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

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Summary

Background 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 variants in the gene encoding DPD (*DPYD*). We assessed the effect of prospective screening for the four most relevant *DPYD* variants (*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]) on patient safety and subsequent *DPYD* genotype-guided dose individualisation in daily clinical care.

Methods In this prospective, multicentre, safety analysis in 17 hospitals in the Netherlands, the study population consisted of adult patients (≥18 years) with cancer who were intended to start on a fluoropyrimidine-based anticancer therapy (capecitabine or fluorouracil as single agent or in combination with other chemotherapeutic agents or radiotherapy). Patients with all tumour types for which fluoropyrimidine-based therapy was considered in their best interest were eligible. We did prospective genotyping for *DPYD**2A, c.2846A>T, c.1679T>G, and c.1236G>A. Heterozygous *DPYD* variant allele carriers received an initial dose reduction of 25% (c.2846A>T and c.1236G>A) or 50% (*DPYD**2A and c.1679T>G), and *DPYD* wild-type patients were treated according to the current standard of care. The primary endpoint of the study was the frequency of severe (National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03 grade ≥3) overall fluoropyrimidine-related toxicity across the entire treatment duration. We compared toxicity incidence between *DPYD* variant allele carriers and *DPYD* wild-type patients on an intention-to-treat basis, and relative risks (RRs) 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 with ClinicalTrials.gov, number NCT02324452, and is complete.

Findings Between April 30, 2015, and Dec 21, 2017, we enrolled 1181 patients. 78 patients were considered non-evaluable, because they were retrospectively identified as not meeting inclusion criteria, did not start fluoropyrimidine-based treatment, or were homozygous or compound heterozygous *DPYD* variant allele carriers. Of 1103 evaluable patients, 85 (8%) were heterozygous *DPYD* variant allele carriers, and 1018 (92%) were *DPYD* wild-type patients. Overall, fluoropyrimidine-related severe toxicity was higher in *DPYD* variant carriers (33 [39%] of 85 patients) than in wild-type patients (231 [23%] of 1018 patients; p=0·0013). The RR for severe fluoropyrimidine-related toxicity was 1·31 (95% CI 0·63–2·73) for genotype-guided dosing compared with 2·87 (2·14–3·86) in the historical cohort for *DPYD**2A carriers, no toxicity compared with 4·30 (2·10–8·80) in c.1679T>G carriers, 2·00 (1·19–3·34) compared with 3·11 (2·25–4·28) for c.2846A>T carriers, and 1·69 (1·18–2·42) compared with 1·72 (1·22–2·42) for c.1236G>A carriers.

Interpretation Prospective *DPYD* genotyping was feasible in routine clinical practice, and *DPYD* genotype-based dose reductions improved patient safety of fluoropyrimidine treatment. For *DPYD**2A and c.1679T>G carriers, a 50% initial dose reduction was adequate. For c.1236G>A and c.2846A>T carriers, a larger dose reduction of 50% (instead of 25%) requires investigation. Since fluoropyrimidines are among the most commonly used anticancer agents, these findings suggest that implementation of *DPYD* genotype-guided individualised dosing should be a new standard of care.

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Introduction

Fluoropyrimidine anticancer drugs, including fluorouracil and its oral prodrug capecitabine, have been widely used for more than 60 years in the treatment of different solid tumour types, such as colorectal, breast, and gastric cancers. Although these drugs are generally well

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Research in context

Evidence before this study

We identified ongoing and published studies before initiation of this study and during the development of the manuscript in ClinicalTrials.gov and PubMed with search terms "fluoropyrimidine* OR 5-fluorouracil OR capecitabine" AND "dihydropyrimidine dehydrogenase OR DPD OR *DPYD*" AND "pre-therapeutic OR pre-emptive OR prospective", with no limitations on language or publication date. Only clinical trials in which fluoropyrimidine dose was prospectively adjusted on the basis of dihydropyrimidine dehydrogenase (DPD) phenotype or the gene encoding DPD (*DPYD*) were selected. We identified three published clinical trials. These studies focused either on a single *DPYD**2A variant (*DPYD**2A), on DPD phenotype (measured as the dihydrouracil to uracil ratio), or on a combined approach. We identified no prospective studies that focused on genotype-guided dosing based on all four *DPYD* variants currently considered clinically relevant (*DPYD**2A, c.1679T>G, c.2846A>T, and c.1236G>A).

Added value of this study

In this study, which to our knowledge represents the first study on *DPYD* genotype-guided dose individualisation of fluoropyrimidine treatment based on screening for four *DPYD* variants, we showed that this strategy is feasible in clinical practice and, importantly, improves patient safety. We furthermore showed that the applied dose reductions of 25% for two of the four *DPYD* variants (c.2846A>T and c.1236G>A) were not sufficient to lower the risk of severe toxicity in these *DPYD* variant allele carriers, indicating that larger dose reductions might be required in these patients. Especially in c.1236G>A carriers, we found a large variation in DPD enzyme activity, clarifying why there is no consensus on the right degree of dose reduction for this variant.

Implications of all the available evidence

This prospective study of *DPYD* genotype-guided dosing of fluoropyrimidines, supported by existing evidence on the clinical validity of the investigated *DPYD* variants, confirms the value of upfront *DPYD* screening and *DPYD*-guided dose individualisation in patients treated with fluoropyrimidines. By implementing the investigated screening strategy in routine clinical care, patient safety of fluoropyrimidine treatment could be substantially improved. Further research is needed to investigate whether toxicity risk can be further reduced by applying larger dose reductions of 50%.

tolerated, up to 30% of patients have severe treatment-related toxicity, including diarrhoea, mucositis, myelosuppression, and hand-foot syndrome.[1–3] Additionally, severe fluoropyrimidine-related toxicity can lead to treatment-related death in up to 1% of patients.[4,5] These severe side-effects can lead to treatment discontinuation and toxicity-related hospital admission, which 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 (eg, around 50% reduced DPD activity compared with normal) is present in 3–5% of the North American and European population. Patients with DPD deficiency have an 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 the gene encoding DPD (*DPYD*). The four *DPYD* variants considered most clinically relevant and with statistically significant 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 around 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 the prevention of severe and potentially fatal fluoropyrimidine-related toxicity, without affecting treatment efficacy. In a previous study,[15] prospective genotyping and dose individualisation for one *DPYD* variant—*DPYD**2A—in a cohort of 1631 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 (*DPYD**2A carriers receiving a 50% dose reduction; n=18; p<0·001). This study[15] showed that by reducing the fluoropyrimidine dose by 50% in *DPYD**2A variant allele carriers, severe toxicity was reduced to a frequency (28%) comparable with that in *DPYD**2A wild-type patients treated with a standard fluoropyrimidine dose (23%).

Patient safety could be further improved by expanding the number of prospectively tested *DPYD* variants beyond *DPYD**2A alone. We assessed the effect of prospective screening for the four most clinically relevant *DPYD* variants on patient safety and subsequent *DPYD* genotype-guided dose individualisation in daily clinical care.

Methods

Study design and participants

This study was a prospective, multicentre, safety analysis in 17 hospitals in the Netherlands. The study population

consisted of adult patients (≥18 years) with cancer who were intended to start fluoropyrimidine-based anticancer therapy, either as a single agent or in combination with other chemotherapeutic agents, radiotherapy, or both. Patients with all tumour types for which fluoropyrimidine-based therapy was considered in their best interest were eligible. Previous chemotherapy was allowed, except for previous 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 (appendix p 2). There were no restrictions on comorbidities, except for diseases expected to interfere with the study or patient's safety. Full inclusion and exclusion criteria are listed in the study protocol (appendix p 10).

The study was approved by the institutional review board of The Netherlands Cancer Institute (Amsterdam, Netherlands) and approval from the board of directors of each individual hospital was obtained for all participating centres. All patients provided written, informed consent before enrolment in the study. Additional informed consent was obtained for *DPYD* variant allele carriers who participated in pharmacokinetic and DPD enzyme activity measurements.

Procedures

Patients were genotyped before the 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] Dose escalation was allowed after the first two cycles to achieve maximal safe exposure 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 was left unchanged at the start of treatment. Homozygous or compound heterozygous *DPYD* variant allele carriers were excluded from the study and could be treated with personalised regimens outside this protocol.[17] Non-carriers of the above-mentioned *DPYD* variants were considered wild-type patients in this study and were treated according to the current standard of care.

Toxicity was graded by participating centres according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03,[18] and severe toxicity was defined as adverse events that were grade 3 or worse. Patients were monitored for toxicity during the entire treatment period and until toxicity was resolved. Toxicities scored by the treating physician or qualified nurse practitioner as possibly, probably, or definitely related to fluoropyrimidine treatment were considered treatment-related (see appendix p 2 for definitions). We also investigated toxicity-related hospital admission and treatment discontinuation due to adverse events. Standard laboratory assessments were done before the start of treatment and each new cycle according to routine clinical care for evaluation of treatment safety.

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

In *DPYD* variant allele carriers who provided written, informed consent for additional tests, plasma concentrations of capecitabine, fluorouracil, and their metabolites were measured at the first day of a capecitabine or fluorouracil cycle (preferably the first cycle) to assess the pharmacokinetic profile in these patients. A validated ultra-performance liquid chromatography tandem mass-spectrometry method was used (appendix pp 2–3). We calculated the results of pharmacokinetic parameters, including the area under the plasma concentration–time curve and half-life ($t_{1/2}$), using non-compartmental analysis, and compared these with control values derived from the literature.[20]

We measured DPD enzyme activity in peripheral blood mononuclear cells in a pretreatment sample in *DPYD* variant allele carriers and compared these values with DPD enzyme activity measured in wild-type patients in this study using a validated assay.[21]

We compared the incidence of severe toxicity in *DPYD* variant allele carriers treated with reduced dose fluoropyrimidine and in wild-type patients treated with standard dose fluoropyrimidine. We also calculated the relative risk (RR) for severe toxicity of these *DPYD* variant allele carriers treated with reduced dose fluoropyrimidine compared with non-carriers. We made a comparison between our calculated RR and a similarly calculated RR for *DPYD* variant allele carriers treated with full dose fluoropyrimidine in a historical cohort derived from a previously published meta-analysis.[10]

Outcomes

The primary endpoint of the study was the frequency of severe overall fluoropyrimidine-related toxicity across the entire treatment duration. Secondary endpoints were pharmacokinetics of capecitabine and fluorouracil in *DPYD* variant allele carriers, measurements of DPD enzyme activity, and a cost analysis on individualised dosing based on upfront *DPYD* genotyping (the results of which will be reported separately).

Statistical analysis

The sample size was based on a one stage A'Hern (phase 2) 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 fluoropyrimidine)[10,15] to 20% by

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See Online for appendix

individualised dosing in *DPYD* variant allele carriers. This assumption resulted in a required sample size of 11 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 an expected sample size of 1100 evaluable patients. Detailed information on the sample size calculation is in the appendix (p 2). We considered patients evaluable when they met the inclusion criteria, and if they received at least one fluoropyrimidine drug dose.

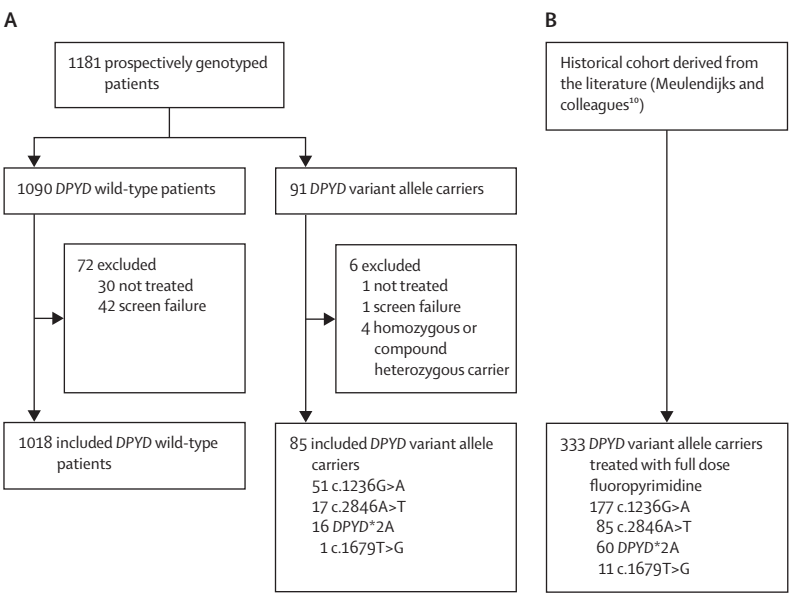

Figure 1: **Trial profile**
(A) Our study. (B) Previous meta-analysis by Meulendijks and colleagues.[10] *DPYD*=gene encoding dihydropyrimidine dehydrogenase.

	DPYD variant allele carriers (n=85)	Wild-type patients (n=1018)	Total (n=1103)	p value*
Sex				0·68
Male	48 (56%)	545 (54%)	593 (54%)	
Female	37 (44%)	473 (46%)	510 (46%)	
Age, years	63 (54–71)	64 (56–71)	64 (56–71)	0·61
Ethnic origin				0·61
White	84 (99%)	964 (95%)	1048 (95%)	
Black	0	19 (2%)	19 (2%)	
Asian	1 (1%)	23 (2%)	24 (2%)	
Other†	0	12 (1%)	12 (1%)	
Tumour type				0·48
Non-metastatic colorectal cancer	32 (38%)	440 (43%)	472 (43%)	
Metastatic colorectal cancer	24 (28%)	208 (20%)	232 (21%)	
Breast cancer	10 (12%)	131 (13%)	141 (13%)	
Gastric cancer	6 (7%)	57 (6%)	63 (6%)	
Other‡	13 (15%)	182 (18%)	195 (18%)	

(Table 1 continues on next page)

We tested associations between dichotomous outcomes (eg, occurrence of severe toxicity or hospital admission) and genotype status using χ^2 or Fisher's exact test (Fisher's exact test was chosen when the smallest cell count was five or lower; for this test, we reported the double one-tailed exact probability). We compared baseline characteristics between *DPYD* variant allele carriers and wild-type patients with either the χ^2 test, Fisher's exact test, or the Kruskal-Wallis rank-sum test, depending on the type of variable. We compared DPD enzyme activity between carriers of individual *DPYD* variants and wild-type patients using Student's *t* tests. $p<0·05$ was considered to indicate a statistically significant difference. We did statistical analyses on an intention-to-treat basis. Analyses were done with SPSS (version 22.0) and R (version 3.1.2). This study is registered with ClinicalTrials.gov, number NCT02324452.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. LMH and JHMS had full access to all the data in the study, and JHMS had final responsibility for the decision to submit for publication.

Results

Between April 30, 2015, and Dec 21, 2017, we enrolled 1181 patients intended to start fluoropyrimidine-based treatment. 78 patients were considered non-evaluable (figure 1), because they were identified as not meeting inclusion criteria after enrolment, did not start fluoropyrimidine-based treatment, or were homozygous or compound heterozygous *DPYD* variant allele carriers. 1103 patients were evaluable, of whom 85 (8%) were heterozygous *DPYD* variant allele carriers and 1018 (92%) were *DPYD* wild-type patients. Baseline characteristics of *DPYD* variant allele carriers and *DPYD* wild-type patients are described in table 1 and the appendix (p 4). The most common tumour type was colorectal cancer. 915 (83%) of 1103 patients were treated with a capecitabine-based regimen.

Generally, 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% for c.1236G>A, 73% for c.2846A>T, 51% for *DPYD**2A, and 50% for c.1679T>G (table 2). Dose reductions were therefore in line with the prespecified 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 with *DPYD* variants, dose reductions were not applied at the start of treatment (appendix p 3). One of these patients (c.2846A>T carrier) was treated with a full capecitabine dose for the first two cycles by mistake, which caused fatal fluoropyrimidine-related toxicity. Although dosing recommendations were not followed in these four patients, they were included in the intention-to-treat analysis.

Doses were escalated during treatment in 11 (13%) of 85 *DPYD* variant allele carriers. In five of these patients (two *DPYD**2A carriers and three c.1236G>A carriers), the higher dose was not well tolerated, leading to a dose reduction. One patient (c.2846A>T carrier) discontinued treatment after dose escalation because of toxicity. Five patients (one c.2846A>T carrier, one c.1236G>A carrier, one c.1679T>G carrier, and two *DPYD**2A carriers) were able to continue treatment with the escalated dose. In *DPYD* wild-type patients, dose escalations were uncommon in clinical practice (3% in our study, mostly in patients who started with an initially reduced dose as a precaution measure).

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

33 (39%) of 85 *DPYD* variant allele carriers had severe (grade ≥3) fluoropyrimidine-related toxicity, which was significantly higher than the proportion of wild-type patients with severe fluoropyrimidine-related toxicity (231 [23%] of 1018; p=0·0013; table 2). The incidence of grade 4 or greater toxicity was low, but similar between both groups (four [5%] of 85 *DPYD* variant allele carriers *vs* 29 [3%] of 1018 wild-type patients; p=0·49; table 2).

Toxicity in *DPYD* variant allele carriers was mainly driven by the two most common variants—c.1236G>A and c.2846A>T—carriers of which also had higher toxicity frequencies. 20 (39%) of 51 c.1236G>A carriers and eight (47%) of 17 c.2846A>T carriers had severe (grade ≥3) toxicity. For *DPYD**2A carriers, five (31%) of 16 patients had severe toxicity. The single c.1679T>G carrier who received reduced-dose treatment tolerated treatment well and did not have severe treatment-related toxicity over the course of treatment (three cycles).

For 16 (19%) of 85 *DPYD* variant allele carriers, fluoropyrimidine-related toxicity resulted in hospital admission, compared with 140 (14%) of 1018 wild-type patients (p=0·26). Median duration of hospital admission was 5 days (IQR 3–7) for *DPYD* variant allele carriers and 5 days (3–10) for wild-type patients. For 15 (18%) of 85 *DPYD* variant allele carriers, fluoropyrimidine treatment was stopped because of fluoropyrimidine-related toxicity, compared with 175 (17%) of 1018 wild-type patients.

When disregarding the c.2846A>T carrier who had fatal fluoropyrimidine-related toxicity because of crucial protocol violation, no treatment-related deaths occurred in *DPYD* variant allele carriers. In the wild-type cohort, three patients died because of fluoropyrimidine-related toxicity (<1%), which is similar to previously reported data.[4,5]

We compared the RR for severe toxicity of *DPYD* variant allele carriers with genotype-guided dosing with the RR for severe toxicity of *DPYD* variant allele carriers from a historical cohort from a meta-analysis.[10] In the meta-analysis,[10] *DPYD* variant allele carriers were not identified before the start of treatment and were therefore treated with a full dose of fluoropyrimidine. We compared RRs for severe toxicity for each *DPYD* variant obtained in the meta-analysis[10] with calculated RRs for this study (table 3, appendix p 5). We found that genotype-guided dosing reduced the RR of severe toxicity in *DPYD**2A carriers from 2·87 (95% CI 2·14–3·86)[10] when treated with full dose fluoropyrimidine to 1·31 (0·63–2·73) when treated with individualised dose, thus showing a clinically relevant reduction of toxicity risk.

For c.1236G>A and c.2846A>T we did not identify a reduction in toxicity risk comparable with that of *DPYD*

	DPYD variant allele carriers (n=85)	Wild-type patients (n=1018)	Total (n=1103)	p value*
(Continued from previous page)				
Type of treatment regimen				0·40
Capecitabine monotherapy (with or without bevacizumab)	14 (16%)	191 (19%)	205 (19%)	
Capecitabine combined with radiotherapy (with or without mitomycin)	18 (21%)	246 (24%)	264 (24%)	
Capecitabine combined with oxaliplatin (with or without bevacizumab)	31 (36%)	343 (34%)	374 (34%)	
Capecitabine combined with other anticancer drugs	5 (6%)	67 (7%)	72 (7%)	
Fluorouracil monotherapy	1 (1%)	1 (<1%)	2 (<1%)	
Fluorouracil combined with radiotherapy (with or without mitomycin)	6 (7%)	57 (6%)	63 (6%)	
Fluorouracil combined with oxaliplatin and folinic acid (with or without bevacizumab)	5 (6%)	38 (4%)	43 (4%)	
Fluorouracil combined with other anticancer drugs	5 (6%)	75 (7%)	80 (7%)	
Body surface area	1·9 (1·8–2·1)	1·9 (1·8–2·1)	1·9 (1·8–2·1)	0·60
WHO performance status				0·68
0	39 (46%)	515 (51%)	554 (50%)	
1	36 (42%)	412 (40%)	448 (41%)	
2	4 (5%)	38 (4%)	42 (4%)	
Not specified§	6 (7%)	53 (5%)	59 (5%)	
Number of treatment cycles	4 (1–8)	3 (1–8)	3 (1–8)	0·97
DPYD status				NA
Wild-type	0	1018 (100%)	1018 (92%)	
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 (<1%)	

Data are n (%) or median (IQR). *DPYD*=gene encoding dihydropyrimidine dehydrogenase. NA=not applicable. *p value comparing *DPYD* variant allele carriers to *DPYD* wild-type patients. We used a Kruskal-Wallis rank-sum test for age, body surface area, and number of treatment cycles; a Fisher's exact test for ethnic origin and WHO performance status; and a χ^2 test for sex, tumour type, and treatment regimen. †Other ethnic origins included Hispanic descent, mixed racial parentage, and unknown ethnic origin. ‡Other tumour types included anal cancer, oesophageal cancer, head and neck cancer, pancreatic cancer, bladder cancer, unknown primary tumour, vulva carcinoma, and several rare tumour types. §WHO performance status was not specified for these patients, but was either 0, 1, or 2, as required by the study inclusion criteria.

Table 1: **Baseline characteristics**

	DPYD variant allele carriers (n=85)	Wild-type patients (n=1018)	p value	c.1236G>A (n=51)	c.2846A>T (n=17)	*DPYD**2A (n=16)	c.1679T>G (n=1)
Relative dose intensity whole treatment*	69% (37–97) [11]	94% (49–128) [10]	NA	74% (51–97) [7]	72% (49–96) [11]	53% (37–74) [9]	54%
Relative dose intensity first cycle*	69% (25–96) [12]	96% (37–128) [10]	NA	74% (51–88) [4]	73% (55–96) [9]	51% (25–82) [11]	50%
Overall grade ≥3 toxicity†	33 (39%)	231 (23%)	0·0013‡	20 (39%)	8 (47%)	5 (31%)	0
Grade ≥3 gastrointestinal toxicity	17 (20%)	86 (8%)	0·00089‡	11 (22%)	4 (24%)	2 (13%)	0
Grade ≥3 haematological toxicity	13 (15%)	65 (6%)	0·0043‡	7 (14%)	4 (24%)	2 (13%)	0
Grade 3 hand-foot syndrome§	1 (1%)	36 (4%)	0·41¶	0	1 (6%)	0	0
Grade ≥3 cardiac toxicity	1 (1%)	9 (1%)	1·0¶	1 (2%)	0	0	0
Grade ≥3 other treatment-related toxicity	9 (11%)	78 (8%)	0·45‡	7 (14%)	1 (6%)	1 (6%)	0
Overall grade ≥4 toxicity‡	4 (5%)	29 (3%)	0·49¶	3 (6%)	1 (6%)	0	0
Grade ≥4 gastrointestinal toxicity	1 (1%)	8 (1%)	1·0¶	1 (2%)	0	0	0
Grade ≥4 haematological toxicity	1 (1%)	12 (1%)	1·0¶	1 (2%)	0	0	0
Grade ≥4 cardiac toxicity	0	1 (<1%)	NA	0	0	0	0
Grade ≥4 other treatment-related toxicity	3 (4%)	9 (1%)	0·12¶	2 (4%)	1 (6%)	0	0
Fluoropyrimidine-related hospital admission	16 (19%)	140 (14%)	0·26‡	10 (20%)	4 (24%)	2 (13%)	0
Stop of fluoropyrimidines because of adverse events	15 (18%)	175 (17%)	1·0‡	8 (16%)	3 (18%)	4 (25%)	0
Fluoropyrimidine-related death	1 (1%)‖	3 (<1%)	0·55¶	0	1 (6%)‖	0	0

Data are mean (range) [SD] or n (%). *DPYD*=gene encoding dihydropyrimidine dehydrogenase. NA=not applicable. *Relative dose intensity was calculated as the given dose in mg/m² divided by the standard dose in mg/m² given for the indication and treatment schedule that was applicable for the patient. We calculated the relative dose intensity for the first cycle alone and for the entire treatment duration. †Overall toxicity included all toxicities evaluated as possibly, probably, or definitely related to fluoropyrimidine treatment. ‡p value determined with χ^2 test with Yates' continuity correction. §Defined as palmar-plantar erythrodysesthesia syndrome by the Common Terminology Criteria for Adverse Events version 4.03.[18] ¶p value determined with Fisher's exact test with one-sided probability (with the p value multiplied by 2). ‖One patient (c.2846A>T carrier) was wrongly treated with a full capecitabine dose for two cycles, which resulted in fatal fluoropyrimidine-related toxicity.

Table 2: **Treatment outcomes for patients included in this study**

	DPYD variant carriers treated with reduced dose (current study)*	*DPYD* variant carriers treated with full dose† (previous meta-analysis)
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‡	4·30 (2·10–8·80)

Data are RR (95% CI). RR=relative risk. *DPYD*=gene encoding dihydropyrimidine dehydrogenase. NA=not applicable. *RR for overall grade ≥3 fluoropyrimidine-related toxicity compared with non-carriers of this variant as described in table 2. †RR for overall grade ≥3 fluoropyrimidine-related toxicity compared with non-carriers of this variant, as determined in a random-effects meta-analysis by Meulendijks and colleagues.[10] Unadjusted RRs for the meta-analysis are depicted, as the RR in the current study was also calculated as an unadjusted value (because patient numbers were low). ‡RR cannot be calculated as only one patient who carried c.1679T>G was present. This patient did not have severe toxicity.

Table 3: **RR for grade ≥3 severe toxicity of *DPYD* variant carriers compared with a historical cohort**

wild-type patients. For c.1236G>A, the RR in the historical cohort was 1·72 (95% CI 1·22–2·42)[10] and in our study it was 1·69 (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 in the meta-analysis was 3·11 (2·25–4·28),[10] which decreased to 2·00 (1·19–3·34) after 25% dose reduction in this study. However, this RR of 2·00 (1·19–3·34) was still increased compared with non-carriers of this variant. For the c.1679T>G variant, we could not calculate a RR in our study because only one patient with this variant was included. In the historical cohort the RR was 4·30 (2·10–8·80).

26 *DPYD* variant allele carriers (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 for blood to be collected for pharmacokinetic analysis. Mean exposure to capecitabine and all metabolites, including fluorouracil, was similar between patients dosed based on *DPYD* genotype and controls (figure 2),[20] suggesting that mean drug exposure of all combined *DPYD* variant allele carriers treated with a reduced dose was adequate. However, in line with our toxicity data, area under the curve values for fluorouracil were markedly higher for c.1236G>A carriers and c.2846A>T carriers compared with *DPYD**2A carriers and c.1679T>G carriers (appendix p 6).

We investigated pretreatment DPD enzyme activity in 56 *DPYD* variant allele carriers and 82 wild-type patients (participating in a subgroup of the study in which DPD phenotyping tests were investigated; figure 3). Mean DPD activity in *DPYD* wild-type patients was 9·4 nmol/mg per h (SD 3·6), similar to previously published data.[23] For the 35 c.1236G>A variant carriers, mean DPD activity was 7·5 nmol/mg per h (2·8; a 20% reduction compared with wild-type). The mean

DPD activity for the 12 c.2846A>T carriers was 6·2 nmol/mg per h (1·9; a 34% reduction), and for the eight *DPYD**2A carriers was 5·2 nmol/mg per h (0·6; a 45% reduction). The single patient with c.1679T>G had a DPD enzyme activity of 3·8 nmol/mg per h (a 60% reduction). Mean DPD enzyme activity was significantly lower for the *DPYD* variants (c.1236G>A, p=0·0050; c.2846A>T, p=0·0034; and *DPYD**2A, p=0·0012) than for wild-type. Statistical analysis was not possible for c.1679T>G. We found no correlation between DPD enzyme activity and the occurrence of severe fluoropyrimidine-related toxicity in *DPYD* variant allele-carrying patients (figure 3, appendix p 7).

Discussion

To our knowledge, this is the first prospective study to investigate the effect of dose individualisation on the basis of four *DPYD* variants on fluoropyrimidine-related toxicity. Our results show that genotype-guided dosing is feasible in clinical practice. Dose individualisation reduced the risk of severe (grade 3 or worse) toxicity for *DPYD**2A carriers, was safe in the single c.1679T>G carrier, and decreased the toxicity risk in c.2846A>T carriers, although the risk was still higher for c.2846A>T carriers than wild-type patients. For c.1236G>A carriers, a 25% dose reduction was not enough to decrease severe treatment-related toxicity. The findings from this study show that *DPYD* genotype-guided dose individualisation has the potential to improve patient safety, because toxicity risk was reduced for three of the four variants in our study. Although the sample sizes of variant allele carriers were modest and not all reductions in toxicity risk were statistically significant, our findings have high clinical relevance. Also, implementation of *DPYD* genotype-guided dosing resulted in similar frequencies of toxicity-related hospital admission and discontinuation of treatment because of fluoropyrimidine-related toxicity for wild-type patients and *DPYD* variant allele carriers.

For *DPYD**2A carriers, the frequency of severe toxicity recorded in this study was 31%, markedly lower than the frequency in the historical cohort (72%).[10] DPD enzyme activity measurements in our study showed that activity for *DPYD**2A carriers was reduced by 45% compared with wild-type patients, which supports the dose recommendation of 50% for this variant.

As only one carrier of the rare c.1679T>G variant was identified in our study statistical comparisons were not possible. However, although a RR for severe toxicity of 4·30 has been reported in the literature,[10] we showed that this patient did not have severe toxicity after completed treatment with a 50% reduced dose. DPD enzyme activity was also decreased by about 60% 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 variants, the risk of severe toxicity remained relatively high despite dose individualisation on the basis of our dosing recommendations (25% reduction). In this study, 39% of c.1236G>A carriers and 47% of c.2846A>T carriers had severe toxicity. For these two variants, an initial dose reduction of 25% was applied in our study because they 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 scarce 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

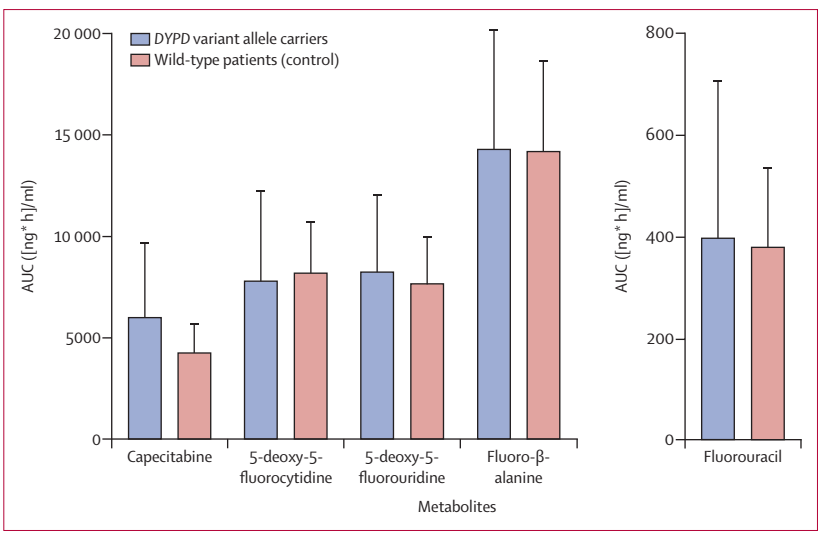

Figure 2: **Pharmacokinetics of *DPYD*-guided capecitabine dosing**
Mean AUCs of capecitabine and the metabolites 5-deoxy-5-fluorocytidine, 5-deoxy-5-fluorouridine, fluorouracil, and fluoro-β-alanine for *DPYD* variant allele carriers treated with *DPYD* genotype-guided dose (blue) and control values from wild-type patients from a previously published study (red).[20] Error bars represent the SD. AUC=area under the plasma concentration–time curve. *DPYD*=the gene encoding dihydropyrimidine dehydrogenase.

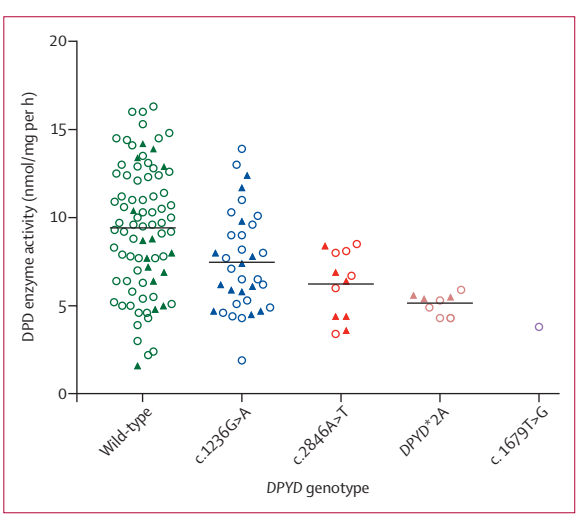

Figure 3: **DPD enzyme activity in peripheral blood mononuclear cells for *DPYD* variant allele carriers and wild-type patients**
Patients with grade 3 or worse fluoropyrimidine-related toxicity are depicted by triangles and patients with grade 0–2 toxicity by circles. DPD=dihydropyrimidine dehydrogenase. *DPYD*=the gene encoding DPD.

based on one small retrospective study.[25] Therefore, the CPIC advises a 25–50% dose reduction in heterozygous c.1236G>A and c.2846A>T carriers.[13] Our results suggest that applying a 25% dose reduction might be insufficient for some patients, as toxicity risk was increased for c.1236G>A and c.2846A>T carriers compared with wild-type patients. In line with these findings, our pharmacokinetic analyses showed that exposure to fluorouracil was markedly higher in c.2846A>T carriers than in *DPYD* wild-type controls. Exposure to fluorouracil in variant allele carriers was at least equal to exposure observed in wild-type patients receiving the standard dose, which suggests that applied genotype-guided dose reduction will not result in undertreatment. However, these pharmacokinetic results should be interpreted with caution. In patients with reduced DPD activity, fluorouracil metabolism is affected, with fluorouracil being the third metabolite derived from the parent compound capecitabine, which restricts interpretation of fluorouracil exposure. Furthermore, pharmacokinetics of capecitabine and its metabolites have a high inter-individual variability in exposure—even in wild-type patients—and are therefore difficult to interpret. Additionally, because of the small number of patients with a *DPYD* variant for whom we also obtained pharmacokinetic data (appendix p 6), firm conclusions based on pharmacokinetic measurements alone cannot be drawn.

The mean DPD enzyme activity for c.1236G>A was reduced by around 20%, but we found large variation in this activity, suggesting that a proportion of patients need a larger dose reduction, while other patients might tolerate a full dose. These findings are in line with the large variation in pharmacokinetic exposure found 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 reduced by around 35% compared with wild-type. These DPD activity measurements show that a 25% dose reduction might not be sufficient for most of these 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 also be considered in these patients.

Our study was done in a daily clinical care setting in regional general hospitals and a few academic centres, showing the feasibility of implementation of upfront *DPYD* screening. To make *DPYD*-guided dosing feasible in all hospitals, the turnaround time for *DPYD* genotyping must be short to prevent a delay in the start of treatment. Participating laboratories in our study had a turnaround time of a few days to a maximum of 1 week.

A limitation of this study is that we used a historical cohort of *DPYD* variant allele carriers treated with full dose as a control, and no direct comparison was made with a control cohort within our study. Inherent to this chosen design, differences between the study populations could have influenced the observed toxicity outcomes. However, we chose this study design because a randomised clinical trial would be 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.[26] A previous clinical study[27] was stopped prematurely because a patient in the group without dose individualisation died due to treatment-related toxicity.

Our study focused on toxicity and did not evaluate survival or other effectiveness outcomes, as this was considered not feasible because of the large variation in tumour types and treatment regimens. However, we did pharmacokinetic measurements, findings of which suggested that applied dose reductions in *DPYD* variant allele carriers did not result in underdosing.

The four *DPYD* variants investigated in this study are especially relevant to white populations. For other ethnicities, more research on the frequency and clinical relevance of these and other *DPYD* variants is recommended.[28] In our study, homozygous and compound heterozygous *DPYD* variant allele carriers were not included and were treated with individualised fluoropyrimidine dosing or alternative treatment outside the study.[17] However, for this group of patients, *DPYD* genotype-guided dosing is of even greater importance than for heterozygous *DPYD* variant allele carriers, because these patients generally have less remaining—or even complete absence of—DPD activity, and a full fluoropyrimidine dose when such a patient is not identified as DPD deficient is 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 *DPYD* variant allele carriers, but also in *DPYD* wild-type patients.

In conclusion, we showed that safety of patients treated with fluoropyrimidines was improved by dose individualisation on the basis of *DPYD* genotype. Dose reduction of 50% in heterozygous *DPYD**2A and c.1679T>G carriers markedly reduced toxicity risk. Applied dose reductions of 25% in heterozygous c.1236G>A and c.2846A>T carriers were insufficient to lower the risk of fluoropyrimidine-related toxicity to the observed 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, could therefore be considered.

Contributors

LMH, CATCL, FMdM, DM, RHJM, JJS, HG, AC, H-JG, and JHMS designed the study. LMH, CATCL, FMdM, G-JC, AB, VOD, ALTI, FJFJ, JEAP, RLHJ, PH, AJtT, HJD, MK, PN, MHWvdP, CMPWM, RHJM, HG,

AC, and JHMS were involved in patient inclusion and collection of individual patient data. LMH, CATCL, FMdM, EK, JJS, HR, JHB, ABPvK, and RHNvS were responsible for collection and measurement of patient samples. LMH, CATCL, and FMdM led the data interpretation and writing of the report. GWJF and EvW gave support for data analysis. All authors contributed to data interpretation and preparation of the report for publication and approved the final version of the manuscript.

Declaration of interests

LMH and CATCL report grants from the Dutch Cancer Society. CATCL was previously supported by an unrestricted grant from Roche Pharmaceuticals. JHMS reports grants from the Dutch Cancer Society during the conduct of the study, and personal fees from Modra Pharmaceuticals outside the submitted work. All other authors declare no competing interests.

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