Original Investigation

DPYD Genotyping to Predict Adverse Events Following Treatment With Flourouracil-Based Adjuvant Chemotherapy in Patients With Stage III Colon Cancer

A Secondary Analysis of the PETACC-8 Randomized Clinical Trial

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IMPORTANCE Previous pharmacogenetic studies have shown the prognostic impact of several rare dihydropyrimidine dehydrogenase gene (*DPYD*) variants on fluorouracil-related adverse events (fluorouracil AEs). However, conflicting results highlight the need for prospective validation in large, homogeneous patient populations uniformly treated with current standard combination therapies used in colon cancer (CC).

OBJECTIVE To determine the impact of *DPYD* variants on fluorouracil AEs in patients with stage III CC treated with a fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) regimen.

DESIGN, SETTING, AND PARTICIPANTS Pharmacogenetic substudy of 1545 patients who participated from December 2005 to November 2009 in the European Pan-European Trials in Alimentary Tract Cancer (PETACC)-8 randomized phase 3 clinical trial.

INTERVENTIONS Patients with resected stage III CC were randomized to receive standard adjuvant FOLFOX4 alone or FOLFOX4 combined with cetuximab for 6 months.

MAIN OUTCOMES AND MEASURES Patients were genotyped on 25 *DPYD* variants. We tested the individual associations between each *DPYD* variant and grade 3 or greater fluorouracil AEs.

RESULTS A total of 1545 patients (57.6% male; median [range] age, 60 [19-75] years) were included in the analysis. The incidence of grade 3 or greater fluorouracil AEs in *D949V* and *V732I* (*DPYD*6*) carriers was 18 in 21 (85.7%) and 121 in 199 (60.8%), respectively. After adjusting for multiple variables, statistically significant associations were identified between grade 3 or greater fluorouracil AEs and both *D949V* (odds ratio [OR], 6.3 [95% CI, 2.0-27.0]; $P < .001$) and *V732I* variants (OR, 1.7 [95% CI, 1.3-2.4]; $P < .001$). Grade 3 or greater overall hematologic adverse events were associated with *V732I* (OR, 1.9 [95% CI, 1.4-2.6]) and *D949V* (OR, 5.2 [95% CI, 2.0-16.0]), and *V732I* was associated with grade 3 or greater neutropenia (OR, 1.8 [95% CI, 1.3-2.4]). The association of *V732I* with the occurrence of grade 3 or greater fluorouracil AEs and overall hematologic adverse events was validated in an independent cohort of 339 patients with metastatic colorectal cancer receiving FOLFOX4 in the Fédération Francophone de Cancérologie Digestive 2000-05 phase 3 trial.

CONCLUSIONS AND RELEVANCE In this large phase 3 study, statistically significant associations were found between *DPYD* variants (*D949V* and *V732I*) and increased incidence of grade 3 or greater fluorouracil AEs in patients treated with adjuvant fluorouracil-based combination chemotherapy. Further studies are warranted to confirm and quantitate these associations.

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Invited Commentary

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Since the 1990s, fluorouracil-based adjuvant chemotherapy has been the standard of care for patients with stage III colon cancer (CC) after curative surgical resection. The MOSAIC study[1] showed significant improvements in disease-free survival and overall survival in patients with stage III CC receiving infused fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) compared with fluorouracil and leucovorin alone, with 5-year disease-free survival of 66.4% and 6-year overall survival of 72.9% in the experimental group. This degree of benefit was confirmed by the NSABP C-07 study[2] in patients receiving FLOX (bolus fluorouracil, leucovorin, and oxaliplatin) compared with those receiving bolus fluorouracil and leucovorin alone. As in the metastatic setting, the addition of oxaliplatin to fluorouracil therefore results in better efficacy compared with fluorouracil alone but also increases overall and severe adverse events (AEs).[1,3]

There is a substantial interindividual variation in the occurrence and/or severity of AEs in patients receiving a similar chemotherapy schedule. Some interpatient differences in AEs can be explained by clinical factors, such as age, sex, and performance status.[4] Although much of the variability in AEs remains unexplained, it may be partly driven by individuals' genetic inheritance, leading to the hypothesis that some patients have germline polymorphisms in genes encoding drug target, drug-metabolizing, and DNA repair enzymes that may influence the safety profile of fluorouracil-based chemotherapy. In this regard, pharmacogenetics may be a useful strategy to personalize and optimize chemotherapy in patients with CC. Routine upfront screening based on specific genotyping according to the treatment provided may avoid severe, even fatal drug-related AEs in a substantial proportion of patients. This seems to be critical, especially for patients treated in the adjuvant setting, because approximately 50% of patients with stage III CC are cured with surgery alone. Pharmacogenetic studies related to fluorouracil-based chemotherapy have mainly focused on the main enzyme of the fluorouracil catabolic pathway, dihydropyrimidine dehydrogenase (DPD), which catabolizes approximately 85% of the administered fluorouracil. Several *DPYD* gene variants are known to affect DPD activity.[5] Previous retrospective and prospective studies have identified associations between the increased incidence of fluorouracil-related AEs and *DPYD*2A* (c.1905 + 1 G>A, previously IVS14 + 1 G>A; rs3918290), *D949V* (c.2846A>T, rs67376798), and I560S (c.1679 t > G, *DPYD*13*, rs55886062). Owing to their relatively low minor allele frequencies across the general population, these results have limited their usefulness in current clinical practice to predict AEs,[6-9] even if dose reductions are advised in recent guidelines for patients carrying any of these 3 *DPYD* variants.[10] Furthermore, there is only limited evidence that genetic variants are generalizable as predictors of AEs across fluorouracil regimens.[11]

Most previous studies suffered from insufficient power to detect associations with AEs because the numbers of patients were often limited, the disease populations were heterogeneous in terms of disease stage and treatment regimens, and few *DYPD* single-nucleotide polymorphisms (SNPs) were studied. One could thus expect to improve the sensitivity of genotyping by considering an expanded number of relevant *DPYD* mutations. In fact, more than 50 polymorphisms in *DPYD* have been identified to date.[12] Although very few of these polymorphisms have been associated with an increased risk of AEs, the clinical relevance of most of these polymorphisms remains low or unclear. Given the need to increase the sensitivity of *DPYD* genotyping and validate *DPYD* screening in large patient cohorts uniformly treated with the current standard combination therapies, we genotyped 25 *DPYD* SNP variants in a large cohort of patients with stage III CC treated in a randomized clinical trial of adjuvant FOLFOX4 chemotherapy alone or combined with cetuximab, with the aim of testing the individual associations between these variants and AEs.

Key Points

Question: What is the clinical impact of 25 *DPYD* germline polymorphisms on adverse effects of adjuvant chemotherapy with fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) in patients with stage III colon cancer?

Findings: Significant associations were identified between grade 3 or greater fluorouracil-related adverse events and *D949V* and *V732I* variants. Association between grade 3 or greater fluorouracil-related adverse events and *V732I* was validated in an independent patient cohort.

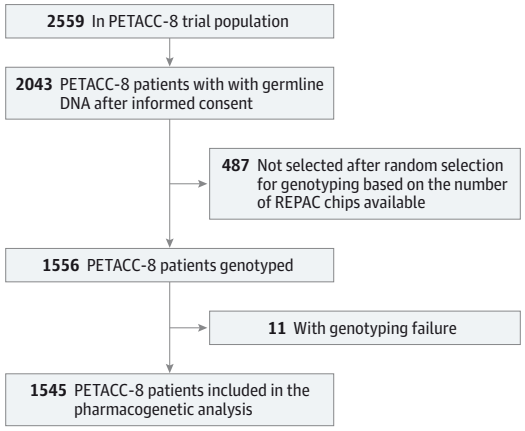
Meaning: If confirmed by independent validation, incorporating *V732I* genetic testing in addition to previous known at-risk *DPYD* variants may be justified to identify patients at increased risk of FOLFOX-induced AEs.

Methods

Patients

The PETACC-8 randomized phase 3 clinical trial allocated 2559 patients with resected (R0) stage III CC to receive FOLFOX4 every 2 weeks (1 cycle) with (arm B) or without (arm A) cetuximab as follows: oxaliplatin, 85 mg/m^2 (2 hours of infusion), on day 1; leucovorin, 200 mg/m^2, on days 1 and 2, followed by fluorouracil (bolus), 400 mg/m^2; then fluorouracil, 600 mg/m^2 (continuous infusion over 22 hours), with or without weekly cetuximab, which was given on day 1, 400 mg/m^2 (2 hours of infusion) the first week, then every week at 250 mg/m^2 (1 hour of infusion) for subsequent infusions. Full details of the current study have previously been published.[13] Patients were carefully monitored biweekly for AEs and graded according to National Cancer Institute–Common Toxicity Criteria for Adverse Events (NCI-CTCAE), version 3.0. Among those, overall severe (≥ grade 3) gastrointestinal (GI) tract AEs, including diarrhea, mucositis, and nausea and/or vomiting, as well as overall grade 3 or greater hematologic AEs, were deemed as related to fluorouracil treatment and selected for further correlation with genotypes. Among the 2559 patients included in this trial, 2043 (80%) gave their written informed consent for germline DNA analysis on blood sample (**Figure**). Among these, 1545 randomly selected patients (76%) were in

Figure. Study Flowchart



REPAC indicates Réseau de Pharmacogénétique des Anticancéreux.

fact genotyped owing to hardware limitations. Their main characteristics did not differ from those of the whole study population (eTable 1 in the Supplement). The study was approved by the appropriate ethics committees, and written informed consent was obtained from the patients.

Genotyping

Genomic DNA was purified using the QIAamp DNA purification system (Qiagen). DNA samples were genotyped for 16 561 SNPs using a customized Illumina SNP genotyping assay designed by the Réseau de Pharmacogénétique des Anticancéreux (REPAC) to capture the genetic variation of 1653 key drug pathway genes (including phase 1 and 2 drug metabolism enzymes; drug transporters; drug targets; drug receptors; and DNA repair-, apoptosis-, and angiogenesis and/or lymphangiogenesis-related proteins).[14,15] The SNPs were selected by tagging functional SNPs with tagSNP[16] using the hapmap database[17] with an r^2 pairwise tagging cutoff of 0.8. The SNPs were selected to characterize the main haplotypes within the white population (95% of haplotypic diversity) according to the following criteria: genes were defined by their position on human genome build 36 (National Center for Biotechnology Information); the minor allelic frequency had to be at least 5%, except for specific genes such as *DPYD*, for which rare variants were also included on the basis of previous knowledge; for SNPs carrying the same information, a "score design" allowed us to select the final SNP (defined by technical criteria related to the chip and established by the manufacturer). Microarrays were processed by Integragen using Illumina technology and Infinium iSelect custom genotyping. Laboratory members were blinded to clinical data. The 25 *DPYD* variants, their potential functional effect on DPD activity, and their frequencies among the genotyped population are detailed in eTable 2 in the Supplement. The present study focused on the 12 SNPs that were not invariant in our population. All but 1 of these 12 SNPs displayed weak linkage disequilibrium (eFigure in the Supplement). The remaining 13 present on the chip were discarded from the analysis because the minor allele was not present in any of the patients.

Statistical Analysis

The primary end point of the study was the development of any grade 3 or greater fluorouracil-related AEs combining grade 3 or greater overall hematologic and GI AEs. Secondary end points were grade 3 or greater overall hematological AEs, grade 3 or greater overall GI AEs, as well as grade 3 or greater diarrhea, mucositis, nausea and/or vomiting, and neutropenia, considered separately. Any other AE was not included in the analysis. Logistic regression modeling was used to test the hypothesis of associations between each of the 12 SNPs and the end points. The modeling was based on the hypothesis of an additive effect of allelic dosage. Associations of tested SNPs were assessed using a likelihood ratio test in which the likelihood ratio followed a χ^2_1 distribution (equal to the difference in the number of variables in the compared full and null models).

We used a hierarchical procedure to test the different hypotheses. Associations between each of the 12 SNPs with the secondary end points were analyzed only if the null hypothesis was rejected for the association with the primary end point. A Bonferroni correction was applied according to this gatekeeping procedure.[18] Analyses were performed on the intent-to-treat population. Using a sample size of 1548 patients, associations with an OR equal to 5 for an overall grade 3 or greater toxicity-related allele of 1% can be detected with an $\alpha = .004$ and a power of 0.80.

To account for potential confounding factors, multivariate models were used. We systematically used a set of 4 relevant clinical variables (age, sex, treatment randomization, and World Health Organization performance status [WHO PS]) and also tested 10 additional population stratification variables. The relevance of these additional covariables was determined for each end point according to the outcome of an association test; $P = .01$ was considered significant. To test and potentially control population stratification in our pan-European sample, a principal component analysis (PCA) was performed using EIGENSOFT software (http://www.hsph.harvard.edu/alkes-price/software/). All available genotyping data were included in the PCA and produced 10 additional variables (the 10 first axes of the PCA) describing population stratification. No association between these 10 variables and the study end points was observed. Therefore, we did not use any of the 10 stratification variables in the multivariate models. A model that included the polymorphisms found to be associated with the primary end point was compared with each single polymorphism model to assess its superiority.

Finally, the cohort of the independent Fédération Francophone de Cancérologie Digestive (FFCD) 2000-05 trial was used as a validation set for these SNPs. The same gatekeeping procedure was used in the validation cohort but this time was restricted to the SNPs selected previously from the PETACC-8[19] cohort analysis. PLINK and R software was used to carry out the association testing and the population-based linkage analysis (http://pngu.mgh.harvard.edu/~purcell/plink/).

Results

Among the 1545 patients included in the pharmacogenetic analysis, 57.6% were male, the median (range) age was 60 (19-75) years, and 79.7% had a WHO PS of 0. The main patient characteristics and median dose intensity of fluorouracil, oxaliplatin, and cetuximab are listed in **Table 1**. More than 74% of the patients received 12 chemotherapy cycles. At least 1 fluorouracil dose modification during treatment was required in 46% of the patients. Among the clinical characteristics, age, sex, and WHO PS had a significant impact on the occurrence of grade 3 or greater fluorouracil-related overall AEs in univariate analysis. Associations between clinical variables and AEs are shown in **Table 2**. Older age and, to a lesser extent, higher WHO PS were associated with a higher risk of grade 3 or greater overall hematologic AEs and neutropenia. Women reported higher risk of fluorouracil-related AEs, including grade 3 or greater overall hematologic AEs, neutropenia, overall GI AEs, and nausea and/or vomiting. As expected, patients in the cetuximab arm had significantly more frequent overall grade 3 or greater GI AEs, diarrhea, and mucositis.

The genotypic analysis adjusted for relevant clinical variables revealed that 2 SNPs, rs1801160 (*V732I*, *DPYD*6*) and rs67376798 (*D949V*), were significantly associated with grade 3 or greater fluorouracil-related overall AEs ($P < .001$ for both) (**Table 3** and eTable 3 in the Supplement). The absolute difference (ie, attributable risk) in the grade 3 or greater overall AE rate was more than 13% in *V732I*, and more than 36.7% in *D949V* carriers. Both SNPs were associated with grade 3 or greater overall hematologic AEs and V732I with neutropenia. The absolutes difference in grade 3 or greater overall hematologic AEs were 15% in *V732I* and 37% in *D949V* carriers. Moreover, the at-risk alleles *V732I* and *D949V* contributed to 29% and 48% of grade 3 or greater overall hematologic AEs (attributable fraction) in the patients carrying these variants, respectively.

The statistical model containing the 2 SNPs showed a significant association with grade 3 or greater overall AEs compared with the one including each SNP separately ($P < .001$ for both), suggesting an independent effect of each SNP. The performance of the 2 combined genotypes as potential biomarkers predicting overall grade 3 or greater AEs was 18%, 90%, 63%, and 53% for sensitivity, specificity, positive predictive value, and negative predictive value, respectively (**Table 4**).

In an attempt to validate our results, we tested the association for *V732I* and *D949V* with the occurrence of grade 3 or greater fluorouracil AEs and overall hematologic AEs in an in-

Table 1. Patient Characteristics

Characteristic	Treatment Arm	
	A, FOLFOX4 (n = 780)	B, FOLFOX4 Plus Cetuximab (n = 765)
Age, median (range), y	60 (21-75)	60 (19-75)
Sex ratio (female/male)	0.79	0.68
WHO PS, %		
0	632 (81)	599 (78)
1	128 (16)	143 (19)
2	1 (0.1)	2 (0.3)
Receipt of 12 chemotherapy cycles, No. (%)	625 (80)	565 (74)
Dose intensity, median (total dose mg/cycle)		
Fluorouracil bolus	1528	1512
Fluorouracil continuous infusion	2368	2366
Oxaliplatin	80	80
Cetuximab	NA	1006
>1 dose modification, No. (%)		
Fluorouracil bolus	354 (45)	355 (46)
Fluorouracil continuous infusion	354 (45)	355 (46)
Oxaliplatin	317 (41)	312 (41)
Cetuximab	NA	86 (11)
Grade ≥3, No. (%)		
Overall fluorouracil-related adverse events	380 (49)	385 (50)
Hematologic adverse events	322 (41)	295 (39)
Neutropenia	288 (37)	274 (36)
Gastrointestinal adverse events	98 (13)	171 (22)
Diarrhea	74 (10)	118 (15)
Mucositis	12 (2)	61 (8)
Nausea/vomiting	19 (2)	15 (2)

Abbreviations: FOLFOX4, folinic acid-fluorouracil-oxaliplatin; NA, not applicable; WHO PS, World Health Organization performance status.

Table 2. Testing Association Hypotheses of Relevant Clinical Variables With the Primary and Secondary End Points Considered[a]

Grade ≥3 Fluorouracil-Related Adverse Events	Age (/10 y)[b,c]		Sex (Male/Female)[b]		Treatment[d]		WHO PS[c]	
	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Overall	1.2 (1.1-1.3)	<.001	0.33 (0.2-0.4)	<.001	1.1 (0.9-1.3)	.50	1.4 (1.1-1.8)	.008
Overall gastrointestinal	1.1 (0.95-1.3)	.20	0.65 (0.5-0.8)	.001	2 (1.5-2.6)	<.001	1.1 (0.8-1.5)	.60
Diarrhea	1.1 (0.95-1.3)	.20	0.7 (0.5-0.9)	.02	1.7 (1.3-2.4)	<.001	1 (0.7-1.5)	>.99
Nausea	0.8 (0.6-1.1)	.20	0.4 (0.2-0.8)	.008	0.8 (0.4-1.6)	.50	1.4 (0.6-3)	.40
Mucositis	1.2 (0.9-1.5)	.30	0.75 (0.5-1.2)	.20	5.5 (3.1-11)	<.001	1.1 (0.6-1.9)	.80
Overall hematological	1.2 (1.1-1.4)	<.001	0.35 (0.3-0.4)	<.001	0.9 (0.7-1.1)	.30	1.4 (1.1-1.8)	.01
Neutropenia	1.2 (1-1.3)	.007	0.4 (0.3-0.5)	<.001	0.95 (0.77-1.2)	.70	1.5 (1.1-1.9)	.005

Abbreviations: OR, odds ratio; WHO PS, World Health Organization performance status.

[a] Restricted to fluorouracil-related adverse events.

[b] Reported OR, male vs female.

[c] Reported ORs correspond to a unit increase of the predictor variable (ie, per 10 y for the age column, per grade in the WHO PS column).

[d] Reported OR, arm receiving FOLFOX4 plus cetuximab vs arm receiving FOLFOX4 only.

Table 3. Logistic Regression Analysis of Polymorphisms *V732I* (rs1801160) and *D949V* (rs67376798) Found to Be Associated With Grade 3 or Greater Fluorouracil-Related Adverse Events[a]

Grade ≥3 Fluorouracil-Related Adverse Events	SNP	Cases[b]	Controls[b]	m/M[c]	OR (95% CI)	*P* Value
Overall	*V732I*	644/116/5	702/77/1	A/G	1.7 (1.3-2.4)	<.001
	D949V	747/18/0	777/3/0	A/T	6.3 (2-27)	<.001
Overall gastrointestinal tract	*V732I*	229/35/5	1117/158/1	A/G	1.3 (0.92-1.9)	.10
	D949V	262/7/0	1262/14/0	A/T	2 (0.73-4.9)	.20
Diarrhea	*V732I*	161/27/4	1185/166/2	A/G	1.4 (0.96-2.1)	.08
	D949V	189/3/0	1335/18/0	A/T	0.99 (0.23-3.0)	>.99
Nausea	*V732I*	27/6/1	1319/187/5	A/G	2.1 (0.92-4.4)	.08
	D949V	34/0/0	1490/21/0	A/T	9.7e-07 (NA-1.7e16)	.30
Mucositis	*V732I*	65/8/0	1281/185/6	A/G	0.75 (0.33-1.5)	.40
	D949V	68/5/0	1456/16/0	A/T	4.9 (1.5-13)	.01
Overall hematological	*V732I*	511/102/4	835/91/2	A/G	1.9 (1.4-2.6)	<.001
	D949V	601/16/0	923/5/0	A/T	5.2 (2.0-16.0)	<.001
Neutropenia	*V732I*	466/93/3	880/100/3	A/G	1.8 (1.3-2.4)	<.001
	D949V	549/13/0	975/8/0	A/T	2.9 (1.2-7.5)	.02

Abbreviations: OR, odds ratio; NA, not applicable; SNP, single-nucleotide polymorphism.

[a] Multivariate models including relevant clinical covariates (age, sex, treatment, and World Health Organization performance status) were used. The reported ORs are adjusted for covariates, and correspond to a unit increase in the dosage of the minor allele (ie, OR of minor homozygous vs major homozygous corresponds to the square of the indicated OR). *P* = .004 was considered significant.

[b] Cases were patients who experienced any grade 3 or greater fluorouracil-related adverse events; controls, patients who did not. Genotypes are detailed as follows: wild-type/heterozygous carrier/homozygous carrier.

[c] M is the major allele; m, the minor one.

Table 4. Clinical Performance of *V732I* and *D949V* Genotype to Predict Fluorouracil-Related Adverse Events

Grade ≥3 Fluorouracil-Related Adverse Events	*DPYD* Allele	%			
				Predictive Value	
		Sensitivity	Specificity	Positive	Negative
Overall	*V732I*	16	90	61	52
	D949V	2	100	86	51
	V732I/D949V	18	90	63	53
Hematologic	*V732I1*	17	90	53	62
	D949V1	3	99	76	61
	V732I/D949V1	20	89	55	63
Gastrointestinal tract	*V732I2*	15	88	20	83
	D949V2	3	99	33	83
	V732I/D949V2	17	87	21	83

Abbreviation: DPYD, dihydropyrimidine dehydrogenase gene.

dependent cohort of 339 patients with metastatic colorectal cancer receiving FOLFOX in the FFCD 2000-05 trial,[3] 300 of whom we had previously genotyped using the same REPAC chips. Because we first tested for the association of 2 polymorphisms with the primary end point (grade ≥3 fluorouracil-related AEs), to get an overall α of 5%, the 2 tests were performed using α = 2.5%. The significant effect of *V732I* could be replicated (OR, 2.7 [95% CI, 1.2-6.7]) but not that of *D949V*. The association of *V732I* and grade 3 or greater overall hematologic AEs was also confirmed (OR, 3.8 [95% CI, 1.6-9.2]; *P* = .002) with a deleterious effect of the rare variant of *V732I* found in 18 heterozygous patients among 90 patients with grade 3 or greater hematologic AEs vs 9 of the 183 control patients (eTable 4 in the Supplement).

Discussion

Through the analysis of 1545 fluorouracil-treated patients from the PETACC-8 adjuvant trial for 25 *DPYD* genetic variants, we identified statistically significant associations for both *D949V* and *V732I* (*DPYD**6*) variants with overall grade 3 or greater fluorouracil AEs. The genotyped patient population was homogeneous, in terms of disease stage and treatments, with well-characterized clinicopathological factors and uniformly assessed, treatment-related AEs. According to previous results of mainly retrospective and/or underpowered studies that included patients with various CC stages and treatment schedules, 3 *DPYD* variants, *DPYD*2A*, *D949V*, and *I560S*, were sug-

gested as having a potential impact on fluorouracil AEs based on their deleterious effects on DPD activity.[10]

In the recent meta-analysis of QUASAR2 and 16 published studies (n = 4855 patients) by Rosmarin et al,[11] global capecitabine AEs were associated with *DPYD*2A* and *D949V* (combined odds ratio [OR], 5.5; *P* = .001), but there was weaker evidence that these polymorphisms predicted AEs from bolus and infusional fluorouracil monotherapy. By contrast, both *DPYD*2A* and *D949V* had a strong effect when fluorouracil was given in combination. Therefore, concomitant drugs may enhance the effect of *DPYD* risk alleles. Regarding our study, one of the hypotheses is that SNP-related lower *DPYD* activity may lead to an increase in the FOLFOX-related background AEs through a synergistic effect of oxaliplatin on specific fluorouracil-related AEs. In accordance with this, a recent large pharmacogenetic analysis of 2886 patients with stage III CC treated adjuvantly in a randomized phase 3 clinical trial (North Central Cancer Treatment Group [NCCTG] N0147) with FOLFOX or irinotecan with fluorouracil and folinic acid, alone or combined with cetuximab, found statistically significant associations between *DPYD*2A* and *D949V* and the increased incidence of grade 3 or greater fluorouracil AEs.[20] Our results therefore confirmed the significant impact of *D949V* but not that of *DPYD*2A* in patients treated with FOLFOX with or without cetuximab. The low frequency of *DPYD*2A* (11 heterozygous patients [0.7%] in our population) may partly explain this result. The same may be true for *I560S* (4 heterozygous patients [0.2%]).

The second most relevant finding of our analysis was the impact of *V732*I on overall grade 3 or greater fluorouracil-related AEs. *V732I* is a nonsynonymous *DPYD* variant. In contrast to the well-known deleterious effect of *D949V* and *DPYD*2A* on DPD enzymatic activity, the effect of *V732I* remains unclear. In a previous study,[21] in which *DPYD* variants were expressed in mammalian cells, and the enzymatic activity of expressed protein was determined relative to wild type, *V732I* did not significantly affect enzyme activity. By contrast, in 94 African American volunteers, *V732I* was significantly associated with altered DPD enzyme activity measured in circulating mononuclear cells.[22] However, *V732I* was shown to be in linkage disequilibrium with *Y186C*, and the exclusion of *Y186C* carriers from the analysis led to nonsignificant *P* values for *V732*I. Further phenotypic data are needed in larger populations. *V732I* has been poorly assessed and is inconsistently shown to contribute to fluorouracil-related AEs. A previous case-control analysis[23] identified a strong association between *V732I* and leucopenia (OR, 8.17 [95% CI, 2.44-27.31]) and neutropenia, while several other reports have shown no association.[6,24,25] In a more recent case-cohort analysis carried out in 568 previously untreated patients with advanced CC participating in the CAIRO2 trial[26] and assigned to capecitabine combined with oxaliplatin, and bevacizumab with or without cetuximab, *V732I* was significantly associated with grade 3 to 4 diarrhea but with a rather low predictive value of 41%. Finally, a recent meta-analysis[27] concluded that *V732I* might contribute to the development of fluorouracil-induced hematologic and GI tract AEs among Asians but not among whites. All these discrepancies may be a result of methodological differences between studies that included various ethnicities, variable doses, and schedules of fluorouracil-based therapy, concomitant administration of various cytotoxic drugs, and variable tumor types. Furthermore, the minor allele frequency of *V732I* was found to be low in several underpowered studies.[11] In the prospective study by Schwab et al,[25] which included 683 patients with different tumor types treated with various fluorouracil monotherapy regimens, *V732I* was not associated with fluorouracil-related severe AEs. However, most patients received weekly high-dose infusional or bolus fluorouracil, with a higher rate of severe AEs in patients receiving bolus-based fluorouracil than in patients receiving continuous infusion, thus suggesting a dose- and schedule-dependent effect of fluorouracil. In addition, *V732I* was not found to be associated with AEs in the pharmacogenetic analysis of the QUASAR II trial,[11] but all the patients were treated with capecitabine alone, and its metabolism and AE profile differ from those of fluorouracil. As previously reported in the meta-analysis by Rosmarin et al,[11] potentially relevant *DPYD* genetic variants may be not generalizable as predictors of AEs across all fluoropyrimidines/fluorouracil regimens.

Heterogeneity in the overall proportion of AEs explained by *DPYD* variants across different studies may be also attributed to differences in the extent of the examination of the *DPYD* gene. The 25 SNPs selected for our REPAC chip aimed to characterize the main haplotypes within the white population (95% of haplotypic diversity). By contrast, the study by Lee et al[20] focused on 25 *DPYD* variants displaying functionally deleterious effects on DPD activity from the current literature. Unfortunately, 21 of these functionally deleterious *DPYD* variants were absent from the study population, and only *DPYD*2A* and *D949V* were present in frequencies suitable to assess associations with grade 3 or greater AEs. Only 11 SNPs were common to our SNP selection, and *V732I* was not included for genotyping.

Although we cannot exclude the possibility that fine mapping may detect additional causal variants of neighboring unknown genes in linkage disequilibrium, the fact that *V732I* added information in the model that included our 2 SNPs and was validated as a predictive SNP in the FFCD 2000-05 trial reinforces our hypothesis that this *DPYD* variant may account for FOLFOX-induced AEs.

Although genome-wide association studies seem to be an attractive approach, such recent analyses have led to rather inconclusive results in patients treated with fluorouracil either alone or in combination with oxaliplatin (FOLFOX).[28,29] In contrast to candidate-gene strategies, significance is often not reached in genome-wide studies given the threshold *P* value required by the multiple testing. Furthermore, the interpretation of the results, in terms of description of the underlying processes, is not often straightforward, and the real biological mechanisms underlying the potential predictive associations remain unknown.

Otherwise, although our data set was relatively large and the power was good enough to detect AE variants with relatively large effects, the power may have been too low to de-

tect variants with low allele frequency (including *DPYD*2A* and *I560S*) and/or smaller effect sizes. Similarly, because our validation cohort contained far fewer patients than the PETACC-8 cohort, the power of our validation analysis did not allow multiple testing. Therefore, we chose to restrict our validation to the strongest associations found in the PETACC-8 cohort, which was a grade 3 or greater hematologic AE, but not our primary end point. Before genetic testing can be used in clinical practice, there is a need to identify and characterize additional fluorouracil AE variants in larger patient cohorts and to investigate the potential associations between combinations of rare and common *DPYD* variants and severe AEs, which may provide a more comprehensive *DPYD* variant model for fluorouracil AE prediction. Such variants should be added to the panel of polymorphisms identified in our study so as to develop a genetic test that might well make it possible to closely monitor patients who are at increased risk of experiencing AEs. It would be worth demonstrating whether such a strategy would be cost-effective.

Conclusions

We have determined that the rare *D949V* and the more common *V732I* variants are associated with fluorouracil-related AEs in a large cohort of patients with stage III CC treated with adjuvant FOLFOX4 chemotherapy. If confirmed by independent validation, incorporating *V732I* genetic testing, in addition to *DPYD*2A* and *D949V* variants previously identified in the NCCTG N0147 trial that analyzed a comparable patient population, may be justifiable to highlight patients at increased risk of FOLFOX-induced AEs. The FOLFOX regimen is the most frequently used regimen in the treatment of CC both in adjuvant and metastatic settings worldwide, thus highlighting the need to identify high-risk patients. To our knowledge, our study is the first to test and reveal the *V732I* at-risk allele in a prospective large randomized clinical trial using this regimen. Further studies are warranted to confirm and quantitate these associations in additional data sets.

ARTICLE INFORMATION

Group Information: The PETACC-8 study investigators are listed in the eAppendix in the Supplement.

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Author Contributions: Dr Boige and Mr Vincent had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Dr Boige, Mr Vincent, and Dr Taieb contributed equally.

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