

## Upfront Genotyping of *DYPD\*2A* to Individualize Fluoropyrimidine Therapy: A Safety and Cost Analysis

Maarten J. Deenen, Didier Meulendijks, Annemieke Cats, Marjolein K. Sechterberger, Johan L. Severens, Henk Boot, Paul H. Smits, Hilde Rosing, Caroline M.P.W. Mandigers, Marcel Soesan, Jos H. Beijnen, and Jan H.M. Schellens

### A B S T R A C T

#### Purpose

Fluoropyrimidines are frequently prescribed anticancer drugs. A polymorphism in the fluoropyrimidine metabolizing enzyme dihydropyrimidine dehydrogenase (DPD; ie, *DYPD\*2A*) is strongly associated with fluoropyrimidine-induced severe and life-threatening toxicity. This study determined the feasibility, safety, and cost of *DYPD\*2A* genotype-guided dosing.

#### Patients and Methods

Patients intended to be treated with fluoropyrimidine-based chemotherapy were prospectively genotyped for *DYPD\*2A* before start of therapy. Variant allele carriers received an initial dose reduction of  $\geq 50\%$  followed by dose titration based on tolerance. Toxicity was the primary end point and was compared with historical controls (ie, *DYPD\*2A* variant allele carriers receiving standard dose described in literature) and with *DYPD\*2A* wild-type patients treated with the standard dose in this study. Secondary end points included a model-based cost analysis, as well as pharmacokinetic and DPD enzyme activity analyses.

#### Results

A total of 2,038 patients were prospectively screened for *DYPD\*2A*, of whom 22 (1.1%) were heterozygous polymorphic. *DYPD\*2A* variant allele carriers were treated with a median dose-intensity of 48% (range, 17% to 91%). The risk of grade  $\geq 3$  toxicity was thereby significantly reduced from 73% (95% CI, 58% to 85%) in historical controls ( $n = 48$ ) to 28% (95% CI, 10% to 53%) by genotype-guided dosing ( $P < .001$ ); drug-induced death was reduced from 10% to 0%. Adequate treatment of genotype-guided dosing was further demonstrated by a similar incidence of grade  $\geq 3$  toxicity compared with wild-type patients receiving the standard dose (23%;  $P = .64$ ) and by similar systemic fluorouracil (active drug) exposure. Furthermore, average total treatment cost per patient was lower for screening (€2,772 [\$3,767]) than for nonscreening (€2,817 [\$3,828]), outweighing screening costs.

#### Conclusion

*DYPD\*2A* is strongly associated with fluoropyrimidine-induced severe and life-threatening toxicity. *DYPD\*2A* genotype-guided dosing results in adequate systemic drug exposure and significantly improves safety of fluoropyrimidine therapy for the individual patient. On a population level, upfront genotyping seemed cost saving.

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### INTRODUCTION

Fluoropyrimidines are anticancer drugs including intravenous fluorouracil (FU), its oral prodrug capecitabine, and the oral prodrug tegafur (component of tegafur-uracil and Teysuno [S-1; Taiho Pharmaceutical, Tokyo, Japan]). They are commonly prescribed for adjuvant as well as palliative treatment of various types of solid malignancies, including GI, breast, and head and neck cancers. Treatment with fluoropyrimidines is generally well tolerated, except in approximately 5% to 10% of the treated population, who develop

severe, potentially life-threatening toxicity early during treatment.<sup>1-3</sup> Treatment of severe toxicity is usually associated with interruption or even discontinuation of potentially effective anticancer therapy and often requires hospitalization. This has a great impact on a patient's prognosis and quality of life and also causes significant health care costs. Intolerance of fluoropyrimidines is mostly associated with deficiency of the primary FU detoxifying enzyme dihydropyrimidine dehydrogenase (DPD).<sup>4</sup> DPD inactivates approximately 80% to 90% of the administered or formed amount of FU into 5,6-dihydro-fluorouracil

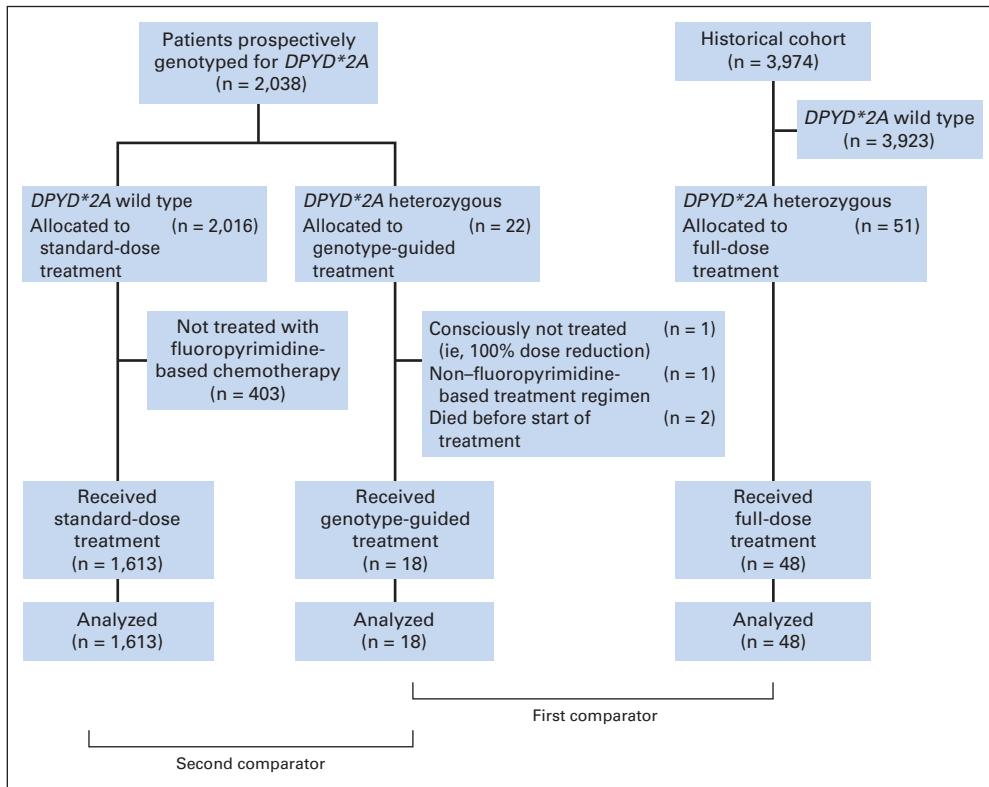


Fig 1. CONSORT diagram.

(FUH<sub>2</sub>).<sup>5</sup> The prevalence of DPD deficiency in whites is approximately 3% to 5%.<sup>6</sup>

Genetic polymorphism in its encoding gene *DPYD* is the best recognized cause of DPD deficiency, with the clinically most relevant polymorphism being *DPYD*\*2A (c.1905+1G>A; IVS14+1G>A; rs3918290). The frequency of *DPYD*\*2A is 1% to 2% in the Western world.<sup>7</sup> The loss of functional DPD activity induced by *DPYD*\*2A results from alternate splicing creating a truncated protein without residual enzyme activity.<sup>8,9</sup> Consequently, the likelihood of severe toxicity in genetically determined poor metabolizers, when receiving standard-dose fluoropyrimidine therapy, is significantly increased, as evidenced by numerous case reports<sup>7,10-13</sup> and retrospective<sup>14-19</sup> and prospective studies<sup>20-23</sup> and further supported by two recent systematic reviews and meta-analyses.<sup>24,25</sup> On the basis of these observations, we hypothesized that upfront genotyping of *DPYD*\*2A followed by individualized dose adjustment would improve safety of fluoropyrimidine therapy for patients and reduce overall treatment cost.

## PATIENTS AND METHODS

### Patient Population

The study population consisted of patients with cancer intended to undergo treatment with fluoropyrimidine-based anticancer therapy, either as single agent or in combination with other chemotherapy or radiotherapy, according to existing standard of care. Prior chemotherapy was allowed. Germline DNA was prospectively obtained and genotyped for *DPYD*\*2A before start of therapy. Heterozygous variant allele carriers were treated with an initial fluoropyrimidine starting dose reduced by  $\geq 50\%$  during the first two cycles, followed by further dose individualization based on tolerability; in the rare case a homozygous variant allele carrier was identified, treating phy-

sicians were advised to start with a minimal dose reduction of 85%. Further dose escalation was allowed up to 100% of the conventional dose for the intended treatment, provided that previous cycles were fully completed and no grade  $\geq 3$  toxicity had occurred. Dose escalation had to be determined in the best interest of the patient and was left to the discretion of the treating oncologist. Doses of nonfluoropyrimidine drugs or radiotherapy were standard and left unchanged at start of treatment. No intervention was applied in *DPYD*\*2A wild-type patients; they were treated according to existing standard-of-care treatment regimens.

### Study Design

This was a prospective, multicenter study conducted in one tertiary referral center (Netherlands Cancer Institute, Amsterdam, the Netherlands) and two large regional hospitals (Slotervaart Hospital, Amsterdam, and Canisius Wilhelmina Hospital, Nijmegen, the Netherlands). The primary end point was toxicity; secondary end points included total treatment cost of *DPYD*\*2A-guided dosing and determination of the pharmacokinetics and DPD enzyme activity in *DPYD*\*2A variant allele carriers.

Because a randomized trial was considered unethical, toxicity of *DPYD*\*2A genotype-guided dosing was compared with toxicity observed in historical controls (ie, patients with *DPYD*\*2A variant genotype previously treated with standard-dose fluoropyrimidine-based chemotherapy). The CONSORT diagram is shown in Figure 1. Historical controls were selected from published studies in which unselected cohorts of patients were genotyped for *DPYD*\*2A and treated with fluoropyrimidine-based chemotherapy. Appropriate trials were identified by a computerized PubMed literature search (search definition provided in Data Supplement). To avoid selection bias, patients described in case reports, case-control studies, review articles, and studies without patients polymorphic for *DPYD*\*2A were excluded from the historical cohort. The analysis was conducted using the pooled data of studies fulfilling the inclusion criteria for the historical cohort.

In addition to comparison with historical controls receiving the standard dose, toxicity of *DPYD*\*2A genotype-guided dosing was also compared with toxicity experienced by wild-type patients receiving the standard dose in our

study, under the assumption that these groups would experience similar frequency and severity of toxicity.

The study was approved by the medical ethical committees of all institutes, and all patients polymorphic for *DYPD*\*2A provided written informed consent before study registration. Toxicity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3.0).<sup>26</sup> In case of measurable disease, tumor measurements were evaluated according to RECIST (version 1.1).<sup>27</sup> A cost-minimization analysis was conducted using a decision analytic model from a health care payer perspective and was restricted to direct medical costs only. The model compared the screening versus nonscreening strategy. Costs of both strategies were calculated, based on costs of screening and subsequent drug treatment. Parameter estimations incorporated in the model were derived from data from our trial, such as patient demographics and treatment characteristics and costs, but also included relevant data from literature (when available), including population frequencies of *DYPD*\*2A and individual patient data on treatment outcome of *DYPD*\*2A variant allele carriers receiving the standard dose. The Data Supplement provides all parameter estimates. For the *DYPD*\*2A genotyping cost, a Dutch standard rate of €75 (\$102) per patient was maintained. Costs for patients who were genotyped for *DYPD*\*2A but eventually not treated with fluoropyrimidine-based chemotherapy were also taken into account. Parameter uncertainty was evaluated by one-way and probabilistic sensitivity analyses. Additional details of the pharmacologic and economic methods are provided in the Data Supplement.

### Statistical Analysis

The sample size calculation was based on the initial hypothesis that the intervention would reduce the incidence of grade  $\geq 3$  toxicity in *DYPD*\*2A variant allele carriers from 85% to 20%. An exact binomial test with a nominal .050 one-sided significance level had 94% power to detect this difference with a total of five variant allele carriers, corresponding to approximately 500 patients for genotyping. Obviously, power and clinical experience of *DYPD*\*2A genotype-guided dosing increased with increasing number of patients; for this reason, we aimed to genotype at least 2,000 patients (100% power).

All data were analyzed according to a per-protocol analysis. Associations between dichotomous outcomes and genotype status were tested using  $\chi^2$  or Fisher's exact test, where appropriate; 95% CIs were calculated using the exact method. Analyses with  $P$  values  $< .05$  were considered significant. All statistical analyses were conducted using SPSS software (version 20.0; SPSS, Chicago, IL).

## RESULTS

### Overall Patient and Treatment Characteristics

Between May 2007 and October 2011, a total of 2,038 consecutive patients with cancer for whom treatment with fluoropyrimidine-based chemotherapy was intended were prospectively genotyped for *DYPD*\*2A before start of therapy. In total, 22 patients (1.1%) proved to be heterozygously polymorphic for *DYPD*\*2A; no homozygous polymorphic carriers were identified. In total, 1,631 (80%) of the 2,038 screened patients were actually treated with fluoropyrimidine-based chemotherapy. Main reasons for not receiving fluoropyrimidine-based chemotherapy were indication for another chemotherapeutic regimen, poor performance status of the patient, or screening of patients referred for second opinion. Table 1 lists the patient demographics and treatment characteristics of the 1,631 treated patients. Colorectal cancer was the most prevalent tumor type. Most patients (90%) were treated with capecitabine, and 10% were treated with intravenous FU.

### Treatment Characteristics of *DYPD*\*2A Variant Allele Carriers

Of the 22 patients who were prospectively identified as *DYPD*\*2A variant allele carriers, 18 (82%) were treated with initially reduced doses of capecitabine (individual treatment data provided in Data Supplement). Four variant allele carriers were not treated with fluoropyrimidine-based chemotherapy: fluoropyrimidine treatment was withheld for one woman (ie, 100% dose reduction) in view of the identified *DYPD*\*2A polymorphism combined with her older age (79 years); one patient received non-fluoropyrimidine-based chemotherapy; and two patients died before start of fluoropyrimidine therapy, one as a result of postoperative complications and one as a result of rapid disease progression.

The genotype-guided dosing strategy resulted in two (11%), 11 (61%), and five (28%) of the 18 variant allele carriers experiencing grade 0, 1 to 2, and  $\geq 3$  toxicity, respectively. Toxicity was short in duration and well controlled using standard supportive care.

The median fluoropyrimidine dose-intensity per treatment cycle for *DYPD*\*2A genotype-guided dosing was 48% (range, 17% to 91%) of the standard indicated dose. In six variant allele carriers (33%), the fluoropyrimidine dose was escalated during treatment, in one patient up to the maximum 91%; in two patients with dose escalations, the dose was later reduced again because of toxicity. Despite starting dose reduction by  $\geq 50\%$ , in three patients (17%), the initial reduced dose was still too high and was further reduced to the minimum 17%. A total of four *DYPD*\*2A carriers were evaluable for response according to RECIST. Two patients achieved a partial response, and two patients had stable disease. In four (80%) of the five patients with rectal cancer treated with chemoradiotherapy, downstaging of the tumor from pT3-4 to ypT0-2 was reached.

### Toxicity of *DYPD*\*2A Genotype-Guided Dosing Versus Standard Dosing

A total of 14 studies fulfilled the inclusion criteria for the historical cohort, which together resulted in 3,974 patients. Of these patients, 51 (1.3%) carried the *DYPD*\*2A variant allele, of whom 48 patients actually received fluoropyrimidine-based treatment at the standard dose (ie, 100%). The Data Supplement lists the individual patient characteristics of the historical cohort. Compared with our prospective patient cohort, historical controls were more often treated with FU-based treatment regimens than with capecitabine-based treatment regimens. Furthermore, the observed prevalence of *DYPD*\*2A was slightly higher in the historical cohort, which may be the result of coincidence or a difference in prevalence in the studied population. Table 2 summarizes the overall treatment outcomes of *DYPD*\*2A genotype-guided dosing versus standard dosing. The incidence of grade  $\geq 3$  toxicity was reduced from 73% (95% CI, 58% to 85%) in variant allele carriers receiving the standard dose to 28% (95% CI, 10% to 53%) by genotype-guided dosing ( $P < .001$ ). Furthermore, the observed toxicity was short in duration, in contrast to the long-lasting toxicity usually observed in variant allele carriers receiving the full dose.<sup>34</sup> Importantly, this finding is also reflected by the absolute reduction in drug-induced death (grade 5 toxicity); as many as five (10%; 95% CI, 3% to 23%) of the 48 historical control patients receiving the standard dose had died as a result of fluoropyrimidine-induced grade 5 toxicity, compared with none (0%; 95% CI, 0% to 19%) who underwent genotype-guided dosing.

**Table 1.** Demographic and Clinical Characteristics of Treated Patients (n = 1,631)

Characteristic	DPYD*2A Wild-Type Patients (n = 1,613)		DPYD*2A Variant Allele Carriers (n = 18)		Overall Population (n = 1,631)	
	No.	%	No.	%	No.	%
Sex						
Male	724	45	7	39	731	45
Female	889	55	11	61	900	55
Race/ethnicity						
White	1,547	96	17	94	1,564	96
Asian	18	1	1	6	19	1
Other	48	3	0	0	48	3
Age, years						
Median	61		62		61	
Range	21-93		44-76		21-93	
BSA, m <sup>2</sup>						
Median	1.9		1.9		1.9	
Range	1.1-2.7		1.7-2.2		1.1-2.7	
Disease status						
Locally advanced CRC	538	33	7	39	545	33
Metastatic CRC	319	20	1	6	320	20
Metastatic BC	251	16	6	33	257	16
Gastric or gastroesophageal cancer	229	14	1	6	230	14
Locally advanced BC	119	7	1	6	120	7
Other	157	10	2	11	159	10
Previously treated with anticancer chemotherapy	412	26	5	28	417	26
Type of treatment regimen						
FU monotherapy	16	1	0	0	16	1
FU-based combination chemotherapy	98	6	0	0	98	6
FU plus radiotherapy	54	3	0	0	54	3
Capecitabine monotherapy	436	27	7	39	443	27
Capecitabine plus platinum agent	382	24	1	6	383	24
Capecitabine triplet regimen	115	7	0	0	115	7
Capecitabine plus other chemotherapeutics	86	5	3	17	89	5
Capecitabine plus radiotherapy	426	26	7	39	433	27
No. of treatment cycles						
Median	4		5		4	
Range	1-52		1-19		1-52	

Abbreviations: BC, breast cancer; BSA, body-surface area; CRC, colorectal cancer; FU, fluorouracil.

The genotype-guided dosing strategy resulted in toxicity rates comparable to those experienced by the second comparator group (ie, patients wild type for *DPYD\*2A* receiving standard-dose therapy; Table 3). This suggests that heterozygous *DPYD\*2A* variant allele carriers are not underexposed when treated with fluoropyrimidines at a starting dose reduced by 50%. Details of the adverse events that occurred in wild-type patients are provided in the Data Supplement.

### Pharmacokinetics of *DPYD\*2A* Genotype-Guided Dosing

From 16 (89%) treated *DPYD\*2A* variant allele carriers, whole blood was obtained for pharmacokinetic analysis. Figure 2 plots the dose-normalized area under the plasma concentration-time curve (AUC) and corresponding 95% CIs of capecitabine and its main metabolites for the *DPYD\*2A* genotype-guided cohort in comparison with pharmacokinetic data obtained from literature. Because patients were treated at various dosages, all AUCs were dose normalized to a dose of 1,250 mg/m<sup>2</sup>, which is allowed because capecitabine exhibits dose-proportional pharmacokinetics.<sup>35,36</sup> In line with the hy-

pothesis, the dose-normalized AUC of FU proved to be twice as high in patients with the *DPYD\*2A* variant genotype compared with the wild-type patient population, as a direct result of the lower DPD enzyme activity (Data Supplement). The dose-normalized AUCs of all other metabolites were overlapping in both patient populations, except for the last inactive metabolite FBAL, which seemed lower compared with one study.<sup>36</sup> Other pharmacokinetic parameters, including times to maximum concentration and apparent half-lives of all metabolites, were also comparable but tended to be slightly lower in reference to the population data (Data Supplement). In summary, the pharmacokinetic data demonstrate that *DPYD\*2A*-induced DPD deficiency increases the exposure to FU by approximately two-fold, underscoring that an average dose reduction of 50% in variant allele carriers results in common therapeutic exposure.

### Decision Analysis

The Data Supplement shows the decision tree used for the cost analysis, with the end nodes of the model being nonsevere (grade 0 to

**Table 2.** Treatment Outcome of *DPYD*\*2A Variant Allele Carriers Treated by Genotype-Guided Versus Standard Dosing (historical controls)

Outcome	<i>DPYD</i> *2A Genotype-Guided Dosing (our study)		<i>DPYD</i> *2A Full Dosing (historical controls) <sup>14-17,20-23,28-33</sup>		<i>P</i>
	No.	%	No.	%	
Total No. of patients polymorphic for <i>DPYD</i> *2A	22		51		
Evaluable No. of patients with <i>DPYD</i> *2A	18		48		
Dose-intensity, %					
First cycle					NA
Median	46				
Range	29-60				
All cycles					NA
Median	48				
Range	17-91				
Grade $\geq$ 3 hematologic toxicity					< .001
Yes	3	17	27	66	
95% CI, %	4 to 41		49 to 80		
No	15	83	14	34	
Missing data	—		7		
Grade $\geq$ 3 GI toxicity					.001
Yes	2	11	23	56	
95% CI, %	1 to 35		40 to 72		
No	16	89	18	44	
Missing data	—		7		
Overall grade $\geq$ 3 toxicity					< .001
Yes	5	28	35	73	
95% CI, %	10 to 53		58 to 85		
No	13	72	13	27	
Grade 5 toxicity (drug-induced death)	0	0	5	10	.19
95% CI, %	0 to 19		3 to 23		

Abbreviation: NA, not applicable.

<sup>†</sup>In genotype-guided dosing cohort, 22 of 2,038 prospectively screened patients were heterozygously polymorphic for *DPYD*\*2A, of whom 18 were actually treated with fluoropyrimidine-based chemotherapy (described in Results and Data Supplement); 51 historical controls were derived from total of 3,974 patients, of whom 48 patients were treated at 100% standard dose; three patients were treated with initially reduced fluoropyrimidine doses and were therefore excluded from historical cohort (Data Supplement).

<sup>‡</sup>Started with standard dose (ie, 100%).

2) versus severe (grade 3 to 5) toxicity. Values of the cost and probability estimates used in the model are listed in the Data Supplement.

In the base-case cost analysis, the expected total cost per patient in the screening strategy was €2,772 (\$3,767) compared

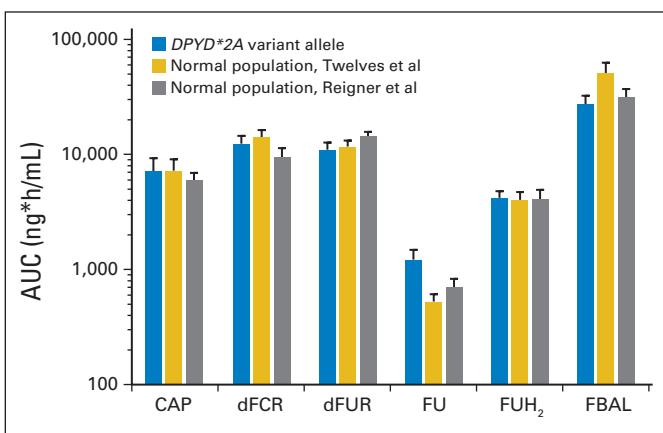
with €2,817 (\$3,828) in the nonscreening strategy, resulting in a cost savings of €45 (\$61) per patient. The probabilistic sensitivity analysis using 1,000 Monte Carlo simulations resulted in an average cost savings of €44 (range, −€74 to €331) per patient. The tornado diagram (Fig 3) shows the effect on the cost savings of screening when all model parameters are varied individually by  $\pm$  20%. The model was shown to be most sensitive to the likelihood of toxicity-related hospitalization of *DPYD*\*2A variant allele carriers receiving the standard dose, followed by the polymorphism frequency of *DPYD*\*2A and genotyping costs.

## DISCUSSION

The results of this study show that upfront genotyping of *DPYD*\*2A is feasible, improves safety of fluoropyrimidine therapy for patients, and is more likely cost saving. Genotype-guided dosing in a daily-life patient population significantly reduced the incidence of grade  $\geq$  3 toxicity, from 73% in historical controls to 28% in the genotype-guided treatment cohort. In contrast to the long-lasting and life-threatening toxicity that typically occurs with full dosing, the observed toxicity with genotype-guided dosing was short in duration and well controlled with general supportive care. This is clearly demonstrated by absolute risk reduction in the incidence of drug-induced death

**Table 3.** Adverse Events in Variant Allele Carriers Receiving *DPYD*\*2A Genotype-Guided Dosing Versus *DPYD*\*2A Wild-Type Patients Receiving Standard Dose

Adverse Event	<i>DPYD</i> *2A Wild-Type Patients (n = 1,613)		<i>DPYD</i> *2A Genotype-Guided Dosing (n = 18)		<i>P</i>
	No.	%	No.	%	
Hematologic grade 1-2 toxicity	562	35	3	17	.11
Hematologic grade $\geq$ 3 toxicity	159	10	3	17	.34
Grade 1-2 diarrhea	474	29	8	44	.16
Grade $\geq$ 3 diarrhea	133	8	1	6	.68
Grade 1-2 hand-foot syndrome	445	28	8	44	.11
Grade $\geq$ 3 hand-foot syndrome	86	5	2	11	.28
Any grade 1-2 toxicity	871	54	11	61	.55
Any grade $\geq$ 3 toxicity	373	23	5	28	.64



**Fig 2.** Pharmacokinetics of *DPYD\*2A* pharmacogenetic-guided dosing. Dose-normalized average (95% CI) area under the plasma concentration-time curves (AUCs) of capecitabine (CAP) and metabolites in *DPYD\*2A* variant allele carriers (blue bars) versus population values (Reigner et al<sup>35</sup> study, gray bars; Twelves et al<sup>36</sup> study, gold bars). Because patients were treated at various dosages, all AUCs were dose normalized to a dose of 1,250 mg/m<sup>2</sup>. dFCR, 5'-deoxy-5-fluorocytidine; dFUR, 5'-deoxy-5-fluorouridine; FBAL, fluoro-beta-alanine; FU, fluorouracil; FUH<sub>2</sub>, 5-fluoro-5,6-dihydrouracil.

from 10% to 0%. DPD enzyme activity measurements confirmed the partial DPD deficiency induced by *DPYD\*2A*, and the pharmacokinetic analysis showed that adequate systemic exposure to FU was achieved after an average dose reduction of 50%. In addition, the cost analysis showed that the screening strategy was more likely cost saving, and there is, based on the uncertainty estimates, only a low probability that this may not be the case.

Although two recent systematic reviews and meta-analyses clearly showed the clinical relevance of screening for *DPYD\*2A*,<sup>24,25</sup> others still debate whether screening for DPD deficiency should become standard of care in treatment with fluoropyrimidines.<sup>37</sup> To our knowledge, ours is the first trial to prospectively evaluated the safety, pharmacokinetics, and costs of *DPYD\*2A* genotype-guided dosing in fluoropyrimidine-based chemotherapy. The data from the historical cohort clearly demonstrate that standard-dose treatment with fluoro-

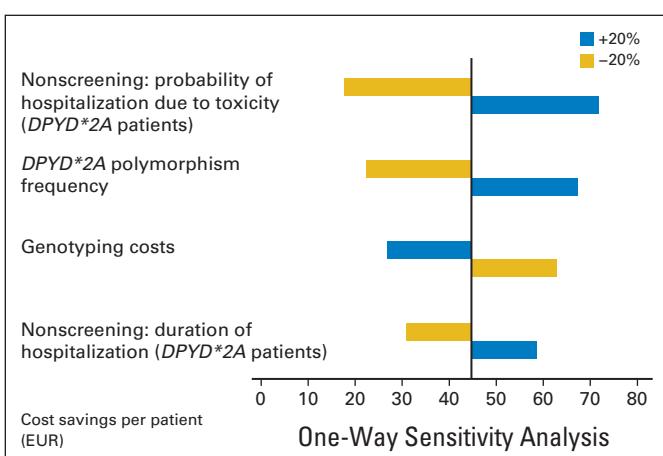
pyrimidines in *DPYD\*2A* variant allele carriers results in unacceptably high rates of severe toxicity and is lethal in approximately 10% of these patients. In comparison, the incidence of fluoropyrimidine-induced death in the overall patient population is only 0.5% to 1%, which is thereby explained for at least 10% to 20% of all toxic deaths by *DPYD\*2A* alone. Given this strong association with severe and lethal toxicity, a randomized trial was considered unethical, and therefore, toxicity data observed in historical controls obtained from appropriate trials were used as the primary comparator instead.

In a recent pilot study, an adequate dosing algorithm in *DPYD\*2A* variant allele carriers could not be defined.<sup>38</sup> In our trial, we demonstrated that patients can be safely treated with starting doses reduced by 50%. Furthermore, in a retrospective study encompassing 568 patients with colorectal cancer, we demonstrated that *DPYD\*2A* is significantly associated with fluoropyrimidine-induced toxicity and toxicity-related dose reductions.<sup>15</sup> In that study, the mean dose-intensity in the seven *DPYD\*2A* variant allele carriers (although started at 100% dose) decreased from 89% in cycle one to 62% in cycle two to 49% in cycle three as a result of toxicity-induced dose reductions; in contrast, dose-intensity in the wild-type patients remained high (96%, 94%, and 93% in cycles one, two, and three, respectively;  $P < .001$ ).<sup>15</sup> The strength of this finding is that at the time of dose prescription, the treating physicians were unaware of the patients' genotype. These results independently support the observation in the current study that an initial 50% fluoropyrimidine dose reduction followed by further individualization based on tolerability is a valid strategy.

The primary objective of this study was to determine the safety of *DPYD\*2A* genotype-guided dosing. Clearly, given the relatively low frequency of *DPYD\*2A*, a study with efficacy as the co-primary or secondary end point is not feasible, because a sample size of tens of thousands of patients would be necessary. However, we argue that it is unlikely that antitumor activity is affected by genotype-guided dosing, because the pharmacokinetic analysis demonstrated similar FU exposure. Also, the incidence of adverse events was comparable to that observed in wild-type patients receiving the standard dose. In addition, although underpowered, overall and progression-free survival were not different between *DPYD\*2A* variant and wild-type patients in our previous retrospective analysis, despite the significant dose reductions applied in variant allele carriers.<sup>15</sup>

A limitation of screening only for *DPYD\*2A* is that only approximately 25% of all DPD-deficient patients are identified, given the fact that 3% to 5% of the population is DPD deficient. Sensitivity could be increased by testing for additional *DPYD* polymorphisms, such as c.2846A>T, c.1679T>G, or c.1236G>A, or by phenotypic approaches.<sup>15,21,24,25,39</sup> At the time this prospective study was started, associations of polymorphisms other than *DPYD\*2A* with toxicity were not yet known or sufficiently established and therefore not taken into consideration.

This study demonstrates for the first time to our knowledge the feasibility of upfront genotyping in daily practice, without delaying start of treatment. Worldwide, hundreds of thousands of patients receive fluoropyrimidine-based chemotherapy each year; genotype-based dose adaptation could prevent thousands of patients from developing fluoropyrimidine-induced severe and potentially lethal toxicity. Our current follow-up study (ClinicalTrials.gov identifier NCT02324452) addresses the safety and cost savings of genotyping for



**Fig 3.** One-way sensitivity analysis of upfront *DPYD\*2A* genotyping versus nonscreening. Vertical line indicates base-case cost savings per patient of screening. All parameters were individually varied by  $\pm 20\%$ , effects of which on cost savings are indicated by horizontal bars.

*DYPD\*2A* plus additional polymorphisms in *DYPD* and upfront phenotyping in 2,000 new patients.

In conclusion, prospective screening for *DYPD\*2A* is life saving, feasible, and cost saving, outweighing screening costs. It should therefore become standard of care in treatment with fluoropyrimidines.

## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at [www.jco.org](http://www.jco.org).

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## AUTHOR CONTRIBUTIONS

**Conception and design:** Maarten J. Deenen, Annemieke Cats, Johan L. Severens, Caroline M.P.W. Mandigers, Marcel Soesan, Jos H. Beijnen, Jan H.M. Schellens

**Provision of study materials or patients:** Annemieke Cats, Henk Boot, Caroline M.P.W. Mandigers, Marcel Soesan, Jan H.M. Schellens

**Collection and assembly of data:** Maarten J. Deenen, Didier Meulendijks, Jan H.M. Schellens

**Data analysis and interpretation:** Maarten J. Deenen, Didier Meulendijks, Annemieke Cats, Marjolein K. Sechterberger, Henk Boot, Paul H. Smits, Hilde Rosing, Jos H. Beijnen, Jan H.M. Schellens

**Manuscript writing:** All authors

**Final approval of manuscript:** All authors

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**AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST**

**Upfront Genotyping of *DPYD*\*2A to Individualize Fluoropyrimidine Therapy: A Safety and Cost Analysis**

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**Maarten J. Deenen**

No relationship to disclose

**Didier Meulendijks**

No relationship to disclose

**Annemieke Cats**

No relationship to disclose

**Marjolein K. Sechterberger**

No relationship to disclose

**Johan L. Severens**

No relationship to disclose

**Henk Boot**

No relationship to disclose

**Paul H. Smits**

No relationship to disclose

**Hilde Rosing**

No relationship to disclose

**Caroline M.P.W. Mandigers**

No relationship to disclose

**Marcel Soesan**

No relationship to disclose

**Jos H. Beijnen**

No relationship to disclose

**Jan H.M. Schellens**

No relationship to disclose