

REVIEW

SAFETY MATTERS: THE TROUBLED AND FINALLY SUCCESSFUL STORY OF DIHYDROPYRIMIDINE DEHYDROGENASE PHARMACOGENETIC TEST IN CANCER PATIENTS

S. Crucitta ¹, F. Sciandra ¹, A. Cerbioni ¹, F. Cucchiara ¹, R. Arici ¹, C. Rafaniello ², A. Capuano ², R. van Schaik ³, R. Danesi ¹, M. Del Re ¹

¹ Unit of Clinical Pharmacology and Pharmacogenetics, Department of Clinical and Experimental Medicine, University Hospital of Pisa, Pisa, Italy

² Department of Experimental Medicine, University of Campania Luigi Vanvitelli, Naples, Italy

³ Department of Clinical Chemistry, Erasmus University Medical Center, Rotterdam, Netherlands

CORRESPONDING AUTHOR:

Romano Danesi
Unit of Clinical Pharmacology and Pharmacogenetics
Department of Clinical and Experimental Medicine
University Hospital of Pisa
55 via Roma
56126 Pisa, Italy
E-mail: romano.danesi@unipi.it
ORCID: 000-0002-4414-8934

Doi: 10.48286/aro.2021.09

History

Received: Jan 10, 2021

Accepted: Feb 12, 2021

Published: Mar 1, 2021

ABSTRACT

Pharmacogenetics investigates the molecular basis of inter-individual differences in drug metabolism and response. Sequence variations in genes encoding for metabolic enzymes may influence drug's pharmacokinetics and/or pharmacodynamics, resulting in reduced efficacy and/or adverse drug reactions. The dihydropyrimidine dehydrogenase (DPD) defi-

ciency is a clear example of how gene variants may affect fluoropyrimidines metabolism, and its evaluation has been recently recommended from regulatory agencies for its implementations in clinical routine. This review provides a summary of pharmacogenetic research on DPD and fluoropyrimidines metabolism, and its involvement in adverse drug reactions.

KEY WORDS

DPYD; 5-FU; capecitabine; chemotherapy; pharmacogenetic.

IMPACT STATEMENT

Dihydropyrimidine dehydrogenase (DPD) is the major responsible for fluoropyrimidines metabolism and its deficiency may lead to life-threatening toxicities. Although the frequency of DPD deleterious variants is low, their screening is clinically relevant to avoid severe toxicities or death in patients treated with fluoropyrimidine-based chemotherapy. While for the most studied variants the dose reduction to apply is already well known, future researches are necessary to understand the role of other DPD mutations, including the c.2194G > A variant, for which interest data have been recently published.

INTRODUCTION

Fluoropyrimidines, including 5-fluorouracil (5-FU) and its prodrug capecitabine (1), are the backbone of chemotherapy regimens for treatment of solid tumors, such as breast (2), colorectal (3), head and neck (4), gastrointestinal (5) and pancreatic cancers (6). They can be used either alone or in combination with irinotecan, platinum derivatives, cyclophosphamide, epirubicin and monoclonal antibodies (e.g., cetuximab and bevacizumab) (7-10).

Owing to the widespread use of these drugs, severe fluoropyrimidine-associated toxicity have been reported, including gastrointestinal toxicity (stomatitis, nausea/vomiting, diarrhea), skin toxicity (pigmentary abnormalities, conjunctivitis, hand-foot syndrome [HFS], skin rashes and hair loss in severe cases), haematological toxicity (neutropenia, leucopenia, anemia), and cardiac toxicity (arrhythmias and cardiac ischemia) (11). Neurologic abnormalities (cerebellar ataxia and changes in cognitive function) have also been reported in less than 1% of the population (11, 12). These toxicities may be the responsible of delays in drug administration or even treatment discontinuation, compromising its therapeutic benefit. Of note, capecitabine seems to display a different profile of toxicity compared to 5-FU, being characterized by a better tolerability, but higher incidence of HFS (13). Its oral administration has some advantages, particularly in patient's quality of life, and for this reason its use is becoming more diffuse in USA and Europe (14). Despite this, a number of patients develop severe, life-threatening toxicity due to fluoropyrimidine-related adverse events. It is well known that dihydropyrimidine dehydrogenase

(DPD) deficiency is at the basis of this toxicity (15), therefore, over the past years a growing number of research addressed the clinical effects of genetic variants in the DPD gene (DPYD), claiming their screening as pre-emptive strategy in patients candidate to fluoropyrimidines treatment. This review provides a summary of pharmacogenetic research on DPD and fluoropyrimidines metabolism and its involvement in adverse drug reactions, including relevant information about clinical implementation of DPD testing.

Fluoropyrimidines: metabolism and mechanism of action

Over 80% of 5-FU is transformed to inactive metabolites in the liver, and about 5% is the responsible for the therapeutic effect (16). The DPD enzyme catabolizes the first step of 5-FU metabolism, transforming the drug into the dihydrofluorouracil (5-FDHU) metabolite. The 5-FDHU is converted to fluoro-β-alanine (FBAL) by two additional enzymes, the dihydropyrimidinease (DPYS) and the beta-ureidopropionase (UPB1) (17), to be lastly excreted by the kidneys. The remaining 5-FU is converted directly or indirectly into fluorouridine monophosphate (FUMP). The phosphorylation of FUMP leads to the formation of FUTP or FdUDP, which is subsequently phosphorylated or dephosphorylated into the active metabolites FdUTP and FdUMP, respectively. FdUMP inhibits the thymidylate synthase (TS), leading to the inhibition of DNA replication (18) (**figure 1**).

Fluoropyrimidines, by acting as analogues of uracil, are able to interfere with the DNA synthesis at

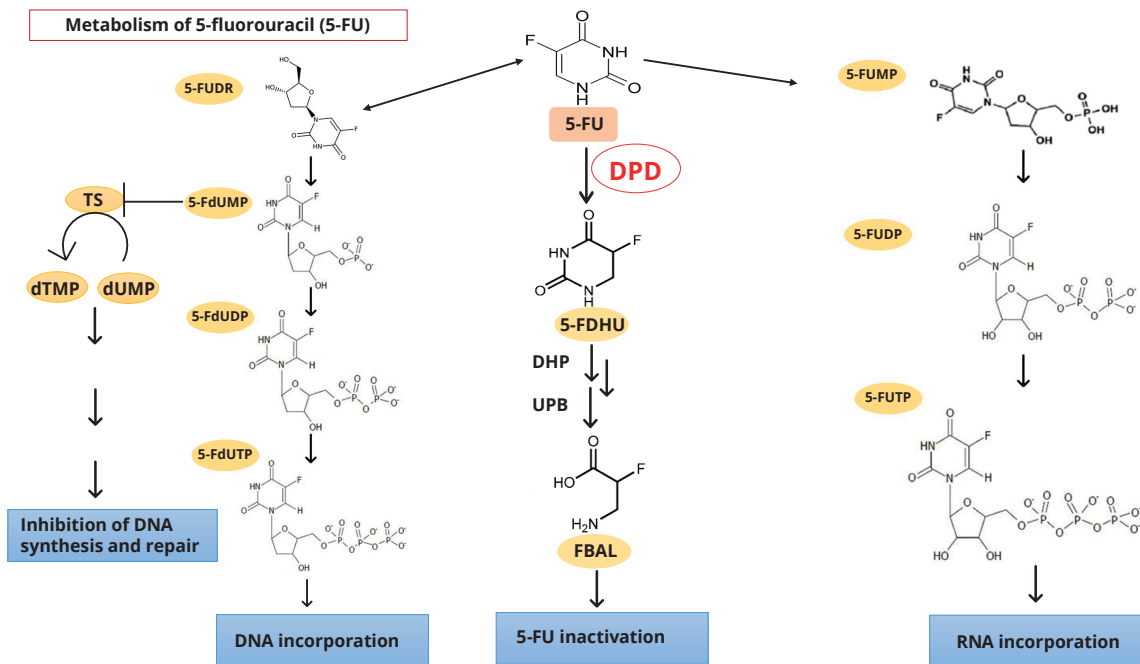


Figure 1. Metabolic pathway of 5-fluorouracil (5-FU).

DPD, dihydropyrimidine dehydrogenase; 5-FDHU, dihydrofluorouracil; DHP, dihydropyrimidinease; UPB, beta-ureidopropionase; F-b-AL, fluoro-b-alanine, FdUDP, fluorodeoxyuridine diphosphate; FdUMP, fluorodeoxyuridine monophosphate; FdUTP, fluorodeoxyuridine triphosphate; FUDP, fluorouridine diphosphate; FUDR, fluorodeoxyuridine; FUMP, fluoro-b-ureidopropionate; FUTP, fluorouridine triphosphate; TS, thymidylate synthase; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate.

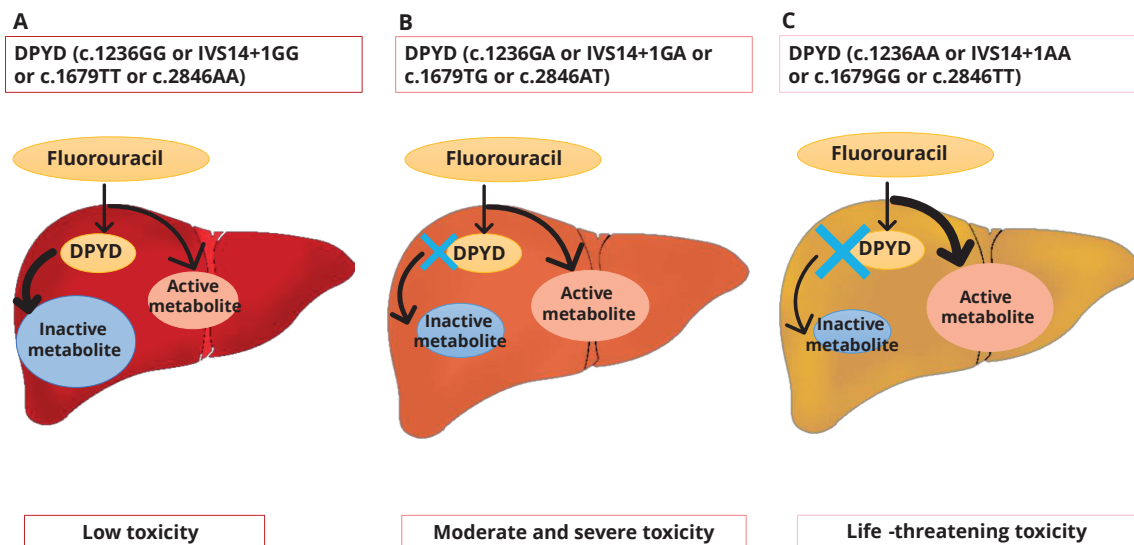


Figure 2. a. Schematic overview of fluoropyrimidine metabolisms and treatment related-toxicity in patients carrying wild-type; **b.** heterozygous or **c.** homozygous clinically relevant variants for DPYD gene.

different levels: 1) the antifolate 5-fluorodeoxyuridine monophosphate (5-FdUMP) covalently binds and inhibits TS, leading to a reduction of thymidine synthesis and of the DNA molecule; 2) the 5-fluorodeoxyuridine triphosphate (5-FdUTP) metabolite can be directly incorporated into genomic DNA causing damage; 3) the 5-fluorouridine triphos-

phate (5-FUTP) can be directly incorporated into genomic RNA causing damage (19).

DPYD variants conferring high risk for fluoropyrimidines toxicity

There is a link between a DPD deficiency and the occurrence of severe or life-threatening toxicity

due to fluoropyrimidines treatment. Patients with a significant DPD deficiency, receiving standard dose of fluoropyrimidine result in overexposure of 5-FU, with development of severe haematological and gastrointestinal toxicities (20) (**figure 2**). In these cases, the toxicity often occurs early after the first cycles of chemotherapy and it is characterized by grade 4 (WHO) symptoms and potentially death. High-grade of diarrhoea, mucositis and neutropenia are the most frequent reported side effects (21). DPD enzyme is encoded by the DPYD gene (22), which is located on the human chromosomal region 1p22, and is composed of 23 exons, spanning 950 Kbs (23). Over 90 among single nucleotide polymorphisms (SNPs) and mutations, including also deletion/insertion, have been described in the DPYD gene, with relevant functional consequences on enzymatic activity for some of them (20). The most clinically relevant DPYD variants reported with statistically significant association with severe toxicity include: c.1236G > A (E412E; rs56038477, haplotype B3), DPYD*13 (rs55886062, c.1679T > G, I560S), DPYD*2A (rs3918290, c.1905 + 1G > A, IVS14 + 1G > A), c.2846A > T (rs67376798, D949V) (24-26). Of these variants, DPYD*13, DPYD*2A and c.2846A > T have the most deleterious impact on DPD activity, whereas c.1236G > A results in moderate reduction of DPD activity and consequent toxicity (26, 27). The frequency of reduced DPD activity in the healthy population is estimated between 3-5% (28, 29). Indeed, a significant variability between ethnic subgroups was observed, and data obtained from phenotype and genotype analyses showed that Asian (30-32), African (33), and Caucasian (34) population present DPD deficiency at variable rates. DPYD*2A is the most studied mutation of the DPYD gene. It is associated with a significantly reduced enzyme activity of 50% in heterozygous patients, and a complete deficiency of enzyme activity in homozygous subjects, causing a life-threatening toxicity due to 5-FU accumulation (35). This splice site mutation in intron 14 changes the invariant junction donor site from G to A. As a result, a deletion of the entire exon 14 occurs and the truncated protein is catalytically inactive (36). Allele frequencies of DPYD*2A have been described to differ between ~ 0.1 and 1.0% in African-American and Caucasian population, respectively (37-39). Similarly, DPYD *13 and c.2846A > T substitution have been reported to be associated with partial or total loss enzyme activity, in heterozygous or homozygous patients, respectively (24, 34, 40). DPYD*13 is

characterised by an Ile560Ser amino acid change in the DPD binding domain, which lead to the destabilization of the protein (22, 41). In vitro study by Offer *et al.* showed that the homozygous expression of this variant resulted in a 75% reduction in enzyme activity respect to the wild-type (36). Allele frequencies of DPYD*13 were found to vary from 0.07 to 0.2% in the Caucasian population (38, 42). DPYD c.2846A > T results in an Asp949Val amino acid change localized near an iron-sulfur motif (41). Homozygous expression of the c.2846A > T variant results in 59% of activity compared to wild-type (37). Reported allele frequencies of c.2846A > T vary from 0.1 to 1.1% in African-Americans and Caucasians, respectively (34, 37, 38, 42). The synonymous variant c.1236G > A occurs in exon 11 and it is in complete linkage with haplotype B3 variants (c.483 + 18G > A, c.680 + 139G > A, c.959-51T > G and c.1129-5923C > G), resulting in a partial non-functional transcript, ranging from 44 to 50% of DPD activity for homozygous carriers (43, 44). The frequency of heterozygous patients in Caucasian population was reported to vary between 2.6 and 6.3% (44-47). Recently, a new variant, the DPYD*6 (rs1801160, c.2194G > A, p.V732I), has been associated with 5-FU related toxicity (27). Allele frequencies of DPYD*6 was found to vary from 1 to 7% in Caucasians, Asians and African Americans population (22, 48-50). However, conflicting results concerning the reduction of enzyme activity of this DPD variant have been published (36, 51, 52). Several studies published in literature highlighted the correlation between 5-FU toxicities and the above reported DPD variants (24, 38, 40, 53-57). A comprehensive pharmacogenetic analysis on 5-FU toxicity was conducted by Rosmarin *et al.* in 927 colorectal cancer patients enrolled in the QUASAR2 trial (55). The authors tested candidate polymorphisms for their associations with capecitabine-dependent toxicity, including diarrhea, nausea/vomiting, stomatitis, neutropenia, thrombocytopenia, and HFS. DPYD c.2846T > A and DPYD*2A mutations were associated with capecitabine-related overall (\geq grade 3) toxicity with a significant odds ratio of 5.51 ($p = 0.0013$) (55). Similarly, a meta-analysis by Terrazzino *et al.* highlighted a strong correlation between DPYD*2A and c.2846A > T variants and the development of high-grade toxicities (odds ratio 5.42, $p < 0.001$) (40), confirming the clinical validity of these SNPs as risk factors of 5-FU-related toxicities. A recent meta-analysis involving 7365 cancer patients with severe toxicity

related to 5-FU treatment (fluorouracil, capecitabine, or tegafur-uracil as single agents, in combination with other anticancer drugs or radiotherapy) showed that DPYD*13 and c.1236G > A/HapB3 DPYD variants, in addition to DPYD*2A, and c.2846A > T, were independent predictors of severe gastrointestinal and haematological fluoropyrimidine-associated toxicity (24). Patients carrying the DPYD*2A, c.1236G > A/HapB3, DPYD*13 and c.2846A > T had a relative risk for toxicity of 2.9 (95%CI: 1.8–4.6), 1.6 (95%CI: 1.3–2.0), 4.4 (95%CI: 2.1–9.3) and 3.0 (95%CI: 2.2–4.1), respectively (24). In contrast, in a study conducted on 603 cancer patients treated with fluoropyrimidine-based chemotherapy as neo-adjuvant/adjuvant or as first-line setting, retrospectively tested for 8 DPYD polymorphisms (c.496A > G, *2A, *4, *5, *6, *13, c.1896 T > C, c.2846A > T), showed that the association between DPYD*13 variant and severe toxicity was not statistically significant, probably because of the low frequency of the mutation (0.3%) (58). However, the study found DPYD*2A and c.2846A > T significantly associated to grade ≥ 3 toxicity ($p = 0.003$, $p = 0.048$), including neutropenia, diarrhea, leukopenia, stomatitis and nausea/vomiting (58). The retrospective DPYD analysis of the TOSCA trial, evaluated 10 DPYD variants for their associations with high-grade fluoropyrimidine-related adverse events in colorectal cancer patients, undergoing adjuvant fluoropyrimidine/oxaliplatin combination chemotherapy. The time-to-toxicity analysis highlighted the contribution of the DPYD*6 (rs1801160, c.2194G > A) variant to 5-FU toxicities, in particular, neutropenia (59). Similarly, in a secondary analysis of the PETACC-8 trial, the DPYD*6 was significantly associated with high-grade adverse events (60). Recently, also evaluated the role of 8 DPYD variants (c.496A > G, c.1236G > A/HapB3, c.1601G > A, c.1627A > G, DPYD*13, c.1896T > C, DPYD*2A, DPYD*6, c.2846A > T) in a cohort of 1254 patients, treated with fluoropyrimidine-containing regimens. A significant association between DPYD*6 variant, in addition to DPYD*2A and c.2846A > T, with gastrointestinal and hematological toxicities was found. Moreover, the study compared the DPYD variants found in the cohort of 982 patients with toxicity, to a control group of 272 patients receiving standard doses of fluoropyrimidine-based therapies, who had no dose reduction, delay or discontinuation of therapy due to toxicity. The association between the most frequent DPYD

polymorphisms c.496A > G, c.1601G > A, c.1627A > G, c.1896T > C and toxicity was not statistically significant, since those SNPs were also found in the control group (27). However, several studies highlighted the importance of other DPYD variants, including DPYD*9A, *3, and *4. DPYD*9A (rs1801265, c.85T > C) results in a Cys29Arg conversion in the crystal structure of the protein. Controversial results have been reported concerning the effect of this variant (61–64), as well as different effects in individuals of different ethnic background have been described. A study on Asian patients receiving fluorouracil-based chemotherapy, showed an association of the variant allele with treatment-related toxicity (65), however a reduced DPD activity was not observed when DPD activity was measured in blood samples (50). Indeed, studies in Caucasian patients did not find any effect of this variant on 5-FU clearance and toxicity (61, 66, 67). The DPYD*3 variant was reported at very low frequency (68), and has not been identified in any of the key studies of fluoropyrimidine toxicity or population studies (33, 48, 50, 69). The DPYD*4 (c.1601G > A, p.Ser534Asn) variant has been found at low frequencies of < 2% in Caucasians, Asians and African Americans (22, 48, 50). Controversial results have been published for this variant phenotype, with no correlation with DPD activity (48) or with case series where the variant was associated with severe fluorouracil-related toxicity (61, 70). Additional polymorphisms have been described in non-Caucasian population. Among African and American individuals, the common variant c.557A > G (rs115232898, p.Y186C) has been identified as a potential risk factor for 5-FU-related toxicity (71). DPYD*5 (rs1801159, c.1627A > G, p.Ile543Val) variant, located in exon 13, is reported in the Asian population with the highest frequency rate (72). Researches identified DPYD*5 and DPYD*9A, together with DPYD*2A as the common frequent SNPs in the Chinese population (73, 74). Several other variants have been frequently associated with fluoropyrimidine toxicities, including, c.257C > T, c.496A > G (p.M166V), c.680G > A, c.1801G > C, c.1850C > T, c.1896T > C, and c.2509-2510insC (21, 75–78). Falvella *et al.* investigated the association between DPYD variants c.496A > G, c.1129-5923C > G, c.1896T > C and capecitabine-related toxicity in 64 metastatic colorectal cancer patients enrolled in phase II trials, showing a significant association between DPYD c.496A > G and high grade (> 3) adverse events, including diarrhoea and neutropenia

(79). However, no association between c.1129-5923C > G and toxicity was found. Accordingly, the multivariate analysis reported by Lee *et al.*, failed to show a significant association of this DPYD variant with 5-FU related toxicity, suggesting a limited predictive value for severe toxicity to 5-FU-based chemotherapy (80). Similarly, Zhang *et al.* investigated the association between 4 DPYD SNPs (c.496A > G, c.1627A > G, c.2194G > A, c.*274T > C) and clinical outcomes of 362 Chinese gastric cancer patients treated with fluorouracil-based adjuvant chemotherapy. The authors found a significant association between c.1627A > G and clinical outcome after 5-FU-based regimens. However, no association between the occurrence of toxicities and the evaluated SNPs was found (81). Several studies identified novel rare DPYD variants associated with fluoropyrimidine toxicity (75, 77, 82-85). Three novel DPYD variants (c.2509-2510insC, c.1801G > C, and c.680G > A), together with other known sequence variants, were detected in patients experiencing unexpected life-threatening toxicities after treatment with 5-FU or capecitabine. The non-synonymous nature of these variants result in a conformational changes of the enzyme affecting DPD activity (76). Similarly, a treatment-related death case regarding a breast cancer patient treated with capecitabine and trastuzumab was reported (75). Four DPYD variants after sequencing analysis were identified: the above-mentioned c.496A > G and c.2194G > A, c.257C > T, causing deficient enzyme activity, and the c.1805C > T, leading to threonine-methionine amino acid change associated with reduced DPD activity (75). This report highlighted the dangerous effect of the combination of new DPYD variants as a possible cause of death in a patient treated with fluoropyrimidines. Likewise, van Kuilenburg *et al.* showed the effect of 2 DPYD variants (c.61C > T and *2A), located on different alleles, as responsible of lethal toxicity after the administration of 5-FU. The 2 DPYD mutations, presented in heterozygous manner, caused a complete deficiency of enzyme activity (86). Recently, Ly *et al.* reported a case of a patient with a rare variant of unknown significance in DPYD (rs755416212, c.704G.A, p.R235Q) who had a life-threatening toxicity capecitabine-related. An in silico tool (DPYD-Varifier (87)) confirmed the deleterious impact on the enzyme activity, and in vitro analysis confirmed the significantly reduced DPD activity by 88% compared to the wild-type DPD (88). Interestingly, the effect of the DPYD p.R235Q

variant was similar to the p.R235W (37), previously detected in a patient with DPD deficiency (62), and CPIC's guidelines for DPYD genotype-guided dosing incorporated this variant as a non-functional allele (26). DPYD-Varifier was also used by Shrestha *et al.* to predict the deleterious impact of DPYD c.394A > G (p.T132A) variant, discovered in a rectal cancer patient who experienced severe FU-associated toxicity during neoadjuvant therapy with capecitabine. In vitro and ex vivo approaches have been used to validate the deleterious function of this new variant. Based on these results the authors determined an activity score for the patient that was used to calculate a safe adjusted dose of FU for adjuvant therapy (83). Nevertheless, no confirmatory studies have been reported and their association with toxicity remains unclear.

Different approaches for DPD screening

DPD deficiency can be tested out by using different techniques, including the phenotype test (direct or indirect measurement of enzymatic activity) or by genotype testing (searching for the main functional polymorphisms of the DPYD gene). The phenotypic approach evaluates the activity of the enzyme (89). Several DPD activity measurement have been tested, including dosage in peripheral mononuclear blood cells (28, 90), and dosage by physiological ratio dihydrouracil/uracil (UH2/U) in plasma, serum, saliva, or urine (66, 91-94). Nevertheless, the application of these methods in clinical practice is complex, due to the special technical equipment and expertise required (95, 96). Indeed, DPD activity in peripheral blood mononuclear cells (PBMCs) may be influenced by several factors, including sampling and storage and cell heterogeneity (97). Conversely, the genotyping approach is more reliable, fast and there are fewer factors that can influence the result. The advantages of a genotyping test include that only a small blood sample is required for DNA extraction, and particular precautions (such as storage condition) are not necessary. The detection of DPYD genetic variants may be carried out by multiple technologies, usually available in laboratories involved in molecular analysis, such as Sanger Sequencing, Real Time PCR, Pyrosequencing, High Resolution Melting PCR. Several reports comparing the phenotyping and genotyping approach to predict DPD deficiency, reported conflicting results (89, 98-101). Coenen *et al.* provided an overview of 8 years of DPD testing in a single center (99), by using different methods,

including radiochemical and non-radiochemical assay by ultra HPLC-MS in PBMCs with uracil, and a combined enzymatic and genetic test by Sanger sequence analysis of 4 DPYD variants. The analysis showed that 18% of patients with a genetic variant had decreased enzyme activity ($p < 0.001$), suggesting a combination of the genetic and the enzymatic test for diagnostic use. Similarly, Pallet *et al.*, comparing the phenotype and genotype in a pretherapeutic screening of DPD deficiency, observed that the use of UH2/U might better reflect the impact of genetic variants on DPD activity (101). A multicenter prospective cohort study assessed the clinical benefit of pretherapeutic screening for DPD deficiency using a multiparametric approach by the calculator 5-FUODPM Tox™ (89, 102). The pre-therapeutic DPD assessment reduced the incidence of early severe toxicities associated with 5-FU, and avoided early toxic death (100). Accordingly, Capitain *et al.* showed a comparison of 4 screening methods (genotyping, phenotyping via plasma U and plasma UH2/U, and a multi-parametric approach) for detecting 5-FU toxicity risk in 472 cancer patients. A lower false negative rate (4.7%) resulted from the multi-parametric methods ($p < 0.001$), compared to genotype (59.8%) and phenotype (36.1% and 21.3% for U and UH2/U, respectively), resulting as the most effective method for limiting G4-5 toxicity. Recently, Etienne-Grimaldi *et al.* showed that the combined phenotyping and genotyping approach increased sensitivity to both grade 3-4 toxicity (16.7% for genotyping versus 20.8% for the combined approach) and grade 4 toxicity (20% for genotyping versus 66.7% for the combined approach) (100).

Pre-treatment DPYD genotyping and clinical implementations

DPYD genotyping pre-treatment screening, and a dose reduction in patients carrying a deleterious variant, is a useful strategy to prevent severe and potentially lethal fluoropyrimidine-related toxicity, without diminishing the treatment efficacy. A reduced fluoropyrimidine dose of 50% in DPYD*2A carriers is the necessary strategy to prevent the risk of severe toxicity, as reported by Deenen *et al.* (103). In fact, fluoropyrimidine-induced mortality rate is reported as reduced from 10% to 0% by genotype-guided dose assessment (103). In a prospective, multicentre, safety analysis, 1.103 patients were screened for 4 different DPYD variants (DPYD*2A, DPYD*13, c.2846A > T, c.1236G > A), with a genotype-guided dose reduction of 25-50%

based on DPYD genotype (50% for *2A or *13, 25% for c.2846A > T or c.1236G > A), improving patient safety with fluoropyrimidine treatment, and suggesting its genetic test implementation in routine clinical practice. As a result, the relative risk for severe fluoropyrimidine-related toxicity was lower in the cohort with the genotype-guided dosage compared to the historical cohort with no genotype-guided dosing. The authors also demonstrated a higher fluoropyrimidine-related severe toxicity in those patients carrying DPYD variants, than in wild-type patients (39% vs. 23%, $p = 0.0013$) (25). A small retrospective study successfully reported the implementation of routine pre-treatment DPYD genetic testing in patients with metastatic breast cancer treated with capecitabine (104). Seventy-two patients were eligible for capecitabine therapy and were tested for 4 DPYD genetic variants (DPYD*2A, DPYD*4, DPYD*13, c.2846A > T), based on their frequency in the British population, resulting associated with severe capecitabine-related toxicities. Five (8.4%) patients were found to carry a DPYD variant (*2A, *4 or c.2846A > T) associated with reduced DPD activity; of these, two received a 50% dose-reduction of capecitabine during their first cycle of treatment with no complications (104). A cohort of patients treated with fluoropyrimidine were tested for DPYD variants (DPYD*2A, DPYD*13, c.2846A > T, c.1236G > A) as part of routine practice. Two hundred and seventy-three (89.6%) out of 314 patients had a pre-treatment DPYD test result. Fourteen patients (5.1%) carried one or more DPYD gene variant and an initial dose reduction was recommended based on their genotype. None of these patients experienced severe toxicity (grade ≥ 3) (105).

The above-mentioned systematic meta-analyses (24, 40, 55) led researchers to evaluate the effective role of DPYD variants and their possibility to be screened as pre-treatment test in clinical routine. The Royal Dutch Association for the Advancement of Pharmacy's 'Pharmacogenetics Working Group' published a guideline supporting fluoropyrimidine-dose reduction for 14 DPYD variants (106). This guideline has been updated in a recent expert consensus guideline, by the Clinical Pharmacogenomics Implementation Consortium (CPIC), which reduced the number of variants to only those with robust supporting data (26). Accordingly, the Group of Clinical Pharmacology in Oncology (GPCO)-UNICANCER and the French Network of Pharmacogenetics (RN-PGx) (107), the German Society for Haematology and Medical Oncology (108), the Italian Association

DPYD-GENOTYPING GUIDELINES	DPYD GENOTYPE	5-FU DOSE-REDUCTION SUGGESTED
CLINICAL PHARMACOGENOMICS IMPLEMENTATION CONSORTIUM (CPI)	Two normal function alleles (*1/*1)	0%
	One normal function allele plus one no function allele or one decreased function allele (*1/2A; *1/13, *1/c.2846A > T, *1/c.1236G > A) or two decreased function allele (c.2846A > T/ c.2846A > T, c.1236G > A/ c.1236G > A)	25-50%
	Two decreased function alleles (*2A/*2A, *13/13) or one no function allele plus one decreased function allele (*2A/c.1236G > A, *2A/ c.2846A > T)	100%
GROUP OF CLINICAL PHARMACOLOGY IN ONCOLOGY (GPCO)-UNICANCER AND THE FRENCH NETWORK OF PHARMACOGENETICS (RNPGX)	Two normal function alleles (*1/*1)	0%
	One normal function allele plus one decreased function allele (*1/c.1236G > A or *1/c.2846A > T)	25-50%
	One normal function allele plus one no function allele (*1/*2A or *1/*13)	50%
	Two decreased function allele (*1/c.1236G > A and *1/c.2846A > T) or one reduced function allele plus one no function allele (combination of c.1236G > A or *1/c.2846A > T with *2A or *13, c.2846A > T)	Strongly reduced initial doses with drug monitoring
	Two no function alleles (*2A/*2A, *13/*13)	100%
ITALIAN ASSOCIATION OF MEDICAL ONCOLOGY AND THE ITALIAN SOCIETY OF PHARMACOLOGY (AIOM-SIF WORKING GROUP)	Two normal function alleles (*1/*1)	0%
	One normal function allele plus one decreased function allele (*1/c.1236G > A)	25%
	Patients who developed toxicity carrying *1/c.2194G > A alleles	15%
	One normal function allele plus one no function allele (*1/*2A, *1/*13 or *1/c.2846A > T)	50%
	Two decreased function alleles (c.1236G > A)	50%
	Two no function alleles (*2A/*2A, *13/*13, c.2846A > T)	100%
	Patients who developed toxicity carrying two decreased function alleles (c.2194G > A)	30%
DUTCH PHARMACOGENETICS WORKING GROUP (DPWG)	Two normal function alleles (*1/*1)	0%
	One normal function allele plus one decreased function allele (*1/c.1236G > A or *1/c.2846A > T)	25%
	One normal function allele plus one no function allele (*1/*2A or *1/*13)	50%
	Two decreased function alleles (*2A/*2A, *13/*13)	100%

Table I. DPYD-genotyping guidelines.

of Medical Oncology and the Italian Society of Pharmacology (AIOM-SIF working group) (109), and the Dutch Pharmacogenetics Working Group (DPWG)

(110) published national recommendations or consensus papers for DPYD screening (**table I**). Recently, the European Medicines Agency (EMA) published

a recommendation on DPD testing for patients prior to treatment with fluorouracil, capecitabine, tegafur (111), highlighting the importance of DPYD screening. Interestingly, a practitioner-friendly guide for clinicians to decide about DPYD genotyping testing prior to starting fluoropyrimidine-based chemotherapy has been published (112).

CONCLUSIONS

DPD-deficiency is an important leading cause of fluoropyrimidine-associated severe toxicity. Because of the extensive use of these anticancer agents, and the availability of feasible genotyping methods (*i.e.*, SNP genotyping by Real Time), the application of DPD variants screening has become easily accessible for clinical laboratories. To date, several consensuses have been published concerning the clinical application of DPD screening in the population of patients candidates to fluoropyrimidine therapy, since patients carrying the deleterious DPYD*2A, DPYD*13 and c.2846T > C variant alleles display severe toxicities, which may be life-threatening in homozygous subjects. Although their frequencies are low, the screening for DPD variants is clinically relevant to avoid severe toxicities or death

in patients treated with fluoropyrimidine-based chemotherapy. Future studies are necessary to highlight the correlation with toxicity of other DPYD SNPs, including the c.2194G > A variant, for which interesting data have been recently reported.

ETHICS

Fundings

There were no institutional or private funding for this article.

Conflict of interests

The authors have declared no conflict of interests.

Availability of data and material

N/A

Authors' contribution

SC, FS, AC, FC, CR draft the manuscript; all authors revised the draft and contributed to the final version.

Ethical approval

N/A

REFERENCES

- Miura K, Kinouchi M, Ishida K, et al. 5-fu metabolism in cancer and orally-administrable 5-fu drugs. *Cancers (Basel)* 2010;2(3):1717-30.
- Cameron DA, Gabra H, Leonard RC. Continuous 5-fluorouracil in the treatment of breast cancer. *Br J Cancer* 1994;70(1):120-4.
- Vodenkova S, Buchler T, Cervena K, Veskrnova V, Vodicka P, Vymetalkova V. 5-fluorouracil and other fluoropyrimidines in colorectal cancer: Past, present and future. *Pharmacol Ther* 2020;206:107447.
- Fu KK. Radiation Therapy With 5-fluorouracil in Head and Neck Cancer. *Semin Radiat Oncol* 1997;7(4):274-82.
- Van Cutsem E, Peeters M. Developments in fluoropyrimidine therapy for gastrointestinal cancer. *Curr Opin Oncol* 1999;11(4):312-7.
- Conroy T, Desseigne F, Ychou M, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med* 2011;364(19):1817-25.
- Fischel JL, Rostagno P, Formento P, Dubreuil A, Etienne MC, Milano G. Ternary combination of irinotecan, fluorouracil-folinic acid and oxalipatin: results on human colon cancer cell lines. *Br J Cancer* 2001;84(4):579-85.
- Periti P, Pannuti F, Della Cuna GR, et al. Combination chemotherapy with cyclophosphamide, fluorouracil, and either epirubicin or mitoxantrone: a comparative randomized multicenter study in metastatic breast carcinoma. *Cancer Invest* 1991;9(3):249-55.
- Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004;350(23):2335-42.
- Min BS, Kim NK, Ahn JB, et al. Cetuximab in combination with 5-fluorouracil, leucovorin and irinotecan as a neoadjuvant chemotherapy in patients with initially unresectable colorectal liver metastases. *Onkologie* 2007;30(12):637-43.

11. Wigmore PM, Mustafa S, El-Beltagy M, Lyons L, Umka J, Bennett G. Effects of 5-FU. *Adv Exp Med Biol* 2010;678:157-64.
12. Han R, Yang YM, Dietrich J, Luebke A, Mayer-Proschel M, Noble M. Systemic 5-fluorouracil treatment causes a syndrome of delayed myelin destruction in the central nervous system. *J Biol* 2008;7(4):12.
13. Walko CM, Lindley C. Capecitabine: a review. *Clin Ther* 2005;27(1):23-44.
14. McGavin JK, Goa KL. Capecitabine: a review of its use in the treatment of advanced or metastatic colorectal cancer. *Drugs* 2001;61(15):2309-26.
15. Boisdron-Celle M, Morel A, Gamelin E. Dihydropyrimidine dehydrogenase deficiency and toxicity to fluoropyrimidine. *Ann Biol Clin (Paris)* 2010;68(1):27-32.
16. Longley DB, Harkin DP, Johnston PG. 5-fluorouracil: mechanisms of action and clinical strategies. *Nat Rev Cancer* 2003;3(5):330-8.
17. van Kuilenburg AB, Meinsma R, Zonnenberg BA, et al. Dihydropyrimidinase deficiency and severe 5-fluorouracil toxicity. *Clin Cancer Res* 2003;9(12):4363-7.
18. Houghton JA, Morton CL, Adkins DA, Rahman A. Locus of the interaction among 5-fluorouracil, leucovorin, and interferon-alpha 2a in colon carcinoma cells. *Cancer Res* 1993;53(18):4243-50.
19. Noordhuis P, Holwerda U, Van der Wilt CL, et al. 5-Fluorouracil incorporation into RNA and DNA in relation to thymidylate synthase inhibition of human colorectal cancers. *Ann Oncol* 2004;15(7):1025-32.
20. Omura K. Clinical implications of dihydropyrimidine dehydrogenase (DPD) activity in 5-FU-based chemotherapy: mutations in the DPD gene, and DPD inhibitory fluoropyrimidines. *Int J Clin Oncol* 2003;8(3):132-8.
21. Gross E, Busse B, Riemenschneider M, et al. Strong association of a common dihydropyrimidine dehydrogenase gene polymorphism with fluoropyrimidine-related toxicity in cancer patients. *PLoS One* 2008;3(12):e4003.
22. Wei X, Elizondo G, Sapone A, et al. Characterization of the human dihydropyrimidine dehydrogenase gene. *Genomics* 1998;51(3):391-400.
23. Takai S, Fernandez-Salguero P, Kimura S, Gonzalez FJ, Yamada K. Assignment of the human dihydropyrimidine dehydrogenase gene (DPYD) to chromosome region 1p22 by fluorescence in situ hybridization. *Genomics* 1994;24(3):613-4.
24. Meulendijks D, Henricks LM, Sonke GS, et al. Clinical relevance of DPYD variants c.1679T > G, c.1236G > A/HapB3, and c.1601G > A as predictors of severe fluoropyrimidine-associated toxicity: a systematic review and meta-analysis of individual patient data. *Lancet Oncol* 2015;16(16):1639-50.
25. Henricks LM, Lunenburg C, de Man FM, et al. DPYD genotype-guided dose individualisation of fluoropyrimidine therapy in patients with cancer: a prospective safety analysis. *Lancet Oncol* 2018;19(11):1459-67.
26. Amstutz U, Henricks LM, Offer SM, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Dihydropyrimidine Dehydrogenase Genotype and Fluoropyrimidine Dosing: 2017 Update. *Clin Pharmacol Ther* 2018;103(2):210-6.
27. Del Re M, Cinieri S, Michelucci A, et al. DPYD*6 plays an important role in fluoropyrimidine toxicity in addition to DPYD*2A and c.2846A > T: a comprehensive analysis in 1254 patients. *Pharmacogenomics J* 2019;19(6):556-63.
28. Etienne MC, Lagrange JL, Dassonville O, et al. Population study of dihydropyrimidine dehydrogenase in cancer patients. *J Clin Oncol* 1994;12(11):2248-53.
29. Lu Z, Zhang R, Carpenter JT, Diasio RB. Decreased dihydropyrimidine dehydrogenase activity in a population of patients with breast cancer: implication for 5-fluorouracil-based chemotherapy. *Clin Cancer Res* 1998;4(2):325-9.
30. Ogura K, Ohnuma T, Minamide Y, et al. Dihydropyrimidine dehydrogenase activity in 150 healthy Japanese volunteers and identification of novel mutations. *Clin Cancer Res* 2005;11(14):5104-11.
31. Yamaguchi K, Arai Y, Kanda Y, Akagi K. Germline mutation of dihydropyrimidine dehydrogenase gene among a Japanese population in relation to toxicity to 5-Fluorouracil. *Jpn J Cancer Res* 2001;92(3):337-42.
32. Saif MW, Mattison L, Carollo T, Ezzeldin H, Diasio RB. Dihydropyrimidine dehydrogenase deficiency in an Indian population. *Cancer Chemother Pharmacol* 2006;58(3):396-401.
33. Hamdy SI, Hiratsuka M, Narahara K, et al. Allele and genotype frequencies of polymorphic cytochromes P450 (CYP2C9, CYP2C19, CYP2E1) and dihydropyrimidine dehydrogenase (DPYD) in the Egyptian population. *Br J Clin Pharmacol* 2002;53(6):596-603.
34. Seck K, Riemer S, Kates R, et al. Analysis of the DPYD gene implicated in 5-fluorouracil catab-

- olism in a cohort of Caucasian individuals. *Clin Cancer Res* 2005;11(16):5886-92.
35. Van Kuilenburg AB, Meinsma R, Zoetekouw L, Van Gennip AH. Increased risk of grade IV neutropenia after administration of 5-fluorouracil due to a dihydropyrimidine dehydrogenase deficiency: high prevalence of the IVS14 + 1g > a mutation. *Int J Cancer* 2002;101(3):253-8.
 36. Offer SM, Wegner NJ, Fossum C, Wang K, Diasio RB. Phenotypic profiling of DPYD variations relevant to 5-fluorouracil sensitivity using real-time cellular analysis and in vitro measurement of enzyme activity. *Cancer Res* 2013;73(6):1958-68.
 37. Offer SM, Fossum CC, Wegner NJ, Stuflessen AJ, Butterfield GL, Diasio RB. Comparative functional analysis of DPYD variants of potential clinical relevance to dihydropyrimidine dehydrogenase activity. *Cancer Res* 2014;74(9):2545-54.
 38. Lee AM, Shi Q, Pavey E, et al. DPYD variants as predictors of 5-fluorouracil toxicity in adjuvant colon cancer treatment (NCCTG N0147). *J Natl Cancer Inst* 2014;106(12).
 39. van Kuilenburg AB, Muller EW, Haasjes J, et al. Lethal outcome of a patient with a complete dihydropyrimidine dehydrogenase (DPD) deficiency after administration of 5-fluorouracil: frequency of the common IVS14 + 1G > A mutation causing DPD deficiency. *Clin Cancer Res* 2001;7(5):1149-53.
 40. Terrazzino S, Cargnin S, Del Re M, Danesi R, Canonico PL, Genazzani AA. DPYD IVS14 + 1G > A and 2846A > T genotyping for the prediction of severe fluoropyrimidine-related toxicity: a meta-analysis. *Pharmacogenomics* 2013;14(11):1255-72.
 41. van Kuilenburg AB, Dobritzsch D, Meinsma R, Haasjes J, Waterham HR, Nowaczyk MJ, et al. Novel disease-causing mutations in the dihydropyrimidine dehydrogenase gene interpreted by analysis of the three-dimensional protein structure. *Biochem J* 2002;364(Pt 1):157-63.
 42. Caudle KE, Thorn CF, Klein TE, et al. Clinical Pharmacogenetics Implementation Consortium guidelines for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing. *Clin Pharmacol Ther* 2013;94(6):640-5.
 43. Meulendijks D, Henricks LM, van Kuilenburg AB, et al. Patients homozygous for DPYD c.1129-5923C > G/haplotype B3 have partial DPD deficiency and require a dose reduction when treated with fluoropyrimidines. *Cancer Chemother Pharmacol* 2016;78(4):875-80.
 44. van Kuilenburg AB, Meijer J, Mul AN, et al. Intragenic deletions and a deep intronic mutation affecting pre-mRNA splicing in the dihydropyrimidine dehydrogenase gene as novel mechanisms causing 5-fluorouracil toxicity. *Hum Genet* 2010;128(5):529-38.
 45. Amstutz U, Farese S, Aebi S, Largiader CR. Dihydropyrimidine dehydrogenase gene variation and severe 5-fluorouracil toxicity: a haplotype assessment. *Pharmacogenomics* 2009;10(6):931-44.
 46. Deenen MJ, Tol J, Burylo AM, 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(10):3455-68.
 47. Sistonen J, Buchel B, Froehlich TK, et al. Predicting 5-fluorouracil toxicity: DPD genotype and 5,6-dihydrouracil:uracil ratio. *Pharmacogenomics* 2014;15(13):1653-66.
 48. Ridge SA, Sludden J, Brown O, et al. Dihydropyrimidine dehydrogenase pharmacogenetics in Caucasian subjects. *Br J Clin Pharmacol* 1998;46(2):151-6.
 49. Maekawa K, Saeki M, Saito Y, et al. Genetic variations and haplotype structures of the DPYD gene encoding dihydropyrimidine dehydrogenase in Japanese and their ethnic differences. *J Hum Genet* 2007;52(10):804-19.
 50. He YF, Wei W, Zhang X, et al. Analysis of the DPYD gene implicated in 5-fluorouracil catabolism in Chinese cancer patients. *J Clin Pharm Ther* 2008;33(3):307-14.
 51. Offer SM, Lee AM, Mattison LK, Fossum C, Wegner NJ, Diasio RB. A DPYD variant (Y186C) in individuals of african ancestry is associated with reduced DPD enzyme activity. *Clin Pharmacol Ther* 2013;94(1):158-66.
 52. Kleibl Z, Fidlerova J, Kleiblova P, et al. Influence of dihydropyrimidine dehydrogenase gene (DPYD) coding sequence variants on the development of fluoropyrimidine-related toxicity in patients with high-grade toxicity and patients with excellent tolerance of fluoropyrimidine-based chemotherapy. *Neoplasma* 2009;56(4):303-16.
 53. Saif MW, Diasio R. Is capecitabine safe in patients with gastrointestinal cancer and dihydropyrimidine dehydrogenase deficiency? *Clin Colorectal Cancer* 2006;5(5):359-62.

54. van Kuilenburg AB, Largiader CR. SNPs and Haplotypes in DPYD and Outcome of Capecitabine-Letter. *Clin Cancer Res* 2011;17(17):5837; author reply 5-6.
55. Rosmarin D, Palles C, Church D, et al. Genetic markers of toxicity from capecitabine and other fluorouracil-based regimens: investigation in the QUASAR2 study, systematic review, and meta-analysis. *J Clin Oncol* 2014;32(10):1031-9.
56. Gbeto CC, Quaranta S, Mari R, et al. Lethal toxicities after capecitabine intake in a previously 5-FU-treated patient: why dose matters with dihydropyrimidine dehydrogenase deficiency. *Pharmacogenomics* 2019;20(13):931-8.
57. Shakeel F, Fang F, Kwon JW, et al. Patients carrying DPYD variant alleles have increased risk of severe toxicity and related treatment modifications during fluoropyrimidine chemotherapy. *Pharmacogenomics* 2021.
58. Toffoli G, Giodini L, Buonadonna A, et al. Clinical validity of a DPYD-based pharmacogenetic test to predict severe toxicity to fluoropyrimidines. *Int J Cancer* 2015;137(12):2971-80.
59. Ruzzo A, Graziano F, Galli F, et al. Dihydropyrimidine dehydrogenase pharmacogenetics for predicting fluoropyrimidine-related toxicity in the randomised, phase III adjuvant TOSCA trial in high-risk colon cancer patients. *Br J Cancer* 2017;117(9):1269-77.
60. Boige V, Vincent M, Alexandre P, et al. DPYD Genotyping to Predict Adverse Events Following Treatment With Fluorouracil-Based Adjuvant Chemotherapy in Patients With Stage III Colon Cancer: A Secondary Analysis of the PETACC-8 Randomized Clinical Trial. *JAMA Oncol* 2016;2(5):655-62.
61. Gross E, Ullrich T, Seck K, et al. Detailed analysis of five mutations in dihydropyrimidine dehydrogenase detected in cancer patients with 5-fluorouracil-related side effects. *Hum Mutat* 2003;22(6):498.
62. Vreken P, Van Kuilenburg AB, Meinsma R, van Gennip AH. Dihydropyrimidine dehydrogenase (DPD) deficiency: identification and expression of missense mutations C29R, R886H and R235W. *Hum Genet* 1997;101(3):333-8.
63. Khushman M, Patel GK, Hosein PJ, et al. Germline pharmacogenomics of DPYD*9A (c.85T > C) variant in patients with gastrointestinal malignancies treated with fluoropyrimidines. *J Gastrointest Oncol* 2018;9(3):416-24.
64. Detailleur S, Segelov E, Re MD, Prenen H. Dihydropyrimidine dehydrogenase deficiency in patients with severe toxicity after 5-fluorouracil: a retrospective single-center study. *Ann Gastroenterol* 2021;34(1):68-72.
65. Zhang H, Li YM, Zhang H, Jin X. DPYD*5 gene mutation contributes to the reduced DPYD enzyme activity and chemotherapeutic toxicity of 5-FU: results from genotyping study on 75 gastric carcinoma and colon carcinoma patients. *Med Oncol* 2007;24(2):251-8.
66. Boisdron-Celle M, Remaud G, TrARoe S, et al. 5-Fluorouracil-related severe toxicity: a comparison of different methods for the pretherapeutic detection of dihydropyrimidine dehydrogenase deficiency. *Cancer Lett* 2007;249(2):271-82.
67. Morel A, Boisdron-Celle M, Fey L, et al. Clinical relevance of different dihydropyrimidine dehydrogenase gene single nucleotide polymorphisms on 5-fluorouracil tolerance. *Mol Cancer Ther* 2006;5(11):2895-904.
68. Van Kuilenburg AB, Vreken P, Abeling NG, et al. Genotype and phenotype in patients with dihydropyrimidine dehydrogenase deficiency. *Hum Genet* 1999;104(1):1-9.
69. Hsiao HH, Yang MY, Chang JG, et al. Dihydropyrimidine dehydrogenase pharmacogenetics in the Taiwanese population. *Cancer Chemother Pharmacol* 2004;53(5):445-51.
70. Lazar A, Mau-Holzmann UA, Kolb H, Reichenmiller HE, Riess O, Schomig E. Multiple organ failure due to 5-fluorouracil chemotherapy in a patient with a rare dihydropyrimidine dehydrogenase gene variant. *Onkologie* 2004;27(6):559-62.
71. Cunha-Junior GF, Bastos-Rodrigues L, Azevedo PG, et al. Prevalence of the DPYD variant (Y186C) in Brazilian individuals of African ancestry. *Cancer Chemother Pharmacol* 2019;84(6):1359-63.
72. Teh LK, Hamzah S, Hashim H, et al. Potential of dihydropyrimidine dehydrogenase genotypes in personalizing 5-fluorouracil therapy among colorectal cancer patients. *Ther Drug Monit* 2013;35(5):624-30.
73. Li GY, Duan JF, Li WJ, Liu T. DPYD*2A/*5A/*9A and UGT1A1*6/*28 polymorphisms in Chinese colorectal cancer patients. *J Cancer Res Ther* 2016;12(2):782-6.
74. Hariprakash JM, Vellarikkal SK, Keechilat P, et al. Pharmacogenetic landscape of DPYD variants in south Asian populations by integration of genome-scale data. *Pharmacogenomics* 2018;19(3):227-41.

75. Del Re M, Qua Quarini E, Sottotetti F, et al. Uncommon dihydropyrimidine dehydrogenase mutations and toxicity by fluoropyrimidines: a lethal case with a new variant. *Pharmacogenomics* 2016;17(1):5-9.
76. Del Re M, Michelucci A, Di Leo A, et al. Discovery of novel mutations in the dihydropyrimidine dehydrogenase gene associated with toxicity of fluoropyrimidines and viewpoint on preemptive pharmacogenetic screening in patients. *EPMA J* 2015;6(1):17.
77. Palmirotta R, Lovero D, Delacour H, Le Roy A, Cremades S, Silvestris F. Rare Dihydropyrimidine Dehydrogenase Variants and Toxicity by Fluoropyrimidines: A Case Report. *Front Oncol* 2019;9:139.
78. Hamzic S, Scharer D, Offer S, et al. Haplotype structure defines effects of common DPYD variants c.85T > C (rs1801265) and c.496A > G (rs2297595) on DPD activity: implication for 5-fluorouracil toxicity. *Br J Clin Pharmacol* 2021.
79. Falvella FS, Cheli S, Martinetti A, et al. DPD and UGT1A1 deficiency in colorectal cancer patients receiving triplet chemotherapy with fluoropyrimidines, oxaliplatin and irinotecan. *Br J Clin Pharmacol* 2015;80(3):581-8.
80. Lee AM, Shi Q, Alberts SR, et al. Association between DPYD c.1129-5923 C > G/hapB3 and severe toxicity to 5-fluorouracil-based chemotherapy in stage III colon cancer patients: NCCTG N0147 (Alliance). *Pharmacogenet Genomics* 2016;26(3):133-7.
81. Zhang XP, Bai ZB, Chen BA, et al. Polymorphisms of dihydropyrimidine dehydrogenase gene and clinical outcomes of gastric cancer patients treated with fluorouracil-based adjuvant chemotherapy in Chinese population. *Chin Med J (Engl)* 2012;125(5):741-6.
82. van Kuilenburg AB, Meijer J, Maurer D, 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(3):721-30.
83. Shrestha S, Tapper EE, Trogstad-Isaacson CS, Hobday TJ, Offer SM, Diasio RB. Dose modification for safe treatment of a compound complex heterozygous DPYD variant carrier with 5-fluorouracil. *JCO Precis Oncol* 2018;2.
84. Tong CC, Lam CW, Lam KO, Lee VHF, Luk MY. A Novel DPYD Variant Associated With Severe Toxicity of Fluoropyrimidines: Role of Pre-emptive DPYD Genotype Screening. *Front Oncol* 2018;8:279.
85. Garcia-Gonzalez X, Kaczmarczyk B, Abarca-Zaballa J, et al. New DPYD variants causing DPD deficiency in patients treated with fluoropyrimidine. *Cancer Chemother Pharmacol* 2020;86(1):45-54.
86. van Kuilenburg AB, Baars JW, Meinsma R, van Gennip AH. Lethal 5-fluorouracil toxicity associated with a novel mutation in the dihydropyrimidine dehydrogenase gene. *Ann Oncol* 2003;14(2):341-2.
87. Shrestha S, Zhang C, Jerde CR, et al. Gene-Specific Variant Classifier (DPYD-Varifier) to Identify Deleterious Alleles of Dihydropyrimidine Dehydrogenase. *Clin Pharmacol Ther* 2018;104(4):709-18.
88. Ly RC, Schmidt RE, Kiel PJ, et al. Severe Capecitabine Toxicity Associated With a Rare DPYD Variant Identified Through Whole-Genome Sequencing. *JCO Precis Oncol* 2020;4.
89. Boisdron-Celle M, Capitain O, Faroux R, et al. Prevention of 5-fluorouracil-induced early severe toxicity by pre-therapeutic dihydropyrimidine dehydrogenase deficiency screening: Assessment of a multiparametric approach. *Semin Oncol* 2017;44(1):13-23.
90. Bocci G, Barbara C, Vannozzi F, et al. A pharmacokinetic-based test to prevent severe 5-fluorouracil toxicity. *Clin Pharmacol Ther* 2006;80(4):384-95.
91. Ciccolini J, Mercier C, Evrard A, et al. A rapid and inexpensive method for anticipating severe toxicity to fluorouracil and fluorouracil-based chemotherapy. *Ther Drug Monit* 2006;28(5):678-85.
92. Wettergren Y, Carlsson G, Odin E, Gustavsson B. Pretherapeutic uracil and dihydrouracil levels of colorectal cancer patients are associated with sex and toxic side effects during adjuvant 5-fluorouracil-based chemotherapy. *Cancer* 2012;118(11):2935-43.
93. Meulendijks D, Henricks LM, Jacobs BAW, et al. Pretreatment serum uracil concentration as a predictor of severe and fatal fluoropyrimidine-associated toxicity. *Br J Cancer* 2017;116(11):1415-24.
94. van Staveren MC, van Kuilenburg AB, Guchelaar HJ, et al. Evaluation of an oral uracil loading test to identify DPD-deficient patients using a limited sampling strategy. *Br J Clin Pharmacol* 2016;81(3):553-61.
95. Di Paolo A, Danesi R, Falcone A, et al. Relationship between 5-fluorouracil disposition, toxicity and dihydropyrimidine dehydrogenase activity in cancer patients. *Ann Oncol* 2001;12(9):1301-6.

96. Van Kuilenburg AB, Van Lenthe H, Van Gennip AH. Radiochemical assay for determination of dihydropyrimidinase activity using reversed-phase high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 1999;729(1-2):307-14.
97. Van Kuilenburg AB, van Lenthe H, Blom MJ, Mul EP, Van Gennip AH. Profound variation in dihydropyrimidine dehydrogenase activity in human blood cells: major implications for the detection of partly deficient patients. *Br J Cancer* 1999;79(3-4):620-6.
98. Capitain O, Seegers V, Metges JP, et al. Comparison of 4 Screening Methods for Detecting Fluoropyrimidine Toxicity Risk: Identification of the Most Effective, Cost-Efficient Method to Save Lives. *Dose Response* 2020;18(3):1559325820951367.
99. Coenen MJH, Paulussen ADC, Breuer M, et al. 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.
100. Etienne-Grimaldi MC, Boyer JC, Beroud C, et al. New advances in DPYD genotype and risk of severe toxicity under capecitabine. *PLoS One* 2017;12(5):e0175998.
101. Pallet N, Hamdane S, Garinet S, et al. A comprehensive population-based study comparing the phenotype and genotype in a pretherapeutic screen of dihydropyrimidine dehydrogenase deficiency. *Br J Cancer* 2020;123(5):811-8.
102. Available at: <http://www.odpm.fr>. Accessed: 04/02/2021.
103. Deenen MJ, Meulendijks D, Cats A, et al. Upfront Genotyping of DPYD*2A to Individualize Fluoropyrimidine Therapy: A Safety and Cost Analysis. *J Clin Oncol* 2016;34(3):227-34.
104. Stavraka C, Pouptsis A, Okonta L, et al. Clinical implementation of pre-treatment DPYD genotyping in capecitabine-treated metastatic breast cancer patients. *Breast Cancer Res Treat* 2019;175(2):511-7.
105. Lunenburg C, Henricks LM, Guchelaar HJ, et al. Prospective DPYD genotyping to reduce the risk of fluoropyrimidine-induced severe toxicity: Ready for prime time. *Eur J Cancer* 2016;54:40-8.
106. Swen JJ, Nijenhuis M, de Boer A, et al. Pharmacogenetics: from bench to byte--an update of guidelines. *Clin Pharmacol Ther* 2011;89(5):662-73.
107. Lorient MA, Ciccolini J, Thomas F, et al. Dihydropyrimidine dehydrogenase (DPD) deficiency screening and securing of fluoropyrimidine-based chemotherapies: Update and recommendations of the French GPCO-Unicancer and RNPgX networks. *Bull Cancer* 2018;105(4):397-407.
108. Wormann B, Bokemeyer C, Burmeister T, et al. Dihydropyrimidine Dehydrogenase Testing prior to Treatment with 5-Fluorouracil, Capecitabine, and Tegafur: A Consensus Paper. *Oncol Res Treat* 2020;43(11):628-36.
109. AIOM-SIF Working group. Recommendations 2019 for pharmacogenetic analyses, 2019- Available at: https://www.aiom.it/wp-content/uploads/2019/10/2019_Racc-analisi-farmacogenetiche_v26.3.2020.pdf. Accessed: 10/01/2021.
110. Lunenburg C, van der Wouden CH, Nijenhuis M, et al. Dutch Pharmacogenetics Working Group (DPWG) guideline for the gene-drug interaction of DPYD and fluoropyrimidines. *Eur J Hum Genet* 2020;28(4):508-17.
111. EMA/367286/2020 (2020). EMA recommendations on DPD testing prior to treatment with fluorouracil, capecitabine, tegafur and flucytosine. Available at: https://www.ema.europa.eu/en/documents/referral/fluorouracil-fluorouracil-related-substances-article-31-referral-new-testing-treatment_en.pdf. Accessed: 10/01/2021.
112. Innocenti F, Mills SC, Sanoff H, Ciccolini J, Lenz HJ, Milano G. All You Need to Know About DPYD Genetic Testing for Patients Treated With Fluorouracil and Capecitabine: A Practitioner-Friendly Guide. *JCO Oncol Pract* 2020;16(12):793-8.