# Dihydropyrimidine dehydrogenase gene variants for predicting grade 4-5 fluoropyrimidine-induced toxicity: FUSAFE individual patient data meta-analysis

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**Key words**:

- dihydropyrimidine dehydrogenase,
- *DPYD*,
- fluoropyrimidine,
- toxicity
- pharmacogenetics,
- meta-analysis.

Abstract word count: 200

Total word count (without Abstract or References): 4062

Number of references: 52

Number of tables: 2

Number of figures: 4

Number of supplemental materials: 3 (Appendix, Sup Figures, Sup Tables)

## Abstract

**Background**: Dihydropyrimidine dehydrogenase (DPD) deficiency is the main known cause of life-threatening fluoropyrimidine (FP)-induced toxicities. We conducted a meta-analysis on individual patient data to assess the contribution of deleterious *DPYD* variants *2A/D949V/*13/HapB3 (recommended by EMA) and clinical factors, for predicting G4-5 toxicity. **Methods**: Study eligibility criteria included recruitment of Caucasian patients without DPD-based FP-dose adjustment. Main endpoint was 12-week haematological or digestive G4-5 toxicity. The value of *DPYD* variants *2A/p.D949V/*13 merged, HapB3, and *MIR27A* rs895819 was evaluated using multivariable logistic models (AUC). **Results:** Among 25 eligible studies, complete clinical variables and primary endpoint were available in 15 studies (8,733 patients). Twelve-week G4-5 toxicity prevalence was 7.3% (641 events). The clinical model included age, sex, body mass index, schedule of FP-administration, concomitant anticancer drugs. Adding *2A/p.D949V/*13 variants (at least one allele, prevalence 2.2%, OR 9.5 [95%CI 6.7-13.5]) significantly improved the model ($p<0.0001$). The addition of HapB3 (prevalence 4.0%, 98.6% heterozygous), in spite of significant association with toxicity (OR 1.8 [95%CI 1.2-2.7]), did not improve the model. *MIR27A* rs895819 was not associated with toxicity, irrespective of *DPYD* variants. **Conclusions**: FUSAFE meta-analysis highlights the major relevance of *DPYD* *2A/p.D949V/*13 combined with clinical variables to identify patients at risk of very severe FP-related toxicity.

## INTRODUCTION

Fluoropyrimidines (FP), 5-fluorouracil (5FU) and capecitabine, remain the most prescribed chemotherapies worldwide in patients with solid tumours. FP-based chemotherapies induce grade (G) 3-4 toxicities in 10-40%, G4 toxicities in 4-9%, and G5 (death) in 0.2-0.5% of patients [1,2]. A real-world study reported that true incidence of death, life-threatening prognosis or incapacity/disability is 1.4% during the first two cycles of FP-based chemotherapy [3]. The main identified cause of early FP-related toxicities is a deficiency of the rate-limiting enzyme of 5FU catabolism, dihydropyrimidine dehydrogenase (DPD), encoded by the *DPYD* gene [4]. In Caucasians, the estimated prevalence of partial DPD deficiency is 3-7% [5,6] and that of complete deficiency is 0.05-0.1% [5,7,8]. DPD deficiency screening is based on genotyping and/or phenotyping (plasma uracil ± dihydrouracil concentrations, or enzyme activity measured in blood mononuclear cells) [6,7,9]. As compared to phenotyping, genotyping has nearly no pre-analytical constraints, favouring implementation. Among more than 300 *DPYD* exonic variants, only a minority are known to significantly decrease enzyme activity [10] and increase severe FP-related toxicities [11]. Upfront *DPYD* testing of variants *2A (c.1905+1G>A, rs3918290), p.D949V (p.Asp949Val, c.2846A>T, rs67376798), *13 (p.Ile560Ser, c.1679T>G, rs55886062) and more common Haplotype B3 (HapB3 defined by the presence of c.1236G>A rs56038477 and/or c.1129-5923C>G rs75017182) is currently recommended by the European Medicines Agency (EMA) [12] and the *Clinical Pharmacogenetic Implementation Consortium* (CPIC) [9], along with subsequent FP dose reduction in variant carriers [13–15]. However, due to their scarcity (6-8% of Caucasians carry at least one variant), the sensitivity of this approach is low, these 4 variants explaining at best 20-30% of early FP-related severe G3-4-5 toxicities [9,16–18]. Of note, the toxicity risk associated with HapB3 is consistently smaller than those of the 3 other variants [2,18], or not significant [19,20]. EMA and CPIC

recommendations are based on 3 meta-analyses (MA) conducted in Caucasians, investigating each variant on G3-4-5 toxicities [16–18] (**Table S1**): two were based on summary data [16,17] and one on both summary and individual patient data (IPD) [18]. A more recent MA on summary data investigated the 4 *DPYD* variants on lethal toxicities [2]. No MA has specifically analysed very severe G4-5 toxicities while they may be the more relevant in the context of DPD deficiency.

Despite decades of research on *DPYD* pharmacogenetics, performance of *DPYD* testing to predict the more severe G4-5 FP-related toxicity is not documented. Sensitivity of *DPYD* testing should be improved by considering the less frequent life-threatening G4-5 toxicities, which are by far the most relevant to prevent. Moreover, toxicity prediction should be further improved by considering clinical covariates related to patients and treatments. We presented herein the largest meta-analysis on individual patient data (IPD-MA) published so far assessing the contribution of deleterious *DPYD* variants *2A/D949V/*13/HapB3, and clinical factors, for predicting very severe G4-5 toxicities. In addition (exploratory analysis), we have analyzed the impact of *MIR27A* rs895819 which has been associated with toxicity risk in patients carrying a *DPYD* risk variant [21,22]. This FUSAFE (Fluoropyrimidine safe) project was initiated by the French GPCO (Groupe de Pharmacologie Clinique Oncologique) Unicancer and RNPGx (Réseau Francophone de Pharmacogénétique).

## METHODS

### Search strategy and selection criteria

The FUSAFE IPD-MA was registered on PROSPERO CRD N°42015025021 (protocol available on https://www.gustaveroussy.fr/sites/default/files/fusafe-protocol.pdf). A literature search was performed on May 2014 (last update on March 2017) to identify studies reporting associations between *DPYD* consensual variants and FP-related toxicities since January 1990 (**Appendix 2**). Main

study inclusion criteria were: i- unbiased patient recruitment (prospective studies or retrospective cohorts) and prospective collection of toxicity data evaluated at least at cycle 1; ii- patients receiving 5FU or capecitabine irrespective of solid tumour localization, stage, administration schedule, chemotherapy regimen and treatment setting; iii- no FP-dose adjustment based on DPD status or FP pharmacokinetics; iv- available *DPYD* genotype (at least variants *2A, p.D949V, *13) and/or pre-treatment DPD phenotype; v- studies in Caucasians, or including at least 50 Caucasians with ethnicity information at patient level. Treatments with chemo-radiotherapy were excluded (**Appendix 3**).

**Data collection, quality control and risk bias evaluation**

Individual patient data were requested for each eligible study. Collected clinical data were age, sex, performance status (PS), body mass index (BMI), pre-treatment renal function, cancer type and stage, FP-naive status, FP dose, FP regimen, schedule of FP administration, concomitant anticancer drugs, and treatment setting. Collected genetic data were *DPYD* variants *2A, *13, p.D949V, HapB3 (c.1236G>A and/or c.1129-5923C>G), and *MIR27A* variants rs895819 and rs11671784. Data checking is described in **Appendix 4.**

**Clinical endpoints**

The main endpoint was G4-5 haematological (anaemia, leukopenia, neutropenia, febrile neutropenia, thrombocytopenia) or digestive (diarrhoea, nausea, vomiting, mucositis) toxicities (CTCAE or WHO criteria) during the first 12 weeks of treatment. Secondary endpoints were: i- 12-week G3-4-5 haematological or digestive toxicities; ii- 4-week G4-5 haematological or digestive toxicities and iii- 4-week G3-4-5 haematological or digestive toxicities (endpoint definitions detailed in **Table S2a**).

### Statistical analysis

We firstly developed an explanatory core logistic regression model based on clinical information only using a pragmatic approach for variable selection (M1 model, see **Appendix 5**) and accounting for clustering by study. Secondly, we added to M1 model a *DPYD*-aggregated binary variable defined by the presence of at least one allele among *2A, *13 or p.D949V variants that were merged given their very low prevalence and strong impact on toxicity (M2 model). We further added HapB3 (dominant coding, M3 model). The three models were fitted on P1, P2 and P3 populations, respectively (**Figure 1**). In addition, we added each variant alone to M1 model to assess the performance of each variant separately. Adjusted odds ratio (OR) measuring the association with the endpoints were reported with 95% confidence intervals (CIs). Between-study heterogeneity was assessed. The Area Under the Curve (AUC) and other derived statistical measures were used to compare the models' performance. Internal-external cross-validation was used for M2 model (main analysis). Pre-planned subsets (i.e. interaction with study characteristics), subgroups (i.e. interaction with patient characteristics) and sensitivity analyses were performed. Exploratory analyses were performed to assess the interaction between *DPYD* and *MIR27A* variants. Data checking and reporting followed PRISMA and STREGA guidelines. Data were analyzed using SAS 9.4 and R 4.1.1 software. Statistical analysis is detailed in **Appendix 5**.

## RESULTS

### Study, patient and genetic data description

Among the 25 eligible studies (**Appendix 6**), IPD was available for 18 studies (10,669 patients, see **Figure 1** and **Table S2b** for risk bias evaluation), leading to 9,610 patients (P0 population) after

excluding patients with chemo-radiotherapy and non-Caucasian patients. Among these 18 studies [13,17,19,21–35], 7 were clinical trials, 8 were prospective cohorts and 3 retrospective cohorts. The primary cancer was colorectal in 86% (n=8,293) of patients and breast in 7% (n=712). Twenty eight % of patients had metastatic disease (n=2713; unknown in 9% n=832). PS (WHO) was 0-1 in 76% (n=7,326) and 2-3 in 4% (n=342) of patients (unknown in 20% n=1,942) (**Table S3**). FP was 5FU in 65% of patients (n=6,234) and capecitabine in 35% (n=3,376). Concomitant anti-cancer drugs were administered in 74% of patients (n=7,059): either one cytotoxic chemotherapy (CT) or one targeted therapy (TT) in 42% (n=4,021), one CT plus one or two TT in 29% (n=2,766, including one CT plus two TT (n=287)), or two CT in 3% (n=272) of patients. Main regimens were FOLFOX (n=4,141) including FOLFOX plus TT (n=1,839) (**Tables S4a-b**). Among the 9,250 patients (15 studies) with at least one *DPYD* variant data (**Table S5**), 0.92% of patients (85/9,202) carried the*2A variant, 0.21% (18/8,435) the *13 variant and 1.13% (104/9,176) the p.D949V variant (all heterozygous). Among the 8,171 patients with HapB3 data, 3.98% (325) carried HapB3 (3.93% heterozygous, 0.05% homozygous (n=4)) (combinations described in **Table S6**). Among the 3,287 patients tested for MIR27A rs895819 [17,21,22,24,28], 54% carried rs895819 (1,437 heterozygous, 337 homozygous) (**Table S5**). Among the 15 studies [13,17,19,21–29,33–35] with complete selected clinical variables and primary endpoint (8,733 patients, P1 population), *DPYD* *2A, *13 and p.D949V genotype data were available in 12 studies [13,17,19,21–29] (7,788 patients, P2 population), and HapB3 in 9 studies [13,17,19,21–27] (6,940 patients, P3 population) (**Figure 1**, **Table 1**). The percentage of patients in the different clinical covariate strata was quite similar across P0, P1, P2 and P3 populations (**Table 2**, **Table S14**).

**Description of toxicity and univariate association with genetic variants**

In P1 population, 12-week G4-5 (primary endpoint) and G3-4-5 haematological and/or digestive toxicity rates were 7.3% (641/8,733) and 25.1% (2,192/8,733), respectively; corresponding 4-week toxicity rates were 2.3% (125/5,538) and 10.3% (569/5,538), respectively. Wide between-study variability was observed (**Figures S1-S2** for P1-P2 populations). Lethal toxicity rate was 0.54% (55/10,138) among the 15 studies with recorded toxic deaths (**Table S7**). Relationships between primary endpoint and *DPYD* and *MIR27A* variants are reported in **Table S8**.

**Toxicity modelling based on clinical variables (M1 model)**

The clinical core model for 12-week G4-5 toxicity (M1, 15 studies, P1 population n=8,733) included age (<50, 50-59, 60-69, ≥70), sex, BMI (normal, underweight, overweight and obesity), schedule of FP administration (bolus ± continuous infusion, continuous infusion alone or *per os*) and concomitant anti-cancer drugs (no concomitant CT, one CT or TT, CT and TT, two CT). Twelve-week G4-5 toxicity was significantly increased in older patients (p<.0001), women (p<.0001), underweight patients (p<0.005), with 5FU bolus-containing regimen (p=0.021) and with concomitant anticancer drugs (p<.0001) (**Table 2**)**.** Odds ratios were rather similar and stable among secondary endpoints (**Table S9**), with the exception of "two concomitant CT" showing smaller OR for 4-week G4-5 (OR= 4.5, 95%CI 1.8-11.6) and G3-4-5 toxicity (OR=4.5, 95%CI 2.6-7.8) relative to 12-week G4-5 (OR=7.4, 95%CI 3.8-14.4) and G3-4-5 (OR=7.4, 95%CI 5.1-10.9). Although age-sex interaction was significant (p=0.039), this interaction was not included in the M1 model since it did not impact model performance (post-hoc analysis, data not shown).

**Added prognostic value of combined *DPYD* *2A/*13/p.D949V variants (M2 model)**

Toxicity rates on P2 population (7.6% [593/7,788] 12-week G4-5 toxicities) were similar to those of P1, except in the G-5FU-TSG study (**Figure S2**). ORs of clinical covariates remained stable between P1 and P2 populations (**Table 2**). Aggregated *DPYD* variants *2A/*13/p.D949V (prevalence 2.2% [175/7,788]) were significantly associated with 12-week G4-5 toxicity ($OR_{mut/wt}$ = 9.5, 95%CI 6.7-13.5, p<.0001) and improved the discriminant ability (AUC=0.73 [95%CI 0.71-0.75] for M1 *vs* 0.75 [95%CI 0.73-0.77] for M2, p<0.0001) (**Table S10**), without significant between-study heterogeneity (p=0.32) (**Figure 2**, left panel). When corrected for overfitting, the average cross-validated AUC was 0.62, varying from 0.47 (95%CI 0.26-0.69, PHRC DPD Breast) to 0.74 (95%CI 0.62-0.87 FFCD 2000-05) and remained higher than that of the clinical M1 model (corrected AUC=0.58) (**Figure S3**). The AUC only based on *DPYD* variants *2A/*13/p.D949V was 0.70 [95%CI 0.68-0.72] (**Table S8**) and corrected AUC was 0.55 (**Figure S3**). In multivariable models, *DPYD* *2A/*13/p.D949V remained strongly significantly associated with secondary endpoints: OR varied from 3.8 [95%CI 2.5-6.0] for 4-week G3-4-5 to 9.6 [95%CI 5.2-17.6] for 4-week G4-5 (**Table S9**, **Figures S4-6-8**) with AUCs higher than 0.70.

**Added prognostic value of *DPYD* HapB3 (M3 model)**

Addition of HapB3 (prevalence 4.0% [277/6,940] including 273 heterozygous and 4 homozygous patients, P3 population, 7.9% 12-week G4-5 toxicities [547/6,940]) did not improve M2 model performance (AUCs difference, p=0.42) (**Figure 3, Table S10**), although HapB3 was significantly associated with G4-5 toxicity. OR was 1.8 [95%CI 1.2-2.7] for HapB3 and 10.1 [95%CI 7.0-14.6] for combined *DPYD* *2A/*13/p.D949V (**Table 2**). The forest plot indicated no significant between-study heterogeneity (p=0.45) (**Figure 2**, right panel). Regarding secondary endpoints, HapB3 OR varied from 1.6 [95%CI 1.0-2.5] for 4-week G3-4-5 to 2.3 [95%CI 1.0-5.3] for 4-week G4-5 toxicity (**Table**

S9, Figures S4-S6-S8), but did not significantly improve AUCs relative to the M2 model (Figures S5-S7-S9). Testing each *DPYD* variant separately (adjusted on clinical variables) showed that p.D949V is associated with the higher toxicity risk (OR between 4.7 and 10.9, depending on the end-point), followed by variant *2A (OR between 3.9 and 8.7), variant *13 (OR between 2.3 and 7.0), and HapB3 (OR between 1.6 and 2.3) (Table S15).

**Impact of *DPYD* variants on hematologic and digestive toxicities separately (M3 model)**

Due to low frequencies, differential impact of *DPYD* variants on hematologic and digestive toxicity was assessable on G3-4-5 toxicity only (13.6% haematological, N=947; 14.9% digestive, N=1037). HapB3 had a similar impact on digestive and hematologic toxicity (OR=1.6 [95%CI 1.2-2.2] *vs* OR=1.6 [95%CI 1.1-2.3]) while merged *2A/*13/p.D949V variants showed a lower impact on digestive toxicity (OR=4.2 [95%CI 3.0-6.0]) *versus* hematologic toxicity (OR=7.9 [95%CI 5.4-11.4]) (Table S16).

**Subset, subgroup and sensitivity analyses for the main endpoint**

The impact of *DPYD* variants did not differ significantly between capecitabine and 5FU (Figure 2), nor between early/locally advanced *vs* advanced/metastatic studies (Table S11). Subgroup analyses revealed the lack of significant interaction between aggregated variants *2A/*13/p.D949V and age (p=0.77), sex (p=0.10) and BMI (p=0.48) (data not shown). Sensitivity analyses showed similar results to those of the main analyses (Tables S13) except for HapB3 with a non-significant impact in patients treated by 5FU alone.

Adding PS (leading to exclude around 1,400 patients) significantly improved the discriminant ability of M1, M2 and M3 models, but marginally improved AUC (difference of 0.01), and confirmed that PS ≥2 was significantly associated with a higher risk of 12-week G4-5 toxicities, with OR ranging

from 2.9 [95%CI 1.7-4.9] for M2 to 5.5 [95%CI 2.8-10.7] for M3; ORs for combined *2A/*13/p.D949V and HapB3 were 10.3 [95%CI 7.1-14.9] and 1.8 [95%CI 1.2-2.7], respectively (**Table S12**).

**Relevance of *MIR27A* polymorphisms (exploratory analysis)**

Univariate analysis (3,287 patients) showed no significant association between *MIR27A* rs895819 and 12-week G4-5 toxicity (**Table S8**) or secondary endpoints (data not shown). No significant, or marginal interaction (p=0.09), were observed between rs895819 (dominant coding) and *DPYD* variants (either 3 variants, 4 variants, or HapB3 alone, **Figures 4**). As compared with patients not carrying any polymorphism, patients carrying one *DPYD* *2A/*13/p.D949V variant had a significantly increased risk of developing 12-week G4-5 toxicities, irrespective of the presence of *MIR27A* variant (adjusted OR=8.1 [95%CI 2.2-29.7] for rs895819 wild-type patients and 8.2 [95%CI 2.7-25.0] for rs895819 carriers) (interaction p=0.76, **Figure 4A**). Adding HapB3 (at least one mutated allele) in the aggregated *DPYD* variable markedly reduced the impact of combined *DPYD* variants, which remained significant only in patients carrying *MIR27A* rs895819 (adjusted OR=3.8 [95%CI 1.6-8.8], interaction p=0.11, **Figure 4B**). Considering HapB3 alone, a similar impact was observed only in patients carrying the MIR27A rs895819 (adjusted OR 3.4 [95%CI 1.2-9.2], interaction p=0.09, **Figure 4C**). Similar results were obtained with additive or recessive MIR*27A* rs895819 coding, as well as for secondary endpoints (data not shown). *MIR27A* rs11671784 variant (1,445 patients) did not show any association with 12-week G4-5 toxicity (**Table S8**).

## DISCUSSION

FUSAFE is the largest individual patient data meta-analysis evaluating *DPYD* *2A/*13/p.D949V and HapB3 variants, and to a lesser extent *MIR27* rs895819, for predicting severe FP-induced toxicities, and the first one focused specifically on life-threatening G4-5 toxicities. This unique database gathers IPD from 10,669 patients without FP dose-adjustment based on DPD status, among whom 6,940 patients were characterized for *DPYD* variants *2A/*13/p.D949V and HapB3. The overall prevalence of 12-week G4-5 haematological and/or digestive toxicity was 7-8%, with a wide between-study variability likely due to FP regimen heterogeneity (**Figure S1, Tables S4a-b**) and difference in toxicity data collection (**Table S2a**). The lethal toxicity rate was 0.54% (55/10,138), in line with literature data [1–3]. Main results are: i- the importance of clinical factors to identify patients at risk of toxicity; ii- the toxicity risk associated with *DPYD* variants *2A/*13/p.D949V is much greater for G4-5 toxicities than for G3-4-5 toxicities; iii- the risk associated with HapB3 heterozygosity is much lower, similar for G4-5 and G3-4-5 toxicities, and HapB3 (at least one mutated allele, 98.6% heterozygous) did not further improve the "clinical + 3 variants" model.

This IPD-MA confirms the prognostic value of previously identified clinical covariates, namely sex, age, BMI, FP administration schedule and associated chemotherapies for FP-induced severe toxicity (**Table 2**). The increased toxicity risk previously described in women [19,24,36] is in line with lower lymphocytic-DPD enzyme activity [6] and 5FU clearance [37] in women. Aging [19,38] and low BSA (correlated with BMI) [38] are known predictors of FP toxicity, as well as use of 5FU bolus [24] and associated anticancer drugs [19,38,39]. The results for the secondary endpoints showed the robustness of the effects of clinical covariates with fair discriminant ability (**Table S9**).

Combined *DPYD* *2A/*13/p.D949V variants significantly improved the clinical model, with an AUC of 0.75 (95%CI 0.73-0.78) and a sensitivity at 0.76, relative to an AUC of 0.73 (95%CI 0.71-0.75) and a sensitivity at 0.70 for the clinical model (main end-point, **Figure 3**).The highest AUC was observed for 4-week G4-5 toxicity (**Figure S7**). The prognostic value of *2A/*13/p.D949V *DPYD* variants did not differ according to FP (5FU *vs* capecitabine, **Figure 2**) nor disease stage (**Table S11**). Sensitivity analyses of 12-week G4-5 toxicity on more homogeneous populations less sensitive to bias (**Table S13**) confirmed the consistency of *2A/*13/D949V *DPYD* effects. Considering the main endpoint, AUC of *2A/*13/D949V variants alone (0.70, 95%CI 0.68-0.72) is of the same order of magnitude as the clinical model (0.73, 95%CI 0.71-0.75), with the largest AUC observed for the combined clinical + 3 *DPY*D variants model (0.75 [95%CI 0.73-0.77], **Figure S3a**). The performance ranking of these 3 models was the same after correction for overfitting by internal-external cross-validation (**Figure S3a**). This result highlights the potential interest of integrating clinical covariates in addition to *DPYD* genotyping to increase the performance of a future toxicity risk prediction for patients treated with FP with or without concomitant treatment.

Multivariable analyses adjusted on clinical variables (all end-points, **Table S15**) showed that toxicity risks of *2A, *13 and p.D949V variants are in the same order of magnitude: for the main end-point OR are 8.6, 7.0 and 10.9, respectively, while that of HapB3 is much lower at 1.7. Although HapB3 was significantly associated with toxicity, addition of HapB3 (at least one mutated allele) to the previous combined “clinical + 3 variants” model did not significantly further improve discrimination ability, whatever the toxicity endpoint (**Figures 3** & **S5-7-9**). These results remain stable across the subsets and different sensitivity analyses. Additional unplanned analyses comparing all possible models (with or without clinical factors) show that HapB3 adds no further statistical information relative to the “clinical + 3 variants” model. The impact of HapB3 homozygosity (4 patients), or

HapB3 combined with other variants (3 patients) may have a greater impact but we could not test it due to very low prevalence. The modest OR of HapB3 agrees with OR or RR reported in the literature, consistently lower than those of *2A, *13 and p.D949V variants [18] (**Table S1**). This modest effect may explain conflicting published results, with a non-significant impact of HapB3 in a large NCCTG N0147 study conducted on 1953 colon cancers (OR=1.33, 95%CI 0.79-2.22) [19], or in a pooled analysis gathering 185 gastric cancers (OR=0.8, 95%CI 0.3-2.4) [20]. HapB3 causal mutation is likely a deep c.1129-5923C>G mutation in intron 10 that creates a splice donor site inducing the inconsistent production of a non-functional transcript [40]. Based on ALPE study results from Henricks et al. [14] showing that reducing the FP dose by 25% in HapB3 carriers did not translate into a reduction of toxicity risk (RR at 1.69 [95%CI 1.18-2.42] *vs* 1.72 [95%CI 1.22-2.42] in patients receiving full FP dose), the DPWG [41] and CPIC (updated CPIC guideline for FP and *DPYD* available from https://cpicpgx.org/guidelines/guideline-for-fluoropyrimidines-and-dpyd/) currently recommend to reduce the FP dose by 50%, or by 25% to 50%, for DPWG and CPIC respectively, in HapB3 heterozygous carriers. The recent publication of survival data of ALPE study [42] reported that HapB3 carriers (N=61), all receiving a 25% FP dose reduction, exhibited a significantly shorter PFS (median 9.1 vs 13.8 months, HR 1.43 95%CI 1.10-1.86, p=0.007) and a non-significant shorter OS, as compared with 183 matched *DPYD* wild-type patients. Altogether, the presently demonstrated low impact of HapB3 heterozygosity on G4-5 and G3-4-5 toxicity, along with ALPE study results, seriously question the relevance of a 25-50% dose reduction in HapB3 heterozygous carriers. Additional pharmacokinetics study may help to refine the right starting dose to recommend according to the patient *DPYD* genotype. In case of starting dose reduction, a rapid FP dose escalation according to tolerance is of major importance to avoid a possible lack of efficacy resulting from underdosing, in line with the CPIC recommendations [9].

DPD expression is regulated by microRNA miR-27A and a common polymorphism (rs895819) in *MIR27A* carried by 55% of Caucasians has been associated with FP-related G3-4 toxicity, only in patients carrying a *DPYD* risk variant [21,22]. Our results did not confirm the relevance of *MIR27A* rs895819, both for 12-week G4-5 toxicities (**Figures 4**) and secondary endpoints (data not shown). A lack of significant interaction was observed between *MIR27A* and *2A/*13/D949V *DPYD* variants. When considering HapB3, either alone or combined with the 3 variants, the interaction showed marginal significance, rendering it difficult to conclude on the interest of combined MIR27A-HapB3 genotyping. Discrepancies between published data and present results may be explained by the time span considered for toxicity, the different studied populations, and different covariates used for adjustment. Our result may also suggest the non-relevance of *MIR27A* rs895819 to help refine FP-toxicity prediction.

Although the strength of this IPD-MA lies in the large number of patients with relevant individual data allowing data checking, assessment of heterogeneity, internal-external cross validation, flexibility of analyses, and inclusion of unpublished data, several limitations must be highlighted. One limitation is the heterogeneity of toxicity recording that forced to use toxicities over the whole treatment as a proxy of 12-week toxicities, for two studies. This weakness is lessened by the stability of results after excluding these two studies. Another limitation is that performance status was not included in our clinical model due to a large proportion of missing data. Nevertheless, the analysis restricted to patients with documented PS showed that PS did not impact the regression coefficients of clinical covariates (data not shown) or variants (**Table S12**). Also, doses of FP and other anticancer therapies, as well as renal function, known to be associated with toxicity [38], were not included due to missing data. Since the majority of FUSAFE population included colorectal

cancer patients (mainly receiving FOLFOX), caution must be taken in extrapolating results to different populations and treatment regimens.

The relevance of current recommendations for *DPYD* 2A/*13/p.D949V/HapB3 genotyping is mainly based on 2 interventional studies in which FP dose was reduced in *DPYD* variant carriers, showing decreased toxicity in variants carriers as compared with historical variant carriers receiving full FP dose [13,14] . Despite accumulation of data supporting the association between deleterious *DPYD* variants and increased risk of severe toxicities, along with studies demonstrating the cost-effectiveness of upfront *DPYD* genotyping [13,43], U.S. guidelines still do not recommend pre-treatment *DPYD* testing [44]. The clinical usefulness of clinical factors combined with *DPYD* variants will need to be confirm by additional studies, as well as validation of FP-dose adjustments based on both clinical and *DPYD* variants [45]. The limited impact of *2A, *13 and p.D949V variants on AUC values is not due to a lack of performance of these variants (OR around 10), but is the result of their low prevalence (2-3% carriers). Despite its modest AUCs, M2 and M3 models may be considered as clinically relevant since they both reduce the 12-week grade 4-5 toxicity risk by 62% as compared to the observed prevalence (i.e. from 7.9% to 4.9%) (**Table S17**). Importantly, 2A/*13/p.D949V and HapB3 variants are not carried by African and Asian populations [46]. In total, the absence of these *DPYD* variants does not ensure complete FP safety. Accordingly, French Health Authorities (ANSM) made mandatory pre-treatment DPD deficiency screening based on measurement of endogenous plasma uracil concentrations, along with strict pre-analytical requirements, as recently illustrated [47]. A French study conducted on 3,680 patients reported that only 10.7% of patients with a deficient DPD phenotype (uracil ≥ 16 ng/ml) carried one of the 4 consensual deleterious *DPYD* variants [5]. Another study reported that 25.3% of patients with a low DPD enzyme activity in blood mononuclear cells carried a consensual variant [48]. Optimal DPD-deficiency screening strategy

should be the combination of phenotyping and genotyping approaches. Other strategies for identifying patients at risk of toxicity is to analyze full *DPYD* exome [23,49], additional FP-related pharmacogenes [17,24,25,31,32,50,51], or full exome [52]. Despite a large literature, no consensus has emerged so far for improving pre-emptive strategies to identify patients at risk of FP-related severe toxicities. A large IPD-MA is awaited to identify and validate additional relevant polymorphisms in *DPYD* gene, its regulation, and other pharmacogenes, in order to build a multigene-signature for predicting very severe FP-related toxicities based on clinical variables and germinal polymorphisms.

**Acknowledgements**

The authors would like to thank the Gustave Roussy library team (current head: Alexia Nerfié) for its support in the literature search.

**Author's contributions**

GLT, JPP, JCB, MCEG, NC and VB with the help of the steering committee members designed and supervised the study. MCEG, and JPP obtained funding. JPP, MCEG, JCB and the DPD working group (cf. appendix 2) searched and selected the studies. Steering committee members contributed to the identification and selection of the studies. JPP, NC, MCEG and VB collected and checked data with the help of the investigators who validated the re-analysis of their trials. GLT, JPP, and NC did the statistical analyses. GLT, JCB, JPP, MCEG, NC and VB wrote the draft, with revisions from the other investigators. All authors contributed to the interpretation of the results during the investigator meeting and the revision of the manuscript. All investigators listed in Web-Appendix 1 received the manuscript for revision.

**Ethics approval**

Included clinical studies were conducted in accordance with the Declaration of Helsinki.

**Consent for publication**: Not applicable

**Data availability**

Individual patient data are not available for sharing. Agreement of the investigators from each study would be necessary to transfer such data. Some summary study-level data are available in

this paper. Additional study-level summary data corresponding to the analyses mentioned in the paper may be provided on request.

**Competing interests**

G Milano reports consulting fees from Servier. JHM Schellens reports patents on oral taxanes. M Schwab reports payment and honoraria for lectures for CED Service GmbH. V Boige reports consulting fees from Merck, Serono, Bayer, Roche, Eisai and BMS. Q Shi reports grants from Janssen, BMS, Genetech, Novartis, Celgene and fees from Regeneron Pharmaceuticals and Chugai Pharmaceuticals. MC Etienne-Grimaldi reports honoraria for lectures for AMGEN. All other authors report no conflicts of interest.

**Funding information**

This study was supported by the French Ministry of Health (PHRC-K 14-193 FUSAFE) and the French « Ligue Nationale Contre le Cancer ». The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

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# Figure legends

**Figure 1:** Flow chart of the FUSAFE meta-analysis

**Figure 2**: Forest plot of prognostic value of *DPYD* *2A/*13/p.D949V [1] (11 studies, P2, n= 7,730 after excluding Newcastle 2001, left panel) and *DPYD* HapB3 [2] (9 studies, P3, n=6,940, right panel) on 12-week grade 4-5 toxicities (main endpoint) adjusted on clinical variables and study.

[1] Combination of the 3 *DPYD* variants *2A/*13/p.D949V is defined as the absence of mutation for the 3 variants *vs* at least one mutation, in patients with information for these 3 variants. Study-specific prognostic value was estimated by the Firth's penalized maximum likelihood method from a logistic regression including an interaction term *DPYD* x Study after excluding Newcastle 2001 (n=58, with 13 toxicities of 12-weeks grade 4-5) due to convergence issues. Estimates obtained on all studies were combined to provide the overall prognostic value (2-step approach). By using a 1-step approach, the overall prognostic value of *DPYD* estimated after excluding Newcastle 2001 (OR=9.5 [6.7-13.5]) was identical to that estimated from the 12 studies (OR=9.5 [6.7-13.5], see Table 2).

[2] HapB3 is defined by the presence of at least one c.1236G>A (rs56038477) allele and/or c.1129-5923C>G (rs75017182) allele, in patients with at least one non-missing value for one of these variants, both in linkage disequilibrium. Study-specific prognostic value was estimated by the Firth's penalized maximum likelihood method from a logistic regression including an interaction term HapB3 x Study due to convergence issues. Estimates obtained on all studies were combined to provide the overall prognostic value (2-step approach). By using a 1-step approach, the overall prognostic value of HapB3 was 1.8 [1.2-2.7] (9 studies) (Table 2).

WT= wild-type; Mutant= mutated (at least one allele).

**Figure 3**: Area under the curve (AUC), Youden index, sensitivity, specificity, positive predictive positive value and negative predictive value of clinical model (M1, blue line), clinical + *DPYD*-based marker model (M2, red line) and clinical + *DPYD*-based marker + HapB3 model (M3, green line) for 12-week grade 4-5 (main endpoint) fitted on P3 analysis set (9 studies, 6,940 patients).

Sensitivity, specificity, positive predictive positive value and negative predictive values are estimated for the cut-off maximizing the Youden index. M1: explanatory clinical core logistic regression model, M2: M1 + *DPYD*-based marker, M3: M2 + HapB3-based marker, CI: confidence interval.

**Figure 4**: Forest plots of prognostic value of *MIR27A* rs895819 (dominant coding) combined with *DPYD* *2A/*13/p.D949V$^{\Delta}$ combination, *DPYD* *2A/*13/D949V/HapB3$^{€}$ combination, and HapB3 alone, from multivariable analyses adjusted on clinical factors on 12-week G4-5 toxicities (main endpoint)$^{\perp}$

**A:** Analysis of 3 *DPYD* variants and *MIR27A* rs895819 + interaction with adjustment on clinical factors (P4, 5 studies, 2,724 patients, 108 toxicities i.e. 4.0%). *MIR27A* rs895819 was carried by 53.4% of patients (9.7% homozygous) and *2A/*13/D949V *DPYD* by 1.5% of patients (n=41).
**B:** Analysis of 4 *DPYD* variants and *MIR27A* rs895819 + interaction with adjustment on clinical factors (P5, 4 studies, 2,396 patients, 93 toxicities i.e. 3.9%). *MIR27A* rs895819 was carried by 54.0% of patients (9.7% homozygous) and *2A/*13/D949V/HapB3 *DPYD* by 5.6% of patients (n=133).

**C:** Analysis of *DPYD* HapB3 and *MIR27A* rs895819 + interaction with adjustment on clinical factors (4 studies, n=2,663 patients, 94 toxicities i.e. 3.5%). *MIR27A* rs895819 was carried by 54% of patients (10% homozygous) and *DPYD* HapB3 by 4.1% of patients (n=109).

[Δ] *DPYD* - : wild-type; *DPYD* +: mutated. *DPYD* is wild-type when 2A, *13 and p.D949V are wild-type, and mutated when at least one mutation is present.
[€]*DPYD*_HapB3 -: wild-type; *DPYD*_HapB3 +: mutated. *DPYD*_HapB3 is wild-type when the 4 variants (*2A, *13, p.D949V and HapB3) are wild-type, and mutated when at least one mutation is present.
*MIR*27A -: wild-type, *MIR*27A +: mutated.
[⊥] The 18 patients (NCT00838370 study) with FP-dose reduction based on *DPYD* status were excluded from these analyses for the following reasons: 7 patients received radiotherapy, one was not Caucasian, one had missing BMI and 9 patients had missing data for rs895819.
CI: confidence interval

# Table legends

**Table 1:** Description of the 15 studies used for the prognostic models[⊥]

[⊥] The last three columns indicate the studies that define the populations P1, P2 and P3 for estimating the clinical (M1), clinical + *DPYD* (M2) and clinical + *DPYD* + HapB3 (M3) regression models, respectively (**Appendix 5**).
[€] Additional references for some studies are described in **Appendix 6**.
* Genotype studies, ** Phenotype studies, *** Studies with both phenotype and genotype data (*DPYD* pharmacogenetic data of Biocolon and ACCORD-13 unpublished)
[£] 423 patients lost between P1 population and P2 population for G-5FU-TSG study related to missing *DPYD* (majority of patients n=413 patients had missing information for SNP *13).
Details on treatments for the whole population are presented on **Table S4b**.

**Table 2:** Multivariable logistic regression models for 12-week grade 4-5 toxicities (main endpoint) [⊥]

[⊥] See **Table S10** for comparison of model performance (M2 vs M1 and M3 vs M2 vs M1 in P2 and P3 populations, respectively) and **Table S14** for the comparison of the distribution of all available covariate strata in P0, P1, P2 and P3 populations.
[Δ] Note that performance status (PS), stage, cancer localization (colorectal, breast, other), fluoropyrimidine (FP) drug, fluoropyrimidine naïve status, treatment line were not retained in the M1 clinical model (see statistical methods in supplemental material).
[£] Main analysis.
[*] Normal: 18.5-24.9, underweight: <18.5, overweight: 25-29.9, obesity: ≥30.
[1] Defined as the presence of no mutation for the 3 variants *vs* at least one mutation in patients with information for these 3 variants.
[2] Defined as wild-type or mutated in patients with at least one non-missing value for one of the 2 variants because of the linkage disequilibrium between these 2 variants.
OR: odds ratio, CI: Confidence interval, BMI: body mass index, CT: chemotherapy, ref: reference, TT: targeted therapy, AUC: Area Under the Curve.