Overview of participating centers in this study**

| Center | Principal investigator | Number of eligible patients included |
|---|---|---|
| Erasmus Medical Center, Rotterdam, the Netherlands | Prof. Ron H.J. Mathijssen, MD | 264 |
| The Netherlands Cancer Institute, Amsterdam, the Netherlands | Prof. Jan H.M. Schellens, MD | 210 |
| Catharina Hospital, Eindhoven, the Netherlands | Geert-Jan Creemers, MD | 118 |
| Leiden University Medical Center, Leiden, the Netherlands | Prof. Hans Gelderblom, MD | 93 |
| Hospital Gelderse Vallei, Ede, the Netherlands | Arnold Baars, MD | 88 |
| Reinier de Graaf Hospital, Delft, the Netherlands[a] | Vincent O. Dezentjé, MD / Annelie J.E. Vulink, MD | 79 |
| Haaglanden Medical Center, the Hague, the Netherlands | Frank J.F. Jeurissen, MD | 46 |
| Deventer Hospital, Deventer, the Netherlands | Alexander L.T. Imholz, MD | 41 |
| Haga Hospital, the Hague, the Netherlands[a] | Prof. Johanna E.A. Portielje, MD / Danny Houtsma, MD | 35 |
| Maastricht University Medical Center, Maastricht, the Netherlands | Rob L.H. Jansen, MD | 28 |
| Franciscus Gasthuis and Vlietland, Rotterdam, the Netherlands | Paul Hamberg, MD | 24 |
| Amphia Hospital, Breda, the Netherlands | Albert J. ten Tije, MD | 20 |
| Bravis Hospital, Roosendaal, the Netherlands | Helga J. Droogendijk, MD | 17 |
| University Medical Center, Utrecht, the Netherlands | Prof. Miriam Koopman, MD | 14 |
| Wilhelmina Hospital, Assen, the Netherlands | Peter Nieboer, MD | 13 |
| Laurentius Hospital, Roermond, the Netherlands | Marlène H.W. van de Poel, MD | 9 |
| Canisius-Wilhelmina Hospital, the Netherlands | Caroline M.P.W. Mandigers, MD | 4 |

[a] In these centers the principal investigator was switched during the study.

## References

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