Survival of patients with cancer with DPYD variant alleles and dose-individualized fluoropyrimidine therapy: a matched-pair analysis

Knikman, J.E.; Wilting, T.A.; Lopez-Yurda, M.; Henricks, L.M.; Lunenburg, C.A.T.C.; Man, F.M. de; ... ; Cats, A.

Survival of Patients With Cancer With *DPYD* Variant Alleles and Dose-Individualized Fluoropyrimidine Therapy—A Matched-Pair Analysis

Jonathan E. Knikman, MSc[1,2]; Tycho A. Wilting, MSc[1]; Marta Lopez-Yurda, PhD[3]; Linda M. Henricks, PhD[4]; Carin A.T.C. Lunenburg, PhD[5]; Femke M. de Man, PhD[6]; Didier Meulendijks, PhD[1,7,8]; Peter Nieboer, PhD[9]; Helga J. Droogendijk, PhD[10]; Geert-Jan Creemers, PhD[11]; Caroline M.P.W. Mandigers, PhD[12]; Alexander L.T. Imholz, PhD[13]; Ron H.J. Mathijssen, PhD[6]; Johanneke E.A. Portielje, PhD[5,14]; Liselot Valkenburg-van Iersel, PhD[15]; Annelie Vulink, PhD[16]; Marlene H.W. van der Poel, PhD[17]; Arnold Baars, PhD[18]; Jesse J. Swen, PhD[19]; Hans Gelderblom, PhD[5]; Jan H.M. Schellens, PhD[20]; Jos H. Beijnen, PhD[2,21]; Henk-Jan Guchelaar, PhD[19]; and Annemieke Cats, PhD[22]

DOI https://doi.org/10.1200/JCO.22.02780

ABSTRACT

PURPOSE *DPYD*-guided fluoropyrimidine dosing improves patient safety in carriers of *DPYD* variant alleles. However, the impact on treatment outcome in these patients is largely unknown. Therefore, progression-free survival (PFS) and overall survival (OS) were compared between *DPYD* variant carriers treated with a reduced dose and *DPYD* wild-type controls receiving a full fluoropyrimidine dose in a retrospective matched-pair survival analysis.

METHODS Data from a prospective multicenter study (ClinicalTrials.gov identifier: NCT02324452) in which *DPYD* variant carriers received a 25% (c.1236G>A and c.2846A>T) or 50% (*DPYD**2A and c.1679T>G) reduced dose and data from *DPYD* variant carriers treated with a similarly reduced dose of fluoropyrimidines identified during routine clinical care were obtained. Each *DPYD* variant carrier was matched to three *DPYD* wild-type controls treated with a standard dose. Survival analyses were performed using Kaplan-Meier estimates and Cox regression.

RESULTS In total, 156 *DPYD* variant carriers and 775 *DPYD* wild-type controls were available for analysis. Sixty-one c.1236G>A, 25 *DPYD**2A, 13 c.2846A>T, and—when pooled—93 *DPYD* variant carriers could each be matched to three unique *DPYD* wild-type controls. For pooled *DPYD* variant carriers, PFS (hazard ratio [HR], 1.23; 95% CI, 1.00 to 1.51; $P = .053$) and OS (HR, 0.95; 95% CI, 0.75 to 1.51; $P = .698$) were not negatively affected by *DPYD*-guided dose individualization. In the subgroup analyses, a shorter PFS (HR, 1.43; 95% CI, 1.10 to 1.86; $P = .007$) was found in c.1236G>A variant carriers, whereas no differences were found for *DPYD**2A and c.2846A>T carriers.

CONCLUSION In this exploratory analysis, *DPYD*-guided fluoropyrimidine dosing does not negatively affect PFS and OS in pooled *DPYD* variant carriers. Close monitoring with early dose modifications based on toxicity is recommended, especially for c.1236G>A carriers receiving a reduced starting dose.

ACCOMPANYING CONTENT

Appendix

Accepted July 11, 2023
Published August 28, 2023

J Clin Oncol 41:5411-5421

View Online Article

INTRODUCTION

Since the introduction of fluorouracil (5-FU) and more recently capecitabine (oral prodrug of 5-FU), fluoropyrimidine-based chemotherapy has become a cornerstone in the treatment of many solid tumors.[1] Dihydropyrimidine dehydrogenase (DPD) plays a key role in fluoropyrimidine-related toxicity.[2,3] DPD deficiency leads to decreased catabolism of 5-FU, and consequently, to a shift toward its active metabolites.[4,5] Single-nucleotide variants in the *DPYD* gene (*DPYD**2A, c.2846A>T, c.1236G>A, and c.1679T>G), encoding for DPD, are a dominant cause of decreased DPD enzyme activity, thereby increasing the exposure to fluoropyrimidines and the risk of developing severe fluoropyrimidine-related toxicity including diarrhea, mucositis, nausea, vomiting, and hand-foot syndrome.[6-10] In a large prospective clinical trial (the Alpe-DPD study; ClinicalTrials.gov identifier: NCT02324452), we showed that by reducing the starting fluoropyrimidine dose to 50% for heterozygous *DPYD**2A carriers, these patients could be

CONTEXT

Key Objective

To determine whether reduced fluoropyrimidine dosing in *DPYD* variant carriers influences progression-free survival (PFS) and overall survival compared with *DPYD* wild-type patients.

Knowledge Generated

DPYD-guided dose individualization of fluoropyrimidine-based chemotherapy likely can be performed without compromising effectiveness. However, PFS for c.1236G>A treated with a 25% reduced dose was significantly shorter compared with *DPYD* wild-type patients treated with a full dose. Close monitoring and early dose modifications are recommended when treating c.1236G>A variant carriers with a reduced starting dose.

Relevance *(A.H. Ko)*

This analysis provides some reassuring evidence that dose reducing fluoropyrimidine-based chemotherapy for patients who are carriers of pathogenic *DPYD* variants does not appear to compromise treatment efficacy, although confirmation is needed in larger cohorts and for specific *DPYD* genotypes.*

*Relevance section written by *JCO* Associate Editor Andrew H. Ko, MD, FASCO.

safely treated.[10] However, the application of a 25% dose reduction in heterozygous c.2846A>T and c.1236G>A carriers was not accompanied by a significant decrease in severe toxicity. Consequently, the need for a larger dose reduction with toxicity-guided dose titration in heterozygous carriers of c.2846A>T and c.1236G>A was considered and is currently recommended by the Clinical Pharmacogenetics Implementation Consortium (CPIC).[11,12] In another study, we showed that progression-free survival (PFS) and overall survival (OS) were not negatively affected by an initial dose reduction of 50% in 37 *DPYD**2A carriers.[13] Nonetheless, the impact of a reduced fluoropyrimidine dose on the survival in a larger cohort of *DPYD* variant carriers, consisting of c.2846A>T, c.1236G>A, and c.1679T>G carriers as well, has yet not been reported. A traditional approach using a randomized clinical trial comparing survival in *DPYD* variant carriers treated with a full dose would be unethical and unfeasible because of the known increased risk of severe fluoropyrimidine-related toxicity, as was recently also addressed by both Hertz and Baker et al[6,14,15] Therefore, we compared the effectiveness of fluoropyrimidine treatment after dose reduction in patients carrying a *DPYD**2A, c.2846A>T, c.1236G>A, or c.1679T>G variant with *DPYD* wild-type patients treated with a full dose using a matched-pair analysis.

METHODS

Study Design and Participants

The design, study population, and results of the Alpe-DPD (ClinicalTrials.gov identifier: NCT02324452) study have previously been published.[10] In brief, patients treated with fluoropyrimidines were included. Heterozygous *DPYD* variant carriers received an initial dose reduction of 25% (c.2846A>T and c.1236G>A) or 50% (*DPYD**2A and c.1679T>G). *DPYD* wild-type controls were treated with the full dose. A total of 1,103 evaluable patients were enrolled between April 2015 and December 2017, of whom 85 were heterozygous carriers of one of the abovementioned four *DPYD* variants. The Alpe-DPD study was approved by the medical ethical committees of each participating hospital, and all patients provided informed consent before enrollment in the study. This included use of collected data for future studies. The present retrospective analysis investigates the effect of the reduced fluoropyrimidine dose on treatment efficacy in *DPYD* variant carriers (n = 82) treated in 14 of the 17 hospitals (n = 1,019) from the Alpe-DPD study, enriched with all *DPYD* variant carriers (n = 143) who were treated according to a similar protocol with the same dose reductions as part of routine clinical care between February 2013 and March 2020 in the Netherlands Cancer Institute (Amsterdam, the Netherlands). Data regarding disease progression, survival, and treatment (follow-up until February 2021) were either used from the Alpe-DPD study or collected from electronic medical records. Data regarding toxicity were only available from patients included in the Alpe-DPD study.[10] This study was approved by the institutional review board or ethics committee at each hospital and was conducted following the principles of the Declaration of Helsinki and Good Clinical Practice. No additional informed consent was needed as patients from the Alpe-DPD study had already consented to use of their data for future studies, and data from the patients from routine clinical care were anonymized.

Matching

Owing to the large degree of heterogeneity among carriers, specifically in terms of treatment received and primary tumor type, a matched-pair analysis was chosen over a

multivariable regression model accounting for these and other characteristics. In each matched group (pooled *DPYD* variant carriers and the individual *DPYD**2A, c.2846A>T and c.1236G>A carriers), each *DPYD* variant carrier was matched to three unique *DPYD* wild-type controls from the Alpe-DPD study.[10] Patients were matched based on available characteristics that we considered relevant for treatment outcome, that is, sex, age (±10 years), primary tumor type (colorectal, breast, gastric, or others), stage of cancer (local, locally advanced, or metastatic), and treatment regimen (Appendix Table A1, online only). Patients with missing data regarding disease progression or death were excluded before matching. Exact matching without replacement was performed per matching group, using R-package MatchIt version 4.3.0.[16] Therefore, wild-type controls were only used once per matched group.

Statistical Analysis

PFS was defined as the time between initiation of treatment and first signs of disease progression by either clinical signs or radiological imaging or death from any cause, whichever came first. OS was defined as the time between initiation of treatment and death from any cause. Patients not experiencing disease progression or death before the end of follow-up were censored at the last date known to be alive. Standardized differences were used to examine the balance in baseline covariates between carriers and wild-type controls in the matched and unmatched samples. PFS and OS curves were generated using the Kaplan-Meier method. A (stratified) log-rank test was used to compare survival between *DPYD* variant carriers and wild-type controls. Univariable Cox regression analyses were performed to test the association between *DPYD* status and PFS and OS. Hazard ratios (HRs) and their corresponding 95% CI were obtained. To account for the matched nature of the data, Cox regression with standard errors calculated using the jackknife sandwich estimator was used as the primary method and Cox regression stratified for matched groups as the secondary method.[17-19] The first approach results in an estimated HR equivalent to the one obtained by a conventional Cox regression, but with a robust variance estimator accounting for clustering within matched groups. The stratified Cox regression is an approach that conditions the matched groups and assumes a common HR, but different baseline hazards, which might not be realistic in many situations and, therefore, used as the secondary approach. Both approaches could be seen as complementary alternatives.[19] Exploratory analysis was performed for all variants pooled and for the individual genetic subgroups *DPYD**2A, c.2846A>T, and c.1236G>A. In addition, multivariable Cox regression analyses were performed for all available *DPYD* variant carriers and wild-type controls (before matching), adjusted for matching variables. Schoenfeld residuals were used to verify the proportional hazards assumption. Median follow-up was calculated using the reverse Kaplan-Meier method. Given the small numbers of the variant carrier subgroups and the exploratory nature of these analyses, no multiplicity adjustments were performed. All statistical analyses were performed using R v3.6.3,[20] and $P < .05$ were considered statistically significant.

RESULTS

In total, 1,162 patients were available for inclusion. Because of missing data regarding disease progression or death, 231 patients were excluded, resulting in a total number of 931 patients. Survival data from 72 *DPYD* variant carriers (13 *DPYD**2A, 14 c.2846A>T, and 45 c.1236G>A) included from the Alpe-DPD study[10] and 84 *DPYD* variant carriers (31 *DPYD**2A, 7 c.2846A>T, and 46 c.1236G>A) identified during routine clinical care in the Netherlands Cancer Institute and treated with a reduced dose of fluoropyrimidines were available for analysis. No survival data were available of the single c.1679T>G variant allele carrier, inhibiting further analysis of this variant. Survival data from 775 *DPYD* wild-type controls treated with a full dose identified during the Alpe-DPD study were available for matching. The characteristics of the matched groups are listed in Table 1.

In total, 156 *DPYD* variant carriers were available for matching. When pooled, 93 *DPYD* variant carriers (25 *DPYD**2A, 13 c.2846A>T, and 55 c.1236G>A) could be matched to three unique *DPYD* wild-type controls. These 93 *DPYD* variant carriers consisted of 52 carriers from the Alpe-DPD study and 41 from routine clinical care. When matched according to *DPYD* variant allele 25 *DPYD**2A, 13 c.2846A>T, and 61 c.1236G>A, carriers could be matched to three unique *DPYD* wild-type controls (Fig 1). Standardized mean differences were all within 0.1 for the matching variables between carriers and noncarriers, indicating a good balance in baseline covariates. Median follow-up time and outcomes of PFS and OS are shown in Table 2. The Kaplan-Meier–estimated PFS and OS distributions for the matched groups are shown in Figure 2.

Cox regression analysis using the JSE showed no statistically significant difference in PFS (Table 3) for the 93 pooled *DPYD* variant carriers compared with their matched wild-type controls (HR, 1.23; 95% CI, 1.00 to 1.51; $P = .053$), but significantly shorter PFS in the subgroup of 61 c.1236G>A variant carriers (HR, 1.43; 95% CI 1.10 to 1.86; $P = .007$). No statistically significant difference in PFS was found between 25 *DPYD**2A and 13 c.2846A>T variant carriers and matched wild-type controls (Table 3). Alternatively, Cox regression analyses stratified for matched groups were also performed, finding significantly shorter PFS among the pooled *DPYD* group and the c.1236G>A variant carriers. For the subgroup of c.2846A>T carriers, a nonsignificant shorter PFS with an HR of 2.48 was found (Appendix Table A2, online only). Although not significant, the multivariable Cox regression indicated a slightly attenuated difference for PFS for the pooled *DPYD* variants (HR, 1.18; 95% CI 0.95 to 1.46; $P = .135$), more pronounced for the c.1236G>A variant and no shorter PFS in the subgroup of c.2846A>T carriers (Appendix Table A3, online only).

TABLE 1. Patient Characteristics of Matched Groups

Characteristic	Matched Pooled *DPYD* Variants		Matched *DPYD*2A* Variants		Matched c.2846A>T Variants		Matched c.1236G>A Variants	
	DPYD Wild-Type (n = 279)	*DPYD* Variant (n = 93)	*DPYD* Wild-Type (n = 75)	*DPYD*2A* (n = 25)	*DPYD* Wild-Type (n = 39)	c.2846A>T (n = 13)	*DPYD* Wild-Type (n = 183)	c.1236G>A (n = 61)
Sex, No. (%)								
Male	144 (51.6)	48 (51.6)	36 (48.0)	12 (48.0)	24 (61.5)	8 (61.5)	96 (52.5)	32 (52.5)
Female	135 (48.4)	45 (48.4)	39 (52.0)	13 (52.0)	15 (38.5)	5 (38.5)	87 (47.5)	29 (47.5)
Age, median, (IQR)	65 (57-72)	63 (55-71)	65 (58-71)	64 (57-69)	69 (54-71)	69 (53-72)	63 (54-71)	63 (53-71)
Stage of cancer, No. (%)								
Local	33 (11.8)	11 (11.8)	6 (8.0)	2 (8.0)	6 (15.4)	2 (15.4)	27 (14.8)	9 (14.8)
Locally advanced	102 (36.6)	34 (36.6)	30 (40.0)	10 (40.0)	15 (38.5)	5 (38.5)	57 (31.1)	19 (31.1)
Metastatic	144 (51.6)	48 (51.6)	39 (52.0)	13 (52.0)	18 (46.2)	6 (46.2)	99 (54.1)	33 (54.1)
Primary tumor type, No. (%)								
Colorectal	186 (66.6)	62 (66.6)	45 (60.0)	15 (60.0)	24 (61.5)	8 (61.5)	117 (63.9)	39 (63.9)
Breast	60 (21.5)	20 (21.5)	21 (28.0)	7 (28.0)	9 (23.1)	3 (23.1)	36 (19.7)	12 (19.7)
Gastric	3 (1.1)	1 (1.1)	3 (4.0)	1 (4.0)	0 (0)	0 (0)	0 (0)	0 (0)
Other[a]	30 (10.8)	10 (10.8)	6 (8.0)	2 (8.0)	6 (15.4)	2 (15.4)	30 (16.4)	10 (16.4)
Treatment regimen,[b] No. (%)								
CAP/5-FU monotherapy	72 (25.8)	24 (25.8)	21 (28.0)	7 (28.0)	12 (30.8)	4 (30.8)	45 (24.6)	15 (24.6)
CAP/5-FU other	9 (3.2)	3 (3.2)	3 (4.0)	1 (4.0)	0 (0)	0 (0)	6 (3.3)	2 (3.3)
CAPOX regimens	108 (38.7)	36 (38.7)	27 (36.0)	9 (36.0)	15 (38.5)	5 (38.5)	72 (39.3)	24 (39.3)
Chemoradiotherapy	90 (32.3)	30 (32.3)	24 (32.0)	8 (32.0)	12 (30.8)	4 (30.8)	60 (32.8)	20 (32.8)
Treatment cycles								
Median (IQR)	3 (1-8)	3 (1-8)	3 (1-8)	4 (1-8)	4 (1-8)	2 (1-5)	3 (1-8)	3 (1-8)
1, No. (%)	91 (32.6)	29 (25.8)	25 (33.3)	7 (28.0)	13 (33.3)	5 (38.5)	60 (32.8)	19 (31.1)
2, No. (%)	25 (9.0)	12 (12.9)	7 (9.3)	4 (16.0)	3 (7.7)	2 (15.4)	17 (9.3)	6 (9.8)
3, No. (%)	29 (10.4)	9 (9.7)	8 (10.7)	1 (4.0)	3 (7.7)	1 (7.7)	17 (9.3)	8 (13.1)
4, No. (%)	20 (7.2)	6 (6.5)	3 (4.0)	2 (8.0)	5 (12.8)	1 (7.7)	14 (7.7)	4 (6.6)
≥5, No. (%)	114 (40.9)	37 (39.8)	32 (42.7)	11 (44.0)	15 (38.5)	4 (30.7)	75 (41.0)	24 (39.3)
Dose intensity (%), median (IQR)								
First cycle	100 (95.8-100.3)	73.4 (55.9-75.0)	100 (94.5-100.0)	49.4 (47.2-55.9)	100 (94.4-100.0)	72.5 (70.6-77.0)	100 (95.3-101.3)	75.0 (72.5-75.8)
All cycles	98.8 (88.4-100.0)	71.4 (56.5-75.0)	97.1 (87.9-100.0)	50.7 (47.2-56.5)	98.5 (86.8-100.0)	72.5 (62.9-75.0)	98.8 (89.1-100.0)	73.9 (68.4-75.6)
Dose modification after initial dose, No. (%)								
No modification	201 (72.0)	58 (62.4)	56 (74.7)	15 (60.0)	30 (76.9)	6 (46.2)	135 (73.8)	41 (67.2)
Escalation	11 (3.9)	16 (17.2)	4 (5.3)	6 (24.0)	0	3 (23.1)	5 (2.7)	7 (11.5)
Reduction	67 (24.1)	19 (20.4)	15 (20.0)	4 (16.0)	9 (23.1)	4 (30.8)	43 (23.5)	13 (21.3)

Abbreviations: CAP/5-FU monotherapy, capecitabine or fluorouracil monotherapy; CAP/5-FU other, capecitabine or 5-FU in combination with other anticancer drugs; CAPOX regimens, capecitabine and oxaliplatin-based regimens.

[a]Other tumor types included head and neck cancer, esophagogastric cancer, anal cancer, vulva carcinoma, urethral cancer, and several rare tumor types.

[b]CAP/5-FU other includes combinations of capecitabine or 5-FU with cisplatin, carboplatin, docetaxel, irinotecan, vinorelbine, temozolomizde, streptozocin, or monoclonal antibodies (bevacizumab, panitimumab, and trastuzumab); capecitabine and oxaliplatin-based regimens in combination with bevacizumab, panitimumab, or trastuzumab; Chemoradiotherapy regimens in combination with mitomycin, cisplatin, or oxaliplatin.

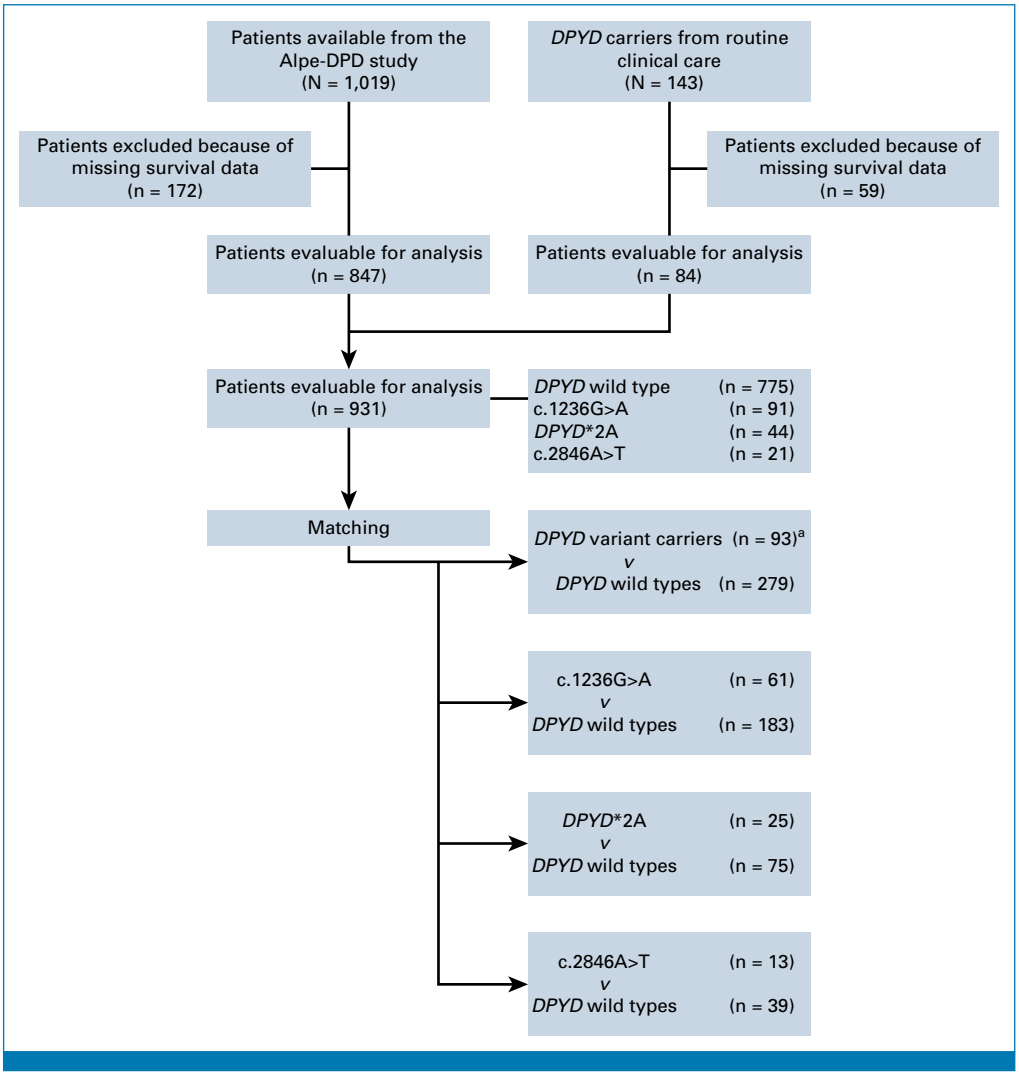

FIG 1. Flow diagram of patient inclusion and matching. [a]Unique controls were used per matched group; therefore, not all *DPYD* variant carriers could be matched, and the total of included *DPYD* variants included in the pooled group (c.1236G>A [n = 55], *DPYD**2A [n = 25], and c.2846A>T [n = 13]) is not the sum of individually matched *DPYD* variant carriers. DPD, dihydropyrimidine dehydrogenase.

Cox regression analysis did not show significant differences in OS (Table 4) for the pooled *DPYD*, c.2846A>T, and c.1236G>A variant carriers compared with matched wild-type controls, and the corresponding HRs were close to the value of 1. In contrast, 25 *DPYD**2A carriers were found to have longer OS than wild types (HR, 0.61; 95% CI, 0.38 to 0.98; $P = .042$). Cox regression analysis stratified for matched groups did not show any differences in OS between all matched groups (Appendix Table A2). The results of the multivariable Cox regression analyses were consistent with those of the primary matched-pair analysis (Appendix Table A4, online only). All Cox regression analyses did not violate proportional hazard assumptions.

To explore the robustness of the results, sensitivity analyses were performed (Appendix Tables A5-A7, online only). Alternative matching strategies were considered including one or two matched controls when less than three matches could be found and 1:2 matching. Adding the date (year) of start of treatment to the matching variables, allowing for a maximum difference of 2 years between matched patients, was also performed to account for possible secular trends. All alternative matching strategies resulted in similar results as compared with the primary analysis. Toxicity data were only available from patients included in the Alpe-DPD study and showed that severe fluoropyrimidine toxicity was substantially more present in *DPYD* variant carriers, despite dose reductions, compared with the matched *DPYD* wild types (Appendix Table A8, online only).

DISCUSSION

The results of our study showed no significant differences in PFS and OS between the pooled *DPYD* variant carriers and matched *DPYD* wild-type patients, suggesting that *DPYD*-guided dose individualization can likely be performed safely

TABLE 2. PFS and OS in Pooled and Subgroup *DPYD* Variant Carriers Matched to *DPYD* Wild-Type Patients

Matched Groups	n	Median Follow-Up Time, Months	95% CI, Months	PFS Events, No. (%)	Median PFS, Months	95% CI, Months	5-Year PFS, %	95% CI, %	Deceased, No. (%)	Median OS, Months	95% CI, Months	5-Year OS, %	95% CI, %
DPYD variant carriers	93	56.9	50.6 to 61.3	66 (71.0)	12.4	9.1 to 18.7	28.7	20.6 to 39.9	50 (53.8)	38.6	26.0 to NE	40.3	30.6 to 53.0
Matched wild type	279	60.7	59.7 to 62.6	177 (63.4)	15.9	12.7 to 24.9	37.1	31.8 to 43.2	164 (58.8)	35.5	26.7 to 53.1	40.9	35.4 to 47.3
*DPYD**2A	25	54.9	29.7 to 66.0	16 (64.0)	17.3	10.2 to NE	37.7	22.2 to 63.9	9 (36.0)	NE	24.2 to NE	59.5	41.9 to 84.5
Matched wild type	75	63.2	61.2 to 66.1	47 (62.7)	17.5	10.0 to NE	37.3	27.8 to 50.0	43 (57.3)	38.4	20.6 to NE	43.4	33.4 to 56.4
c.2846A>T	13	58.1	39.7 to NE	8 (61.5)	21.5	2.5 to NE	38.5	19.3 to 76.5	6 (46.2)	NE	12.6 to NE	52.7	31.2 to 89.2
Matched wild type	39	61.7	59.1 to 65.4	21 (53.8)	25.6	13.2 to NE	48.7	35.3 to 67.2	19 (48.7)	65.2	20.5 to NE	53.9	40.3 to 72.0
c.1236G>A	61	56.9	49.0 to 63.1	48 (78.7)	9.1	7.2 to 17.1	20.2	12.0 to 33.8	40 (65.6)	27.0	22.2 to 48.0	26.9	16.8 to 43.1
Matched wild type	183	60.7	59.4 to 63.7	123 (67.2)	13.8	10.9 to 21.6	33.5	27.2 to 41.2	114 (62.3)	30.3	21.4 to 48.0	38.0	31.3 to 46.1

Abbreviations: NE, not estimable; OS, overall survival; PFS, progression-free survival.

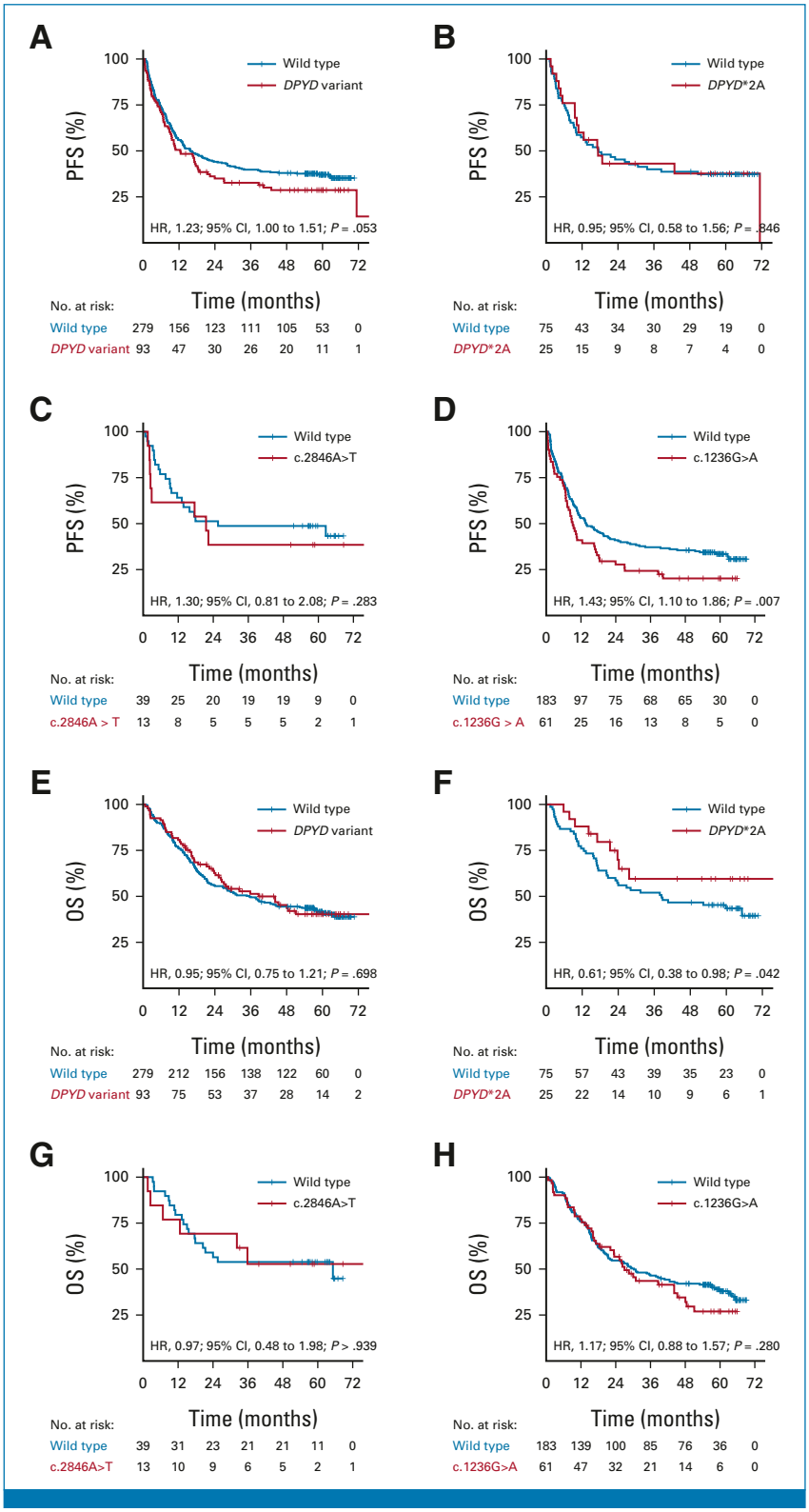

FIG 2. Kaplan-Meier plots and HRs for PFS and OS of pooled (A and E) *DPYD* variant carriers, (B and F) *DPYD**2A, (C and G) c.2846A>T, and (D and H) c.1236G>A carriers. All *P* values indicated on the Kaplan-Meier curves were adjusted *P* values corresponding to the Cox regression analysis using the jackknife sandwich estimator. Censoring is indicated by tick marks. HRs, hazard ratios; OS, overall survival; PFS, progression-free survival.

TABLE 3. HRs for Progression-Free Survival Calculated by Matched-Pair Cox Regression Analysis

		Cox Regression			Cox Regression With Robust Standard Errors		
DPYD Variant Allele	No.	HR	95% CI	*P*	HR	95% CI	*P*
DPYD variant carrier	93	1.23	0.92 to 1.63	.159	1.23	1.00 to 1.51	.053
*DPYD**2A	25	0.95	0.53 to 1.70	.869	0.95	0.58 to 1.56	.846
c.2846A>T	13	1.30	0.57 to 2.93	.535	1.30	0.81 to 2.08	.283
c.1236G>A	61	1.43	1.02 to 2.00	.036*	1.43	1.10 to 1.86	.007*

NOTE. Robust standard errors (95% CI) were obtained using the jackknife sandwich estimator. Results of stratified Cox regression can be found in Appendix Table A2.
Abbreviation: HR, hazard ratio.
*Indicates a significant difference with a *P* value of <.05.

without compromising effectiveness. For PFS, the primary matched-pair analysis using the JSE method was not significantly different, although this was borderline. Although the stratified Cox regression indicated that PFS might be negatively affected by a reduced fluoropyrimidine dose with HRs up to 1.76, the multivariable Cox regression analysis did not show a significant difference in PFS and a lower HR of 1.18. Furthermore, both PFS and OS were not negatively affected in *DPYD**2A variant allele carriers treated with a reduced dose of 50% compared with matched wild-type patients, in line with previous findings.[13,21] Carriers of c.2846A>T were found to trend toward shorter PFS, although not significant, and the results were hampered by low power because of the small sample size. Subgroup analysis revealed a consistently shorter PFS for c.1236G>A carriers. The trend toward shorter PFS accompanied by a borderline *P* value in the pooled *DPYD* variant carriers was, therefore, probably largely driven by the survival outcomes of c.1236G>A, which made up the majority of pooled *DPYD* variant carriers. For OS, no significant difference was found in c.1236G>A carriers. This discrepancy may be caused by differences in administration of other systemic treatment lines or other treatment modalities after fluoropyrimidine-based treatment between all studied patient groups. Unfortunately, this information was not available.

Sensitivity analyses performed to explore the robustness of the results confirmed the abovementioned findings. Nonetheless, the incidence of severe fluoropyrimidine-related toxicity was higher in the c.1236G>A carriers (27.3%) compared with matched *DPYD* wild types (17.2%) in this study (Appendix Table A8) and are in line with results from the Alpe-DPD study.[10] Notably, no dose modifications after initial dose reduction were applied in 75.8% of the c.1236G>A carriers (Appendix Table A8), who remained on the 75% starting dose throughout all treatment cycles, whereas in only 6.1% of c.1236G>A carriers dose escalation was performed. These results suggest that a substantial number of c.1236G>A carriers may benefit from an upward dose titration when treatment is well tolerated, which was recommended in the Alpe-DPD study protocol,[10] but seemingly was applied to a limited extent. It also suggests that DPD enzyme activity is not affected similarly across all c.1236G>A carriers. This is underscored by a large variation in DPD enzyme activity and exposure to 5-FU in c.1236G>A carriers.[10] Depending on the magnitude of impact on the DPD enzyme activity, this could result in both underexposure and overexposure. Moreover, previous research has shown that wild-type mRNA for DPD is still detectable in homozygous c.1236G>A carriers, indicating that at least some normal functional DPD can still be formed in these patients.[22,23] These findings, combined with the presented data regarding the treatment outcome, suggest that a dose reduction of 25% may not be beneficial for all c.1236G>A carriers.

A possible strategy to prevent both severe toxicity and potential subtherapeutic dosing of fluoropyrimidines in c.1236G>A carriers would be individualized early dose

TABLE 4. HRs for Overall Survival Calculated by Matched-Pair Cox Regression Analysis

		Cox Regression			Cox Regression With Robust Standard Errors		
DPYD Variant Allele	No.	HR	95% CI	*P*	HR	95% CI	*P*
DPYD variant carrier	93	0.95	0.69 to 1.31	.770	0.95	0.75 to 1.21	.698
*DPYD**2A	25	0.61	0.30 to 1.26	.184	0.61	0.38 to 0.98	.042*
c.2846A>T	13	0.97	0.39 to 2.44	.953	0.97	0.48 to 1.98	.939
c.1236G>A	61	1.17	0.82 to 1.69	.385	1.17	0.88 to 1.57	.280

NOTE. Robust standard errors (95% CI) were obtained using the jackknife sandwich estimator. Results of stratified Cox regression can be found in Appendix Table A2.
Abbreviation: HR, hazard ratio.
*Indicates a significant difference with a *P* value of <.05.

escalation after a reduced dose in the absence of severe toxicity.[3,15,24] Of note, a larger dose reduction of 50% is currently recommended by the CPIC. This deserves further attention as this could negatively impact PFS in case dose escalation is not applied when treatment is otherwise tolerated well.[12]

A limitation of this study is the use of matching, irrespective of the method used for adjustment. When carriers are left unmatched, like in our study, the estimation of the effect of mutation status is possibly biased, and it is unclear to which population of carriers the results apply. Using less restrictive matching criteria as an alternative leads to matching of less similar patients and introduces residual confounding. Ideally, matching could be avoided altogether performing instead a multivariable Cox regression analysis on all data. However, as in this study, when there is a high degree of heterogeneity in variables such as tumor type and treatment regimen, the estimation of this model and its interpretation is not straightforward. Nonetheless, multivariable Cox regression was performed and showed similar results (Appendix Tables A3 and A4). Owing to the retrospective design of our study, patient data regarding matching variables were not always complete, which hampered the matching and reduced the number of matches. Ideally, matching would be performed using additional variables that influence treatment outcome such as molecular tumor subtypes. However, these data were not available. Furthermore, because of the choice of 1:3 matching without replacement of patients, it was not possible to match each of the *DPYD* variant carriers to three *DPYD* wild-type controls. Hence, additional analyses using alternative matching strategies were also performed to include more *DPYD* variant carriers. Similar results were found using other matching strategies for c.1236G>A (Appendix Tables A5-A7), which further strengthens the assumption that a dose reduction of 25% in c.1236G>A may negatively affect disease progression if dose titration is not applied when patients experience no or minimal toxicity. Another limitation was the use of an additional category other defined for primary tumor type including less prevalent tumor types that otherwise had to be left out of the main analyses because of not finding matches. Similarly, less frequent treatment regimens were also combined into one category to increase the number of matches. Although this could potentially introduce biases, it can reduce incomplete matching. Furthermore, patients with local or locally advanced disease were grouped for the end point PFS while disease-free survival (DFS) may be better suited as the treatment goal in these patients is cure. However, data regarding DFS were not available. A post hoc power analysis showed that our study was underpowered to completely exclude false-negative findings. To detect the currently observed difference in PFS (HR, 1.23) between *DPYD* variant carriers and *DPYD* wild-type controls with 80% power, with a 1:3 ratio, nearly twice as many PFS events would have been needed. All limitations must be taken into account, and the findings presented in this study must, therefore, be interpreted with appropriate caution.

Most limitations could be overcome by performing an adequately powered prospective study using novel trial designs as recently described[14,15] which include similar approaches using *DPYD* wild-type patients treated with a full dose as a comparator and the use of real-world evidence.[14,15] In addition, pharmacokinetic analyses of *DPYD* variant carriers may help to establish the relationship between 5-FU exposure and survival.

In summary, the results of this retrospective exploratory analysis suggest that PFS and OS are not negatively affected by *DPYD*-guided dose individualization in the pooled *DPYD* variant carriers and likely do not hamper the effectiveness of fluoropyrimidines as was also previously shown for *DPYD**2A.[13] However, a shorter PFS in c.1236G>A carriers receiving *DPYD*-guided fluoropyrimidine dosing cannot be excluded. Notwithstanding this, it should be considered that 75.8% of c.1236G>A carriers remained on the same dose throughout all treatment cycles, suggesting that a substantial number of c.1236G>A variant carriers could have benefited from upward dose titration when treatment was well-tolerated, as was dictated in the study protocol. Evidence on the impact on PFS for c.2846A>T was highly dependent on matching strategy and was hampered by the small numbers of carriers available and requires further research with a larger sample size. Notably, both c.1236G>A and c.2846A>T carriers still experienced significantly more severe fluoropyrimidine-induced toxicity after a 25% dose reduction compared with wild-type controls treated with a full dose.[11] Apparently, the 25% dose reduction was not sufficient to protect all variant carriers from developing severe toxicity and, therefore, more research is needed to explain the impact of heterogeneity in DPD enzyme activity in *DPYD* variant allele carriers. Meanwhile, close monitoring with early dose modifications, escalation when possible, and reduction when necessary, based on toxicity, are recommended when treating c.1236G>A variant carriers with a reduced fluoropyrimidine starting dose.

AFFILIATIONS

[1]Division of Pharmacology, The Netherlands Cancer Institute, Amsterdam, the Netherlands
[2]Department of Pharmacy & Pharmacology, The Netherlands Cancer Institute, Amsterdam, the Netherlands
[3]Biometrics Department, The Netherlands Cancer Institute, Amsterdam, the Netherlands
[4]Department of Clinical Chemistry and Laboratory Medicine, Leiden University Medical Center, Leiden, the Netherlands
[5]Department of Medical Oncology, Leiden University Medical Center, Leiden, the Netherlands
[6]Department of Medical Oncology, Erasmus MC Cancer Institute, Erasmus University Medical Center, Rotterdam, the Netherlands
[7]Department of Clinical Pharmacology, Division of Medical Oncology, The Netherlands Cancer Institute, Amsterdam, the Netherlands

[8]Late Development Oncology, AstraZeneca, Cambridge, UK
[9]Department of Internal Medicine, Wilhelmina Hospital Assen, Assen, the Netherlands
[10]Department of Internal Medicine, Bravis Hospital, Roosendaal, the Netherlands
[11]Department of Medical Oncology, Catharina Hospital, Eindhoven, the Netherlands
[12]Department of Internal Medicine, Canisius-Wilhelmina Hospital, Nijmegen, the Netherlands
[13]Department of Internal Medicine, Deventer Hospital, Deventer, the Netherlands
[14]Department of Medical Oncology, Haga Hospital, The Hague, the Netherlands
[15]Department of Internal Medicine, Maastricht University Medical Center, Maastricht, the Netherlands
[16]Department of Medical Oncology, Reinier de Graaf Gasthuis, Delft, the Netherlands
[17]Department of Internal Medicine, Laurentius Hospital, Roermond, the Netherlands
[18]Department of Internal Medicine, Hospital Gelderse Vallei, Ede, the Netherlands
[19]Department of Clinical Pharmacy and Toxicology, Leiden University Medical Center, Leiden, the Netherlands
[20]Department of Pharmaceutical Sciences, Utrecht University, Utrecht, the Netherlands
[21]Division of Pharmacoepidemiology and Clinical Pharmacology, Department of Pharmaceutical Sciences, Utrecht University, Utrecht, the Netherlands
[22]Department of Gastrointestinal Oncology, Division of Medical Oncology, The Netherlands Cancer Institute, Amsterdam, the Netherlands

CORRESPONDING AUTHOR

A. Cats, PhD, Department of Gastrointestinal Oncology, Division of Medical Oncology, The Netherlands Cancer Institute, Plesmanlaan 121, Amsterdam 1066 CX, the Netherlands; e-mail: a.cats@nki.nl.

SUPPORT

C.A.T.C.L. was supported by an unrestricted grant from Roche Pharmaceuticals. L.M.H. and C.A.T.C.L. were sponsored by the Dutch Cancer Society (Alpe-d'HuZes/KWF-fund, NKI2013-6249). There was no involvement from any of the funding sources in the study design, data collection, analysis, or interpretation of the data.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at DOI https://doi.org/10.1200/JCO.22.02780.

AUTHOR CONTRIBUTIONS

Conception and design: Jonathan E. Knikman, Jesse J. Swen, Hans Gelderblom, Jan H.M. Schellens, Jos H. Beijnen, Henk-Jan Guchelaar, Annemieke Cats
Administrative support: Tycho A. Wilting
Provision of study materials or patients: Peter Nieboer, Helga J. Droogendijk, Geert-Jan Creemers, Caroline M.P.W. Mandigers, Alexander L.T. Imholz, Ron H.J. Mathijssen, Johanneke E.A. Portielje, Liselot Valkenburg-van Iersel, Annelie Vulink, Marlene H.W. van der Poel, Arnold Baars, Hans Gelderblom, Jan H.M. Schellens, Annemieke Cats
Collection and assembly of data: Jonathan E. Knikman, Tycho A. Wilting, Linda M. Henricks, Carin A.T.C. Lunenburg, Femke M. de Man, Didier Meulendijks, Peter Nieboer, Helga J. Droogendijk, Geert-Jan Creemers, Carolina M.P.W. Mandigers, Alexander L.T. Imholz, Ron H.J. Mathijssen, Johanneke E.A. Portielje, Liselot Valkenburg-van Iersel, Annelie Vulink, Marlene H.W. van der Poel, Arnold Baars, Jesse J. Swen, Hans Gelderblom, Jan H.M. Schellens, Jos H. Beijnen, Henk-Jan Guchelaar, Annemieke Cats
Data analysis and interpretation: Jonathan E. Knikman, Tycho A. Wilting, Marta Lopez-Yurda, Femke M. de Man, Ron H.J. Mathijssen, Jesse J. Swen, Hans Gelderblom, Jan H.M. Schellens, Jos H. Beijnen, Henk-Jan Guchelaar, Annemieke Cats
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

ACKNOWLEDGMENT

The authors thank all participating centers of the NCT02324452 study for their help in including patients in the study and Prof. E.W. Steyerberg for his critical review of the manuscript.

REFERENCES

1. Longley DB, Harkin DP, Johnston PG: 5-fluorouracil: Mechanisms of action and clinical strategies. Nat Rev Cancer 3:330-338, 2003
2. Diasio RB, Harris BE: Clinical pharmacology of 5-fluorouracil. Clin Pharmacokinet 16:215-237, 1989
3. Knikman JE, Gelderblom H, Beijnen JH, et al: Individualized dosing of fluoropyrimidine-based chemotherapy to prevent severe fluoropyrimidine-related toxicity: What are the options? Clin Pharmacol Ther 109:591-604, 2021
4. van Kuilenburg ABP: Dihydropyrimidine dehydrogenase and the efficacy and toxicity of 5-fluorouracil. Eur J Cancer 40:939-950, 2004
5. van Kuilenburg ABP, Haasjes J, Richel DJ, et al: Clinical implications of dihydropyrimidine dehydrogenase (DPD) deficiency in patients with severe 5-fluorouracil-associated toxicity: Identification of new mutations in the DPD gene. Clin Cancer Res 6:4705-4712, 2000
6. Meulendijks D, Henricks LM, Sonke GS, et al: Clinical relevance of *DPYD* variants c.1679T>G, c.1236G>A/HapB3, and c.1601G>A as predictors of severe fluoropyrimidine-associated toxicity: A systematic review and meta-analysis of individual patient data. Lancet Oncol 16:1639-1650, 2015
7. Terrazino S, Cargnin S, del Re M, et al: *DPYD* IVS14 + 1G > A and 2846A > T genotyping for the prediction of severe fluoropyrimidine-related toxicity: A meta-analysis. Pharmacogenomics 14: 1255-1272, 2013
8. Rosmarin D, Palles C, Church D, et al: Genetic markers of toxicity from capecitabine and other fluorouracil-based regimens: Investigation in the QUASAR2 study, systematic review, and meta-analysis. J Clin Oncol 32:1031-1039, 2014
9. Henricks LM, Lunenburg CATC, Meulendijks D, et al: Translating *DPYD* genotype into DPD phenotype: Using the *DPYD* gene activity score. Pharmacogenomics 16:1277-1286, 2015
10. Henricks LM, Lunenburg CATC, de Man FM, et al: *DPYD* genotype-guided dose individualisation of fluoropyrimidine therapy in patients with cancer: A prospective safety analysis. Lancet Oncol 19: 1459-1467, 2018
11. CPIC® guideline for fluoropyrimidines and *DPYD* [Internet], 2018. https://cpicpgx.org/guidelines/guideline-for-fluoropyrimidines-and-dpyd/
12. Clinical Pharmacogenetics Implementation Consortium: CPIC® guideline for fluoropyrimidines and *DPYD* (February 2020 update) [Internet], 2020. https://cpicpgx.org/guidelines/guideline-for-fluoropyrimidines-and-dpyd/

13. Henricks LM, van Merendonk LN, Meulendijks D, et al: Effectiveness and safety of reduced-dose fluoropyrimidine therapy in patients carrying the *DPYD**2A variant: A matched pair analysis. Int J Cancer 144:2347-2354, 2019
14. Hertz DL: Assessment of the clinical utility of pretreatment *DPYD* testing for patients receiving fluoropyrimidine chemotherapy. J Clin Oncol 40:3882-3892, 2022
15. Baker SD, Bates SE, Brooks GA, et al: *DPYD* testing: Time to put patient safety first. J Clin Oncol 41:2701-2705, 2023
16. Ho DE, Imai K, King G, et al: MatchIt: Nonparametric preprocessing for parametric causal inference [Internet], 2011. http://www.jstatsoft.org/
17. Austin PC: The use of propensity score methods with survival or time-to-event outcomes: Reporting measures of effect similar to those used in randomized experiments. Stat Med 33:1242-1258, 2014
18. Sjölander A, Greenland S: Ignoring the matching variables in cohort studies—When is it valid and why? Stat Med 32:4696-4708, 2013
19. Shinozaki T, Mansournia MA, Matsuyama Y: On hazard ratio estimators by proportional hazards models in matched-pair cohort studies. Emerg Themes Epidemiol 14:6, 2017
20. R Core Team: R: A language and environment for statistical computing. Vienna, Austria, R Foundation for Statistical Computing, 2021. https://www.R-project.org/
21. Deenen MJ, Meulendijks D, Cats A, et al: Upfront genotyping of *DPYD**2A to individualize fluoropyrimidine therapy: A safety and cost analysis. J Clin Oncol 34:227-234, 2016
22. Froehlich TK, Amstutz U, Aebi S, et al: Clinical importance of risk variants in the dihydropyrimidine dehydrogenase gene for the prediction of early-onset fluoropyrimidine toxicity. Int J Cancer 136: 730-739, 2015
23. van Kuilenburg ABP, Meijer J, Mul ANPM, et al: Intragenic deletions and a deep intronic mutation affecting pre-mRNA splicing in the dihydropyrimidine dehydrogenase gene as novel mechanisms causing 5-fluorouracil toxicity. Hum Genet 128:529-538, 2010
24. With M, Knikman J, Man FM, et al: Dihydropyrimidine dehydrogenase phenotyping using pretreatment uracil: A note of caution based on a large prospective clinical study. Clin Pharmacol Ther 112: 62-68, 2022

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Survival of Patients With Cancer With *DPYD* Variant Alleles and Dose-Individualized Fluoropyrimidine Therapy—A Matched-Pair Analysis

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/jco/authors/author-center.

Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

Carin A.T.C. Lunenburg
Employment: BMS GmbH & Co. KG
Stock and Other Ownership Interests: BMS GmbH & Co. KG
Travel, Accommodations, Expenses: BMS GmbH & Co. KG

Didier Meulendijks
Employment: AstraZeneca
Stock and Other Ownership Interests: AstraZeneca

Ron H.J. Mathijssen
Stock and Other Ownership Interests: Galapagos NV
Research Funding: Roche (Inst), Boehringer Ingelheim (Inst), Pfizer (Inst), Cristal Therapeutics (Inst), Pamgene (Inst), Bayer Holding (Inst)

Liselot Valkenburg-van Iersel
Consulting or Advisory Role: Servier, Roche, Pierre Fabre
Travel, Accommodations, Expenses: Servier

Hans Gelderblom
Research Funding: Novartis (Inst), Ipsen (Inst), Deciphera (Inst), AmMax Bio (Inst)

Jan H.M. Schellens
Employment: Modra Pharmaceuticals, Byondis
Stock and Other Ownership Interests: Modra Pharmaceuticals
Honoraria: Debiopharm Group
Consulting or Advisory Role: Debiopharm Group
Research Funding: Dutch Cancer Society (Inst)
Patents, Royalties, Other Intellectual Property: Patent on oral taxanes

Jos H. Beijnen
Employment: Modra Pharmaceuticals
Stock and Other Ownership Interests: Modra Pharmaceuticals
Honoraria: Modra Pharmaceuticals
Research Funding: Modra Pharmaceuticals (Inst)
Patents, Royalties, Other Intellectual Property: Patent dealing with the pharmaceutical formulation of oral taxanes (Inst)
Expert Testimony: Hoyng Rokh Monegier (Inst)

No other potential conflicts of interest were reported.

APPENDIX

TABLE A1. Treatment Regimens Included in Each Treatment Group

Group	Included Treatment Regimens
CAP/5-FU monotherapy	Capecitabine monotherapy
	5-FU monotherapy
CAP/5-FU other	Capecitabine + docetaxel Capecitabine + monoclonal antibodies (trastuzumab, bevacizumab, or panitimumab) Capecitabine + carboplatin or cisplatin Capecitabine + vinorelbine Capecitabine + temozolomide
	5-FU + bevacizumab 5-FU + irinotecan 5-FU + iriniotecan + bevacizumab 5-FU + cisplatin 5-FU + streptozocin
CAPOX regimens	Capecitabine + oxaliplatin
	Capecitabine + oxaliplatin + bevacizumab
	Capecitabine + oxaliplatin + trastuzumab Capecitabine + oxaliplatin + panitumumab
ECX/EOX	Capecitabine + cisplatin + epirubicin
	Capecitabine + oxaliplatin + epirubicin
FEC	5-FU + epirubicin + cyclophosphamide 5-FU + doxorubicin + cyclophosphamide
FOLFOX regimens	5-FU + oxaliplatin + folinic acid
	5-FU + oxaliplatin + folinic acid + bevacizumab
	5-FU + oxaliplatin + irinotecan + folinic acid
FLOT/DOC	5-FU + oxaliplatin + docetaxel Capecitabine + oxaliplatin + docetaxel
Chemoradiotherapy regimens	Radiotherapy + 5-FU + mitomycin
	Radiotherapy + capecitabine
	Radiotherapy + capecitabine + mitomycin
	Radiotherapy + capecitabine + cisplatin
	Radiotherapy + capecitabine + oxaliplatin

Abbreviations: 5-FU, fluorouracil; CAP/5-FU monotherapy, capecitabine or fluorouracil monotherapy; CAP/5-FU other, capecitabine or 5-FU in combination with other anticancer drugs; CAPOX regimens, capecitabine and oxaliplatin-based regimens; ECX/EOX, capecitabine, cisplatin, and epirubicin/capecitabine, oxaliplatin, epirubicin; FEC, 5-fluorouracil, epirubicin, and cyclophosphamide; FLOT/DOC, 5-FU, oxaliplatin, and docetaxel/capecitabine, oxaliplatin, and docetaxel; FOLFOX, infusional fluorouracil, leucovorin, and oxaliplatin.

TABLE A2. HRs From Cox Regression Model for PFS and OS Accounting for Matching by Stratification for Matched Groups

	Cox Regression			Cox Regression Stratified for Matching		
DPYD Variant Allele	HR	95% CI	*P*	HR	95% CI	*P*
PFS						
DPYD variant carrier	1.23	0.92 to 1.63	.159	1.76	1.25 to 2.48	.001*
*DPYD**2A	1.04	0.57 to 1.92	.899	0.86	0.42 to 1.76	.679
c.2846A>T	1.30	0.57 to 2.93	.535	2.48	0.92 to 6.67	.073
c.1236G>A	1.43	1.02 to 2.00	.036*	2.03	1.34 to 3.05	<.001*
OS						
DPYD variant carrier	0.95	0.69 to 1.31	.770	0.94	0.65 to 1.35	.736
*DPYD**2A	0.76	0.37 to 1.58	.465	0.47	0.19 to 1.15	.097
c.2846A>T	0.97	0.39 to 2.44	.953	1.40	0.50 to 3.92	.520
c.1236G>A	1.17	0.82 to 1.69	.385	1.16	0.77 to 1.77	.477

Abbreviations: HR, hazard ratio; OS, overall survival; PFS, progression-free survival.
*Indicates a significant difference with $P < .05$.

TABLE A3. HRs From Cox Regression Model for Progression-Free Survival Calculated by Multivariable Cox Regression Analysis Including the Matching Variables

	Pooled *DPYD* Variants (n = 156)			*DPYD**2A (n = 44)			c.2846A>T (n = 21)			c.1236G>A (n = 91)		
Variable	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
DPYD status												
Wild type (n = 775)	—	—	—	—	—	—	—	—	—	—	—	—
DPYD variant	1.18	0.95 to 1.46	.135	0.96	0.64 to 1.44	.859	0.96	0.55 to 1.71	.900	1.57	1.21 to 2.04	<.001*
Sex												
Men	—	—	—	—	—	—	—	—	—	—	—	—
Women	0.93	0.76 to 1.12	.430	0.99	0.83 to 1.18	.894	0.91	0.74 to 1.13	.400	0.97	0.80 to 1.19	.800
Treatment regimen												
CAP/5-FU monotherapy	—	—	—	—	—	—	—	—	—	—	—	—
CAP/5-FU other	0.90	0.64 to 1.28	.570	0.99	0.65 to 1.49	.946	1.01	0.69 to 1.47	.965	0.99	0.69 to 1.42	.968
CAPOX regimens	0.62	0.45 to 0.84	.002*	0.89	0.74 to 1.07	.200	0.58	0.42 to 0.80	.001*	0.62	0.45 to 0.85	.003*
ECX/EOX	0.70	0.39 to 1.25	.224	0.93	0.60 to 1.44	.737	0.66	0.35 to 1.23	.190	0.90	0.49 to 1.64	.725
FEC	0.22	0.08 to 0.61	.004*	0.78	0.45 to 1.36	.379	0.23	0.09 to 0.65	.005*	0.22	0.08 to 0.62	.004*
FLOT/DOC	0.74	0.18 to 3.11	.684	0.93	0.17 to 5.10	.930	0.70	0.17 to 2.97	.633	0.86	0.20 to 3.61	.834
FOLFOX regimens	0.88	0.60 to 1.30	.533	0.95	0.66 to 1.38	.806	0.78	0.52 to 1.19	.248	0.95	0.64 to 1.42	.816
Chemoradiotherapy	0.61	0.44 to 0.86	.004*	0.91	0.75 to 1.10	.322	0.61	0.42 to 0.87	.007*	0.64	0.45 to 0.91	.012*
Age	1.00	0.99 to 1.01	.740	1.00	0.99 to 1.01	.920	1.00	0.99 to 1.01	.510	1.00	0.99 to 1.01	.713
Stage of cancer												
Local	—	—	—	—	—	—	—	—	—	—	—	—
Locally advanced	1.47	1.07 to 2.01	.018*	1.05	0.88 to 1.26	.574	1.68	1.17 to 2.41	.005*	1.53	1.10 to 2.13	.028*
Metastatic	6.55	4.80 to 8.92	<.001*	1.52	1.25 to 1.84	<.001*	7.76	5.39 to 11.2	<.001*	6.97	5.00 to 9.70	<.001*
Primary tumor type												
Breast	—	—	—	—	—	—	—	—	—	—	—	—
Colorectal	0.67	0.47 to 0.94	.020*	0.90	0.74 to 1.08	.256	0.69	0.48 to 1.00	.052	0.67	0.47 to 0.96	.028*
Gastric	1.62	0.94 to 2.80	.0083*	1.07	0.72 to 1.57	.747	1.71	0.94 to 3.09	.078	1.55	0.88 to 2.72	.013*
Ovarian	17.1	3.89 to 75.2	<.001*	0.00	0.00 to inf	.998						
Pancreatic	1.57	0.99 to 2.48	.054	1.29	0.78 to 2.13	.321	1.96	1.17 to 3.29	.011*	1.63	1.01 to 2.63	.047*
Other	1.01	0.68 to 1.48	.971	0.97	0.75 to 1.26	.834	1.09	0.71 to 1.67	.680	0.89	0.59 to 1.34	.577

Abbreviations: CAP/5-FU monotherapy, capecitabine or fluorouracil monotherapy; CAP/5-FU other, capecitabine or 5-FU in combination with other anticancer drugs; CAPOX regimens, capecitabine and oxaliplatin-based regimens; ECX/EOX, capecitabine, cisplatin, and epirubicin/capecitabine, oxaliplatin, epirubicin; FEC, 5-fluorouracil, epirubicin, and cyclophosphamide; FLOT/DOC, 5-FU, oxaliplatin, and docetaxel/capecitabine, oxaliplatin, and docetaxel; FOLFOX, infusional fluorouracil, leucovorin, and oxaliplatin; HR, hazard ratio.
*Indicates a significant difference with $P < .05$.

TABLE A4. HRs From Cox-Regression Model for Overall Survival Calculated by Multivariable Cox-Regression Analysis Including the Matching Variables

	Pooled *DPYD* Variants (n = 156)			*DPYD**2A (n = 44)			c.2846A>T (n = 21)			c.1236G>A (n = 91)		
Variable	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
DPYD status												
Wild type (n = 775)	—	—	—	—	—	—	—	—	—	—	—	—
DPYD variant	0.95	0.74 to 1.22	.652	0.68	0.42 to 1.10	.119	0.66	0.35 to 1.26	.207	1.25	0.94 to 1.68	.128
Sex												
Men	—	—	—	—	—	—	—	—	—	—	—	—
Women	0.98	0.79 to 1.20	.825	0.95	0.76 to 1.19	.683	0.93	0.74 to 1.17	.540	1.01	0.82 to 1.25	.916
Treatment regimen												
CAP/5-FU monotherapy	—	—	—	—	—	—	—	—	—	—	—	—
CAP/5-FU other	0.71	0.49 to 1.03	.071	0.69	0.47 to 1.02	.065	0.70	0.46 to 1.05	.082	0.70	0.47 to 1.03	.067
CAPOX regimens	0.55	0.40 to 0.76	<.001*	0.50	0.36 to 0.70	<.001*	0.51	0.36 to 0.72	<.001*	0.55	0.39 to 0.77	<.001*
ECX/EOX	1.00	0.55 to 1.82	.996	1.17	0.63 to 2.17	.628	0.86	0.45 to 1.64	.653	1.21	0.65 to 2.24	.550
FEC	0.28	0.09 to 0.90	.032*	0.29	0.09 to 0.94	.039*	0.30	0.09 to 0.96	.042*	0.30	0.09 to 0.96	.043*
FLOT/DOC	1.32	0.31 to 5.59	.706	1.28	0.30 to 5.43	.740	1.15	0.27 to 4.91	.846	1.40	0.33 to 5.97	.648
FOLFOX regimens	1.05	0.71 to 1.55	.821	0.99	0.66 to 1.50	.965	0.94	0.62 to 1.42	.764	1.10	0.73 to 1.65	.660
Chemoradiotherapy	0.56	0.39 to 0.80	.001*	0.56	0.39 to 0.82	.003*	0.57	0.39 to 0.84	.004	0.61	0.42 to 0.88	.009*
Age	1.01	1.00 to 1.01	.173	1.01	1.00 to 1.01	.290	1.00	0.99 to 1.01	.465	1.01	1.00 to 1.01	.174
Stage of cancer												
Local	—	—	—	—	—	—	—	—	—	—	—	—
Locally advanced	1.68	1.16 to 2.43	.006*	1.77	1.18 to 2.64	.005*	1.94	1.28 to 2.93	.002	1.78	1.21 to 2.62	.003*
Metastatic	7.33	5.11 to 10.5	<.001*	8.42	5.64 to 12.6	<.001*	8.76	5.79 to 13.3	<.001*	8.20	5.59 to 12.0	<.001*
Primary tumor type												
Breast	—	—	—	—	—	—	—	—	—	—	—	—
Colorectal	0.96	0.67 to 1.37	.821	1.00	0.68 to 1.46	.999	1.00	0.67 to 1.47	.982	1.01	0.70 to 1.48	.841
Gastric	2.35	1.33 to 4.17	.003*	2.46	1.34 to 4.49	.004*	2.73	1.47 to 5.05	.001*	2.69	1.49 to 4.85	.001*
Ovarian	8.77	2.10 to 36.7	.003*	7.80	1.02 to 59.8	.048*						
Pancreatic	2.88	1.79 to 4.65	<.001*	3.15	1.91 to 5.20	<.001*	3.24	1.93 to 5.43	.001*	3.12	1.90 to 5.14	.001*
Other	1.71	1.14 to 2.55	.009*	1.83	1.18 to 2.83	.007*	1.88	1.19 to 2.96	.006*	1.59	1.03 to 2.46	.036*

Abbreviations: CAP/5-FU monotherapy, capecitabine or fluorouracil monotherapy; CAP/5-FU other, capecitabine or 5-FU in combination with other anticancer drugs; CAPOX regimens, capecitabine and oxaliplatin-based regimens; ECX/EOX, capecitabine, cisplatin, and epirubicin/capecitabine, oxaliplatin, epirubicin; FEC, 5-fluorouracil, epirubicin, and cyclophosphamide; FLOT/DOC, capecitabine, cisplatin, and epirubicin/capecitabine, oxaliplatin, epirubicin; FOLFOX, infusional fluorouracil, leucovorin, and oxaliplatin; HR, hazard ratio.
*Indicates a significant difference with $P < .05$.

TABLE A5. HRs From Cox Regression Model for PFS and OS by Matched-Pair Cox Regression Analysis Using Matching Strategy That Allows for One or Two Matches When Less Than Three Matches Are Available

		Cox Regression			Cox Regression With Robust Standard Errors			Cox Regression Stratified for Matching		
DPYD Variant Allele	No.	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
PFS										
DPYD variant carriers	128	1.22	0.95 to 1.57	.118	1.22	1.01 to 1.47	.034*	1.79	1.31 to 2.45	<.001*
*DPYD**2A	32	0.97	0.56 to 1.66	.900	0.97	0.60 to 1.55	.885	0.95	0.50 to 1.79	.872
c.2846A>T	16	1.23	0.59 to 2.57	.590	1.23	0.79 to 1.91	.370	2.00	0.80 to 5.00	.139
c.1236G>A	78	1.40	1.03 to 1.91	.033*	1.40	1.10 to 1.77	.006*	2.12	1.44 to 3.13	<.001*
OS										
DPYD variant carriers	128	0.98	0.74 to 1.30	.898	0.98	0.79 to 1.21	.866	0.98	0.71 to 1.37	.911
*DPYD**2A	32	0.66	0.34 to 1.28	.221	0.66	0.41 to 1.06	.086	0.48	0.22 to 1.07	.073
c.2846A>T	16	0.86	0.37 to 2.02	.733	0.86	0.42 to 1.77	.685	1.22	0.47 to 3.18	.677
c.1236G>A	78	1.15	0.82 to 1.61	.411	1.15	0.88 to 1.50	.296	1.19	0.80 to 1.77	.396

Abbreviations: HR, hazard ratio; OS, overall survival; PFS, progression-free survival.
*Indicates a significant difference with $P < .05$.

TABLE A6. HRs From Cox Regression Model for PFS and OS Calculated by Matched-Pair Cox Regression Analysis Using 1:2 Matching Ratio

		Cox Regression			Cox Regression With Robust Standard Errors			Cox Regression Stratified for Matching		
DPYD Variant Allele	No.	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
PFS										
DPYD variant carriers	109	1.17	0.89 to 1.56	.265	1.17	0.95 to 1.46	.144	1.67	1.17 to 2.38	.005*
*DPYD**2A	24	0.96	0.51 to 1.83	.908	0.96	0.58 to 1.61	.885	0.73	0.33 to 1.62	.435
c.2846A>T	17	0.92	0.43 to 1.97	.836	0.92	0.55 to 1.55	.763	1.21	0.48 to 3.03	.690
c.1236G>A	67	1.48	1.05 to 2.10	.026*	1.48	1.15 to 1.92	.003*	2.30	1.46 to 3.63	<.001*
OS										
DPYD variant carriers	109	0.94	0.69 to 1.28	.693	0.94	0.74 to 1.19	.608	0.84	0.57 to 1.21	.360
*DPYD**2A	24	0.75	0.35 to 1.61	.460	0.75	0.47 to 1.21	.235	0.40	0.13 to 1.22	.110
c.2846A>T	17	0.65	0.27 to 1.55	.331	0.65	0.31 to 1.35	.246	0.81	0.30 to 2.19	.683
c.1236G>A	67	1.18	0.81 to 1.71	.393	1.18	0.88 to 1.57	.269	1.04	0.66 to 1.63	.877

Abbreviations: HR, hazard ratio; OS, overall survival; PFS, progression-free survival.
*Indicates a significant difference with $P < .05$.

TABLE A7. HRs From Cox Regression Model for PFS and OS Calculated by Matched-Pair Cox Regression Analysis Using 1:3 Matching Ratio and a Maximum Difference of 2 Years Between Start of Treatment

		Cox Regression			Cox Regression With Robust Standard Errors			Cox Regression Stratified for Matching		
DPYD Variant Allele	No.	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
PFS										
DPYD variant carriers	73	1.18	0.85 to 1.63	.320	1.18	0.94 to 1.48	.160	1.61	1.09 to 2.36	.016*
*DPYD**2A	14	0.97	0.45 to 2.06	.927	0.97	0.55 to 1.69	.901	0.93	0.40 to 2.17	.859
c.2846A>T	12	1.18	0.50 to 2.80	.704	1.18	0.73 to 1.90	.491	2.13	0.76 to 5.95	.148
c.1236G>A	48	1.28	0.88 to 1.87	.191	1.28	0.96 to 1.72	.095	1.64	1.04 to 2.58	.033*
OS										
DPYD variant carriers	73	1.00	0.71 to 1.42	.988	1.00	0.78 to 1.29	.983	1.04	0.69 to 1.54	.864
*DPYD**2A	14	0.72	0.29 to 1.76	.468	0.72	0.40 to 1.28	.262	0.60	0.22 to 1.65	.320
c.2846A>T	12	1.03	0.41 to 2.60	.950	1.03	0.50 to 2.11	.936	1.54	0.54 to 4.40	.422
c.1236G>A	48	1.14	0.77 to 1.70	.514	1.14	0.84 to 1.55	.396	1.13	0.71 to 1.79	.614

Abbreviations: HR, hazard ratio; OS, overall survival; PFS, progression-free survival.
*Indicates a significant difference with $P < .05$.

TABLE A8. Toxicity and Dose Modification of Matched Patients (only including *DPYD* variant carriers and wild types from the Alpe-DPD study)

DPYD Variant Allele	No.	No Toxicity, No. (%)	Grade 1 Toxicity, No. (%)	Grade 2 Toxicity, No. (%)	Grade ≥3 Toxicity, No. (%)	No Dose Modification, No. (%)	Dose Escalation, No. (%)	Dose Reduction, No. (%)
DPYD variant carriers	52	6 (11.5)	12 (23.1)	16 (30.8)	18 (34.6)	37 (71.2)	5 (9.6)	10 (19.2)
Matched wild types	156	40 (25.6)	29 (18.6)	62 (39.7)	25 (16.0)	118 (75.6)	5 (3.2)	33 (21.2)
*DPYD**2A	9	0	4 (44.4)	3 (33.3)	2 (22.2)	6 (66.7)	2 (22.2)	1 (11.1)
Matched wild types	27	11 (40.7)	5 (18.5)	8 (29.6)	3 (11.1)	22 (81.5)	2 (7.4)	3 (11.1)
c.2846A>T	9	1 (11.1)	2 (22.2)	0	6 (66.7)	4 (44.4)	1 (11.1)	4 (44.4)
Matched wild types	27	2 (7.4)	10 (37.0)	10 (37.0)	5 (18.5)	23 (85.2)	0	5 (18.5)
c.1236G>A	33	5 (15.2)	6 (18.2)	13 (39.4)	9 (27.3)	25 (75.8)	2 (6.1)	6 (18.2)
Matched wild types	99	24 (24.2)	14 (14.1)	44 (44.4)	17 (17.2)	75 (75.8)	3 (3.0)	21 (21.2)

NOTE. Henricks et al.[10]