



DPYD c.85 T > C, DPYD c.496 A>G, and DPYD c.2194 G>A and severe toxicity to fluoropyrimidines in PREPARE and Alpe-DPD trials. An original study and meta-analysis

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ABSTRACT

Current DPYD genotyping panels used to prevent fluoropyrimidine (FL)-related toxicity explain only a limited proportion of severe adverse events. The clinical relevance, beyond current testing, of additional DPYD variants, including DPYD*6 (c.2194 G>A, rs1801160), DPYD c.496 A>G (rs2297595), and DPYD*9 A (c.85 T > C, rs1801265) remains debated. This study evaluated each polymorphism individually, irrespective of the allelic configuration at the other investigated loci, and as part of three-locus haplotypes in relation to severe FL-related toxicity. For single-variant effects, data from patients enrolled in two trials (PREPARE, Italian cohort; Alpe-DPD) were combined with 31 eligible published studies in a meta-analysis comprising 33 studies and 17,485 patients, with subgroup analyses by ethnicity. Haplotype analyses were performed in the 1296 trial participants. LC-MS was applied to evaluate plasma uracil and dihydrouracil levels. In the meta-analysis, DPYD c.2194 G>A (OR=1.70; 95%CI: 1.48–1.95) and c.496 A>G (OR=1.55; 95%CI: 1.19–2.02) were associated with FL toxicity. No overall association was observed for c.85 T > C; however, it improved c.496 A>G associated risk prediction in a haplotype analysis. Haplotype #3 (minor c.496 G with major c.85 T) was consistently associated with increased severe overall toxicity (PREPARE: OR=2.62, 95%CI: 1.06–6.50; Alpe-DPD: OR=2.09, 95%CI: 1.01–4.37) and gastrointestinal toxicity (PREPARE: OR=5.65, 95%CI: 2.11–15.15; Alpe-DPD: OR=2.71, 95%CI: 1.23–5.97). An exploratory Receiver Operating Characteristic (ROC) curve analysis demonstrated a significant incremental gain in risk discrimination achieved by adding DPYD c.85 T > C to a model that already included c.496 A>G. Haplotype #4 (minor alleles at both loci) was not associated with toxicity, suggesting a functional interaction between the variants. DPYD c.2194 G>A did not add to haplotype-based stratification. In PREPARE, pre-treatment plasma uracil and dihydrouracil confirmed reduced DPD function in haplotype #3 carriers. Overall, DPYD c.2194 G>A and c.496 A>G increased severe FL-toxicity risk when evaluated individually, whereas the integration of c.85 T > C refined risk prediction in a haplotype-based approach, supporting further refinement of pharmacogenetic risk stratification.

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1. Background

A deficiency in the enzymatic conversion of 5-fluorouracil (5-FU) to its inactive metabolite dihydrofluorouracil—mediated by dihydropyrimidine dehydrogenase (DPD; *DPYD*)—is a well-established risk factor for severe to potentially lethal fluoropyrimidine (FL)-related adverse drug reactions. Consequently, both the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA) recommend pre-treatment DPD testing before initiating fluoropyrimidine-based therapy [1,2].

Clinical implementation guidelines issued by the Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group (DPWG) provide recommendations for fluoropyrimidine dose adjustment according to *DPYD* metabolizer phenotype or activity score, as determined by the patient's genotype [3, 4]. More recently, the Pharmacogenomics (PGx) Working Group of the Association for Molecular Pathology (AMP) proposed an expanded *DPYD* variant panel for clinical implementation, categorizing variants into Tier 1 (high-priority) and Tier 2 (lower-priority) based on their clinical relevance [5]. Nevertheless, routine clinical testing in many centers remains limited to the four clinically validated *DPYD* variants (*DPYD**2 A, rs3918290; *DPYD**13, rs55886062; c.2846 A>T, rs67376798; c.1129–5923 C>G [HapB3], rs75017182) which currently form the basis of most pharmacogenetic dosing recommendations [6,7].

Pre-treatment *DPYD* testing has been shown not only to improve treatment safety, but also to positively affect treatment adherence, patients' quality of life, and medical costs [8–11]. Since 2020, pre-treatment DPD testing prior to FL prescription has also been recommended by the European Medicines Agency (EMA), contributing to changes in FL dosing practices across Europe [12,13]. More recently, the U.S. Food and Drug Administration (FDA) updated FL labels to include warnings about the risk of serious adverse reactions in patients with impaired DPD function [1].

Despite these advances, currently recommended *DPYD* panels explain only a minority (about 17%) of observed FL toxicities and do not fully account for the marked interpatient variability in DPD function [6, 14]. A phenotyping approach based on plasma uracil (U) and dihydrouracil (UH2) concentrations as surrogates of DPD function has been proposed as a potentially better predictor of FL toxicity; however, it is affected by critical pre-analytical constraints, lacks robust prospective evidence, and its clinical utility remains controversial [15–17]. Additional *DPYD* variants have been reported as potentially associated with increased FL toxicity risk, but most pharmacogenetic guidelines consider the current evidence insufficient to recommend their routine clinical implementation. Examples include *DPYD**9 A (rs1801265, c.85 T > C, p.Cys29Arg), *DPYD* c.496 A>G (rs2297595, p.Met166Val), and *DPYD**6 (rs1801160, c.2194 G>A, p.Val732Ile) [18]. Although CPIC still classifies these variants as “normal function,” they have been extensively investigated for their potential contribution to FL-related toxicity risk; however, reported effects are inconsistent and appear to depend on treatment regimen, patient ethnicity, and clinical setting [18, 19]. The substantial yet inconclusive evidence available for these variants therefore supported their investigation in the PREPARE and Alpe-DPD cohorts and was sufficiently extensive to also allow a formal quantitative meta-analysis.

Notably, *DPYD* c.85 T > C and *DPYD* c.496 A>G show a high degree of linkage disequilibrium (LD), and it has been hypothesized that their combined effect on DPD function may have confounded earlier association studies focused on single variants. More recently, the predictive value of their haplotype combination—rather than individual variants—has been evaluated with encouraging results [20–22], warranting further investigation. In contrast, *DPYD* c.2194 G>A has been studied largely as an individual biomarker, with limited data on its contribution within haplotype combinations [20].

The primary aim of the present study was to investigate the association of *DPYD* c.85 T > C, *DPYD* c.496 A>G, and *DPYD* c.2194 G>A with

the risk of severe FL-related toxicity, both individually (i.e., each locus being evaluated irrespective of the allelic configuration at the other two loci) and as phased three-locus haplotypes, in two large prospective multicenter pharmacogenetic trials: the PREemptive Pharmacogenomic Testing for Preventing Adverse Drug REactions (PREPARE)-Ubiquitous Pharmacogenomics trial (Italian cohort) [10,23] and the Alpe-DPD trial (Dutch cohort) [6]. To strengthen the evidence and explore variant effects across different patient ethnicities, a systematic literature review and meta-analysis was also performed evaluating the association between individual *DPYD* variants and FL-related toxicity.

2. Patients and methods

2.1. Study design and patient cohorts

This study included patients from two prospective clinical trials, the PREPARE trial and the Alpe-DPD trial, and a systematic review and meta-analysis of the literature evaluating the association between selected *DPYD* germline variants and FL-related toxicity.

2.2. PREPARE cohort

2.2.1. Patient cohorts

The primary study population was derived from the prospective PREPARE trial (NCT03093818, ClinicalTrials.gov), a multicentre study evaluating genotype-guided FL dosing. The overall results of the PREPARE trial have been previously reported [23], and data from Italian participants have been described separately [10].

For the present sub-analysis, patients were eligible if they fulfilled all of the following criteria: (i) enrolment in the PREPARE study in Italy, in either the control or intervention arm; (ii) treatment with capecitabine or 5-FU as the index drug; (iii) availability of residual genomic material; (iv) availability of clinical and toxicity data; and (v) provision of written informed consent for the use of anonymized genetic material for research purposes beyond the original PREPARE study (approval by CEUR – Comitato Etico Unico Regionale, protocol number CRO-2016–51).

Within PREPARE, patients carrying variant alleles of *DPYD* c.1905 + 1 G>A (*DPYD**2 A), c.1679 T > G (*DPYD**13), c.2846 A>T, or c.1236 G>A (tagging *DPYD*-HapB3) received an upfront 50% FL dose reduction according to the study protocol. Because of this dose modification, these patients were included only in LD analyses but were excluded from genotype–toxicity association analyses.

2.2.2. Clinical data collection and toxicity assessment

Clinical information was extracted from patients' medical records and included: (i) baseline tumor characteristics; (ii) chemotherapy regimen, including FL type, combination therapy, and treatment setting; and (iii) toxicity data for each chemotherapy cycle. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

Only toxicities considered biologically plausible for FL–*DPYD* gene–drug interactions were included in the analysis. These comprised hematological toxicities (neutropenia, leukopenia, thrombocytopenia) and non-hematological toxicities (nausea/vomiting, diarrhea, oral mucositis, hand–foot syndrome, and cardiac toxicity). For each patient, the maximum grade of toxicity observed during the entire course of chemotherapy was used for statistical analyses. Clinically relevant toxicity was defined as grade ≥ 3 for non-hematological adverse events or grade ≥ 4 for hematological adverse events. These grade thresholds were selected to capture adverse events with a substantial potential impact on clinical management and were informed by the conventional definition of dose-limiting toxicity used in phase I trials of cytotoxic anticancer agents, in which grade 3–4 non-hematological or grade 4 hematological toxicities are generally considered dose limiting and therefore drive changes in the drug administration [24]. This distinction

reflects the fact that grade 3 non-hematological adverse events are usually clinically manifest and more burdensome for the patients and may directly interfere with treatment continuation, whereas grade 3 hematological abnormalities may be transient or manageable and are not invariably considered dose limiting.

2.2.3. Genotyping procedures in the PREPARE cohort

Genomic DNA was extracted from peripheral blood or saliva samples. Genotyping for *DPYD* c.85 T > C, *DPYD* c.496 A>G, and *DPYD* c.2194 G>A was performed using commercially available TaqMan single-nucleotide polymorphism (SNP) genotyping assays (Thermo Fisher Scientific, Applied Biosystems). Reactions were run on an ABI 7500 real-time PCR system (Thermo Fisher Scientific) following the manufacturer's instructions. The genotype call rate was 100% for all three assays. Positive and negative controls were included in each genotyping run and consistently yielded the expected results. Duplicate samples were included as reproducibility controls and showed 100% genotype concordance. Genotype data for *DPYD**2 A, *DPYD**13, c.2846 A>T, and c.1236 G>A had been previously generated within the PREPARE trial [23].

2.3. Validation cohort: Alpe-DPD study

The findings from the PREPARE cohort were evaluated in an independent validation cohort derived from the Dutch multicenter, prospective Alpe-DPD study (NCT02324452, ClinicalTrials.gov). The study design and primary results of Alpe-DPD have been reported previously [6].

Briefly, adult patients (≥ 18 years) with any cancer type for which FL therapy was indicated were enrolled across 17 hospitals in the Netherlands. Prospective genotyping for *DPYD**2 A, *DPYD**13, c.2846 A>T, and c.1236 G>A was performed prior to treatment initiation, and FL doses were adjusted accordingly. For the present analysis, patients without available genotype data or carrying variant alleles of these four *DPYD* markers were excluded.

The study was approved by the institutional review board (protocol number: M14DPD) of The Netherlands Cancer Institute (Amsterdam, Netherlands) and approval from the board of directors of each individual hospital was obtained for all participating Centers (see [supplemental information](#)). All participants provided written informed consent, including consent for the use of clinical data and remaining biological material for additional *DPYD* sequencing analyses.

Baseline clinical characteristics and toxicity data were collected as previously described [6]. Adverse events were graded according to CTCAE version 4.03, and only the highest-grade adverse events classified as possibly, probably, or definitely related to FL treatment were included. The same grade thresholds used to define clinically relevant toxicity in the PREPARE cohort were applied to the Alpe-DPD analyses.

Genotype data for *DPYD* c.85 T > C, c.496 A>G, and c.2194 G>A were retrieved from next-generation sequencing of *DPYD* exons, using a previously described sequencing pipeline [25]. Because genotyping was performed using TaqMan assays in PREPARE and next-generation sequencing in Alpe-DPD, with no samples analyzed by both methods, direct cross-platform concordance could not be assessed. Nevertheless, both platforms are established genotyping methods, and the available cohort-specific quality-control results supported reliable genotype calling, although minor platform-related differences cannot be completely excluded.

2.4. Systematic review and meta-analysis

2.4.1. Literature search strategy

A systematic review was conducted in accordance with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines and followed the PECO (Patients, Exposure, Comparisons, Outcomes) framework (see [Supplementary Method](#) for details about the

PECO framework). The review protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO 2025; registration number CRD420251034391).

A comprehensive literature search was performed to identify studies evaluating the association between c.85 T > C, c.496 A>G, and c.2194 G>A and the risk of severe FL-related toxicity. MEDLINE (PubMed), the Cochrane Library, and Web of Science Core Collection were searched, with the last update on 1 May 2026. As all relevant articles retrieved from the latter databases were indexed in MEDLINE, analyses were based on MEDLINE records. Search terms included combinations of keywords related to “*DPYD*”, the three polymorphisms (‘*DPYD* c.2194 G>A’, ‘*DPYD* c.496 A>G’ and ‘*DPYD* c.85 T > C’), ‘fluoropyrimidine (5-fluorouracil or capecitabine)’, and ‘toxicity’, combined using Boolean operators (OR/AND) (see [Supplementary Method](#) for the exact algorithms employed in the literature search). Additional studies were identified by manual screening of reference lists.

Study selection was performed independently by two investigators (EDM and MG), with disagreements resolved by a third reviewer (EC). In cases of overlapping populations, only the publication including the largest number of patients was retained.

2.4.2. Eligibility criteria and data extraction

Eligible studies met the following criteria: (i) publication in English in a peer-reviewed journal; (ii) availability of quantitative data on the association between at least one of the three *DPYD* variants and FL-related toxicity; and (iii) evaluation of toxicity of at least grade 2 as a clinical endpoint. Studies were excluded (i) if they were reviews, meta-analyses, case reports, descriptive studies, or conference abstracts; (ii) if quantitative data were insufficient or unclear; (iii) if they did not evaluate FL-related toxicity; or (iv) if only grade ≥ 1 toxicity was assessed.

Data were extracted independently in duplicate (EDM and MG) using a pre-piloted extraction form. Collected variables included study characteristics, patient demographics, cancer type, treatment details, toxicity definitions, and genotyping methods. Results from the PREPARE and Alpe-DPD cohorts were incorporated into the quantitative synthesis.

2.4.3. Quality assessment

The methodological quality of included studies was evaluated independently by two authors using the Newcastle–Ottawa Scale (NOS, The Newcastle–Ottawa Scale, 2025). Scores ranged from 0 to 9, with a score ≥ 7 indicating high methodological quality.

2.5. Measurement of uracil and dihydrouracil concentrations

DPD function was indirectly assessed by measuring plasma concentrations of U and UH2 using the DOSIURA kit (Shimadzu Chemistry & Diagnostics). The plasma was collected before the start of treatment with FLs, and the sample was processed within two hours of collection. Analyses were conducted according to the manufacturer's instructions. Chromatographic separation was performed using a Nexera LC-40 $\times$ 3 HPLC system, coupled to a SCIEX CITRINE 6500 QTrap triple-quadrupole mass spectrometer operating in positive ion mode.

Quantification was based on multiple reaction monitoring, measuring one quantifier and one qualifier per analyte. Data acquisition and processing were performed using Skyline software (version 25.1.0.237). Based on published evidence, impaired DPD function was defined as plasma uracil concentrations > 16 ng/mL and/or a UH2/U ratio < 6 [26].

2.6. Statistical analysis

Linkage disequilibrium among *DPYD* (i.e., c.1905 +1 G>A [*2 A], c.2846 A>T, c.1236 G>A, c.2194 G>A, c.85 T > C, c.496 A>G) was evaluated using the LDheatmap package in R (version 6.4). Lewontin's D' and r^2 -measure were calculated. Haplotypes and diplotypes involving

DPYD c.85 T > C, c.496 A>G, and c.2194 G>A were inferred using PHASE version 2.1.1 using the most likely haplotype ("best" reconstruction) in the analysis.

In single-variant analyses, each of the three investigated variants was evaluated separately, without conditioning on the genotypes at the other two loci. In haplotype analyses, the phased allelic combinations across c.85 T > C, c.496 A>G, and c.2194 G>A were evaluated.

Categorical variables were summarized as counts and percentages, and continuous variables as medians with interquartile ranges (Q₁-Q₃). Odds ratios (ORs) and 95% confidence intervals (CIs) for toxicity were estimated using unconditional logistic regression models adjusted for gender, age, FL type (5-FU or capecitabine), and treatment regimen (monotherapy or combination therapy). These covariates were selected a priori among the variables available in both cohorts, based on previous evidence identifying them as relevant patient- and treatment-related determinants of fluoropyrimidine toxicity [27]. Given the targeted, hypothesis-driven design of the study and the limited number of pre-specified candidate variants, no adjustment for multiple testing was applied. For haplotype analyses, additive genetic models were applied.

Differences in U and UH2 mean concentrations across diplotypes were measured through Cohen's *f* as effect size and tested using analysis of variance (ANOVA) with Tukey's *post hoc* test. Associations between diplotypes and categorical U or UH2/U levels were evaluated with Cramer's *V* and tested using Fisher's exact test. Effect size was considered weak if ≤ 0.2 , moderate when > 0.2 and ≤ 0.6 , or strong when > 0.6 .

A quantitative estimate of toxicity risk was obtained through meta-analysis by extracting ORs and 95% CIs from published eligible studies, preferentially using adjusted estimates when available. When ORs were not reported, they were calculated from genotype-specific toxicity counts, and the SE of log(OR) was derived from CIs or contingency tables. Analyses were restricted to the dominant model, as it was most frequently reported. Pooled ORs and 95% CIs were estimated using DerSimonian–Laird random-effects models [28], weighting studies by the inverse variance of log(OR). Heterogeneity was assessed with *I*² [28], robustness was evaluated via leave-one-out influence analysis, and publication bias was explored using funnel plots [29]. When ≥ 2 studies were available for both cohort and case–control designs, pooled estimates were stratified by study design. Results were presented in forest

plots (squares proportional to study weight; diamonds for pooled effects). The meta-analysis evaluated single-variant associations only and did not include haplotype-based estimates.

Finally, a Receiver Operating Characteristic (ROC) curve analysis was conducted in the whole PREPARE cohort to evaluate the improvement in patients risk stratification due to the implementation of *DPYD* c.496 A>G, *DPYD* c.85 T > C, and *DPYD* c.2194 G>A testing. The discrimination ability of a baseline model including gender, age, FL type (5-FU or capecitabine), treatment regimen (monotherapy or combination therapy), *DPYD**2 A, *DPYD**13, *DPYD* c.2846 A>T and *DPYD* c.1236 G>A (Model 1) was compared with three models that incrementally included *DPYD* c.496 A>G (Model 2), *DPYD* c.85 T > C (Model 3), and *DPYD* c.2194 G>A (Model 4). Differences in the area under the curve (AUCs) of Models 2–4 versus Model 1 were evaluated through DeLong test.

Analyses were conducted in R (version 4.6.1) or SAS, with two-sided $p < 0.05$ considered statistically significant.

3. Results

3.1. Patient characteristics

3.1.1. PREPARE study cohort

Between March 2017 and June 2020, a total of 1232 patients were enrolled in the PREPARE study in Italy under the coordination of CRO-Aviano IRCCS. Of these, 727 patients (59.0%) fulfilled all eligibility criteria for the present analysis (Fig. 1). Baseline sociodemographic and clinical characteristics are summarized in Table 1.

The study population was predominantly of self-reported European ancestry (717 patients, 98.6%). The remaining patients were of African ($n = 2$), Hispanic ($n = 4$), West Asian ($n = 3$) origin, or did not report ethnicity ($n = 1$). Thirty-three patients carried at least one decreased-function *DPYD* variant allele among *DPYD**2 A, c.2846 A>T, or c.1236 G>A (tagging *DPYD*-HapB3), whereas no carriers of *DPYD**13 were identified (Table 1).

All 727 patients were successfully genotyped for *DPYD* c.2194 G>A, c.496 A>G and c.85 T > C. No deviation from Hardy–Weinberg equilibrium was detected for any of the three variants ($P > 0.05$). Minor allele frequencies for these variants are reported in Supplementary

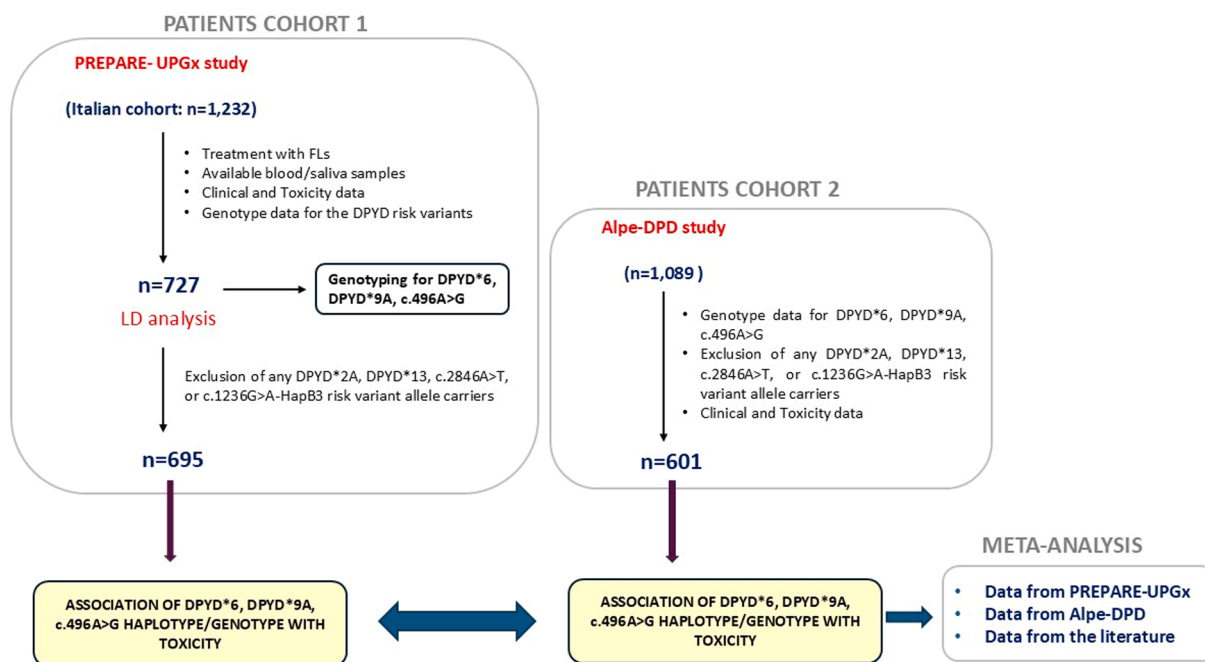


Fig. 1. Flowchart of the study strategy. Abbreviations: FLs, fluoropyrimidines, LD, linkage disequilibrium.

Table 1

Sociodemographic and clinical characteristics of eligible cancer patients from PREPARE study (n = 727) and Alpe-DPD study (n = 1089).

Characteristics	PREPARE study (n = 727)		Alpe-DPD study (n = 1089)	
	n	(%)	n	(%)
Gender				
Female	340	(46.8)	507	(46.0)
Male	387	(53.2)	582	(54.0)
Age (years)				
Median (Q1–Q3)	65	(57–73)	64	(56–71)
Cancer site				
Colorectum/anus	373	(51.3)	690	(63.4)
Head and neck	103	(14.2)	4	(0.4)
Esophagus/stomach	99	(13.6)	105	(9.6)
Breast	60	(8.3)	134	(12.3)
Pancreas	53	(7.3)	47	(4.3)
Other (including multiple sites)	39	(5.4)	104	(9.6)
Unknown	-	-	5	(0.5)
Fluoropyrimidine type				
Fluorouracil	379	(52.1)	172	(15.8)
Capecitabine	348	(47.9)	857	(78.7)
Capecitabine / Fluorouracil	-	-	2	(0.2)
Unknown	-	-	58	(5.3)
Regimen				
Monotherapy	184	(25.3)	164	(15.1)
Combination therapy ^a	543	(74.7)	867	(79.6)
Unknown	-	-	58	(5.3)
DPYD*2 A				
GG	717	(98.6)	1068	(98.1)
GA	10	(1.4)	15	(1.4)
Unknown	-	-	6	(0.6)
DPYD*13				
TT	727	(100)	1082	(99.4)
TA	0	0	1	(0.1)
Unknown	-	-	6	(0.6)
DPYD c.2846 A>T				
AA	723	(99.4)	1066	(97.9)
AT	4	(0.6)	17	(1.6)
Unknown	-	-	6	(0.6)
DPYD HapB3 (c.1236 G>A)				
GG	708	(97.4)	1036	(95.1)
GA	19	(2.6)	47	(4.3)
Unknown	-	-	6	(0.6)

^a Fluorouracil /Capecitabine in association with platinum, irinotecan, and/or biological agents.

Table S1 and were consistent with those observed in reference European populations (1000 Genomes Project and gnomAD genomes). The full cohort was included in LD analyses, while 695 patients were eligible for genotype–toxicity association analyses after exclusion of carriers of DPYD*2 A, c.2846 A>T, or c.1236 G>A (Fig. 1).

A detailed overview of treatment-related toxicities observed during FL therapy in the PREPARE Italian cohort is provided in Supplementary Figure S1.

3.1.2. Alpe-DPD study cohort

Among the 1181 patients prospectively genotyped in the Alpe-DPD study, 1089 had genotype data available for at least one of the three DPYD variants (c.2194 G>A, c.496 A>G, c.85 T > C). Genotype frequencies were comparable to those observed in the PREPARE cohort (Supplementary Table S1). No deviation from Hardy–Weinberg equilibrium was detected for any of the three variants ($P > 0.05$). Baseline clinical and sociodemographic characteristics of the Alpe-DPD patients are reported in Table 1.

After exclusion of patients carrying decreased-function DPYD variants used for dose adjustment and those without complete data, a total of 601 patients were included in the final analyses (Fig. 1).

3.2. Association between single DPYD variants and toxicity

3.2.1. PREPARE cohort

The association between DPYD c.2194 G>A, c.496 A>G, and c.85 T > C and genotypes and the risk of developing FL-related toxicity was evaluated in 695 eligible PREPARE patients (Supplementary Table S2). Homozygosity for the c.496 A>G variant was associated with a significantly increased risk of clinically relevant toxicity (OR=7.14, 95% CI: 1.48–34.33) and non-hematological toxicity (OR=10.38, 95% CI: 2.20–49.01). No statistically significant associations were observed for c.2194 G>A and c.85 T > C as single variants in this cohort.

3.2.2. Alpe-DPD cohort

The predictive role of the three DPYD variants was subsequently assessed in the Alpe-DPD validation cohort (Supplementary Table S2). In the Alpe-DPD cohort, heterozygosity for c.496 A>G was associated with an increased risk of clinically relevant FL-related toxicity compared with the reference genotype (OR=1.62, 95% CI: 1.02–2.59), whereas no significant association was observed among homozygous variant carriers. No statistically significant associations were observed for c.2194 G>A and c.85 T > C when analysed individually.

3.3. Systematic review and meta-analysis

3.3.1. Study selection and characteristics

A total of 203 records were identified through database searches and manual screening of references. After title and abstract screening, 45 articles were excluded. Full-text evaluation of the remaining studies led to the exclusion of 127 articles based on predefined criteria. Ultimately, 31 studies were deemed eligible for inclusion in the meta-analysis (Fig. 2).

Among these, DPYD c.2194 G>A was evaluated in 17 studies [30–46], c.496 A>G in 19 studies [22,31,33–36,38–40,44,46–53], and c.85 T > C in 22 studies [22,33,34,36,38–41,44,45,47–49,52–59]. Study characteristics, including patient populations, treatment regimens, toxicity definitions, genetic markers assessed, and genotyping methods, are summarized in Supplementary Table S3. Twenty-three studies were conducted in European or North American populations, and most were retrospective in design. FLs (5-FU or capecitabine), often in combination with other chemotherapeutic or targeted agents, were predominantly used for gastrointestinal and breast cancers. Severe toxicity was generally defined as grade ≥ 3 and assessed cumulatively throughout treatment.

One study [54] did not meet the predefined quality threshold (Newcastle–Ottawa Scale score <7) and was excluded. The remaining studies were of high methodological quality, with NOS scores ranging from 7 to 9 (Supplementary Table S4).

Sufficient data (i.e., minimum of three legible studies) were available to perform quantitative meta-analyses for overall toxicity (i.e., any type of grade ≥ 2 toxicity), neutropenia, and diarrhea. Analyses for aggregated hematological or non-hematological toxicity could not be performed due to insufficient data.

3.3.2. Meta-analysis results

3.3.2.1. DPYD c.2194 G>A. Twelve studies, including PREPARE and Alpe-DPD datasets [31–34,36–38,40,42,44,60], assessed the impact of DPYD c.2194 G>A on overall toxicity. Carriage of the c.2194 A allele was significantly associated with increased toxicity risk (OR=1.70, 95% CI 1.48–1.95; dominant model; Fig. 3). Significant associations were also observed for neutropenia (OR=2.01, 95% CI 1.55–2.61) and diarrhea (OR=2.07, 95% CI 1.09–3.90) (Supplementary Figures S2 and S3). Ethnicity-stratified analyses were not feasible due to the predominance of Caucasian study populations.

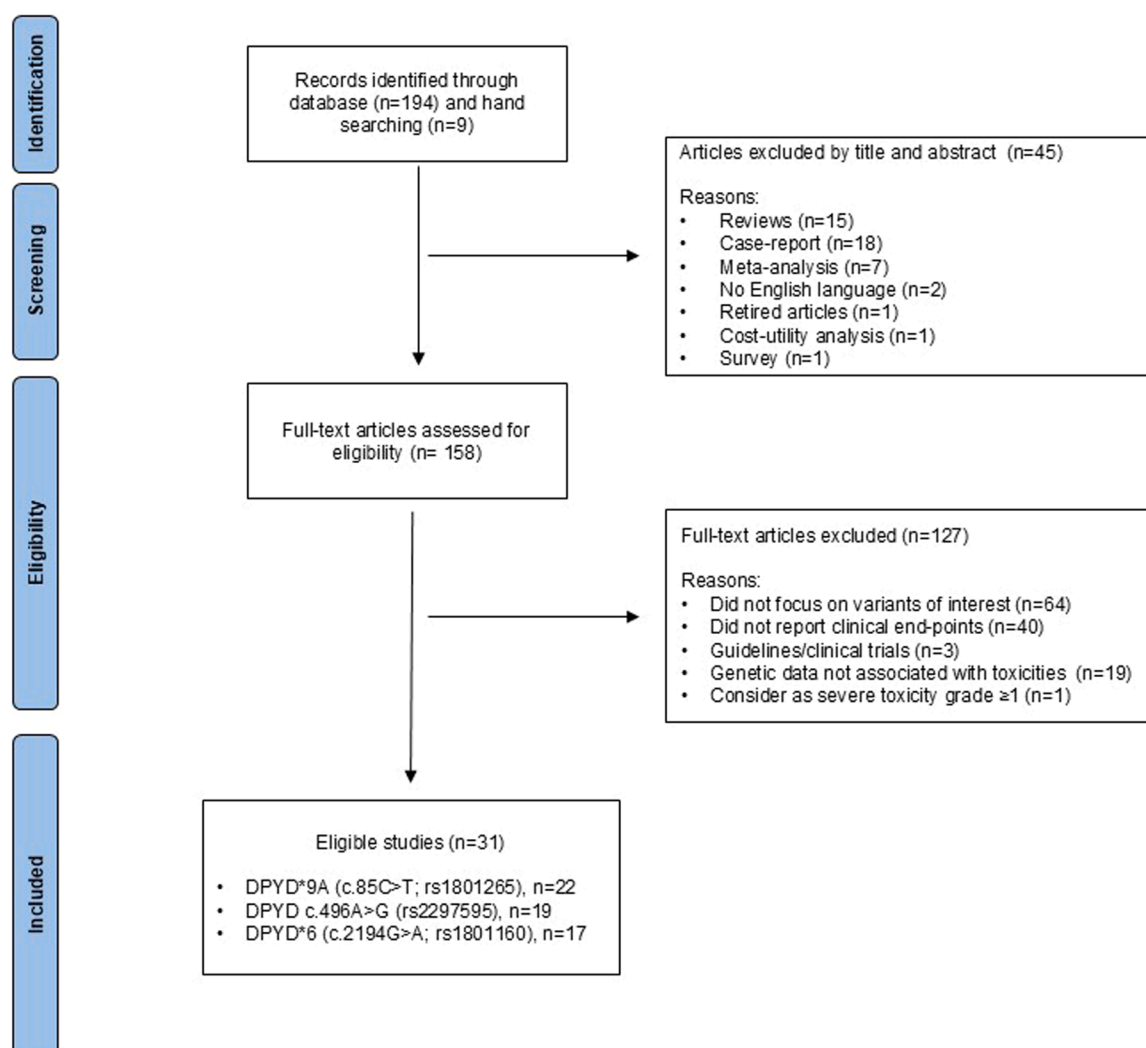


Fig. 2. PRISMA flow diagram.

3.3.2.2. DPYD c.496 A>G. Thirteen studies, including PREPARE- and Alpe-DPD-derived datasets [22,31,33,36,38–40,44,47,50,51], contributed data on the association between c.496 A>G and overall toxicity. The DPYD c.496 G allele was associated with a significantly increased risk of overall toxicity under a dominant model (OR=1.55, 95% CI: 1.19–2.02), with consistent associations in both Caucasian (OR=1.51, 95% CI: 1.14–1.99) and non-Caucasian populations (OR=2.44, 95% CI: 1.01–5.85) (Fig. 3). A deleterious, though non-significant, association was also observed for neutropenia (OR=1.08, 95% CI: 0.77–1.51) and diarrhea (OR=1.23, 95% CI: 0.82–1.85; Supplementary Figures S2 and S3). Ethnicity-stratified analyses for these endpoints were not feasible because all but one included study enrolled Caucasian patients.

3.3.2.3. DPYD c.85 T > C. Ten studies, including PREPARE and Alpe-DPD datasets [22,33,36,38–40,44,47], evaluated the association between DPYD c.85 T > C and overall toxicity. No significant association was observed in the overall analysis (OR=0.93, 95% CI 0.81–1.06; dominant model; Fig. 3). However, stratification by ethnicity revealed divergent effects. In Caucasian populations, the c.85 C allele showed no association with toxicity, with a trend towards a protective effect (OR=0.89, 95% CI 0.78–1.01). In contrast, among non-Caucasian populations, the variant was associated with increased toxicity risk (OR=1.77, 95% CI 1.06–2.95; Fig. 3). Similar patterns were observed for neutropenia and diarrhea (Supplementary Figures S2 and S3).

3.3.3. Sensitivity analyses and publication bias

Publication bias for the main overall-toxicity analyses was evaluated by funnel-plot inspection, with no evidence of bias in any analysis (Supplementary Figure S4). Robustness was assessed via leave-one-out influence analyses (repeating the meta-analysis while excluding one study at a time), yielding consistent pooled estimates: DPYD c.2194 G>A ORs 1.63 (excluding Ruzzo et al. [44]) to 1.74 (excluding the Alpe-DPD or the PREPARE study); DPYD c.496 A>G ORs 1.36 (excluding the Alpe-DPD trial) to 1.63 (excluding Madi et al. [40]); and DPYD c.85 T > C ORs 0.90 (excluding Ruzzo et al. [44]) to 0.97 (excluding Madi et al. [40]).

3.4. Haplotype analysis and toxicity risk

LD analysis was conducted in the 727 PREPARE patients. The LD heatmap, reporting pairwise D' and R^2 values, is shown in Supplementary Figure S5. In this cohort, DPYD c.1236 G>A showed high D' with DPYD c.2194 G>A, c.85 T > C, and c.496 A>G (D' =0.837–0.942), although R^2 values were low (R^2 =0.0006–0.390), likely reflecting the low minor allele frequencies. LD among DPYD c.2194 G>A, c.85 T > C, and c.496 A>G showed D' values ranging from 0.247 to 0.766 and R^2 values from 0.0008 to 0.2336; the strongest correlation was observed between DPYD c.496 A>G and DPYD c.85 T > C (R^2 =0.2336; D' =0.766).

Based on DPYD c.2194 G>A, c.85 T > C, and c.496 A>G, seven

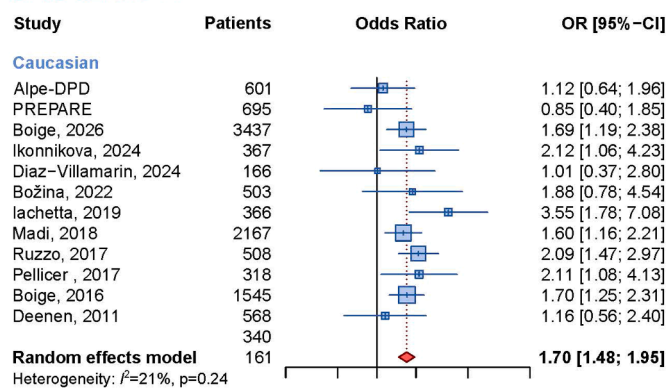
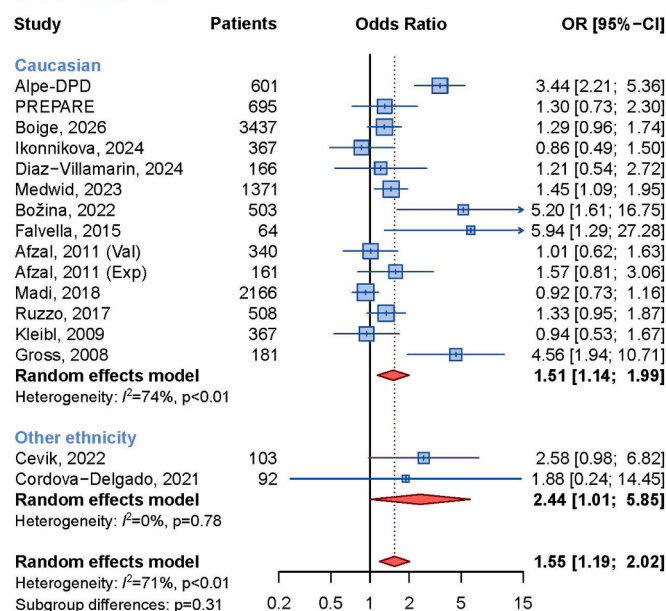
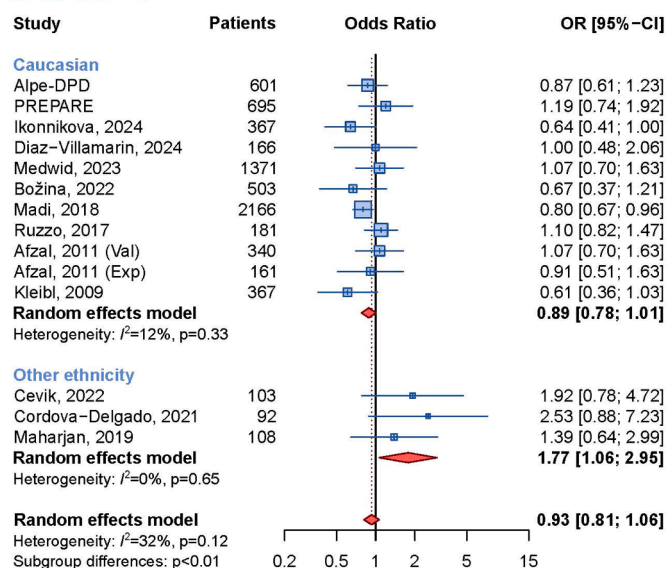
DPYD c.2194G>A**DPYD c.496A>G****DPYD c.85T>C**

Fig. 3. Forest plot for the risk of overall toxicity for *DPYD* c.2194 G>A, *DPYD* c.496 A>G, and *DPYD* c.85 T > C polymorphisms according to dominant genetic model.

haplotypes were inferred and their associations with toxicity were evaluated (Table 2). Haplotype frequencies were similar across the PREPARE and Alpe-DPD cohort. Haplotype #3—carrying the major alleles of *DPYD* c.85 T > C (c.85 T) and *DPYD* c.2194 G>A (c.2194 G) together with the minor allele of *DPYD* c.496 A>G (c.496 G)—was significantly associated with an increased risk of clinically relevant toxicity (OR=2.62, 95% CI: 1.06–6.50) and gastrointestinal toxicity (OR=5.65, 95% CI: 2.11–15.15) under an additive model. By contrast, haplotype #4—combining the minor allele of *DPYD* c.496 A>G (c.496 G) with the minor allele of *DPYD* c.85 T > C (c.85 C) - was not associated with clinically relevant toxicity or gastrointestinal toxicity. The presence of either *DPYD* c.2194 G>A allele within the haplotypes did not appear to modify these effects.

The significant associations observed in the PREPARE cohort were replicated in the Alpe-DPD cohort. In the Alpe-DPD group, haplotype #3 was significantly associated with an increased risk of clinically relevant (OR=2.09, 95% CI: 1.01–4.37) and grade ≥ 3 gastrointestinal toxicity (OR=2.71, 95% CI: 1.23–5.97) under an additive model (Table 2).

3.5. Uracil and dihydrouracil plasma concentrations

To explore the phenotypic impact of the c.496 A>G and c.85 T > C

combinations suggested by the haplotype analysis, a targeted exploratory U/UH2 analysis was performed in selected PREPARE patients with available pretreatment plasma sample and carriers of a diplotype composed of haplotypes #1–#4. These haplotypes shared the reference c.2194 G allele and differed in their c.496 A>G and c.85 T > C configurations.

Pre-treatment plasma U and UH2 levels were measured in a subgroup of 204 PREPARE patients to assess the impact of the *DPYD* haplotypes (#2, #3, and #4) on the DPD phenotype (Fig. 4). Large differences (Cohen's $f=2.620$) in U concentrations emerged according to *DPYD* diplotype ($p=0.018$), with diplotype 1.3/1.3 showing the highest median level (16.0 ng/mL). The proportion of patients with U > 16 ng/mL was also higher in the 1.3/1.3 group (53.9%) than in the other diplotypes (Cramer's $V=0.229$; $p=0.019$). Consistently, the UH2/U ratio was lower in patients with diplotype 1.3/1.3 (median: 5.8 in 1.3/1.3 vs 8.8, 8.1, and 7.0 in 1.1, 1.2/2.2, and 1.4/4.4, respectively) with large effect size (Cohen's $f=0.800$, $p<0.001$), and the proportion of patients with UH2/U < 6 was highest in the 1.3/1.3 group (53.9%; Cramer's $V=0.235$, $p=0.016$).

Table 2
Haplotype combination between *DPYD* c.496 A>G, *DPYD**9 A (c.85 T > C) and *DPYD**6 (c.2194 G>A) and corresponding risk of severe toxicity in PREPARE (n = 695) and Alpe-DPD (n = 601) cohort. Associations with *P*-value < 0.05 are in bold.

Hap	DPYD*6, c.2194 G>A	DPYD c.496 A>G	DPYD*9 A, c.85 T > C	PREPARE cohort			APLE-DPD cohort					
				Allele	Frequency	Clinically relevant toxicity ^a	Gastro- intestinal G3-G5	Neutropenia G4-G5	Allele	Frequency	Clinically relevant toxicity ^a	Gastro- intestinal G3-G5
N°	Allele	Allele	Allele	%	OR (95% CI) ^b				%	OR (95% CI) ^b		
#1	G	A	T	74.3	0.87 (0.60–1.25)	0.79 (0.49–1.30)	1.02 (0.58–1.79)		71.9	0.99 (0.75–1.30)	0.96 (0.66–1.39)	0.58 (0.22–1.52)
#2	G	A	C	10.9	0.98 (0.57–1.69)	0.52 (0.20–1.32)	1.87 (0.92–3.80)		14.8	0.78 (0.54–1.11)	0.79 (0.48–1.33)	2.00 (0.60–6.67)
#3	G	G	T	1.6	2.62 (1.06–6.50)	5.65 (2.11–15.15)	0.59 (0.08–4.32)		2.3	2.09 (1.01–4.37)	2.71 (1.23–5.97)	1.17 (0.12–11.78)
#4	G	G	C	7.4	1.15 (0.62–2.11)	1.24 (0.54–2.81)	0.47 (0.14–1.60)		6.1	1.12 (0.68–1.85)	1.21 (0.64–2.30)	1.11 (0.22–5.53)
#5	A	A	T	5.6	0.95 (0.45–1.99)	1.42 (0.59–3.39)	0.68 (0.20–2.36)		4.0	1.33 (0.71–2.48)	0.96 (0.41–2.24)	1.88 (0.21–16.98)
#6	A	A	C	0.1	---	---	---		0.1	---	---	---
#7	A	G	C	0.1	---	---	---		0.8	0.80 (0.19–3.38)	---	---

Abbreviations: Hap, haplotype
^a Clinically relevant toxicity was defined as grade ≥ 3 for non-hematological adverse events or grade ≥ 4 for hematological adverse events.
^b Odds ratio (OR) and corresponding 95% confidence intervals (CI) were estimated for additive model through multivariable logistic regression model, including term for gender, age, fluoropyrimidine type, and regimen.

3.6. *DPYD* variants in patients' risk stratification

The implementation of *DPYD* variants, c.496 A>G, c.85 T > C, and c.2194 G>A, for patients' risk stratification was evaluated through a ROC analysis. For hematological toxicity (Fig. 5), Model 1 – including *DPYD**2 A, *DPYD**13, *DPYD* c.2846 A>T and *DPYD* c.1236 G>A – reported an AUC= 0.779, which was not substantially improved with the inclusion of the other three *DPYD* variants (Models 2–4). Conversely, for the non-hematological toxicity, the discriminatory ability of Model 1 (AUC=0.604) was significantly improved with the inclusion of *DPYD* c.496 A>G and *DPYD* c.85 T > C (Model 3, AUC=0.660, *p* = 0.026), exceeding the clinically relevant AUC limit of 0.65. For the clinically relevant toxicity, the inclusion of the *DPYD* c.496 A>G, *DPYD* c.85 T > C and *DPYD* c.2194 G>A (Model 4) increased the AUC of Model 1 from 0.685 to 0.713, though the difference was not statistically significant (*p* = 0.051).

4. Discussion

Several well-characterized *DPYD* variants, including *DPYD* c.2194 G>A, c.496 A>G, and c.85 T > C, are currently excluded from recommended FL dose-adjustment genotyping panels because of the absence of consistent and robust clinical validation of their predictive value for toxicity [18]. Although functional and clinical data have accumulated over the years, the effect sizes reported for these variants when analyzed individually have often been modest or inconsistent, limiting their clinical translation. Recent studies have suggested that analyzing haplotype combinations—particularly those involving *DPYD* c.496 A>G and c.85 T > C—may provide a more informative approach than evaluating single variants alone [20–22,33]; however, evidence to support this strategy has remained limited and fragmented.

In this context, the present study provides comprehensive and consistent evidence from two large prospective pharmacogenetic trials, PREPARE and Alpe-DPD, complemented by a systematic review and meta-analysis, supporting a clinically relevant role for *DPYD* c.496 A>G and c.2194 G>A, in predicting FL-related toxicity, as single variants. Importantly, our findings further demonstrate that the predictive effect of *DPYD* c.496 A>G is modified by c.85 T > C when the two variants are considered in combination, identifying a specific haplotype associated with increased toxicity risk. To our knowledge, this is the first study to robustly validate this haplotype-based approach across independent prospective cohorts and to link it with a functional DPD phenotype. By contrast, adding *DPYD* c.2194 G>A to the c.496 A>G–c.85 T > C haplotype did not improve toxicity prediction; however, the low number of carriers of haplotypes containing the *DPYD* c.2194 G>A minor allele (c.2194 A) precludes definitive conclusions regarding its contribution. The main single-variant and haplotype associations across cohorts and toxicity outcomes are summarized in Supplementary Table S5. Because carriers of the four established actionable *DPYD* variants were excluded, the associations observed in the present study specifically reflect residual toxicity risk among patients negative for these variants, in line with the objective of identifying additional predictive markers beyond current guideline-based genotyping. However, this design precluded the evaluation of compound heterozygous genotypes involving the four established actionable *DPYD* variants and limits the generalizability of the risk estimates to unselected FL-treated populations. Further validation in real-world cohorts is therefore warranted.

When evaluated individually in single-variant analyses, the present data showed that *DPYD* c.496 A>G and c.2194 G>A are both associated with an increased risk of FL-related toxicity. Despite being currently classified as a “normal function” allele by CPIC, based largely on in vitro enzyme activity assays [61,62], accumulating in vivo phenotyping data suggest that *DPYD* c.496 A>G and c.2194 G>A may contribute to a clinically meaningful reduction in DPD function in a subset of patients [20,21].

For *DPYD* c.496 A>G, an effect was observed across the PREPARE

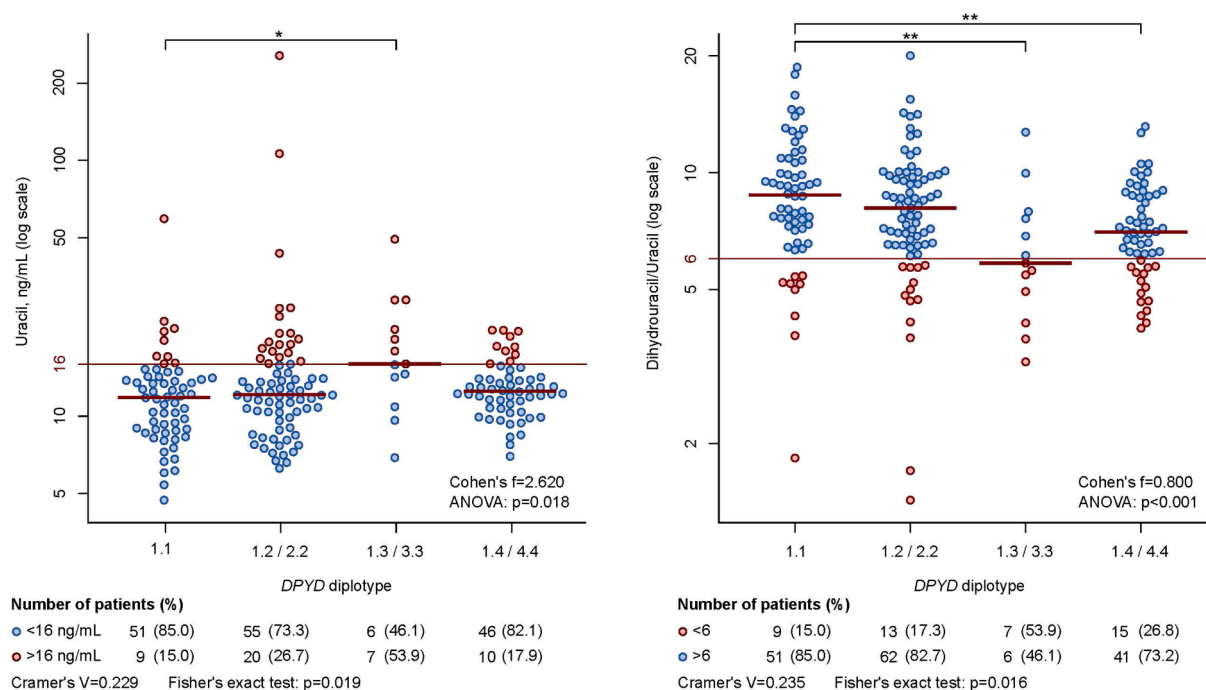


Fig. 4. Distribution of uracil concentration and dihydrouracil/uracil ratio according to *DPYD* haplotype. Asterisks indicate the level of significance according to *post-hoc* Tukey test (*: $p < 0.05$; **: $p < 0.01$).

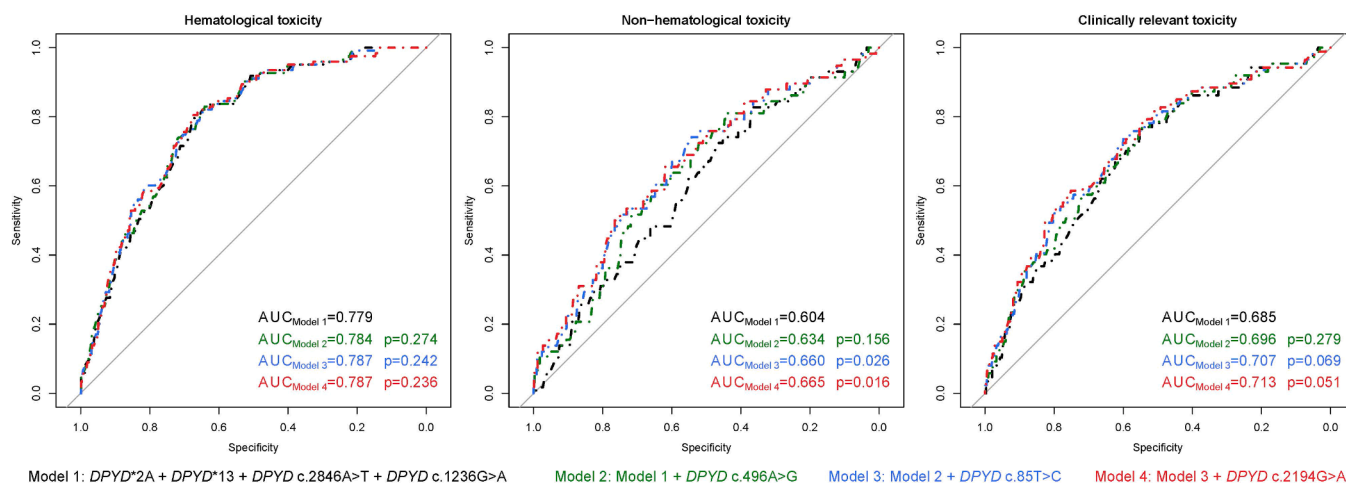


Fig. 5. Receiver Operating Characteristic (ROC) curve for patients' risk stratification using *DPYD* c.496 A>G, *DPYD* c.85 T > C, and *DPYD* c.2194 G>A. Areas Under the Curves (AUCs) were compared through DeLong test.

and Alpe-DPD cohorts and confirmed in the meta-analysis, where carriers of the variant allele showed a significantly higher risk of overall toxicity. Interestingly, the association appeared to be driven primarily by non-hematological toxicity, particularly gastrointestinal adverse events, whereas no statistically significant association was detected for grade 4–5 hematological toxicity, although the lower number of qualifying hematological events may have limited statistical power. Although this distinction could not be formally explored in the meta-analysis due to limited reporting of toxicity subtypes in published studies, it generates the hypothesis that c.496 A>G may exert tissue-specific effects, potentially at the level of the intestinal epithelium, where local DPD function is critical for FL catabolism.

Similarly, in the meta-analysis, *DPYD* c.2194 G>A was associated with an increased risk of overall toxicity, as well as neutropenia and diarrhea, although these associations did not reach statistical

significance in either PREPARE or Alpe-DPD when analyzed separately. These pooled findings are consistent with a recent meta-analysis reporting an approximately two-fold increased risk of FL-related adverse events in carriers of the c.2194 A allele, particularly among European populations [63]. These clinical associations are supported by functional data showing a reduction in 5-fluorouracil degradation rates associated with the *DPYD* c.2194 G>A variant in a dose-dependent manner [20].

A key finding of the present study is the demonstration that the association of c.496 G with toxicity differed according to its haplotypic configuration with c.85 T > C. Specifically, the haplotype combining the minor c.496 G allele with the major allele of c.85 T > C (c.85 T; haplotype #3) was consistently associated with an increased risk of clinically relevant and grade ≥ 3 gastrointestinal toxicity in both the PREPARE and Alpe-DPD cohorts. In contrast, the combination of

c.496 G with the c.85 T > C minor allele (c.85 C; haplotype #4) was not associated with increased toxicity risk, despite the presence of the same c.496 G variant. This observation strongly suggests a functional interaction between these two variants, resulting in differential effects on DPD function and clinical phenotype.

The improvement in the toxicity risk stratification, resulting by the addition of c.2194 G>A, c.496 A>G, and c.85 T > C variants to the standard *DPYD* 4-variant panel (Model 1) was also supported by an exploratory ROC analysis in PREPARE. This analysis showed the clearest incremental improvement in the model AUC when c.85 T > C (Model 3) was added to c.496 A>G (Model 2), particularly for non-hematological and, to a lesser extent, clinically relevant toxicity. The inclusion of c.2194 G>A in the model (Model 4) resulted in a slight increase in predictive power, although the limited number of individuals carrying c.2194 A may have reduced the ability to detect its incremental contribution.

This interpretation is supported by our phenotyping analysis in a subgroup of PREPARE patients, where only carriers of haplotype #3 showed a clinically meaningful reduction in DPD function, as reflected by increased plasma U concentrations and reduced UH2/U ratios. No such effect was observed for other haplotype combinations, including haplotype #4. These findings align with previous phenotyping studies reporting opposing effects of c.496 A>G and c.85 T > C on DPD function, measured by UH2/U ratios in patient plasma [21,39]. Such counteracting effects may partly explain the inconsistent and often conflicting results reported in the literature when these variants are analyzed individually, without accounting for their combined occurrence. These phenotypic findings should nevertheless be regarded as exploratory because of the limited sample size and the lack of independent validation in the Alpe-DPD cohort.

Taken together, these data challenge the current paradigm of *DPYD* genotyping, which is largely based on the independent effect of single variants. Our results highlight that, for relatively common variants such as c.496 A>G and c.85 T > C — which frequently co-occur in the same individual—haplotype-based analyses may provide a more accurate estimation of DPD function and toxicity risk. This concept is particularly relevant given that impaired DPD function is increasingly recognized as a polygenic and quantitative trait rather than a strictly monoallelic condition driven by a limited number of high-impact variants [19, 64–68]. The meta-analysis component of this study further allowed exploration of possible ethnicity-specific effects, particularly for *DPYD* c.85 T > C. While this polymorphism was not associated with overall toxicity risk in the pooled analysis, stratification by ethnicity revealed divergent effects. In studies including predominantly Caucasian populations, the c.85 C allele showed no association with toxicity and even a trend towards a protective effect. In contrast, among populations classified as “Other ethnicity”, the c.85 C was associated with an increased risk of toxicity. Although intriguing, this finding should be interpreted cautiously, and mainly confirms the absence of a significant predictive effect in studies including homogeneous Caucasian populations. On the other hand, the “Other ethnicity” subgroup included a limited number of cohorts with markedly different ancestral backgrounds, including admixed and multiethnic populations. This diversity may have influenced the magnitude and direction of the pooled estimate and limits its generalizability to any specific non-Caucasian population. In this context, the literature data report substantial differences in allele frequencies across ethnic groups, with *DPYD* c.85 T > C being more prevalent in individuals of African ancestry [19,69] and suggest that population-specific linkage disequilibrium patterns with other functional variants may influence the observed phenotype. Additionally, emerging evidence points to potential epigenetic mechanisms, including miRNA-mediated regulation, that may modulate the functional impact of *DPYD* variants in an ethnicity-dependent manner, further underscoring the complexity of DPD regulation [70]. Although not conclusive, our findings support further investigation of a possible population-specific effect of *DPYD* c.85 T > C in larger and more clearly

characterized ancestry groups.

Recent advances in sequencing technologies have shown that comprehensive interrogation of the entire *DPYD* gene can uncover rare and novel variants with clinically relevant effects on DPD function [64–67]. These findings reinforce the notion that impaired DPD function arises from the combined influence of multiple genetic variants, each contributing incrementally to enzyme function. In this context, long-read sequencing approaches may represent an ideal strategy to capture both common and rare variants and to resolve their phasing at the individual level, enabling a more precise assessment of combined genetic effects on DPD function and toxicity risk [19].

Although the present findings support further evaluation of *DPYD* c.2194 G>A, c.496 A>G, and c.85 T > C as components of expanded pharmacogenetic risk stratification, their broad clinical implementation requires additional evidence. Despite the support provided by the meta-analysis, allele frequencies, haplotype structures, and linkage disequilibrium patterns may differ across ancestral populations; consequently, the clinical performance and incremental value of an expanded testing panel may not be directly generalizable from the predominantly European PREPARE and Alpe-DPD cohorts to genetically diverse or admixed populations. Validation in well-characterized populations with different ancestral backgrounds, together with prospective implementation studies assessing clinical utility, analytical standardization, and cost-effectiveness, will be required before these variants can be broadly incorporated into routine pretreatment *DPYD* testing. Moreover, other *DPYD* genetic variants reported to be linked to those investigated here, such as the recently functionally characterized regulatory variant rs4294451 [71–73], may further complicate haplotype interpretation, especially in the context of different ethnic backgrounds and warrant further investigation.

This study has several limitations. Although PREPARE and Alpe-DPD are large, prospective, multicentre trials, the majority of enrolled patients were of European descent. Consequently, the predictive value of the identified haplotype combinations requires validation in more ethnically diverse populations. The meta-analysis was subject to substantial heterogeneity across studies, reflecting differences in study design, treatment regimens, toxicity definitions, follow-up duration, and genotyping methods. This heterogeneity was particularly relevant to toxicity assessment, as the included studies used sometimes inconsistent definitions and grading thresholds. Toxicity data were also not fully harmonized between PREPARE and Alpe-DPD because they were collected according to their respective original study protocols. Random-effects models cannot fully account for this clinical heterogeneity, which may have reduced the comparability of the study-specific estimates and contributed to between-study variability. The meta-analysis was also largely restricted to a dominant genetic model, thereby combining heterozygous and homozygous variant carriers. This approach maximized comparability across studies but precluded the assessment of allele-dose, additive, recessive, or genotype-specific effects and may have obscured associations that do not follow a dominant pattern. Nevertheless, several measures were taken to mitigate potential bias in the meta-analysis: uniform eligibility criteria were applied, study quality was assessed using the Newcastle–Ottawa Scale, and the robustness of the pooled estimates was examined through influence analyses. Another limitation of the meta-analysis is that European and North American populations were pooled as “Caucasian” in ethnicity-stratified analyses, potentially masking finer genetic differences between these groups. For *DPYD* c.2194 G>A, data from non-Caucasian populations remain scarce, limiting generalizability. Regarding the haplotype reconstruction, although only the most likely haplotype was used for ambiguous calls in the analysis, because of the high LD between the polymorphisms, the underestimation of standard errors is likely to be small. Finally, considering that the study was targeted and hypothesis-driven, the tests for genetic, toxicity-related, and subgroup comparisons were not controlled for family-wise error rate. Therefore, false-positive findings cannot be excluded, particularly for marginal

associations or findings not consistently replicated across datasets, which should be interpreted cautiously and require further validation.

In conclusion, this study provides robust evidence that DPYD c.496 A>G and c.2194 G>A when evaluated individually in single-variant analyses, are significantly associated with an increased risk of FL-related toxicity, supporting their potential clinical relevance in refining genotype-guided dosing strategies. Importantly, the present results demonstrate that haplotype-based analysis of DPYD c.496 A>G and c.85 T>C further improves toxicity risk stratification, with consistent validation across two large prospective cohorts and functional confirmation at the DPD phenotype level. These findings argue for a shift from single-variant to haplotype-informed DPYD genotyping approaches and highlight the need for further studies in diverse populations to optimize personalized FL therapy.

Ethics declaration

Written informed consent to take part in the study and to publish the article has been obtained from all participants or their legal representatives. The privacy rights of participants have been observed.

This study included organ or tissue donors. This study includes human biological material and consent was obtained by donors, or their next of kin or legal representatives, for use in this study and for publication of the article. The samples used in this research were not sourced from executed prisoners or prisoners of conscience.

This study was performed in compliance with relevant laws, regulatory frameworks and guidelines where the research took place. This study was approved by the CEUR – Comitato Etico Unico Regionale. (Approval No. CRO-2016–51)

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CRediT authorship contribution statement

Elena De Mattia: Writing – original draft, Visualization, Conceptualization. **Jerry Polesel:** Visualization, Methodology, Formal analysis. **Maaike van der Lee:** Validation, Investigation. **Stefan Bohringer:** Formal analysis. **Martina Gambron:** Investigation. **Samantha Perfler:** Investigation. **Rossana Roncato:** Investigation. **Giovanni Canil:** Investigation. **Bianca Posocco:** Investigation. **Annemieke Cats:** Resources. **Ron H.J. Mathijssen:** Resources. **Henk-Jan Guchelaar:** Resources. **Jan H.M. Schellens:** Resources. **Hans Gelderblom:** Resources. **Michele Spina:** Resources. **Fabio Puglisi:** Resources. **Jesse J. Swen:** Writing – review & editing, Funding acquisition, Conceptualization. **Erika Cecchin:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

Declaration of Competing Interest

JHMS is a patent holder for oral taxanes, holds shares in Modra Pharmaceuticals B.V., and is a shareholder and patent holder as well as an employee of Adcytherix SAS. All other authors declare that they have no competing interests.

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Not applicable

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.phrs.2026.108451.

Data availability

Data will be made available on request.

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