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Effectiveness and safety of reduced-dose fluoropyrimidine therapy in patients carrying the *DPYD**2A variant: A matched pair analysis

Linda M. Henricks[1,2], Lisanne N. van Merendonk[1,2], Didier Meulendijks[1,2,3], Maarten J. Deenen[4,5], Jos H. Beijnen[6,7], Anthonius de Boer[7], Annemieke Cats[8] and Jan H.M. Schellens[1,2,7]

[1]Division of Pharmacology, The Netherlands Cancer Institute, Amsterdam, the Netherlands
[2]Department of Clinical Pharmacology, Division of Medical Oncology, The Netherlands Cancer Institute, Amsterdam, the Netherlands
[3]Dutch Medicines Evaluation Board (CBG-MEB), Utrecht, The Netherlands
[4]Department of Clinical Pharmacy, Catharina Hospital, Eindhoven, The Netherlands
[5]Department of Clinical Pharmacy and Toxicology, Leiden University Medical Center, Leiden, the Netherlands
[6]Department of Pharmacy & Pharmacology, The Netherlands Cancer Institute, Amsterdam, the Netherlands
[7]Division of Pharmacoepidemiology and Clinical Pharmacology, Department of Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, the Netherlands
[8]Department of Gastrointestinal Oncology, Division of Medical Oncology, The Netherlands Cancer Institute, Amsterdam, the Netherlands

Carriers of the genetic *DPYD2A variant, resulting in dihydropyrimidine dehydrogenase deficiency, are at significantly increased risk of developing severe fluoropyrimidine-associated toxicity. Upfront *DPYD**2A genotype-based dose reductions improve patient safety, but uncertainty exists whether this has a negative impact on treatment effectiveness. Therefore, our study investigated effectiveness and safety of *DPYD**2A genotype-guided dosing. A cohort of 40 prospectively identified heterozygous *DPYD**2A carriers, treated with a ~50% reduced fluoropyrimidine dose, was identified. For effectiveness analysis, a matched pair-analysis was performed in which for each *DPYD**2A carrier a matched *DPYD**2A wild-type patient was identified. Overall survival and progression-free survival were compared between the matched groups. The frequency of severe (grade ≥ 3) treatment-related toxicity was compared to 1] a cohort of 1606 wild-type patients treated with full dose and 2] a cohort of historical controls derived from literature, i.e. 86 *DPYD**2A variant carriers who received a full fluoropyrimidine dose. For 37 out of 40 *DPYD**2A carriers, a matched control could be identified. Compared to matched controls, reduced doses did not negatively affect overall survival (median 27 months *versus* 24 months, $p = 0.47$) nor progression-free survival (median 14 months *versus* 10 months, $p = 0.54$). Risk of severe fluoropyrimidine-related toxicity in *DPYD**2A carriers treated with reduced dose was 18%, comparable to wild-type patients (23%, $p = 0.57$) and significantly lower than the risk of 77% in *DPYD**2A carriers treated with full dose ($p < 0.001$). Our study is the first to show that *DPYD**2A genotype-guided dosing appears to have no negative effect on effectiveness of fluoropyrimidine-based chemotherapy, while resulting in significantly improved patient safety.**

Introduction

Fluoropyrimidine drugs, including 5-fluorouracil (5-FU) and its oral prodrug capecitabine, are the cornerstone of chemotherapeutic treatment for multiple solid tumor types, including colorectal, breast and gastric cancer. An estimated two million patients worldwide are treated yearly with this class of anti-cancer drugs.[1] However, these drugs are associated with substantial treatment-related toxicity, with around 30% of treated patients experiencing severe toxicity, (grade 3 or higher according to the Common Terminology Criteria for

Key words: *DPYD*, dihydropyrimidine dehydrogenase, fluoropyrimidines, 5-fluorouracil, capecitabine

Abbreviations: 5-FU: 5-fluorouracil; 95%CI: 95% confidence interval; CTC-AE: Common Terminology Criteria for Adverse Events; DPD: dihydropyrimidine dehydrogenase; *DPYD*: gene encoding dihydropyrimidine dehydrogenase; IRB: institutional review board; SNP: single nucleotide polymorphisms; WHO-status: Performance status according to the World Health Organization

Additional Supporting Information may be found in the online version of this article.

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Correspondence to: Linda M. Henricks, PharmD, PhD. Department of Clinical Pharmacology, Division of Medical Oncology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, the Netherlands, E-mail: l.henricks@nki.nl; Tel.: +31 20 512 6219

What's new?

Genetic variants in the dihydropyrimidine dehydrogenase gene (*DPYD*) enhance toxicity associated with fluoropyrimidine-based chemotherapies and a 50% reduction in drug dosing in affected carriers. Here the authors addressed the fear of "underdosing" by retrospectively matching cancer patients with mutant or wild-type *DPYD* status. No significant difference was seen in overall survival, progression-free survival or disease control between the two groups affirming current clinical guidelines.

Adverse Events (CTC-AE)), often leading to hospitalization and interruption or discontinuation of therapy. The most common adverse events include diarrhea, mucositis, hand-foot syndrome and myelosuppression.[2]

In recent years, it has become clear that fluoropyrimidine-related toxicity is often related to deficiency of the enzyme dihydropyrimidine dehydrogenase (DPD), the main metabolic enzyme of fluoropyrimidines.[2,3] An estimated 3–8% of the population is subject to reduced DPD activity, and when treated at a full dose of 5-FU or capecitabine, exposure to 5-FU is increased, resulting in a higher risk of developing severe toxicity.[3,4] Most often, DPD deficiency is the result of single nucleotide polymorphisms (SNPs) in *DPYD*, the gene encoding DPD. The first *DPYD* variant that was discovered, also considered to be one of the most clinically relevant variants, is the *DPYD**2A variant (IVS14+1G>A, c.1905+1G>A, rs3918290), which results in skipping of exon 14, and hence a non-functional enzyme.[5,6] Heterozygous *DPYD**2A variant allele carriers, with a frequency of ~1% in the Western population, carry one functional allele and one non-functional allele and therefore have approximately 50% DPD enzyme function compared to normal.[7]

In a previously performed large clinical trial we showed that by reducing the fluoropyrimidine starting dose by 50% in heterozygous *DPYD**2A carriers, these patients can be safely treated. The frequency of severe treatment-related toxicity was reduced from 73% in a historical cohort of *DPYD**2A carriers treated with full dose, to 28% by reducing the starting fluoropyrimidine dose by ~50% in *DPYD**2A carriers. The risk of toxicity in these DPD deficient patients was thus found to be reduced to the background risk of toxicity in patients without DPD deficiency, which was 23% in the same study, in the cohort of *DPYD* wild-type patients treated at full dose.[8] Furthermore, pharmacokinetic analyses showed that drug exposure in the heterozygous *DPYD**2A carriers treated at a reduced dose was comparable to control values of wild-type patients treated with standard dose, suggesting that exposure was adequate.

However, upfront screening for DPD deficiency and dose reduction in patients carrying *DPYD* variant alleles remains not standard practice in all treatment centers where patients are treated with fluoropyrimidines. The most critical uncertainty related to dose reduction in DPD deficient patients, as argued by those who are critical in relation to *DPYD* screening, is whether fluoropyrimidine treatment will still be efficacious when doses are reduced, as this could potentially result in underdosing. For this reason, we undertook a study to investigate effectiveness of fluoropyrimidine therapy after dose reduction in DPD deficient patients carrying the *DPYD**2A allele. In the largest study performed in this respect, we investigated a cohort of 40 *DPYD**2A variant allele carriers treated with a reduced dose and determined effectiveness of treatment compared to matched controls of *DPYD**2A wild-type patients treated with a full dose.

Patients and Methods

Patient selection

Our study was performed in a single center in which all patients who were treated with fluoropyrimidine-based therapy as part of routine clinical care, were screened prospectively for the *DPYD**2A variant prior to start of therapy. If patients were identified as heterozygous carriers of *DPYD**2A, the fluoropyrimidine starting dose was reduced by approximately 50%. It was allowed to titrate the dose upwards during treatment after two cycles based on tolerance, as decided by the treating physician.

Patients who were heterozygous carriers of *DPYD**2A were included in this analysis, comprising all *DPYD**2A carriers who were screened between May 2007 and April 2015, who started with fluoropyrimidine-based chemotherapy, either as monotherapy or in combination with other chemotherapeutic agents or radiotherapy. The first 18 patients were identified during a prospective study that enrolled patients from May 2007 to October 2011 (NCT00838370). Safety data of these patients have been published by Deenen *et al.*[8] After closing of the trial, prospective *DPYD**2A screening was continued in our institute (The Netherlands Cancer Institute, Amsterdam, The Netherlands), as part of routine clinical care. Patients identified as *DPYD**2A carriers in this second period, taken together with the first identified *DPYD**2A carriers were considered *group 1*.

Results on effectiveness of fluoropyrimidine treatment and risk of severe toxicity in this group were compared to *group 2*, which consisted of all *DPYD**2A wild-type patients from the study of Deenen *et al.* screened between May 2007 and October 2011. For effectiveness analyses a selection of patients in group 2 was made, based on identified matched controls for the patients in group 1. For toxicity analyses, a comparison was made between group 1 and the entire cohort of group 2, and also between group 1 and a literature cohort (*group 3*). This literature cohort consisted of *DPYD**2A carriers who were treated with a full dose of fluoropyrimidines. For this historical cohort, the same publications as used for the previous clinical trial were included, describing unselected cohort studies of patients

genotyped for *DPYD**2A and treated with fluoropyrimidine-based chemotherapy. Furthermore, using the same search terms, the historical cohort was expanded with publications after February 1, 2014 (end date of search by Deenen *et al.*).[8]

Patients of whom data were included, were treated according to routine clinical care, and data was collected retrospectively, thus institutional review board (IRB) approval was not required. Data from wild-type control patients and a subset of *DPYD**2A carriers were derived from the study of Deenen *et al.*,[8] for which IRB approval was granted by The Netherlands Cancer Institute, Amsterdam, The Netherlands.

Study design

Our study investigated both effectiveness and toxicity. The primary endpoint for effectiveness was overall survival (defined as the time between initiation of treatment and death, by any cause). Secondary endpoints for effectiveness were progression-free survival (defined as the time between initiation of treatment and first signs of disease progression by either radiology or clinical signs, or death, whichever came first) and objective tumor response (according to RECIST 1.1 criteria).

A secondary aim of the study was investigating the incidence of severe (CTC-AE grade ≥ 3) fluoropyrimidine-associated toxicity. Overall fluoropyrimidine-associated toxicity and several subtypes of toxicity such as hematological toxicity (including neutropenia, leukopenia, thrombocytopenia), gastrointestinal toxicity (including diarrhea and mucositis) and hand-foot syndrome were investigated. Other parameters associated with toxicity that were investigated, included hospitalization for treatment-related toxicity, treatment interruptions due to toxicity and incidence of treatment-related death.

Matching

For all *DPYD**2A carriers (group 1) a matched control was identified from the *DPYD**2A wild-type cohort (group 2) for the primary effectiveness analyses. A one-to-one matching procedure was performed. Patients were matched on covariables that were known to have a relevant influence on treatment outcome.

First, automatic matching in the database was performed based on the following criteria: treatment at the same institute, tumor type (colorectal cancer, gastric or esophageal cancer, breast cancer, pancreatic cancer, head and neck cancer or other), disease stage (local and locally advanced or metastatic), sex, treatment received (capecitabine/5-FU, radiotherapy yes/no, monotherapy/combination therapy) and age at first administration of fluoropyrimidine treatment (±5 years).

After automatic matching, a manual selection to identify the best matching control was performed (as automatic matching in the database was not possible for the remaining criteria). The following criteria were used for manual selection: the line of treatment, specification of concomitant chemotherapy, WHO-status at baseline, and if the tumor type was defined as "other" a similar tumor type was selected manually. If more than one wild-type patient was available fulfilling all matching criteria, the paired match was chosen at random. If there was no exact match available fulfilling all matching criteria, a discrepancy on one matching variable was allowed, but this excluded tumor type and disease stage, as those variables were expected to have the largest impact on treatment outcome. The *DPYD**2A wild-type cohort had retrospectively been genotyped for three other *DPYD* variants (c.2846A>T, c.1679T>G, c.1236G>A) for another study.[9] Carriers of these variants were excluded from the matching process.

Statistical analysis

Patient and treatment characteristics were analyzed by group using descriptive statistics. Overall survival and progression-free survival were compared between the matched groups 1 and 2 using Kaplan–Meier estimates and the log-rank test for equality of survival curves. A log-rank hazard ratio was calculated as well. Patients alive at last follow-up were censored. Objective tumor response was compared using the McNemar's test, where the proportions of patients with disease control (complete response, partial response, stable disease) and disease progression were compared.

For toxicity analyses, the Fisher's exact test was used to compare frequencies of severe toxicities, hospitalization, treatment interruptions and treatment-related death between groups (group 1 *versus* group 2 and group 1 *versus* group 3).

For all analyses, p values <0.05 were considered statistically significant. Statistical analyses were performed using SPSS Version 22.0 (IBM SPSS Statistics).

Results

Overall patient characteristics

For the current analysis, 16 out of 18 patients that were identified during the prospective study were included, as the two other patients were treated at another hospital, and survival data for these patients could not be retrieved. An additional 24 heterozygous *DPYD**2A carriers were identified during routine screening and these patients were included in our study as well. This resulted in a total of 40 identified *DPYD**2A carriers who were treated with fluoropyrimidines at a reduced starting dose (Fig. 1). The cohort of *DPYD**2A wild-type patients from the previous prospective clinical trial was used as control group. This cohort included 1613 fluoropyrimidine-treated patients that were prospectively screened as wild-type for *DPYD**2A. As clinical data were incomplete for 7 of these patients, 1606 patients were included in the current analysis.

Baseline characteristics for *DPYD**2A carriers (group 1) and *DPYD**2A wild-type patients (group 2) are depicted in Table 1. The 40 *DPYD**2A carriers were treated with a mean dose intensity of 53.0% (mean dose of the entire treatment duration). The mean dose intensity for the first cycle was 51.6%. In eleven patients, doses were titrated upwards during treatment, in seven patients doses had to be further reduced after the initial dose reduction of 50%.

For the effectiveness analysis, matched controls for the *DPYD**2A carriers were identified in the wild-type cohort. For three *DPYD**2A carriers no suitable match could be identified, thus those three patients were excluded from effectiveness analyses, leaving 37 evaluable patients. Perfect matching was not possible for all remaining patients, mostly as the WHO status was often unknown (which was caused by the retrospective nature of data collection and often incomplete patient files). Small discrepancies on matching factors were then allowed. In Supporting Information Table 1 an overview of these discrepancies is given.

For the literature control cohort, used for comparison of toxicity, a total of 17 published studies were selected, describing clinical data on 86 *DPYD**2A variant allele carriers.[10–26] Patient characteristics of the historical cohort are included in Table 1.

Effectiveness of genotype-guided dosing

Overall survival was compared between the 37 *DPYD**2A carriers receiving genotype-guided dosing and 37 matched wild-type controls (Fig. 2). Median survival of *DPYD**2A carriers was 27 months (2.3 years), with a range of 1 months to 83 months (6.9 years). Median survival of wild-type patients was 24 months (2.0 years) with a range of 0.7 months to 97 months (8.1 years). The log-rank test showed that overall survival was not significantly different between both groups ($p = 0.47$). The hazard ratio comparing *DPYD**2A carriers to wild-type patients for overall survival was 0.82 (95% confidence interval (95%CI): 0.47–1.43).

Also progression-free survival curves were similar for both groups (Fig. 2). Median progression-free survival for *DPYD**2A carriers was 14 months (1.2 years) with a range of 0.7 months to 83 months (6.9 years), and median progression-free survival for wild-type patients was 10 months with a range of 0.2 months to 97 months (8.1 years). Progression-free survival curves were not statistically significantly different ($p = 0.54$). When comparing *DPYD**2A carriers to wild-type patients, the hazard ratio for progression-free survival was 0.83 (95%CI: 0.47–1.50).

There was no statistically significant difference for the proportions of patients with disease control for both groups either ($p > 0.99$, Supporting Information Table 2). 12 out of 37 *DPYD**2A carriers had controlled disease (of whom four had a partial response and eight stable disease), and 10 out of 37 wild-type patients (of whom one with complete response, six patients with partial response and three with stable disease).

Toxicity of genotype-guided dosing

Genotype-guided dosing resulted in 7 out of 40 patients (18%) in group 1 experiencing grade ≥3 overall fluoropyrimidine-related toxicity. The incidence of gastrointestinal toxicity, hematological toxicity and hand-foot syndrome was respectively 10%, 10% and 5% (Table 2). Both for overall toxicity and the subtypes of toxicity, frequencies were highly comparable to the cohort of *DPYD**2A wild-type patients (Table 2). The same accounted for incidence of treatment-related hospitalization, treatment interruptions and treatment-related

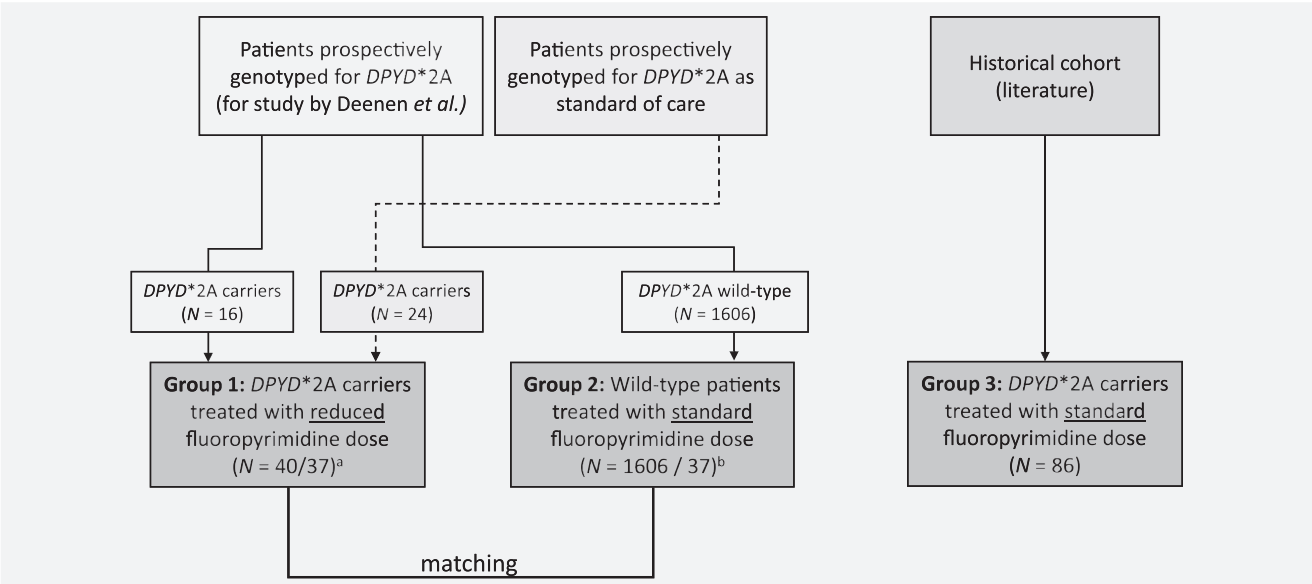

Figure 1. Selection of patients. (*a*) For toxicity analyses all patients from group 1 were included ($N = 40$). As no appropriate matches could be identified for 3 patients, 37 patients were included for effectiveness analyses. (*b*) For toxicity analyses all patients from group 2 were included ($N = 1606$). For effectiveness analyses a subgroup was included ($N = 37$) which consisted of patients that were matched to the patients of group 1.

Table 1. Baseline and treatment characteristics of patients (group 1, 2 and 3)

	Group 1: *DPYD**2A carriers treated with reduced dose (*N* = 40)	Group 2: Wild-type patients treated with standard dose (*N* = 1606)	Group 3: *DPYD**2A carriers treated with standard dose from literature (*N* = 86)
Sex			
Male	14 (35%)	720 (45%)	13 (15%)
Female	26 (65%)	886 (55%)	15 (17%)
Unknown	-	-	58 (67%)
Age, median [range]	61.7 [33.8–90.8]	61.2 [20.8–88.8]	Unknown
Ethnic origin			
Caucasian	39 (98%)	1540 (96%)	Unknown
Southeast Asian	1 (2%)	14 (1%)	Unknown
African	0 (0%)	21 (1%)	Unknown
Other	0 (0%)	31 (2%)	Unknown
BSA, median [range]	1.8 [1.5–2.2]	1.9 [1.1–2.7]	Unknown
Disease status			
Locally advanced CRC	9 (23%)	534 (33%)	6 (7%)
Metastatic CRC	4 (10%)	320 (20%)	57 (66%)
Locally advanced BC	3 (8%)	119 (7%)	1 (1%)
Metastatic BC	12 (30%)	250 (16%)	2 (2%)
GC	2 (5%)	227 (14%)	3 (3%)
Other	10 (25%)	156 (10%)	2 (2%)
Unknown	-	-	15 (17%)
Previously treated with chemotherapy			
Yes	17 (43%)	359 (22%)	Unknown
No	23 (58%)	1247 (78%)	Unknown
Treatment regimen			
CAP mono	15 (38%)	424 (26%)	4 (5%)
CAP + Pt	2 (5%)	378 (24%)	1 (1%)
CAP triplet	0 (0%)	114 (7%)	1 (1%)
CAP + RT	12 (30%)	436 (27%)	0 (0%)
CAP + other	9 (23%)	86 (5%)	8 (9%)
5-FU mono	0 (0%)	16 (1%)	21 (24%)
5-FU + RT	0 (0%)	54 (3%)	0 (0%)
5-FU + other	2 (5%)	98 (6%)	41 (48%)
Unknown	-	-	10 (12%)

Abbreviations: 5-FU mono: 5-fluorouracil monotherapy; 5-FU + other: 5-FU combined with other chemotherapeutics; 5-FU + RT; 5-fluorouracil combined with radiotherapy; BC: breast cancer; CAP mono: capecitabine monotherapy; CAP + other; capecitabine combined with other chemotherapeutics; CAP + Pt: capecitabine plus platinum agent; CAP triplet: capecitabine combined with platinum agent and taxane; CAP + RT: capecitabine combined with radiotherapy; CRC: colorectal cancer; GC: gastric or gastroesophageal cancer.

death. None of these outcomes were significantly different between group 1 and group 2 (Table 2).

Toxicity risk was also compared to the historical literature cohort (group 3). This showed that genotype-guided dosing resulted in a significantly lower risk of severe toxicity, i.e. 77% in group 3 *versus* 18% in group 1 ($P < 0.001$). When calculating a relative risk for the risk of severe overall toxicity of group 3 compared group 1, this resulted in a relative risk of 4.39 (95%CI: 2.22–8.68). Individual patient characteristics of *DPYD**2A carriers in the historical cohort are depicted in Supporting Information Table 3. Treatment-related death was not present in the genotype-guided dosing *DPYD**2A carriers, whereas this was 8% in the historical cohort (7 out of 86 patients). This resulted in a relative risk of 7.07 (95%CI: 0.41–120.82).

Discussion

This is to our knowledge the largest study so far determining whether effectiveness of fluoropyrimidine chemotherapy is affected by dose reduction in DPD deficient patients who are carriers of the *DPYD**2A variant. Due to the heterogeneous patient population receiving fluoropyrimidine therapy and the low frequency of *DPYD**2A (approximately 1%), trials investigating the effectiveness of genotype-guided dosing are difficult to perform, as these require a very large sample size. We prospectively screened

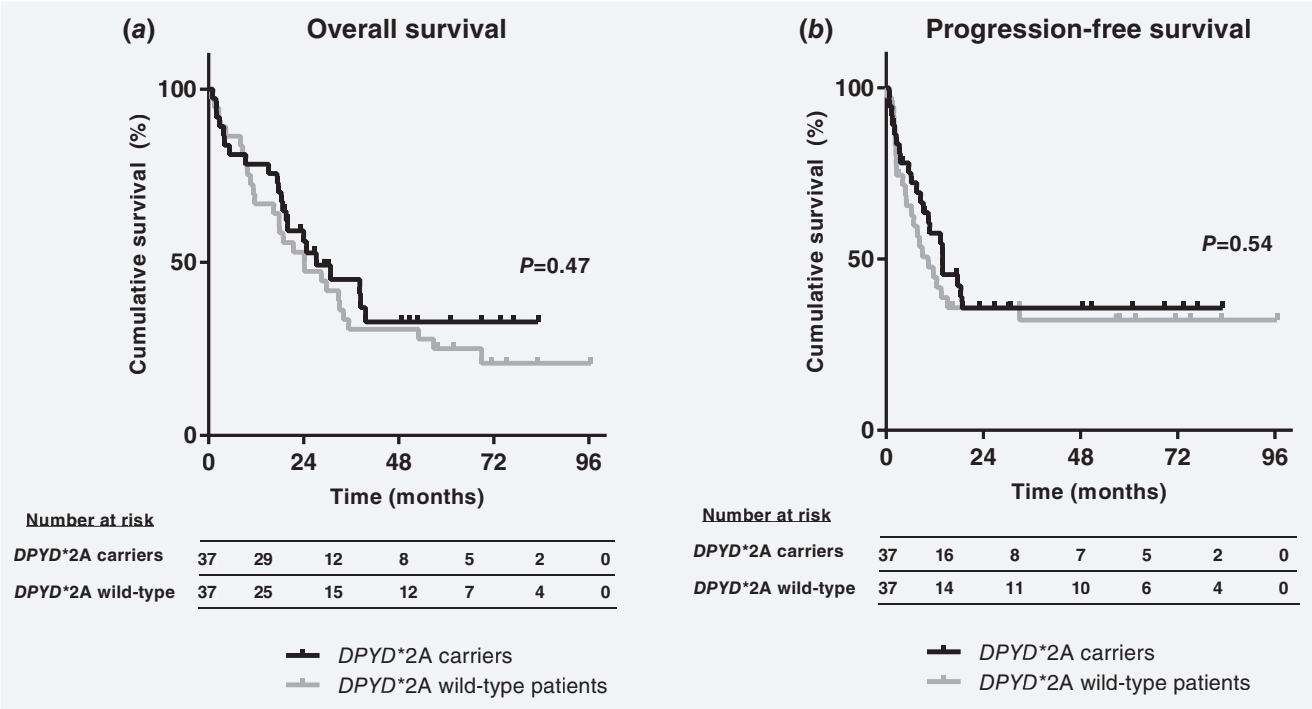

Figure 2. Survival analyses of *DPYD**2A carriers with reduced dose *versus* wild-type patients with standard dose. Shown is the Kaplan–Meier survival curve for overall survival (a) or progression-free survival (b). The *p*-value was calculated using the log-rank test for equality of survival curves. Patients alive at last follow-up were censored.

over 4000 patients to identify 40 patients with the *DPYD**2A variant, of which efficacy and safety data of fluoropyrimidine-based treatment were collected retrospectively. Subsequently, we performed a matched pair analysis using control patients from the same institute. By choosing matching factors known to be associated with effectiveness of fluoropyrimidine chemotherapy, we aimed to make the comparison between *DPYD**2A carriers and wild-type patients as reliable as possible.

Table 2. Treatment outcome of patients (group 1, 2 and 3)

	Group 1: *DPYD**2A carriers treated with reduced dose (*N* = 40)	Group 2: Wild-type patients treated with standard dose (*N* = 1606)	Group 3: *DPYD**2A carriers treated with standard dose from literature (*N* = 86)	*p* Value group 1 *vs* 2[1]	*p* Value group 1 *vs* 3[1]
Mean dose intensity (% of standard dose)	53	92	Unknown[2]	NA	NA
Hand-foot syndrome Grade 3	2 (5%)	84 (5%)	Unknown	>0.99	NA
Hematological toxicity Grade ≥3	4 (10%)	158 (10%)	48 (56%)	>0.99	**<0.001**
Gastrointestinal toxicity Grade ≥3	4 (10%)	150 (9%)	33 (38%)	0.78	**0.001**
Overall toxicity Grade ≥3	7 (18%)	372 (23%)	66 (77%)	0.57	**<0.001**
Treatment interruptions	5 (13%)	309 (19%)	Unknown	0.41	NA
Treatment discontinuation due to toxicity	7 (18%)	262 (16%)	Unknown	0.83	NA
Hospitalization	6 (15%)	179 (11%)	Unknown	0.44	NA
Treatment-related death	0 (0%)	2 (0%)	7 (8%)	>0.99	0.096

Abbreviations: NA: not applicable.
[1]*p* Value calculated using Fisher's Exact test. Values in bold are significant.
[2]Dose intensity is unknown, but patients started with standard dose (~100%).

For the effectiveness analysis, 37 pairs of *DPYD**2A carriers and wild-type patients were identified. When anticipating a 15% decrease in overall survival (from 45% in the wild type group to 30% in the carrier group at the end of the study, with a hazard ratio of wild-type *versus* carriers of 0.66), 154 pairs (192 events in total) will be needed to reach 80% power, with a 5% significance level (log-rank test, Freedman method using Stata v11.0). With the available 37 pairs of patients, only a difference of at least 33% is detectable with 80% power. This shows that our study was therefore underpowered. However, due to the low frequency of *DPYD**2A, it was not feasible to meet the required sample size.

The study shows that both overall and progression-free survival were comparable between *DPYD**2A variant allele carriers receiving reduced dose and wild-type patients receiving standard dose fluoropyrimidines. These results endorse the assumption that dose reductions do not result in inferior treatment outcome in these DPD deficient patients. This assumption has previously been made on the basis of DPD activity which is approximately 50% reduced in *DPYD**2A variant allele carriers, and a 50% fluoropyrimidine reduced dose is therefore expected to result in exposure that is comparable to exposure in *DPYD* wild-type patients receiving standard dose. This has also been shown by the fact that pharmacokinetic analyses in the previous prospective study confirmed that 5-FU exposure was equal between *DPYD**2A genotype-dosed patients and wild-type patients receiving full dose.[8] Furthermore, we found that toxicity risk in the *DPYD**2A carriers receiving a reduced dose was similar to toxicity risk in the cohort of wild-type patients, which further endorses this assumption.

Our study also confirms that upfront genotyping for *DPYD**2A improves patient safety of fluoropyrimidine therapy, in line with what was previously shown in a large prospective trial.[8] In the previous prospective study, grade ≥3 toxicity was found to be decreased from 73% to 28% by genotype-guided dosing, and in our current analysis this risk dropped from 77% to 18%, which is of the same order of magnitude. Treatment-related death decreased from 8% to 0% in our study. Our results are derived from a real-world population, which strengthens the implications of these findings for clinical practice. Importantly, Deenen *et al.* previously showed that genotype-guided dosing is also cost saving, as costs for treatment of severe adverse events and hospitalization are decreased, and outweigh costs of screening of the entire population.[8]

A few other small studies have been performed, that did investigate the effect of fluoropyrimidine dose individualization on effectiveness. In the study by Launay *et al.* 5-FU individualized dosing was based on DPD phenotype (measured as the ratio between uracil, the endogenous substrate of DPD and its product dihydrouracil). Of the 59 included patients with digestive cancer, 15 (25%) were identified as DPD deficient and received a reduced dose of 5-FU (average dose reduction of 35%). These dose reductions did not result in lower effectiveness in this small group of patients compared to the non-DPD deficient patients (p = 0.89 when comparing the number of patients with clinical benefit, stable disease and progressive disease).[27] A drawback of our study is the low sample size.

Our current study focused only on *DPYD**2A genotyping, while it has become clear in recent years that other *DPYD* variants result in DPD deficiency as well. Currently, the three additional variants c.2846A>T, c.1236G>A and c.1679T>G are considered clinically relevant and upfront genotyping for this panel is recommended.[28–30] For these polymorphisms it is expected that similar to *DPYD**2A, genotype-guided dosing does not negatively affect treatment outcome, while safety is significantly improved.

Due to the retrospective design of our study, patient data was not always complete, which hampered the matching of *DPYD**2A carriers to wild-type patients to some extent, as matching factors could not always be retrieved. Ideally additional matching factors which are thought to be relevant would be included, e.g. molecular subtypes of cancer which affect prognosis as well. However, this was not feasible with our current study design, as these data were not available. Due to the large control group of wild-type patients of 1606 patients that was available for matching, all available data were used in the best way possible to make the matching as adequate as possible.

In conclusion, this retrospective and matched-pair analysis supports the hypothesis that dose reductions in *DPYD**2A carriers do not result in inferior effectiveness of fluoropyrimidine-based chemotherapy, and that toxicity risk normalizes to the background toxicity risk in wild-type patients. Although these findings are preferably replicated to strengthen the assumption that effectiveness is not negatively affected, the results support current clinical guidelines which recommend a 50% upfront fluoropyrimidine dose reduction in *DPYD**2A variant allele carriers.[30]

Authors' contributions

Study concept and design: LH, DM, MD, AB, JB, AC, JS.

Acquisition, analysis or interpretation of data: LH, LM, DM, MD.

Drafting of the manuscript: LH.

Critical revision and approval of the manuscript: all authors.

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