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Review

Current diagnostic and clinical issues of screening for dihydropyrimidine dehydrogenase deficiency

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Abstract Fluoropyrimidine drugs (FP) are the backbone of many chemotherapy protocols for treating solid tumours. The rate-limiting step of fluoropyrimidine catabolism is dihydropyrimidine dehydrogenase (DPD), and deficiency in DPD activity can result in severe and even fatal toxicity. In this review, we survey the evidence-based pharmacogenetics and therapeutic recommendations regarding *DPYD* (the gene encoding DPD) genotyping and DPD phenotyping to prevent toxicity and optimize dosing adaptation before FP administration. The French experience of mandatory DPD-deficiency screening prior to initiating FP is discussed.

1. Introduction

Fluoropyrimidine drugs (FP), 5-fluorouracil (5FU), capecitabine, and tegafur-based treatment (UFT, S1) are administered in 50% of patients receiving chemotherapy for solid tumours [1] mostly for treatments of digestive, breast, and head and neck cancers [2,3]. While the majority of patients can be treated safely, 10%–40% of patients will experience severe FP-associated toxicities (grade ≥3 according to the Common Terminology Criteria for Adverse Events (CTCAE)), mainly haematological and digestive, being lethal in 0.1%–1% of patients [1,4–11]. When not directly life-threatening, these toxicities may affect patient quality of life and treatment efficacy related due to discontinuation or postponement of chemotherapy cycles, and generate additional costs such as prolonged hospital stay [12]. The most prevalent biochemical cause of FP toxicities is a deficiency of the catabolic enzyme dihydropyrimidine dehydrogenase (DPD). Patients with partial or complete DPD deficiency have a reduced capacity to catabolize 5FU and are at risk of experiencing severe reactions, such as haematological and/or digestive toxicities [9,13]. These toxicities usually occur during the first treatment cycles, supporting the importance of screening for DPD deficiency and optimizing FP starting dose before initiating therapy. The purpose of this review is to provide an update on the scientific and clinical rationales for the implementation and interpretation of dihydropyrimidine dehydrogenase (*DPYD*) genotyping and phenotyping in order to secure FP-based chemotherapies.

2. FP metabolism and mechanism of action

The anabolic pathways of 5FU are similar to those of uracil, and a small fraction of 5FU (1–5%) is transformed into the cytotoxic metabolites fluorodeoxy uridine-monophosphate (FdUMP), fluorodeoxyuridine-triphosphate (FdUTP) and fluorouridine triphosphate (FUTP). Two anabolic pathways have been described. The first consists to convert 5FU by orotate phosphoribosyltransferase (OPRT) and uridine phosphorylase into fluorouridine-monophosphate (FUMP). FUMP is phosphorylated to fluorouridine-diphosphate (FUDP), which is further phosphorylated to FUTP or converted to fluorodeoxyuridine-diphosphate (FdUDP) by ribonucleotide reductase. In the second pathway, 5FU is converted by thymidine phosphorylase into FUDR, which is converted by thymidine kinase to FdUMP, which is finally phosphorylated to FdUDP and FdUTP (Fig. 1).

5FU cytotoxicity is thus related to different mechanisms. FdUMP, which has a greater affinity for thymidylate synthase (TS) than the natural substrate dUMP, inhibits TS, resulting in the inhibition of DNA synthesis and promotion of thymineless stress, triggering Fas-mediated apoptosis. The ternary complex formed between FdUMP, TS, and 5–10 methylene-tetrahydrofolate is stabilized by the co-administration of folinic acid, favouring TS inhibition. In addition, the incorporation of FUTP in RNA, and FdUTP in DNA (facilitated by the depletion of the dTTP pool as a consequence of TS inhibition), impacts RNA synthesis and damages newly synthetized DNA [10,14].

The large majority (>85%) of 5FU is catabolized by DPD to 5-fluorodihydrouracil (5FUH2), a non-cytotoxic metabolite. DPD is ubiquitously expressed, including in tumour tissues and peripheral blood mononuclear cells (PBMC) [15–17]. Its physiological role is to convert uracil and thymine into dihydrouracil (UH2) and dihydrothymine. 5FUH2 is further converted by dihydropyrimidinase into 5-fluoroureidopropionic acid (5FUPA) in the liver. Finally, ß-ureidopropionase converts 5FUPA to α-fluoro-ß-alanine (FBAL), the major urinary catabolite in humans. In addition to the intensive catabolism of 5FU, ~5% of administered 5FU is excreted in the urine.

FP oral prodrugs (capecitabine, tegafur) are converted into 5FU and catabolized by DPD. Tegafur, initially combined with uracil (UFT) in order to inhibit DPD, is now combined with the DPD inhibitor gimeracil and the OPRT inhibitor oteracil (S-1 is the code name of the combination of the 3 compounds). Oteracil

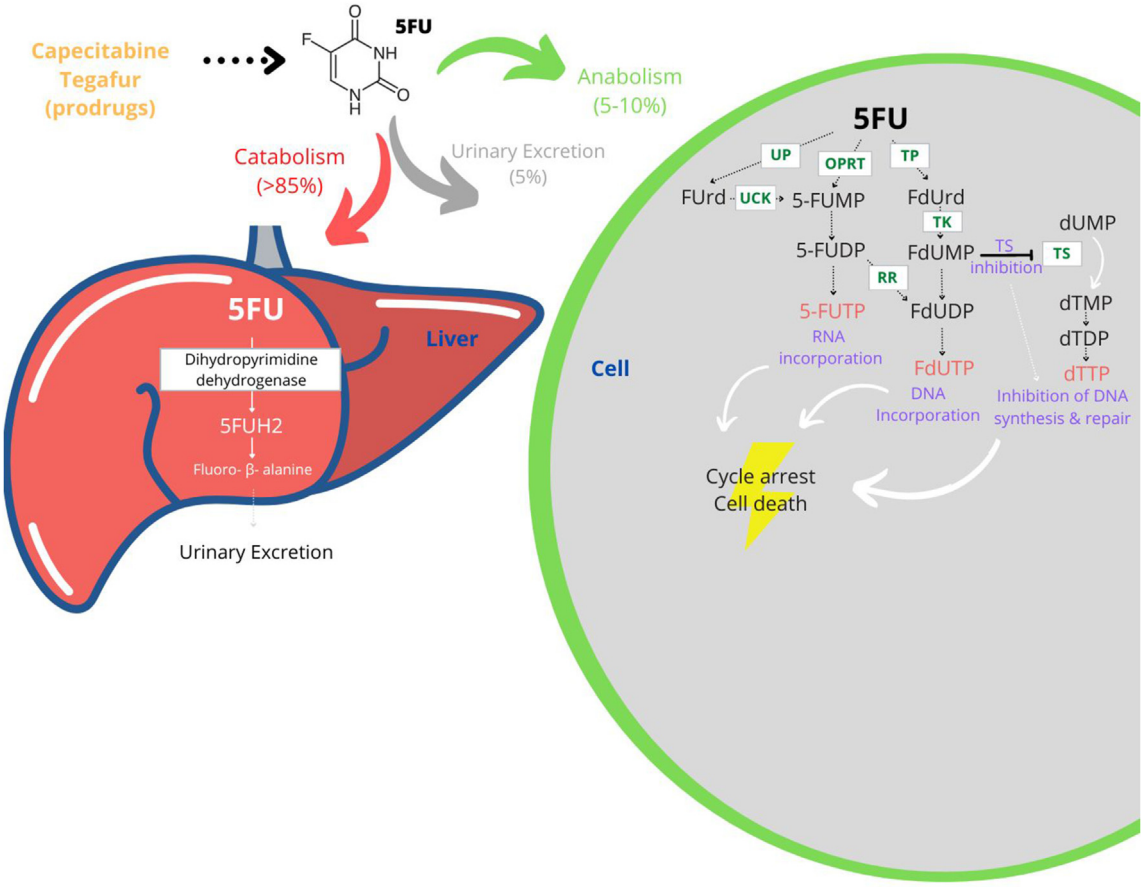

Fig. 1. **5FU metabolism and mechanism of action**. Only a small fraction of 5FU (1–5%) is metabolized to the cytotoxic metabolites fluorodeoxyuridine-monophosphate (FdUMP), fluorodeoxyuridine-triphosphate (FdUTP) and fluorouridine triphosphate (FUTP). The main mechanism of action of FP is thymidylate synthase (TS) inhibition, preventing dTMP formation. Alternative pharmacodynamic pathways involve incorporation of 5FU metabolites into DNA and RNA, resulting in cytotoxity as a consequence of RNA and DNA damage. UP, uridine phosphorylase; OPRT: Orotate phosphoribosyl-transferase; TP, thymidylate phosphorylase; UCK, uridine-cytidine kinase; RR, ribonucleotide reductase; TK, thymidylate kinase; FUrd: fluorouridine; FdUrd: fluorodesoxyuridine; TS: thymidylate synthase; DPD: dihydropyrimidine dehydrogenase.

inhibits the phosphorylation of 5FU to FUMP in the gastrointestinal tract, thereby diminishing the toxic effects of 5FU in normal gastrointestinal mucosa. Gimeracil is a reversible DPD inhibitor that maintains high 5FU concentrations in blood and tumour tissues [18]. The impact of DPD deficiency on toxicities induced by tegafur co-administered with a DPD inhibitor is thus theoretically limited.

3. DPD deficiency and FP toxicity

DPD enzyme activity measured in peripheral blood mononuclear cell (PBMC) exhibits a wide inter-patient variability [19] and a significant correlation was found between DPD enzyme activity measured in liver and in PBMC on a limited set of 27 patients [20]. The wide inter-subject variability in PBMC-DPD activity leads to a large variability in 5FU systemic exposure after administration of a standard dose [4,19,21,22]. Since the publication in 1985 of the first case of a life-threatening toxicity related to 5FU in a DPD-deficient patient [23], numerous publications have demonstrated a causal link between DPD deficiency and the occurrence of severe or fatal FP-related toxicities [24–28] and Supplementary tables. It is estimated that approximately 3%–5% of Caucasians are carriers of partial DPD deficiency and that 0.1–0.3% are completely deficient [29]. Although other genes may play a role in FP toxicity (e.g. *TYMS* and *MTHFR*), and that complete DPD deficiency does not explain all FP toxicity-related deaths [1], *DPYD* non-functional variants are the main identified contributors of 5FU-related severe toxicities [30,31].

4. DPYD defective allelic variants

The *DPYD* gene encoding DPD, spans ~900 kb on chromosome 1 and contains 23 exons [32]. *DPYD* is highly polymorphic, with more than 200 variants

Table 1
Current consensual deleterious *DPYD* variants and prevalence in populations of Caucasian origin.

DPYD variants	Minor allelic frequency	Proportion of carriers		Allele activity score[a]
		heterozygous	homozygous	
***DPYD**2A** (IVS14+1G > A, c.1905G > A)	0.8% [10,83]	1.5%	0.01%	0
***DPYD**13** (c.1679T > G; p.I560S)	0.1% [10,56,61]	0.2%	0.0001%	0
c.2846A > T (p.D949V)	0.6% [9,10,56]	1%	0.004%	0.5
HapB3 (c.1129−5923C > G, c.1236G > A)	2.4% [10]	4.6%	0.06%	0.5

[a] According to Henricks *et al.* [Pharmacogenomics 2015].

described in coding regions, but their effects on DPD enzyme activity are unknown for the majority of them [33–35]. Complete DPD deficiency is a cause of familial pyrimidinemia, a rare disorder of pyrimidine metabolism characterized by a variable phenotype ranging from absence of symptoms to severe neurological involvement with developmental delay, intellectual disability, and seizures. Analysis of urine typically shows high levels of uracil and thymine, and these patients are predisposed to FP-related toxicity [23].

The first described and most studied deleterious *DPYD* variant is the splice-site *DPYD**2A (c.1905+1G > A; rs3918290) found in 1.5% of Caucasians, associated with reduced 5FU clearance and severe or fatal toxicity [25,36,37]. Three additional deleterious variants associated with decreased DPD activity and toxicity risk have been described: *DPYD*∗13 (c.1679T > G; p. Ile560Ser; rs55886062), c.2846A > T (p.Asp949Val, rs67376798), and the more common variant c.1129−5923C > G (rs75017182) linked to haplotype B3 (HapB3) [10](Table 1).

Overall, 6–7% of Caucasians carry one of these four variants at the heterozygous state. Importantly, these four deleterious variants are not present in populations of African or Asian populations [38–40]. Other *DPYD* variants associated with reduced DPD activity (*in vitro* and/or *ex vivo*) have been reported in non-Caucasian populations, including the c.557A > G (p.Tyr186Cys, rs115232898) variant identified in African American/Afro-Caribbean with a minor allelic frequency (MAF) of 1.2% and virtually absent in Asian and European populations. *DPYD*∗5 (c.1627A > G, p.Ile543Val; rs1801159) and *DPYD*∗6 (c.2194G > A, p.Val732Ile, rs18011160) variants are detected in East-Asian populations with MAF of 25% and 18% respectively [41,42].

The four ∗2A, ∗13, c.2846A > T and HapB3 *DPYD* variants are considered clinically actionable:

- The *DPYD*∗2A variant is located in the intron boundary of exon 14 coding for a part of the pyrimidine binding site. This genetic alteration is responsible for the skipping of exon 14 (165 base pairs deletion), resulting in a truncated catalytically inactive enzyme [34,43–45]. Clinical studies have reported an association between *DPYD*∗2A variant and DPD enzyme activity, supporting the decreased function of *DPYD*∗2A. In heterozygous *DPYD*∗2A carriers, DPD activity is reduced by approximately 50%, and in homozygous carriers, DPD activity is completely or nearly abolished [46,47]. Pharmacokinetic studies have demonstrated that patients carrying the *DPYD*∗2A variant are subject to significant increases in systemic 5FU exposure, more than twice the average level [48,49].
- The *DPYD*∗13 variant (c.1679T > G; p. Ile560Ser) is a serine-for-isoleucine substitution in exon 13 corresponding to a highly conserved region of the protein. The precise functional consequence of this missense mutation is not known, but this substitution may reduce the stability of a sensitive region of the protein [50,51]. Experimental data showed that homozygous expression of the *DPYD*∗13 variant resulted in a 75% reduction in DPD enzyme activity compared with the wild-type [44]. Clinical studies have confirmed that patients carrying the *DPYD*∗13 allele (heterozygous) have a markedly reduced DPD activity [51,52].
- The missense mutation c.2846A > T (p. Asp949Val), located on exon 22, affects DPD activity through a structural change that interferes with cofactor binding and electron transport [51]. *In vitro* and *ex vivo* data generally indicate a reduction in enzyme activity in the presence of this variant. An *in vitro* assessment showed that homozygous expression of the *DPYD* c.2846A > T variant resulted in a 40–60% reduction in DPD activity compared to the wild-type [34]. This finding suggests that homozygous carriers would have a ∼50% reduction in DPD activity and heterozygous carriers may have a ∼25% reduction [44].
- Haplotype B3 (Hap B3) spans from intron 5 to exon 11 [53,54]. *DPYD* c.1129−5923C > G (rs75017182) is a deep intronic mutation (intron 10) that introduces a cryptic splice site, resulting in a non-functional transcript. The synonymous variant c.1236G > A (p. E412E, rs56038477) is in complete linkage disequilibrium with c.1129−5923C > G and can be used as a proxy for HapB3 in Caucasians. HapB3 is also in linkage disequilibrium with 3 other intronic variants: c.483 + 18G > A (rs56276561), c.680 + 139G > A (rs6668296), and c.959-51T > G (rs115349832). HapB3 is reported to reduce mRNA splicing efficiency by 30%, and *ex vivo* data suggest a moderately reduced DPD activity of 20–35% on average in heterozygous carriers and 20–60% in homozygous carriers [54,55].

5. DPYD gene activity score

In 2015, Henricks *et al.* proposed a "gene activity score" for translating the *DPYD* genotype into the DPD phenotype, based on the four most relevant *DPYD*-deficient alleles (*DPYD*∗*2A*, c.2846A > T, *DPYD*∗*13*, c.1236G > A/HapB3) (Table 1) [56]. Based on *in vitro* and clinical functionality of each deleterious allele, this approach assigns for each allele a "variant activity score" of zero for completely deficient alleles (*DPYD*∗*2A* and ∗*13*), 0.5 for partially deficient alleles (c.2846A > T and HapB3) and to 1 for the wild-type ∗1 allele. The gene activity score corresponds to the sum of the two lowest variant activity scores. The resulting gene activity score ranges from 0 (two completely deficient alleles) to 2 (two wild-type alleles). This gene activity score was further recommended by the Clinical Pharmacogenetics Implementation Consortium (CPIC) [41] to estimate the risk of developing severe FP-related toxicities. One limitation of this score is its restriction to four variants, since it is anticipated that other rare *DPYD* variants in Caucasians (potentially more frequent in other ethnic groups) may also influence DPD activity and severe FP-related toxicities [46]. An additional caveat is that a single variant not deleterious by itself can nevertheless be significantly associated with DPD deficiency if it forms a haplotype with additional functional variants. Finally, another limitation is the case of patients carrying at least two deleterious *DPYD* alleles because conventional genetic tests do not indicate whether they are located on the same DNA strand; for such patients, the Dutch Pharmacogenetics Working Group (DPWG) recommended an additional phenotyping test to determine DPD enzyme activity [57].

6. Genotyping approaches for DPD deficiency screening

The CPIC and the Dutch Pharmacogenetics Workgroup (DPWG) proposed *DPYD* testing of *DPYD*∗*2A*, *DPYD*∗*13*, c.2846A > T and HapB3 [10,57]. Based on combined genotyping of *DPYD*∗*13*, c.2846A > T and HapB3 (*DPYD*∗*2A* not considered given that carriers received reduced FP dosing), Meulendijks *et al.* reported a sensitivity of 6% and a positive predictive value (PPV) of 13% for predicting global severe toxicity (grades 3–4) in a prospective study including 550 patients [58]. Toffoli *et al.* [33] and Etienne-Grimaldi *et al.* [59] showed that genotyping of *DPYD*∗*2A*, ∗13 et c.2846A > T predicted grade 3–4 toxicities with a sensitivity of 12% and 17% and a PPV of 61% and 71%, respectively. The performance of consensual deleterious *DPYD* variants for predicting the most relevant grade 4–5 haematological and digestive toxicities was reported in the FUSAFE meta-analysis on individual patient data conducted on 6403 patients [60]: the adjusted OR associated with the presence of at least one *DPYD* variant among ∗*2A*, ∗*13* and c.2846A > T was 10.6 (95%CI 7.1–15.6) and the sensitivity and PPV of the multivariate model including these 3 *DPYD* variants and clinical co-variates (age, sex, body mass index, modality of FP administration and associated anticancer drugs) were 75.5% and 15.2%, respectively. Importantly, the addition of HapB3 did not significantly improve the logistic model of grade 4–5 toxicity prediction, even though HapB3 was associated with an OR of 2.1 (95%CI 1.4–3.0). Literature data consistently reported that the toxicity risk associated with HapB3 was much smaller than those associated with ∗*2A*, ∗*13* and c.2846A > T [61], in line with the relatively weak association between HapB3 and DPD phenotype [56]. Of note, a recent meta-analysis on resumed data focused on lethal toxicities reported a RR of death of 25.6 in patients carrying any of the 4 consensual *DPYD* variants with a mortality risk of 2.3%, while excluding HapB3 increased mortality up to 3.7% [5]. The clinical impact of HapB3 is thus conflicting and the above results may question its clinical relevance for predicting grade≥3 FP-related toxicities. Of note, exhaustive exome *DPYD* sequencing led to the identification of additional rare coding variants with potent deleterious activity [59,62], indicating that extending *DPYD* targeted genotyping to whole *DPYD* gene sequencing, which is made possible by next-generation sequencing technologies, may be a promising clinically relevant approach.

7. Phenotyping approaches for DPD deficiency screening

PBMC DPD enzyme activity is considered the reference method for investigating DPD deficiency but it is highly cost- and time-intensive. Alternate methods such as uracil breath test and uracil loading dose [63] are also cumbersome and expensive, and fail to meet current standards for large-scale DPD screening implementation. Measuring plasma uracil [U] and dihydrouracil [UH2] concentrations was proposed 25 years ago as a simple inexpensive way to evaluate DPD activity [64]. Indeed, DPD deficiency will lead to uracil accumulation in plasma, thus making [U] or [UH2]/[U] ratio convenient surrogates for detecting impaired DPD activity in patients. Many improved HPLC-UV, UPLC-UV, GC–MS and LC-MS/MS methods have been developed [65–68]. Beyond monitoring plasma levels, other groups have proposed measuring [U] and [UH2] in saliva samples to simplify the sampling process and pre-analytical steps [69–71].

Measuring [U], regardless of the analytical technique used, is the most convenient method to detect DPD deficiency, and the one retained by French Health Authorities for DPD deficiency screening. This decision was mainly based on consistent results from 3 independent prospective studies: a French study conducted on 252 colorectal cancer patients receiving i.v. 5FU [26], a more recent French study conducted on 286 breast

Table 2
Strengths and limitations of phenotyping and genotyping for DPD-deficiency screening.

	Strengths	Limitations
Genotyping of DPYD *2A, p.D949V, *13 and Haplotype B3	- Low cost	- No relevant for Asian and African populations
	- Easy to perform and implement	- Low sensibility for identifying patients at risk of toxicity
	- Unequivocal result	- Account for genetics only
	- No pre-analytical requirement	- Uncertain enzyme activity reliability
	- Validated genotype-guided FP dosing	
Phenotyping of [U] ± [UH_2]/[U]	- Apply to all populations	- Time-consuming and highly specialized non-standardized analytical method
	- Takes into account all parameters influencing DPD enzyme activity	- Strict pre-analytical requirements
	- Higher sensitivity as compared to genotyping	- Circadian variability
		- Possible influence of food intake
		- Uncertain cutoff value
		- No validated phenotype-guided FP dosing
		- Lack of specificity

cancer patients treated with capecitabine [59,72], and a Dutch study on 550 patients with various cancers receiving capecitabine or 5FU [58]. The last 2 studies [58,59,72] showed that [U] was a better predictor of FP-related toxicities than [UH2]/[U], while the former [26] reported similar performance. The two French studies identified a [U] threshold at risk of severe grade 3–4 toxicity at 15 ng/ml [26] and 16 ng/mL [59,72], respectively, the latter yielding a RR at 3.1 (95%CI 1.7–5.9) [72]. The Dutch study showed that [U] higher than 16 ng/mL was strongly associated with severe grade 3–4 FP toxicity (OR 5.3, 95%CI 1.5–18.7) and fatal toxicities (OR 44.8, 95%CI 4.6–441), the higher the [U], the greater the toxicity risk, with a 2.75-fold higher risk on early global toxicity grade 3–4 and a 5.1-fold higher risk on lethal toxicity, for every 10 ng/mL increase [58].

The major limitation of [U] analysis is the observance of strict pre-analytical rules requiring that blood sample must be centrifuged as quickly as possible (within 1h at ambient temperature, or 4h at +4 °C), and plasma frozen at −20 °C until analysis [73]. Indeed, plasma [U] increases with time likely due to the conversion of blood uridine into uracil by uridine phosphorylase [74] potentially leading to falsely conclude to DPD deficiency. Recent [U] data from de With *et al.* [75] clearly illustrate this limitation: despite centralization of [U] measurement (performed in a single "reference hospital"), in 6 hospitals out of 16, [U] were significantly higher than those measured in the reference hospital, thus undermining the reliability of their results reporting a lack of correlation between [U] and toxicity. Also, exogenous and endogenous factors such as food intake [76], circadian variations [77], renal function [78], sampling conditions [79], and a prior FP treatment [80] may influence [U] and [UH2].

Thanks to the wide experience of [U] testing in France, along with the setting up of an external quality assessment scheme (EQAS) for [U] since 2019, nationwide [U] testing has been successfully implemented in France. A survey by the French Medicines Agency (ANSM) (https://ansm.sante.fr) showed that roughly 70,000 tests are carried out every year in France, which corresponds to the annual number of new patients receiving FP-based chemotherapy [1]. Currently, 47 laboratories from European countries (France, Belgium, Luxemburg, Denmark) participate in this [U] EQAS.

8. Comparison and combination of genotyping and phenotyping approaches

Strengths and limitations of genotyping and phenotyping approaches are listed in Table 2. Due to the scarcity of the 4 deleterious **2A/*13*/c.2846A > T/HapB3 *DPYD* variants (~6–7% of carriers in Caucasians, ~ 2–3% excluding HapB3), the sensitivity of *DPYD* genotyping, i.e. the proportion of patients with deleterious variants among patients experiencing toxicity, is inevitably low, and depends on the toxicity prevalence (severity of toxicity). In contrast, DPD phenotype is by nature a continuous variable, and as such, its performance for predicting FP toxicities depends on the applied threshold.

From a large French cohort study including 3680 patients with both genotyping and phenotyping, only 10.7% of patients with [U]≥16 ng/ml carried one of the 4 deleterious variants; conversely 16% of variants carriers exhibited [U]≥16 ng/ml (26% in excluding HapB3 carriers) [81] Interestingly, the same study reported that 13.3% of patients with [UH2]/[U]<10 carried one of the 4 deleterious variants while 33% of variants carriers exhibited [UH2]/[U]<10, suggesting that [UH2]/[U] may better reflect the impact of deleterious *DPYD* variants than [U]. Regardless of the phenotyping marker, some overlap is observed between deficient populations based on genotyping and phenotyping, one could thus expect to improve screening performance by combining both approaches. Results from the 3 published prospective studies testing a combined *DPYD* genotyping (2–3 consensual variants) plus phenotyping strategy failed to provide an appropriate level of evidence for the superiority of such a combined strategy as compared to each approach alone [26,58,59]. Nevertheless, it must be underlined that complementary genetic testing might help to confirm the genetic origin of enzymatic deficiency, particularly in patients with complete DPD deficiency, and thus justify family counselling.

9. Cost-effectiveness of upfront DPD deficiency testing to guide FP dose adjustment

The cost-benefit of an upfront DPD screening strategy has been evaluated in several studies [82–84] that focused on *DPYD* genotyping only. Although toxicity-related costs may depend on national health systems, these Irish, English and Dutch studies concluded that targeted *DPYD* genotyping is cost-effective. For instance, the cost-efficiency study by Henricks *et al.* conducted on 1103 patients prospectively enrolled in the ALPE trial, showed that upfront genotyping of the 4 *DPYD* variants followed by FP dose reduction according to CPIC guidelines improves patient safety and quality of life, without additional costs (author's conclusion is "*cost-saving or cost-neutra*l") [82]. Cost-effectiveness of phenotyping-guided dosing remains to be evaluated so as to provide a comprehensive comparison of both genotyping, phenotyping, and combined approaches.

10. Pharmacokinetic-guided dosing of 5FU

5FU clearance has a very large inter-patient variability, up to a 10- to 100-fold range, and drug dosing based on body surface area (BSA) does not reduce the resulting inter-patient variability in 5FU systemic exposure [21]. Importantly, many studies (reviewed in Beumer *et al.* [21]) have demonstrated relationships between 5FU systemic exposure (mainly area under the curve (AUC) of concentration x time) and haematologic and/or digestive severe FP-related toxicities, as well as treatment efficacy (tumour response and survival). Irrespective of the 5FU-based containing regimen, an optimal target 5FU AUC of 20–30 ng h/mL has been defined for digestive cancers [21]. Of note, Saam *et al.* [85] reported that BSA-based dosing results in 5FU AUC below 20 ng h/ml in 53% of patients receiving FOLFIRI (29/55) and in 51% of patients treated with FOLFOX6 regimen (153/302), highlighting potential relevance of 5FU therapeutic drug monitoring (TDM) not only for reducing, but also increasing 5FU dosing. Two randomized studies [86,87] have demonstrated that individual 5FU dosing based on a significantly improved 5FU therapeutic index, allowing validation of TDM for continuous i.v. 5FU. In the trial of Gamelin *et al.* [87] in CRC patients treated with a weekly 8-h 5-FU 1500 mg/m^2 single agent dosing regimen, the TDM performed weekly allowed to reach the target AUC in 94% of patients in a mean time of four cycles. 5FU TDM improved overall response rates from 18% with BSA-based dosing of 5FU to 33% with PK-guided dosing of 5-FU while the incidence of toxicity was lower. In the Fety *et al.* [86] study in HNSCC, TDM was performed at cycle 1 to adjust the doses for cycles 2 and 3. Haematological toxicity and mucositis were significantly reduced in the PK-adjusted arm with a comparable objective response rate to the standard treatment arm. A large number of nonrandomized or single-arm clinical studies (reviewed in Beumer *et al.* [21]) have consistently described a reduction of toxicity when applying 5FU TDM. A 5FU dose adjustment algorithm for FOLFOX6 regimen has been proposed [88] and validated [89], based on the 5FU AUC measured at the previous cycle. Of note, the benefit of TDM performed at cycle 1 has been demonstrated in elderly patients [90]: the percentage of elderly patients within the therapeutic range significantly increased from 29% at C1 to 64% at C2 ($p = 0.011$). Since TDM is not a pre-emptive strategy, it does not allow to prevent life-threatening toxicities occurring at first cycle [91]. An alternative is the administration of a 5FU test dose, as described by Bocci *et al.* [92] although a low 5FU dose may induce severe toxicities in case of profound DPD deficiency. Despite being time-consuming and requiring specific resources and expertise, pharmacokinetic-guided dosing of 5FU has proven its clinical relevance and is cost-effective [93]. 5FU TDM is thus a relevant strategy for adjusting 5FU dose as soon as second cycle in patients with partial DPD deficiency.

11. Recommendations

Despite extensive research on FP pharmacogenetics since 1985, controversy remains among the medical

community about the usefulness of systematic DPD deficiency screening prior to the initiation of FP-based treatments.

11.1. Recommendations from scientific consortia

Current CPIC guidelines provide FP dosing recommendations at first cycle for *DPYD* normal, intermediate, and poor metabolizers [10], based on the DPYD activity score proposed by Henricks [56]. Poor metabolizers with a gene activity score at zero should avoid 5FU and prodrugs (Fig. 2). Poor metabolizers with a gene activity score of 0.5 and no alternate therapeutic option should receive <25% of the standard dose with early TDM if feasible, and additional DPD phenotype testing to better assess DPD activity. Intermediate metabolizers with a gene activity score at 1 or 1.5 should receive 50% of the standard dose, with a special attention in patients homozygous for c.2846A > T (gene score 1) who may require a reduction of more than 50% (2018 update). CPIC recommendations only applied for i.v. 5FU and capecitabine.

In 2019 the DPWG published guidelines [57] also based on the DPYD activity score by Henricks [56]. DPWG recommendations differ from CPIC for patients carrying two decreased function alleles (gene activity score 1) or one decreased and one non-functional allele (gene activity score 0.5): DPGW considers that enzyme activity cannot be accurately predicted and that additional phenotyping (ideally DPD enzyme activity measured in PBMCs) is required to adjust FP dosing. For other patients with gene activity score at 1 (i.e. patient with one non-functional allele) or 1.5, dosing recommendations for 5FU (i.v. and cutaneous) and capecitabine are similar to those of CPIC (50% of standard dose). Second difference is that DPWG contraindicates tegafur in patients with a gene activity score at 0, 1 or 1.5.

In 2018, the French National Pharmacogenetic Network (Réseau Francophone de Pharmacogénétique (RNPGx)) and the Clinical Oncopharmacology Group (Groupe de Pharmacologie Clinique Oncologique (GPCO-UNICANCER)) published guidelines for systematic upfront DPD-deficiency screening for 5FU and capecitabine [94]. The RNPGx/GPCO recommended

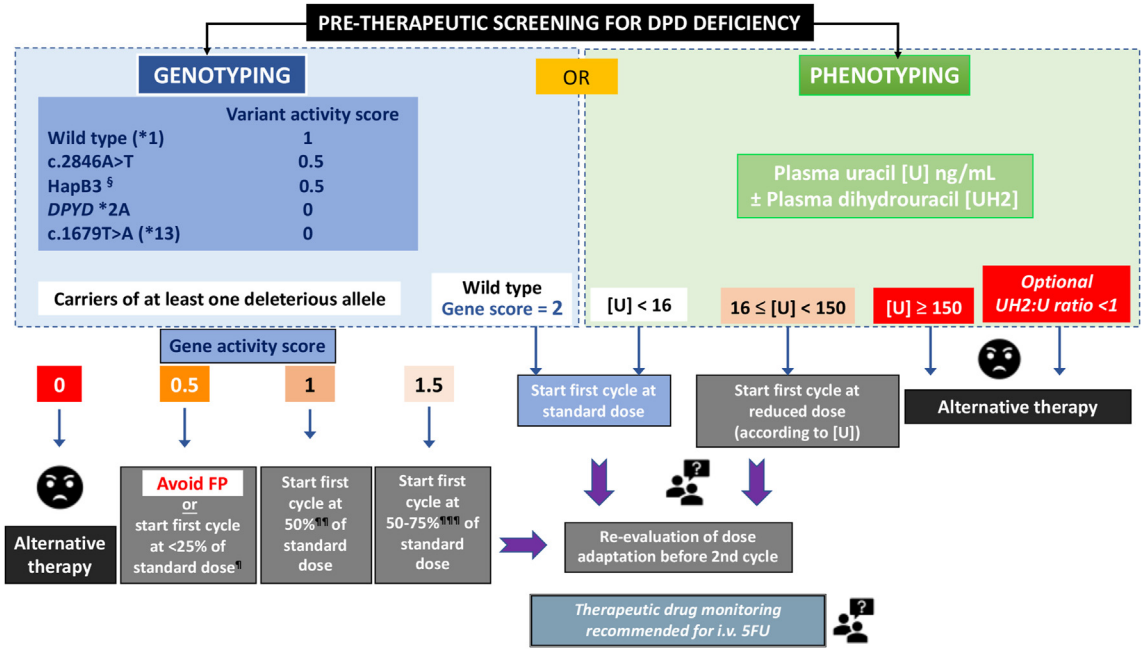

Fig. 2. **Current decision-making algorithms for DPD deficiency screening and subsequent FP dose adaptation at first cycle**. Phenotyping approach corresponds to HAS/INCa French recommendations. Measurement of plasma [UH2]/[U] ratio is not mandatory but may help to classify patients as deficient/not deficient. Genotyping approach corresponds to the most recent CPIC and DPWG recommendations. The gene activity score is obtained by adding the two lowest activity score variants.

¶ For patients with no alternative therapeutic option and a gene score of 0.5, additional DPD phenotyping is recommended, along with TDM to refine FP dose adjustment.

¶¶ For patients with a gene activity score at 1 resulting from homozygosity for HapB3 or c.2846A > T, or the presence of these 2 variants, it is advised to perform DPD phenotyping to refine FP dose adjustment. Literature data suggest that patients homozygous for c.2846A > T should receive a starting dose considerably lower than 50%.

¶¶¶ Based on conflicting data about HapB3 impact on DPD activity, the starting dose to apply for HapB3 heterozygous patients is unclear.

§ HapB3: c.1236G > A in complete linkage disequilibrium with c.1129−5923C > G.

[U] as the preferred approach, with FP contra-indication for [U] >100 ng/mL [94] and FP dose reduction from 25% to 50% for [U] ≥16 ng/mL depending on the [U] and patient profile. Targeted genotyping of the 4 consensual deleterious *DPYD* alleles was recommended in addition to phenotyping, along with dose reductions based on CPIC recommendations.

11.2. Recommendations from medical societies

The National Comprehensive Cancer Network (NCCN) guidelines point out the risk of severe FP toxicities induced by deleterious *DPYD* variants but does not support pre-emptive *DPYD* genotyping due to levels of evidence deemed controversial. The American Society of Clinical Oncology (ASCO) does not provide any guidelines for DPD deficiency testing, arguing lack of standardized approaches. European Society of Molecular Oncology (ESMO) guidelines in localized colon cancer strongly recommend DPD genotyping or phenotyping before initiating FP-based adjuvant therapy, and ESMO guidelines in metastatic colorectal cancer patients states that DPD-deficiency testing should be conducted before initiating 5FU or capecitabine [95,96]. Asian medical societies do not recommend DPD testing since DPD deficiency is rare in Asian patients.

11.3. Recommendations from health authorities

The conflicting positions taken by scientific consortia and medical societies have led health authorities to remain cautious in making clear decisions. Under pressure of 5FU victim patient's associations, particularly in France and the United Kingdom, European health authority's position regarding DPD testing has recently moved.

In 2018, French Health Authorities set up a working group to deeply analyse DPD phenotyping and genotyping performances, with the aim to identify completely DPD-deficient patients and prevent life-threatening toxicities [97]. On April 2019, France was the first country to mandate DPD-deficiency testing in all patients before initiating FP-based chemotherapy, based on [U] along with strict pre-analytical requirements. Since 2021, prescription and dispensation assistance softwares must include a specific pop-up, warning the physician and pharmacist on the need to be aware of [U]. Due to the scarcity of published data on completely deficient patients, the [U] threshold for complete deficiency was set at 150 ng/ml, based on data from 38,862 patients tested in French laboratories between 2000 and 2018, corresponding to 0.08% of patients. The threshold for partial deficiency was set at 16 ng/ml. French HAS-INCa recommendations are the following: i- FP are contra-indicated in patients with [U] ≥150 ng/mL; ii- FP dose reduction should be considered in patients with [U] between 16 and 150 ng/mL on the basis of a clinical-biological reflection depending on the depth of DPD deficiency, on the chemotherapy protocol and the pathophysiological criteria (Fig. 2). In the absence of validated dose-guided reduction according to [U], HAS-INCa recommendations emphasize that FP dose must be re-evaluated as soon as second cycle, depending on tolerance at first cycle, for ensuring an efficient dose-intensity. In addition, for i.v. 5FU, TDM is recommended for adjusting 5FU dose in partially deficient patients. Importantly, since 2020, no preventable toxicity-related death has been declared to the French Pharmacovigilance Network in patients with complete or partial DPD deficiency (data available on https://ansm.sante.fr).

On April 2020, the European Medical Agency (EMA) updated its recommendations on upfront DPD testing for 5FU, capecitabine and tegafur chemotherapy, notifying that DPD deficiency can be tested not only by checking for the presence of certain mutations in the *DPYD* gene, but also by measuring [U] [98]. The EMA recommends considering a reduced starting FP dose in partially-deficient patients, and contra-indicates FP in patients with complete DPD deficiency. The European summaries of product characteristics (SmPCs) of 5FU and capecitabine mention a contra-indication in patients with known complete DPD deficiency, and a special warning and reduced dose recommendation in patients with partial DPD deficiency. In line with EMA, SmPCs of 5FU and capecitabine recommend upfront DPD phenotyping and/or genotyping but do not make it mandatory.

In contrast, in the US package inserts of 5FU and capecitabine, the Food and Drug Administration does not recommend DPD-deficiency testing, and does not contra-indicated these drugs in patients with complete DPD deficiency; the "Warning and Precautions" section indicates an increased risk of serious or fatal reactions in patients with low or absent DPD activity and states that "*There is insufficient data to recommend a specific dose in patients with partial DPD activity as measured by any specific test*" without mention on the tests [99].

12. Benefits and risks for patients

There are no randomized clinical studies comparing upfront DPD deficiency screening (whatever the approach) followed by FP dose adjustment *versus* standard FP dose. Nowadays such a study would not be ethical. A few non-randomized interventional clinical studies have shown that pre-emptive DPD testing, regardless of the approach, contributed to a significantly reduced

incidence of drug-related severe toxicity or death compared with historical groups, or through matched pair study [100–103]. A large prospective study conducted in 17 Dutch hospitals (1103 patients) assessed the effect of pre-emptive screening for the four consensual deleterious *DPYD* variants on patient safety [103]. Heterozygous *DPYD* variant carriers received an initial dose reduction of 25% (c.2846A > T and HapB3) or 50% (*DPYD*∗*2A* and ∗*13*), and *DPYD* wild-type patients were treated according to the current standard of care. Despite dose reduction, overall grade 3–4 toxicity was significantly higher in variant carriers as compared to wild-type patients (39% *versus* 23%, respectively). The authors further compared, for each variant, the relative risk (RR) of severe toxicities between this *DPYD*-guided dose study and a historical patient cohort receiving standard FP doses: for *DPYD*∗*2A,* RR was 1.3 (95%CI 0.6–2.7) for genotype-guided dosing compared with 2.87 (95%CI 2.1–3.9) in the historical cohort; for *DPYD*∗*13* no toxicity occurred with genotype-guided dosing compared with 4.3 (95%CI 2.1–8.8); for HapB3 RR was 1.69 (95%CI 1.2–2.4) compared with 1.72 (95%CI 1.2–2.4); and for c.2846A > T RR was 2.0 (95%CI 1.2–3.3) compared with 3.1 (95%CI 2.3–4.3), suggesting that a dose reduction of 25% is too low for c.2846A > T carriers, in line with sparse pharmacokinetics data collected in five c.2846A > T carriers [103].

A major issue of FP dose reduction in partially deficient patients is its potential deleterious impact on treatment efficacy, this being particularly relevant for adjuvant colorectal cancer [104,105]. Only one retrospective study specifically examined efficacy in DPD-deficient patients receiving reduced FP dose [101]. Efficacy was assessed in 40 prospectively identified heterozygous *DPYD*∗*2A* carriers treated with an ∼50% reduced FP dose (5FU, capecitabine) who were matched (according to hospital, tumour type, treatment, disease stage, age) with wild-type *DPYD*∗2A patients receiving standard dose. Overall and progression-free survivals were not significantly different between ∗2A carriers (medians 27 and 14 months, respectively) and wild-type patients (medians 24 and 10 months, respectively).

The risk of undertreating partially deficient patients with DPD-guided dose is of great concern since pharmacokinetics studies have demonstrated significant relationships between 5FU systemic exposure and treatment efficacy. Two French studies recently investigated 5FU systemic exposure in cancer patients with pre-emptive uracilemia testing and subsequent FP dose reduction [106,107]. In contrast to a previous historical study [26], both reported the lack of significant correlation between 5FU clearance and [U]. The study of Dolat *et al.* involving 169 patients showed that patients with [U] ≥16 ng/mL had significantly lower 5FU AUC than patients with [U] <16 ng/mL (p = .0016), in line with significantly lower 5FU doses administered in deficient patients (mean 3540 mg) relative to non-deficient patients (mean 4050 mg, $p < 0.001$) [106]. Such results show the difficulty to assess the optimal FP starting dose based on [U].

Another aspect of DPD phenotyping is to help identifying DPD-proficient patients. A recent retrospective study conducted on 136 cancer patients treated with various FP-based regimens showed that the 18% of patients with the highest DPD enzyme activity measured in PBMCs had a significantly lower overall survival, progression-free survival and response rate as compared to remaining patients with normal or deficient DPD activity [108].

13. Conclusions

Cancer patients treated with FP might experience severe, life-threatening toxicities due to impaired DPD activity. France remains currently the only country that makes upfront DPD-deficiency testing mandatory. EMA recommends DPD deficiency testing for all patients before FP-based chemotherapy by checking the four consensual deleterious *DPYD* variants or by measuring [U] and contraindicates FP in case of complete deficiency or recommends reducing the FP starting dose in case of partial DPD deficiency. In patients with partial deficiency, the FP dose reduction applied at first cycle should be followed by a dose increase according to tolerance, as soon as cycle 2 in order to ensure treatment efficacy. The impact of *DPD*-guided FP dose reduction on efficacy is poorly documented. FP-dose recommendations should also consider baseline characteristics such as age, sex, performance status, body mass index, FP-based regimen and associated anticancer drugs, since they are significantly related to the toxicity risk. Importantly for i.v. 5FU, FP dose adaptation based on pharmacokinetics should be implemented in partially deficient patients since it is currently the dose-individualization strategy with the highest level of evidence.

Both phenotyping and genotyping approaches have demonstrated their feasibility for large-scale screening. Compared to phenotyping, genotyping has no pre-analytical constraints, is easier to perform, and gives unequivocal results. However, due to scarcity of deleterious *DPYD* variants, consensual genotyping has a lower sensitivity than phenotyping for identifying patients at risk of severe FP-related toxicities. Moreover, current genotyping recommendations are only relevant in Caucasian population. *DPYD* genotype-guided FP dose adaptation has been validated in prospective studies. There is currently no validated dosing guidelines based on [U]. A clinical trial in the Netherlands is ongoing to investigate whether upfront DPD genotyping and phenotyping, and subsequent dose reductions based on CPIC and French guidelines, reduce severe toxicities in patients treated with 5FU or capecitabine (ClinicalTrials.gov NCT04269369). Additional

prospective studies are needed to define a dose reduction algorithm based on [U] and associated with the best clinical benefit for the patient. Hopefully, such future studies will lead to the harmonization of clinical guidelines for DPD testing across oncology organizations.

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Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2022.11.028.

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