Dihydropyrimidine Dehydrogenase (DPD) Pharmacogenetics

Subjects: Pharmacology & Pharmacy

Contributor: Valeria Conti

The dihydropyrimidine dehydrogenase (DPD), encoded by the *DPYD* gene, is the enzyme mainly involved in the catabolism of fluoropyrimidines (FP). *DPYD* polymorphisms increase the risk of severe FP-related toxicity and *DPYD*-pharmacogenetics (*DPYD*-PGx) is recommended before starting the FP-based chemotherapy.

Other factors influence FP safety, therefore phenotyping methods, such as measurement of plasmatic 5-fluorouracil (5-FU) clearance and DPD activity, could complement the *DPYD*-PGx.

Here, authors reported eleven clinical cases in whom a combined genotyping/phenotyping approach, together with careful clinical monitoring was used to optimise the FP-based treatment. In addition, authors performed a systematic review of the literature concerning the use of *DPYD*-PGx, together with phenotyping methods to personalise such a chemotherapy.

DPYD Pharmacogenetics 5-fluorouracil Therapeutic drug monitoring

1. Introduction

Fluoropyrimidines (FP), including 5-fluorouracil (5-FU) and its oral prodrug capecitabine, are cytotoxic antineoplastic agents belonging to the class of antimetabolites. They are commonly used to treat solid cancer types such as gastrointestinal, head-neck and breast cancers associated or not to other chemotherapeutics and both cytotoxic and biologic drugs [1][2]. The administration of the FP may cause severe, even life-threatening, adverse drug reactions (ADR), including myelosuppression, mucositis/stomatitis, diarrhoea and hand–foot syndrome (HFS). Indeed, it has been estimated that an increased risk of severe ADR (grade > 2) involves 10–30% of treated patients, although these data greatly depend on the therapeutic regimen used [1][3][4].

The rate-limiting step of FP catabolism is the conversion of fluorouracil to dihydrofluorouracil, which is catalysed by an enzyme called dihydropyrimidine dehydrogenase (DPD), encoded by a highly polymorphic gene (i.e., *DPYD*). Several single-nucleotide polymorphisms (SNPs) have been associated to an alteration of the *DPYD* sequence, and some of them may determine a partial or complete DPD deficiency, leading to FP severe toxicity [1].

The Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group (DPWG) [5][6] have published guidelines for FP dosing based on the pharmacogenetic testing of four *DPYD* polymorphisms that are *DPYD**2A* (rs3918290), *DPYD**13* (rs55886062), *DPYD c.2846A>T* (rs67376798) and *c.1129-5923C>G* (rs75017182 and *HapB3*). The latter is the most common variant, with ~4% allelic frequency; the *DPYD**2A, c.2846A>T* and *DPYD**13* are present in ~2.0%, 1.4% and 0.1%, respectively, of Caucasian patients [5].

Recently, a new polymorphism, *DPYD*6* (rs1801160), has been associated with both gastrointestinal and haematological FP-ADR [7].

Notably, other *DPYD* genetic variants may lead to dangerous clinical consequences, although their frequency is very low [5][6][8].

With the main aim of reducing the risk of severe FP-induced toxicity, the CPIC and DPWG have implemented a gene activity score (*DPYD*-AS), which ranges from 0 (complete DPD deficiency) to 2 (normal DPD activity). In patients who are homozygous for one or more of the aforementioned SNPs, the recommendation is to avoid the use of FP. However, if alternative drugs are not considered a suitable option, the FP dosage should be markedly reduced while establishing a therapeutic drug-monitoring (TDM) approach. Patients who are heterozygous should receive a 50% dose reduction at the first cycle of chemotherapy, followed by a titration dose, while monitoring the patient's clinical conditions and possibly performing TDM [5][6].

However, it has been estimated that 30–50% of the patients experience severe ADR, despite not having a DPD deficit associated with such *DPYD* polymorphisms. In fact, there are several factors, including comorbidities, polytherapy, other variants in the *DPYD* and other genes, that can play an important role [9]. Besides *DPYD* polymorphisms, two SNPs in the 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene [10] and a tandem repeat in the thymidylate synthase enhancer region (*TYMS-TSER*) could concur in predicting FP-related toxicity [11]. Moreover, a SNP in glutathione S-transferase-p1 (*GSTP1*) has been suggested as a genetic factor able to influence the response to oxaliplatin, a drug frequently administered with FP [12].

Several strategies complementing the *DPYD* pharmacogenetics (*DPYD*-PGx) have been proposed to prevent FP-related severe ADR associated with DPD deficit. Among others, the measurement of the plasmatic dihydrouracil/uracil ratio (UH_2/U) and the monitoring of 5-FU clearance are considered valid approaches [13][14].

2. Cases Presentation

Case 1 was a Caucasian 55-year-old male former smoker with a history of hypertension. The patient had stage IV colorectal adenocarcinoma with metastases in the lymph nodes, lungs, liver and kidneys. The tumour mutational profile identified no mutations in the *KRAS*, *NRAS* or *BRAF* genes. The patient was treated with the combination of 5-FU, leucovorin and oxaliplatin (FOLFOX6) regimen, plus cetuximab. After three cycles of chemotherapy, the patient reported grade 1 thrombocytopenia and paraesthesia; grade 2 stomatitis, rash and leukopenia and grade 3 neutropenia and mucositis.

A post-therapeutic *DPYD*-PGx was performed, revealing that the patient was heterozygous for *DPYD*2A*. Moreover, the patient was homozygous (TT) for *MTHFR-C677T*, homozygous *TYMS-TSER-2R/2R* and homozygous (AA) for *GSTP1-A313G*. The plasmatic UH2/U ratio was 4.52. Based on these results and the reported toxicity, both the 5-FU and cetuximab doses were reduced. Specifically, the total dosage of 5-FU was reduced to 50%, according to the CPIC and DPWG guidelines.

At the fourth cycle of therapy, the pharmacokinetic analysis revealed a trough 5-FU plasma concentration of 950 ng/mL. The CT scan demonstrated an overall stable disease, according to the RECIST criteria v1.1. The patient was still treated with the same doses of 5-FU. At the sixth cycle of therapy, the 5-FU plasma concentration was 400 ng/mL. The following cycles (fifth to eighth) of chemotherapy were administered at the same drug doses. A new CT scan demonstrated no evidence of disease progression. The ADR were grade 1 leukopenia, neutropenia, thrombocytopenia and mucositis and grade 2 HFS. Following a further two cycles of therapy, the reported ADR were grade 1 paraesthesia, erythematous maculopapular rash and grade 2 cutaneous and mucous fissures. Lastly, following a further two treatment cycles, a new CT scan showed disease progression. The treatment was stopped, and the administration of a new chemotherapeutic regimen was planned. Sadly, the patient died before starting a second line of treatment.

Case 2 was a Caucasian 48-year-old male with no comorbidity. He had stage IV colorectal adenocarcinoma with metastases in the lymph nodes and liver. The tumour mutational profile highlighted the presence of a *KRAS* mutation; thus, a treatment with the FOLFOX6 regimen plus bevacizumab was planned. A pretherapeutic *DPYD*-PGx was requested, and the patient was identified as *DPYD*2A* heterozygous. In addition, he was wild type for *MTHFR-C677T* and *MTHFR-A1298C*, heterozygous *TYMS TSER-2R/3R* and homozygous (GG) for *GSTP1-A313G*. The plasmatic UH2/U ratio was 3.22. Based on these results, a 50% dose reduction of 5-FU was planned for the first cycle of FOLFOX administration, according to the CPIC and DPWG guidelines. After the first cycle of treatment, the plasmatic 5-FU clearance was 474 ng/mL. Following three cycles of therapy, a stable disease was found, according to the RECIST criteria v1.1, and no adverse events were reported. The patient was still treated with the same doses of chemotherapeutic agents for an additional seven cycles of therapy. Grade 1 paraesthesia and mucositis and grade 2 HFS but no severe ADR were reported, and the CT scan demonstrated a stable disease. Afterward, the patient was treated up to the twelfth cycle with a FOLFOX regimen plus bevacizumab, still obtaining, at revaluation, a stable disease. Then, he was a candidate for a maintenance therapy with capecitabine plus bevacizumab. Following 16 cycles of this therapy, the patient reported grade 1 paraesthesia and mucositis, and no severe ADR were recorded.

Case 3 was a Caucasian 60-year-old male former smoker with no comorbidities. He had stage IV rectal adenocarcinoma with liver metastases. The tumour mutational profile did not identify mutations in either *KRAS*, *NRAS* or *BRAF*. Based on the tumour profile and stage, the patient was a candidate for a FOLFOX regimen plus cetuximab. A pretherapeutic *DPYD*-PGx was performed, and the patient was found heterozygous for *DPYD c2846A>T* SNP. Therefore, according to the CPIC and DPWG guidelines, he started chemotherapy with a 50% dose reduction of 5-FU. Moreover, he was homozygous (TT) for *MTHFR-C677T*, heterozygous *TYMS TSER-2R/3R* and heterozygous for *GSTP1-A313G*. The plasmatic UH2/U ratio was 1.77.

A grade 2 diffuse maculopapular rash was reported, and, based on such an ADR, the dose of cetuximab was also reduced to 50% for the second cycle of therapy. The plasmatic 5-FU clearance was 811 ng/mL—still high, notwithstanding the 5-FU dose reduction. The patient reported no improvement of the skin rash and grade 2 diarrhoea. At the third cycle of therapy with the same drugs doses, the 5-FU plasma level was 1093 ng/mL. Grade 1 nausea and grade 3 diarrhoea were reported. Based on these results, the 5-FU dose was further reduced by an

additional 10% at the fourth cycle of therapy. However, the 5-FU plasma concentration was still high (1048 ng/mL), and grade 4 diarrhoea was reported. Hence, it was decided not to administer 5-FU in a continuous infusion, leaving the administration of 5-FU in bolus. Nevertheless, the 5-FU plasma concentration was still high (i.e., 934 ng/mL), and grade 3 diarrhoea was reported.

A CT scan showed a partial response according to the RECIST criteria with a reduction of hepatic lesions. It was decided to carry out a further cycle with oxaliplatin plus cetuximab.

After this cycle, the hepatic lesions were resected. After one month from surgery, a CT scan demonstrated the development of a new hepatic lesion. The patient was a candidate to start a new treatment with 5-FU plus irinotecan as a modified 5-FU, leucovorin and irinotecan (FOLFIRI) regimen, since 5-FU was administered with a 50% dose reduction and without continuous infusion. The patient performed six cycles of FOLFIRI plus bevacizumab. Grade 3 diarrhoea was reported. As a consequence, the 5-FU administration was stopped, and only irinotecan and bevacizumab were further administered. After four cycles of this treatment, the CT scan demonstrated a progression of the disease. The patient died after 11.2 months from starting treatment with irinotecan plus bevacizumab.

Table 1 and Table 2 report the main characteristics and the occurrence of grade ≥ 3 ADR of 3/11 and 8/11 clinical cases, respectively. Table 1 describes three clinical cases for whom either pretherapeutic *DPYD*-PGx or post-therapeutic *DPYD*-PGx were performed. As phenotypic characteristics, the UH2/U ratio values and plasmatic 5-FU clearance were reported.

Table 1. Reports of the main characteristics of three patients with the occurrence of grade ≥ 3 ADR.

Pt	Sex	Age (years)	Tumor Type and Stage	Chemotherapy Regimen	Pre-Therapeutic *DPYD*-PGx	Post-Therapeutic *DPYD*-PGx	*DPYD* Genotype	UH2/U Ratio	5-FU Dosage	5-FU Clearance	ADR ≥ 3	Total Toxicity
1	M	55	CRC (metastatic)	Folfox plus cetuximab	/	Yes (C4)	Heterozygous for *DPYD**2A	4.52	C1-C3: 100% C4-C6: 50%	950 ng/mL (C4) 400 ng/mL (C6)	G3 mucositis (C3) G3 neutropenia (C3) (neuthrophils: 830.58 /mm^3)	7
2	M	48	CRC (metastatic)	Folfox plus bevacizumab	Yes	/	Heterozygous for *DPYD**2A	3.22	C1-C8: 50%	474 ng/mL (C1)	/	6
3	M	60	Rectal cancer (metastatic)	Folfox plus cetuximab)	Yes	/	Heterozygous for c.2846A>T	1.77	C1-C3: 50% C4: 40% C5-C6:	811 ng/mL (C2) 1093 ng/mL (C3) 1048	G3 diarrhoea (C3) G4 diarrhoea (C4) G3 diarrhoea (C5)	3

Pt	Sex	Age (years)	Tumor Type and Stage	Chemotherapy Regimen	Pre-Therapeutic *DPYD*-PGx	Post-Therapeutic *DPYD*-PGx	*DPYD* Genotype	UH2/U Ratio	5-FU Dosage	5-FU Clearance	ADR ≥ 3	Total Toxicity
									40% (only bolus)	ng/mL (C4) 934 ng/mL (C5)		

YD-PGx, io; ADR,

Table 2. Reports of 8 clinical cases for whom either pretherapeutic *DPYD*-PGx or post-therapeutic *DPYD*-PGx were performed. As phenotypic characteristics, the UH2/U ratio values were reported.

Pt	Sex	Age (Years)	Tumor Type and Stage	Chemotherapy Regimen	Pre-Therapeutic *DPYD*-PGx	Post-Therapeutic *DPYD*-PGx	*DPYD* Genotype	UH2/U Ratio	5-FU Dosage	ADR ≥3
1	F	63	Stomach cancer (locally advanced)	Folfox	/	Yes (C2)	Heterozygous for *DPYD**2A	7.09	C1-C2: 100% C3: 5-FU withdrawal	G3 vomit (C2)
2	M	43	CRC (metastatic)	Folfiri with bevacizumab	/	Yes (C8)	Heterozygous for c.2846A>T	3.88	C1-C8: 100% C9: 50%	G3 vomit (C8)
3	M	63	Kidney cancer (metastatic)	Xeloda	yes	/	Heterozygous for c.2846A>T	6.57	C1-C6: 50%	/
4	M	68	CRC (metastatic)	Xelox	yes	/	Heterozygous for c.2846A>T	4.4	C1-C7: 50%	/
5	M	78	CRC (local)	Xelox	yes	/	Heterozygous for c.2846A>T	3.37	C1-C2: 50%	/
6	M	72	CRC (locally advanced)	Xelox	yes	/	Heterozygous for *DPYD**2A	5.15	C1-C8: 50%	/
7	F	52	Vulva carcinoma (local)	Xeloda with cisplatin	yes	/	Heterozygous for *DPYD**2A	7.38	C1-C5: 50%	/
8	M	76	Rectosigmoid cancer (locally advanced)	Folfox	yes	/	Heterozygous for *DPYD**2A	2.44	C1-C5: 50%	/

Pt, patient; M, male; F, female; CRC, ColoRectal Cancer; FOLFOX, 5-Fluorouracil plus leucovorin plus oxaliplatin; FOLFIRI, 5-Fluorouracil plus leucovorin plus irinotecan; XELOX, capecitabine plus oxaliplatin; XELODA, Capecitabine; DPYD-PGx, DPYD pharmacogenetics; UH2/U RATIO, dihydrouracil/uracil ratio; ADR, Adverse Drug Reaction; C, cycle; G, Grade.

A pretherapeutic *DPYD*-PGx was performed in two out of three cases, while one patient (case 1) had already started chemotherapy before requesting *DPYD*-PGx. Importantly, the patients were monitored during all treatment cycles.

Besides these three clinical cases, the history of other eight patients is briefly reported below, and their main characteristics are listed in Table 2.

All subjects were monitored for at least four treatment cycles. In two out of eight subjects, the *DPYD*-PGx was required after the occurrence of severe toxicity (post-therapeutic *DPYD*-PGx), while in six out of eight, pharmacogenetic testing was performed before the treatment started (pretherapeutic *DPYD*-PGx). All patients were identified as carriers of *DPYD* variants—precisely, four out of eight were *DPYD**2A* heterozygous, and four out of eight were *DPYD c.2846* heterozygous.

The two patients for whom the *DPYD*-PGx was performed after 5-FU administration experienced grade 3 ADR with a different timing, and both were then revealed as *DPYD*-variant carriers. More in detail, one patient with stage III gastric cancer, treated with FOLFOX, suffered from grade 3 vomit after the second cycle; he was then identified as *DPYD**2A* heterozygous and continued to be treated only with oxaliplatin. Moreover, the patient was homozygous (TT) for *MTHFR*-C677T, homozygous *TYMS TSER*-3R/3R and homozygous (AA) for *GSTP1*-A313G.

The other one with stage IV colon cancer, treated with FOLFIRI plus bevacizumab, showed grade 3 vomit after the eighth cycle of chemotherapy. The patient was identified as *DPYD c.2846* heterozygous, and the 5-FU dosage was halved. With regards to the other SNPs, the patient was homozygous (TT) for *MTHFR*-C677T, heterozygous *TYMS TSER*-2R/3R and wild type for *UGT1A1**28 SNP. The latter polymorphism is routinely analysed in patients treated with irinotecan.

Conversely, in the other patients, a pretherapeutic *DPYD*-PGx was performed; thus they were treated with a starting halved dose of 5-FU, and no severe ADR were reported.

3. Systematic Review

A systematic review was performed to analyse the studies investigating the variability of responses to FP-based chemotherapy by *DPYD* genotyping combined with phenotyping methods and/or clinical monitoring.

Of the potential 112 articles assessed for eligibility, after considering the inclusion and exclusion criteria, 22 studies were included in the analysis [15][16][17][18][19][20][21][22][23][24][25][26][27][28][29][30][31][32][33][34][35][36]. Table 3 shows such studies subdivided with respect to the analysed *DPYD* polymorphisms (*DPYD*-PGx), the used phenotyping methods and the presence of clinical monitoring. A *DPYD-PGx*/clinical monitoring combination was present in 11, and *DPYD-PGx*/phenotyping in three, surveys. A *DPYD-PGx*/phenotyping/clinical monitoring combined approach was made in eight studies (Table 3).

Table 3. The table reports the studies included in the systematic review and subdivided into three groups: DPYD-PGx/clinical monitoring combination, DPYD-PGx/phenotyping and DPYD-PGx/phenotyping/clinical monitoring. Abbreviations: PBMC, peripheral blood mononuclear cells; HPLC-UV, high-performance liquid chromatography-UV detector; LC-MS/MS, liquid chromatography-tandem mass spectrometry; 5-FUDR, 5-FU degradation rate; UHPLC-

MS/MS, ultra-high-performance liquid chromatography-tandem mass spectrometry; PK, pharmacokinetics; FP, fluoropyrimidines; DPD, dihydropyrimidine dehydrogenase; UH2/U ratio, dihydrouracil/uracil ratio and AAS, atomic absorption spectrometry.

First Author's Name (Published Year)	Enrolled Patients (n)	Outcomes	*DPYD*-PGx/Clinical Monitoring	*DPYD*-PGx/Phenotyping	*DPYD*-PGx/Phenotyping/Clinical Monitoring
Kuilenburg et al. (2000) [15]	37	*DPD* activity and overall toxicity; *DPYD* genotyping in patients with reduced DPD activity.			*DPYD**2A, c.2846A>T, *DPYD**6, *DPYD**9A, c.496A>G/ UH2/U ratio in PBMC/ADR until two treatment months.
Schwab et al. (2008) [16]	683	Overall toxicity; *DPYD, TYMS, MTHFR* genotyping; sequencing of *DPYD* exome; influence of sex and promoter methylation on DPD expression in human liver.	*DPYD**2A, c.2846A>T, c.623G>T, *DPYD**4, *DPYD**6, and c.2858G>C/ ADR reported until the second cycle of treatment.		
Kristensen et al.(2010) [17]	68	Relationship between UH_2/U plasma ratio and 5-FU-related early toxicity; relationship between 5-FU concentration and toxicity; IVS14+1G>A mutation screening.			*DPYD**2A/ UH_2/U ratio in plasma 5-FU clearance by HPLC-UV/ ADR reported until the second cycle of treatment.
Deenen et al. (2011) [18]	568	Relationships between SNPs and toxicity, SNPs and dose modification of capecitabine, *DPYD* haplotypes and toxicity, *DPYD* SNPs and	*DPYD**2A, c.2846A>T and c.1236G>A [*HapB3*]/ ADR reported until the second cycle of treatment.		

First Author's Name (Published Year)	Enrolled Patients (n)	Outcomes	*DPYD*-PGx/Clinical Monitoring	*DPYD*-PGx/Phenotyping	*DPYD*-PGx/Phenotyping/Clinical Monitoring
		haplotypes and survival.			
Deenen et al. (2016) [19]	2038	Feasibility, safety and cost of *DPYD**2A genotype-guided dosing.	*DPYD**2A/ADR reported until the sixth cycle of treatment.		
Sistonen et al. (2014) [20]	28	Relationship between UH_2/U plasma ratio and *DPYD* genetic variation; plasma concentration of 5-FU and corresponding AUC; toxicity.			c.234-123G>C, c.496A>G, c.775A>G, c.1129-5923C>G [*Hap B3*], *DPYD**13, *DPYD**2A and c.2846A>T/ UH_2/U ratio in plasma- 5-FU clearance by LC-MS/MS/ ADR reported until the second cycle of treatment.
Lee et al. (2014) [21]	2886	Relationship between *DPYD* variants and toxicity.	*DPYD**2A, *DPYD**13, c.2846A>T/ ADR until the twelfth cycle of treatment.		
Gentile et al. (2015) [22]	156	Correlation between degradation rate of 5-FU with detected SNPs.		*DPYD**2A, *DPYD**13, c.2846A>T/5-FUDR assay in PBMC by HPLC-MS/MS	
Joerger et al. (2015) [23]	140	Quantitative effect of 44 gene polymorphism in 16 drug pathway associated genes on progression free survival (PFS), on chemotherapy toxicity, on objective response rate (ORR), on overall survival (OS).			*DPYD**13, *DPYD**2A, c.2846A>T, *DPYD**9A, c.1896T>C/5-FU clearance by AAS and HPLC/ADR until disease progression.

First Author's Name (Published Year)	Enrolled Patients (n)	Outcomes	*DPYD*-PGx/Clinical Monitoring	*DPYD*-PGx/Phenotyping	*DPYD*-PGx/Phenotyping/Clinical Monitoring
Lunenburg et al. (2016) [24]	275	Evaluation of requests of prospective *DPYD* screening and results with a dose recommendation; estimation of the follow up of the dose recommendations.	*DPYD**2A, *DPYD**13, c.2846A>T, c.1236G>A [HapB3]/ ADR reported until the second cycle of treatment.		
Galarza et al. (2016) [25]	60	Estimation of the use of plasma and saliva; Uracil to UH_2 metabolic ratio and *DPYD* genotyping.			*DPYD* *2A, *13, c.557A>G, *DPYD* *7/ UH_2/U ratio in plasma/ ADR reported until the third cycle of treatment.
Milano et al. (2016) [26]	243	Sequencing of *DPYD* exome and frequence of G3, G4 toxicity over cycle 1-2.	*DPYD**2A, *DPYD**13, c.2846A>T, c.1774C>T, c.1475C>T, D342G/ ADR reported until the second cycle of treatment.		
Boisdron-Celle et al. (2017) [27]	85	*UGT1A1* and *DPYD* genotyping; UH_2/U ratio; follow up of efficacy and tolerance.			*DPYD**2A, *DPYD**13, c.2846A>T, *DPYD**7/ UH_2/U ratio in plasma/ADR every two weeks until three months.
Etienne-Grimaldi et al.(2017) [28]	243	*DPYD* sequencing; relationship between toxicity and *DPYD* variants; *DPD* phenotyping.			*DPYD**2A, *DPYD**13, c.2846A>T/ UH_2/U ratio in plasma/ ADR reported until the second cycle of treatment.
Liu et al. (2017) [29]	661	Relationship between *UGT1A1*	*DPYD**5, c.1896 T > C,		

First Author's Name (Published Year)	Enrolled Patients (n)	Outcomes	*DPYD*-PGx/Clinical Monitoring	*DPYD*-PGx/Phenotyping	*DPYD*-PGx/Phenotyping/Clinical Monitoring
		and *DPYD* polymorphism and incidence of severe neutropenia and diarrhea; relationship between *UGT1A1* and *DPYD* variants and objective response rate, disease control rate, overall and progression free survival.	and *DPYD**2A/ ADR reported every two-three cycles or whenever patient's condition changed.		
Henricks et al.(2018) [30]	1181	Frequency of severe overall FP-related toxicity; pharmacokinetics of fluoropyrimidines in *DPYD* variant allele carriers; *DPD* enzyme activity; cost analysis on individualised dosing by upfront *DPYD* genotyping.			*DPYD**2A, c.2846A>T, *DPYD**13 and c.1236G>A [*Hap B3*]/ UH_2/U ratio in PBMC/PK data by UHPLC-MS/MS/ADR until toxicity resolution.
Cremolini et al. (2018) [31]	443	Relationship between *DPYD* and *UGT1A1* genotyping and toxicity.	*DPYD**2A, *13, c.2846A>T/ADR reported until the fourth cycle of treatment.		
Jacobs et al.(2019) [32]	237	Pharmacokinetics of capecitabine and 5-FU in *DPYD* variant allele carriers.		*DPYD**2A, c.2846A>T, c.1236G>A [*HapB3*]/5-FU clearance by LC MS/MS.	

Among the studies with a DPYD-PGx/clinical monitoring combination, five out of eleven studies confirmed the importance of DPYD variants in predicting FP-related toxicity, although a too-short clinical monitoring was performed (only two treatment cycles) [16][18][24][26][34]. Two studies [19][29] analysed only DPYD*2A of the DPYD SNPs currently recommended. The first confirmed a strong association between DPYD*2A and a severe and

First Author's Name (Published Year)	Enrolled Patients (n)	Outcomes	*DPYD*-PGx/Clinical Monitoring	*DPYD*-PGx/Phenotyping	*DPYD*-PGx/Phenotyping/Clinical Monitoring
Iachetta et al.(2019) [33]	1827	Relationship between *DPYD* and toxicity.	*DPYD**13, *DPYD**2A, c.2846A>T, *DPYD**6 /ADR reported until the eleventh cycle of treatment.		
Kleinjan et al. (2019) [34]	185	*DPYD* genotyping and toxicity.	*DPYD**2A, c.2846A>T, *DPYD**13 and c.1236G>A [HapB3] /ADR reported until the second cycle of treatment.		
Negarandeh et al.(2020) [35]	88	Relationship between *DPYD* genotyping and toxicity.	*DPYD**2A, c.2846A>T, *DPYD**6/ADR reported following 227 cycles for 88 patients.		
Nicolas Pallet et al. (2020) [36]	5886	Relationship between *DPYD* genotyping and [U] and UH_2/U ratio in plasma.		*DPYD**2A, *DPYD**13, c.2846A>T, c.1236G>A [HapB3]/ [U] and UH_2/U ratio in plasma.	

toxicity. Unfortunately, because of the low sample size of the study, they did not show conclusive results about the usefulness of plasmatic 5-FU clearance determination in improving the predictive potential of DPYD-PGx [23].

Galarza et al. found that salivary and plasmatic UH2 concentrations were inversely correlated with the ADR grade. However, given the low number of patients enrolled in the study, no DPYD variant allele carriers were identified [25].

Boisdron et al. conducted a phase II study in 85 patients to test the efficacy of a pharmacogenetic-guiding dosing approach combined with the UH2/U ratio measurement. Despite a very large increase in drug dosages, a low incidence of severe ADR was shown in patients who used a guiding dosing approach. However, also in this case, it was not possible to conclude if this phenotyping analysis enhanced the predictability of DPYD genotyping because of the low sample size of the study [27]. Etienne et al. failed to demonstrate a correlation between DPYD variants and the plasmatic UH2/U ratio values. The authors concluded that only an extension of the genetic panel may

improve the performance of DPYD-PGx for predicting severe and life-threatening ADR associated with capecitabine [28].

Kuilenburg et al. measured the UH2/U ratio in PBMCs as an indirect assessment of DPYD activity. They demonstrated that patients with a low DPD activity experienced a more rapid onset of toxicity as compared to those with a normal enzymatic activity. Moreover, grade 4 neutropenia occurred in a substantial percentage (55%) of the patients with a decreased DPD activity as compared to that (13%) of subjects with a normal DPD activity. Notably, eleven out of fourteen patients suffering from severe ADR with a decreased enzymatic activity were identified as carriers of DPYD polymorphisms. In particular, six, four and one out of eleven patients carried DPYD*2A, DPYD*9A and DPYD*6 in homozygosis, respectively [15]. Kristensen et al. also showed a significant correlation between the plasmatic UH2/U ratio and the presence of DPYD*2A [17].

Finally, in a prospective study, Henricks et al. analysed all the four recommended DPYD SNPs and performed two phenotyping tests by measuring the UH2/U ratio in PBMCs and plasmatic 5-FU pharmacokinetics (PK) by UHPLC-MS/MS. The patients carrying DPYD c.1236G>A and DPYD c.2846A>T were more likely to manifest FP-related severe toxicity as compared to wild-type subjects. In addition, the mean DPD enzyme activity was significantly lower in patients bearing these two genetic variants, as well as DPYD*2A, as compared to other patients. Only one patient carrying DPYD*13 showed a 60% DPD activity reduction. This patient was treated with a reduced 5-FU dosage for three treatment cycles, and no severe ADR occurred.

4. Conclusions

Nowadays, the regulatory agencies recommend carrying out the *DPYD-PGx,* including four *DPYD* polymorphisms (i.e., rs3918290, rs55886062, rs67376798 and rs75017182, HapB3) in patients who need to be treated with FP.

Despite that these *DPYD* variants are strongly associated with treatment toxicity, other genetic and nongenetic factors concur to determine the variable response to FP-based chemotherapy.

A pretherapeutic *DPYD-PGx* offers the possibility to avoid early ADR. Nonetheless, severe and even fatal FP-related toxicity may happen anytime during the therapy also in subjects having no DPD deficit attributable to the four recommended *DPYD* SNPs.

On the other hand, measuring the plasmatic 5-FU clearance—currently, the best method to perform TDM—does not permit to diagnose a possible DPD deficit prior to starting the treatment.

Therefore, because both genetic and phenotypic tests show advantages and disadvantages, a combined genotyping/phenotyping approach, together with careful and continuous clinical monitoring, is the best diagnostic method to optimise the therapy with FP.

References

1. Longley, D.B.; Harkin, D.P.; Johnston, P.G. 5-Fluorouracil: Mechanisms of Action and Clinical Strategies. Nat. Rev. Cancer 2003, 3, 330–338.
2. Venook, A.P.; Niedzwiecki, D.; Lenz, H.-J.; Innocenti, F.; Fruth, B.; Meyerhardt, J.A.; Schrag, D.; Greene, C.; O'Neil, B.H.; Atkins, J.N.; et al. Effect of First-Line Chemotherapy Combined with Cetuximab or Bevacizumab on Overall Survival in Patients With KRAS Wild-Type Advanced or Metastatic Colorectal Cancer: A Randomized Clinical Trial. JAMA 2017, 317, 2392–2401.
3. Hoff, P.M.; Ansari, R.; Batist, G.; Cox, J.; Kocha, W.; Kuperminc, M.; Maroun, J.; Walde, D.; Weaver, C.; Harrison, E.; et al. Comparison of Oral Capecitabine versus Intravenous Fluorouracil plus Leucovorin as First-Line Treatment in 605 Patients with Metastatic Colorectal Cancer: Results of a Randomized Phase III Study. J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 2001, 19, 2282–2292.
4. Van Kuilenburg, A.B.P.; Dobritzsch, D.; Meijer, J.; Meinsma, R.; Benoist, J.-F.; Assmann, B.; Schubert, S.; Hoffmann, G.F.; Duran, M.; de Vries, M.C.; et al. Dihydropyrimidinase Deficiency: Phenotype, Genotype and Structural Consequences in 17 Patients. Biochim. Biophys. Acta 2010, 1802, 639–648.
5. Amstutz, U.; Henricks, L.M.; Offer, S.M.; Barbarino, J.; Schellens, J.H.M.; Swen, J.J.; Klein, T.E.; McLeod, H.L.; Caudle, K.E.; Diasio, R.B.; et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Dihydropyrimidine Dehydrogenase Genotype and Fluoropyrimidine Dosing: 2017 Update. Clin. Pharmacol. Ther. 2018, 103, 210–216.
6. Lunenburg, C.A.T.C.; van der Wouden, C.H.; Nijenhuis, M.; Crommentuijn-van Rhenen, M.H.; de Boer-Veger, N.J.; Buunk, A.M.; Houwink, E.J.F.; Mulder, H.; Rongen, G.A.; van Schaik, R.H.N.; et al. Dutch Pharmacogenetics Working Group (DPWG) Guideline for the Gene–Drug Interaction of DPYD and Fluoropyrimidines. Eur. J. Hum. Genet. 2020, 28, 508–517.
7. Del Re, M.; Cinieri, S.; Michelucci, A.; Salvadori, S.; Loupakis, F.; Schirripa, M.; Cremolini, C.; Crucitta, S.; Barbara, C.; Di Leo, A.; et al. DPYD*2A and c.2846A>T: A Comprehensive Analysis in 1254 Patients. Pharm. J. 2019, 19, 556–563.
8. Van Kuilenburg, A.B.P.; Meijer, J.; Mauer, D.; Dobritzsch, D.; Meinsma, R.; Los, M.; Knegt, L.C.; Zoetekouw, L.; Jansen, R.L.H.; Dezentjé, V.; et al. Severe Fluoropyrimidine Toxicity Due to Novel and Rare DPYD Missense Mutations, Deletion and Genomic Amplification Affecting DPD Activity and MRNA Splicing. Biochim. Biophys. Acta Mol. Basis Dis. 2017, 1863, 721–730.
9. Coenen, M.J.H.; Paulussen, A.D.C.; Breuer, M.; Lindhout, M.; Tserpelis, D.C.J.; Steyls, A.; Bierau, J.; van den Bosch, B.J.C. Evolution of Dihydropyrimidine Dehydrogenase Diagnostic Testing in a Single Center during an 8-Year Period of Time. Curr. Ther. Res. Clin. Exp. 2019, 90, 1–7.

10. Ulvik, A.; Ueland, P.M.; Fredriksen, A.; Meyer, K.; Vollset, S.E.; Hoff, G.; Schneede, J. Functional Inference of the Methylenetetrahydrofolate Reductase 677C > T and 1298A > C Polymorphisms from a Large-Scale Epidemiological Study. Hum. Genet. 2007, 121, 57–64.
11. Lecomte, T.; Ferraz, J.-M.; Zinzindohoué, F.; Loriot, M.-A.; Tregouet, D.-A.; Landi, B.; Berger, A.; Cugnenc, P.-H.; Jian, R.; Beaune, P.; et al. Thymidylate Synthase Gene Polymorphism Predicts Toxicity in Colorectal Cancer Patients Receiving 5-Fluorouracil-Based Chemotherapy. Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res. 2004, 10, 5880–5888.
12. McLeod, H.L.; Sargent, D.J.; Marsh, S.; Green, E.M.; King, C.R.; Fuchs, C.S.; Ramanathan, R.K.; Williamson, S.K.; Findlay, B.P.; Thibodeau, S.N.; et al. Pharmacogenetic Predictors of Adverse Events and Response to Chemotherapy in Metastatic Colorectal Cancer: Results from North American Gastrointestinal Intergroup Trial N9741. J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 2010, 28, 3227–3233.
13. Boisdron-Celle, M.; Remaud, G.; Traore, S.; Poirier, A.L.; Gamelin, L.; Morel, A.; Gamelin, E. 5-Fluorouracil-Related Severe Toxicity: A Comparison of Different Methods for the Pretherapeutic Detection of Dihydropyrimidine Dehydrogenase Deficiency. Cancer Lett. 2007, 249, 271–282.
14. Van Staveren, M.C.; Jan Guchelaar, H.; Van Kuilenburg, A.B.P.; Gelderblom, H.; Maring, J.G. Evaluation of Predictive Tests for Screening for Dihydropyrimidine Dehydrogenase Deficiency. Pharm. J. 2013, 13, 389–395.
15. Van Kuilenburg, A.B.; Haasjes, J.; Richel, D.J.; Zoetekouw, L.; Van Lenthe, H.; De Abreu, R.A.; Maring, J.G.; Vreken, P.; van Gennip, A.H. Clinical Implications of Dihydropyrimidine Dehydrogenase (DPD) Deficiency in Patients with Severe 5-Fluorouracil-Associated Toxicity: Identification of New Mutations in the DPD Gene. Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res. 2000, 6, 4705–4712.
16. Schwab, M.; Zanger, U.M.; Marx, C.; Schaeffeler, E.; Klein, K.; Dippon, J.; Kerb, R.; Blievernicht, J.; Fischer, J.; Hofmann, U.; et al. Role of Genetic and Nongenetic Factors for Fluorouracil Treatment-Related Severe Toxicity: A Prospective Clinical Trial by the German 5-FU Toxicity Study Group. J. Clin. Oncol. 2008, 26, 2131–2138.
17. Kristensen, M.H.; Pedersen, P.; Mejer, J. The Value of Dihydrouracil/Uracil Plasma Ratios in Predicting 5-Fluorouracilrelated Toxicity in Colorectal Cancer Patients. J. Int. Med. Res. 2010, 38, 1313–1323.
18. Deenen, M.J.; Tol, J.; Burylo, A.M.; Doodeman, V.D.; De Boer, A.; Vincent, A.; Guchelaar, H.-J.; Smits, P.H.M.; Beijnen, J.H.; Punt, C.J.A.; et al. Relationship between Single Nucleotide Polymorphisms and Haplotypes in DPYD and Toxicity and Efficacy of Capecitabine in Advanced Colorectal Cancer. Clin. Cancer Res. 2011, 17, 3455–3468.
19. Deenen, M.J.; Meulendijks, D.; Cats, A.; Sechterberger, M.K.; Severens, J.L.; Boot, H.; Smits, P.H.; Rosing, H.; Mandigers, C.M.P.W.; Soesan, M.; et al. Upfront Genotyping of DPYD*2A to

Individualize Fluoropyrimidine Therapy: A Safety and Cost Analysis. J. Clin. Oncol. 2016, 34, 227–234.

20. Sistonen, J.; Büchel, B.; Froehlich, T.K.; Kummer, D.; Fontana, S.; Joerger, M.; Van Kuilenburg, A.B.P.; Largiadèr, C.R. Predicting 5-Fluorouracil Toxicity: DPD Genotype and 5,6-Dihydrouracil:Uracil Ratio. Pharmacogenomics 2014, 15, 1653–1666.
21. Lee, A.M.; Shi, Q.; Pavey, E.; Alberts, S.R.; Sargent, D.J.; Sinicrope, F.A.; Berenberg, J.L.; Goldberg, R.M.; Diasio, R.B. DPYD variants as predictors of 5-fluorouracil toxicity in adjuvant colon cancer treatment (NCCTG N0147). J. Natl. Cancer Inst. 2014, 106.
22. Gentile, G.; Botticelli, A.; Lionetto, L.; Mazzuca, F.; Simmaco, M.; Marchetti, P.; Borro, M. Genotype-Phenotype Correlations in 5-Fluorouracil Metabolism: A Candidate DPYD Haplotype to Improve Toxicity Prediction. Pharm. J. 2016, 16, 320–325.
23. Joerger, M.; Huitema, A.D.R.; Boot, H.; Cats, A.; Doodeman, V.D.; Smits, P.H.M.; Vainchtein, L.; Rosing, H.; Meijerman, I.; Zueger, M.; et al. Germline TYMS Genotype Is Highly Predictive in Patients with Metastatic Gastrointestinal Malignancies Receiving Capecitabine-Based Chemotherapy. Cancer Chemother. Pharmacol. 2015, 75, 763–772.
24. Lunenburg, C.A.T.C.; Van Staveren, M.C.; Gelderblom, H.; Guchelaar, H.-J.; Swen, J.J. Evaluation of Clinical Implementation of Prospective DPYD Genotyping in 5-Fluorouracil- or Capecitabine-Treated Patients. Pharmacogenomics 2016, 17, 721–729.
25. Galarza, A.F.A.; Linden, R.; Antunes, M.V.; Hahn, R.Z.; Raymundo, S.; da Silva, A.C.C.; Staggemeier, R.; Spilki, F.R.; Schwartsmann, G. Endogenous Plasma and Salivary Uracil to Dihydrouracil Ratios and DPYD Genotyping as Predictors of Severe Fluoropyrimidine Toxicity in Patients with Gastrointestinal Malignancies. Clin. Biochem. 2016, 49, 1221–1226.
26. Milano, G. Highlight on DPYD Gene Polymorphisms and Treatment by Capecitabine*. Scand. J. Clin. Lab. Invest. 2016, 76, S30–S33.
27. Boisdron-Celle, M.; Metges, J.P.; Capitain, O.; Adenis, A.; Raoul, J.L.; Lecomte, T.; Lam, Y.H.; Faroux, R.; Masliah, C.; Poirier, A.L.; et al. A Multicenter Phase II Study of Personalized FOLFIRI-Cetuximab for Safe Dose Intensification. Semin. Oncol. 2017, 44, 24–33.
28. Etienne-Grimaldi, M.-C.; Boyer, J.-C.; Beroud, C.; Mbatchi, L.; Van Kuilenburg, A.; Bobin-Dubigeon, C.; Thomas, F.; Chatelut, E.; Merlin, J.-L.; Pinguet, F.; et al. New Advances in DPYD Genotype and Risk of Severe Toxicity under Capecitabine. PLoS ONE 2017, 12.
29. Liu, D.; Li, J.; Gao, J.; Li, Y.; Yang, R.; Shen, L. Examination of Multiple UGT1A and DPYD Polymorphisms Has Limited Ability to Predict the Toxicity and Efficacy of Metastatic Colorectal Cancer Treated with Irinotecan-Based Chemotherapy: A Retrospective Analysis. BMC Cancer 2017, 17.

30. Henricks, L.M.; Lunenburg, C.A.T.C.; de Man, F.M.; Meulendijks, D.; Frederix, G.W.J.; Kienhuis, E.; Creemers, G.-J.; Baars, A.; Dezentjé, V.O.; Imholz, A.L.T.; et al. DPYD Genotype-Guided Dose Individualisation of Fluoropyrimidine Therapy in Patients with Cancer: A Prospective Safety Analysis. Lancet Oncol. 2018, 19, 1459–1467.
31. Cremolini, C.; Del Re, M.; Antoniotti, C.; Lonardi, S.; Bergamo, F.; Loupakis, F.; Borelli, B.; Marmorino, F.; Citi, V.; Cortesi, E.; et al. DPYD and UGT1A1 Genotyping to Predict Adverse Events during First-Line FOLFIRI or FOLFOXIRI plus Bevacizumab in Metastatic Colorectal Cancer. Oncotarget 2018, 9, 7859–7866.
32. Jacobs, B.A.W.; Deenen, M.J.; Joerger, M.; Rosing, H.; de Vries, N.; Meulendijks, D.; Cats, A.; Beijnen, J.H.; Schellens, J.H.M.; Huitema, A.D.R. Pharmacokinetics of Capecitabine and Four Metabolites in a Heterogeneous Population of Cancer Patients: A Comprehensive Analysis. CPT Pharmacomet. Syst. Pharmacol. 2019, 8, 940–950.
33. Iachetta, F.; Bonelli, C.; Romagnani, A.; Zamponi, R.; Tofani, L.; Farnetti, E.; Nicoli, D.; Damato, A.; Banzi, M.; Casali, B.; et al. The Clinical Relevance of Multiple DPYD Polymorphisms on Patients Candidate for Fluoropyrimidine Based-Chemotherapy. An Italian Case-Control Study. Br. J. Cancer 2019, 120, 834–839.
34. Kleinjan, J.P.; Brinkman, I.; Bakema, R.; Van Zanden, J.J.; Van Rooijen, J.M. Tolerance-Based Capecitabine Dose Escalation after DPYD Genotype-Guided Dosing in Heterozygote DPYD Variant Carriers: A Single-Center Observational Study. Anticancer Drugs 2019, 30, 410–415.
35. Negarandeh, R.; Salehifar, E.; Saghafi, F.; Jalali, H.; Janbabaei, G.; Abdhaghighi, M.J.; Nosrati, A. Evaluation of Adverse Effects of Chemotherapy Regimens of 5-Fluoropyrimidines Derivatives and Their Association with DPYD Polymorphisms in Colorectal Cancer Patients. BMC Cancer 2020, 20, 560.
36. Pallet, N.; Hamdane, S.; Garinet, S.; Blons, H.; Zaanan, A.; Paillaud, E.; Taieb, J.; Laprevote, O.; Loriot, M.-A.; Narjoz, C. A Comprehensive Population-Based Study Comparing the Phenotype and Genotype in a Pretherapeutic Screen of Dihydropyrimidine Dehydrogenase Deficiency. Br. J. Cancer 2020.