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Prevention of 5-fluorouracil-induced early severe toxicity by pre-therapeutic dihydropyrimidine dehydrogenase deficiency screening: Assessment of a multiparametric approach

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ABSTRACT

5-Fluorouracil (5-FU)–based treatments can lead to early-onset severe (4%–5%) even fatal (0.3%) toxicities in patients with dihydropyrimidine dehydrogenase (DPD) deficiency. This multicenter prospective cohort study aimed to assess the clinical benefit of pretherapeutic screening for DPD deficiency using a multiparametric approach. Two parallel cohorts of patients treated with 5-FU–based chemotherapy for colorectal carcinoma were compared in a prospective nonrandomized study. In arm A, patients had DPD deficiency screening before treatment, whereas in arm B no pretherapy screening was performed. Dosing was based on 5-FU administration guidelines of each institution. DPD deficiency screening was performed using a combined multiparametric approach (5-FU$^{ODPM\ Tox}$). The frequency of early grade 4–5 toxic events potentially induced by 5-FU was compared in the two groups. At total of 1,142 patients (n = 1,116 evaluable) were enrolled. In arm A, out of 718 evaluable patients, nine grade 4 early toxicities potentially related to 5-FU were reported in nine patients (1.2%) with no toxic death despite one complete DPD deficiency and 24 partial deficiencies. The 24 patients with partial deficiency had safe pharmacokinetics (PK)-monitored 5-FU. In arm B, among 398 evaluable patients, 17 grade 4–5 toxic early events potentially related to 5-FU were reported in 12 patients (4.2%). The incidence of early severe toxicity was significantly higher in arm B ($P = .0019$), confirming the positive impact of pretherapeutic DPD assessment. The percent of patients with a toxicity grade 3 or higher observed in arm A was 10.8% (n = 78) compared to 17.55% (n = 69) in arm B ($P = .0497$). The percentage of death was reduced from 2.5/1,000 in arm B to 0 in arm A. The time to occurrence of all grade ≥ 3 toxicities was determined in both arms and the

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difference between the two arms was significant ($P = .047$). Overall, one patient with complete DPD deficiency confirmed retrospectively died within 13 days from grade 5 multivisceral toxicity. Enrollment was prematurely closed after external experts' decision. In conclusion, multiparametric pretherapeutic DPD deficiency screening significantly lowered the risk of early severe toxicity and avoided an early toxic death. This approach should be used for safe administration of 5-FU–based treatments.

1. Background

Fluoropyrimidines are widely used in oncology as the backbone of a large percentage of current chemotherapy regimens across a broad spectrum of cancers (Table 1). Most treatment combinations, especially for digestive tract cancers, are based on 5-fluorouracil (5-FU), combined with other cytotoxic drugs, such as oxaliplatin, irinotecan, or docetaxel and targeted therapies, such as epidermal growth factor receptor (EGFR) or vascular endothelial growth factor (VEGF) monoclonal antibodies. The published literature suggests that approximately 2 million patients receive 5-FU worldwide each year, with approximately 250,000 to 300,000 patients in the United States receiving 5-FU–based regimens [1]. An estimated 10%–20% of treated patients develop serious, sometimes life-threatening, 5-FU toxicity [2]. It is estimated that in 3%–5% of patients, early, severe adverse events occur even with conventional moderate doses of fluoropyrimidines. These adverse events includ hematological, mucosal, cutaneous, and digestive toxic side effects occurring in patients receiving therapy for metastatic disease, as well as those receiving adjuvant therapy, the majority of whom have potentially been cured with surgery [3,4]. It is also estimated that approximately 0.5% of patients die from these early toxic effects, most often polyvisceral, which has been related to a pre-existing partial or complete deficiency of the catabolic enzyme dihydropyrimidine dehydrogenase (DPD) [3–5]. DPD is the initial and rate-limiting enzyme responsible for the reduction of the endogenous pyrimidine bases, uracil and thymine into 5,6-dihydrouracil and 5,6-dihydrothymine, respectively (Fig. 1) [6]. DPD also catabolizes more than 80% of an administered dose of 5-FU to the inactive metabolite 5,6-dihydrofluorouracil, the first step of the catabolic cascade. Remarkably, DPD enzyme activity is subject to a wide variability, resulting in a possible range of enzymatic deficiencies that span from partial to complete loss of enzyme activity, with approximately 3%–5% of the entire population demonstrating partial deficiency and 0.2%–0.3% demonstrating complete deficiency (Fig. 2) [6,7]. DPD deficiency is partly linked to a genetic polymorphism since about 50% of patients who experienced early highly toxic and sometimes lethal effects were genotypically heterozygous or homozygous for known mutant alleles of the DPYD gene (Table 2) [8–11]. More than 30 variant DPD alleles have been published, and about half of them were considered to have potentially deleterious impact on DPD enzyme activity [12,13]. In these cases, the single-nucleotide polymorphisms (SNPs) are located in regions that are important for enzyme activity: the uracil binding site, NADP, or the FAD binding site, resulting in truncated, nonfunctional proteins, deletion of important binding sites, interference with cofactor binding or electron transport, or alteration of the [4Fe-4S] function (Figure 3) [12,14]. The SNP most often reported in the literature is the splice-site mutation IVS14+1G>A identified as occurring in the 14th intronic region and resulting in a protein that has a partial deletion [15]. However, we have previously shown that this is not the most frequent SNP resulting in early severe adverse events [12]. Out of 22 potentially relevant SNPs reported in the literature, we could determine the following four SNPs most often implied in clinical DPD deficiency, and their functional relevant impact on DPD activity (Table 3) [12]:

- D949V—rs67376798—exon 22: 1.8%
- IVS14+1 G>A—*DPYD*2A*—rs3918290, intron 14: 1.2%
- I560S—*DPYD**13—rs55886062—exon 13: 0.3%
- Del TCAT—*DPYD**7—exon 4: 0.3%

We showed that these four SNPs were highly predictive of decreased 5-FU plasma clearance ($P < .001$) and severe toxicity (60%, 70%, 100%, and 100%, respectively) ($P < .001$) [12,16]. The pretherapeutic screening for *DPYD* gene SNPs could be valuable for preventing early severe 5-FU–induced toxicity. However, screening for the detection of these four mutations, currently not performed in practice [17], has important limitations. We have demonstrated that genotyping alone could not explain all cases of clinical DPD deficiency and could not suffice to predict with high accuracy patients at high risk of early severe toxic events induced by fluoropyrimidines [12,16].

Another approach recommended by some authors [18–21] is the individual dose adjustment based on pharmacokinetic monitoring, but the objective of this approach is different from the early detection and is actually complementary. It does not permit a pre-therapeutic detection of a DPD deficiency but it will allow for the adjustment of dosing to the level of metabolism of 5-FU. And one must keep in mind that in contrast to DPD-deficient patients, some patients have an accelerated catabolism of 5-FU and are at risk of underdosing [20,22,28].

Beside the genotyping, we developed a phenotypic approach, assessing the activity of the enzyme, and we identified certain features clearly involved in the clinical status of DPD deficiency. We demonstrated that the determination of the dihydrouracil/uracil (UH_2/U) ratio in blood was a promising complementary approach in practice [16,23–25]. Furthermore, we showed that neither DPYD genotyping nor phenotyping alone was sufficient by themselves. Combining on the one hand *DPYD* gene genotyping with SNP detection to on the other hand, the determination of UH_2/U ratio, i.e. uracil and dihydrouracil concentrations in blood, along with certain demographic characteristics, such as age and gender, in a multiparametric approach with a dedicated algorithm appeared to be the best approach to detect DPD deficiency with 96% sensitivity and 96% specificity, to assess DPD activity and to determine the optimum 5-FU dosage [26].

The objective of the present study was to demonstrate the accuracy of this multi-parametric approach for detecting DPD deficiency, before treatment, in patients planned to be treated with a 5-FU–based regimen with adaptation of the treatment according to the results. Two groups of patients were compared in a controlled non-randomized study: arm A with systematic pretherapeutic detection of DPD deficiency and arm B without detection. The primary endpoint was the percentage of early (occurring before cycle 3) grade 4–5 toxic events potentially related to 5-FU administration. Their frequency is 3%–4% according to the literature in the patients treated with 5-FU–based regimen, with a conventional protocol, with no pre-therapeutic detection of DPD deficiency and 5-FU dose based on body

Table 1
Regimens that include 5-FU or capecitabine and their indications.

Anal cancer	Locoregional	Mitomycin + 5-FU + RT
		5-FU + cisplatin + external beam RT
	Advanced	Cisplatin + 5-FU by CIV
Gallbladder cancer and Cholangiocarcinoma	Advanced	Gemcitabine + capecitabine
		Capecitabine + oxaliplatin
		Mitomycin + capecitabine
Breast cancer	Adjuvant	Cyclophosphamide + epirubicin + 5-FU (FEC) → docetaxel
		Cyclophosphamide + epirubicin + 5-FU (FEC) → weekly paclitaxel
		Docetaxel + trastuzumab followed by cyclophosphamide + epirubicin + 5-FU (FEC)
	Adjuvant / metastatic	Cyclophosphamide + epirubicin + 5-FU (FEC 100)
		Cyclophosphamide + methotrexate + 5-FU (CMF)
	Metastatic	Capecitabine
		Docetaxel + capecitabine
		Ixabepilone + capecitabine
		Capecitabine + trastuzumab
Carcinoma of unknown primary	Refractory or recurrent	Oxaliplatin + capecitabine
Cervical cancer	Initial	RT + cisplatin + 5-fluorouracil
Colorectal cancer	Locally advanced	RT + capecitabine
	Adjuvant	Capecitabine + oxaliplatin (XELOX)
	Adjuvant / Advanced	Bolus fluorouracil + leucovorin (Roswell Park regimen)
		Bolus 5-fluorouracil
		Capecitabine
	Metastatic / adjuvant	Leucovorin + infusional 5-FU + oxaliplatin (FOLFOX)
	Metastatic	Infusional 5-fluorouracil
		Irinotecan + bolus 5-FU + leucovorin (IFL)
		Leucovorin + infusional 5-FU + irinotecan (FOLFIRI)
		Cetuximab + FOLFOX-4
		Cetuximab + FOLFIRI
		Panitumumab + FOLFOX-4
		Panitumumab + FOLFIRI
		Ziv-aflibercept + FOLFIRI
		Bevacizumab + FOLFIRI
		Bevacizumab + FOLFOX
Esophageal cancer	Locally advanced	5-FU + cisplatin + RT
		Oxaliplatin + protracted infusion 5-FU + RT prior to surgery
		Cisplatin + capecitabine
	Recurrent / metastatic	Epirubicin + cisplatin + 5-FU (ECF)
		5-FU + cisplatin
		Docetaxel + cisplatin + 5-FU (DCF)
		Oxaliplatin + 5-FU + leucovorin (FOLFOX)
		Epirubicin + oxaliplatin + capecitabine (EOC, EOX)
		Irinotecan + 5-FU + leucovorin (FOLFIRI)
		Cisplatin + capecitabine + trastuzumab
Gastric cancer	Adjuvant	5-fluouracil + leucovorin + RT
		Epirubicin + cisplatin + 5-FU (ECF)
		Capecitabine + oxaliplatin after D2 gastrectomy
	Advanced disease	Epirubicin + cisplatin + 5-FU (ECF)
		Docetaxel + cisplatin + 5-FU (DCF)
		Epirubicin + cisplatin + 5-FU (ECF)
		Epirubicin + cisplatin + capecitabine (ECX)
		Epirubicin + oxaliplatin + 5-FU (EOF)
		Epirubicin + oxaliplatin + capecitabine (EOX)
		Cisplatin + 5-FU (FUP)
		Cisplatin + 5-FU (CF)
		Irinotecan + 5-FU (IF)
Head and neck cancer	Chemoradiation	Carboplatin + 5-FU + RT
		Cisplatin + RT followed by cisplatin + 5-fluorouracil
	Advanced disease	Docetaxel + cisplatin + 5-FU (TPF)
		Cisplatin + 5-FU (PF)
	High risk	Postoperative RT + cisplatin + 5-fluorouracil
	Metastatic / recurrent	Cisplatin + 5-fluorouracil
		Carboplatin + 5-fluorouracil
		Cisplatin or carboplatin + 5-FU + cetuximab
PNETS	Advanced / metastatic	Streptozocin + 5-fluorouracil
Pancreatic cancer	Advanced / metastatic	Oxaliplatin + irinotecan + 5-FU + leucovorin (FOLFIRINOX)
		Oxaliplatin + folinic acid (leucovorin) + 5-FU (OFF) + BSC (best supportive care)
Vaginal cancer	Advanced	Cisplatin + 5-FU + RT

From Boyiadzis et al [54].
RT = radiation therapy; CIV = continuous intravenous infusion.

surface area [5,11,14]. We assumed this frequency could be reduced to 1% with pretherapeutic assessment of DPD activity [16]. The secondary endpoints were early grade ≥ 3 adverse events potentially related to 5-FU, grade ≥ 3 adverse events occurring during the first 3 months of treatment, respective frequencies of the relevant DPYD SNPs and correlation with 5-FU toxic events.

In the present study, we could demonstrate a significantly reduced frequency of early severe toxic side effects in the group of patients with pre-therapeutic assessment of DPD activity;

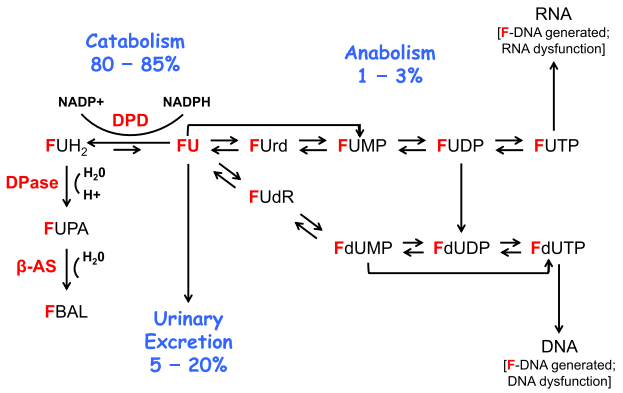

Fig. 1. Metabolic pathways of 5-fluorouracil.

furthermore, the study had to be stopped early, after consulting a group of independent experts, in conformity with the protocol, due to a toxic death in a patient in arm B, with no detection, retrospectively attributed to DPD deficiency.

2. Study oversight

The study was approved by the independent ethics committee at each participating institution and was conducted in accordance with the International Conference on Harmonisation E6 requirements for Good Clinical Practice and with the ethical principles outlined in the Declaration of Helsinki. All the patients provided written informed consent before the initiation of the study. The reference of the study in Current Controlled Trials NCT01547923.

All of the authors vouch for the adherence of the study to the protocol. The first draft of the manuscript was written by the first author, with input from trial investigators, and by clinical researchers and a biostatistician employed by the sponsor, all of whom are authors. No one who is not an author or who is not otherwise acknowledged contributed to the manuscript, other than giving constructive feedback. The first author made the decision to submit the manuscript for publication, which was agreed on by all the authors.

The sponsor monitored the study and provided the DPD deficiency detection at no charge. Data were collected by the investigators and analyzed by a statistician, employed by the sponsor, who is also an author and who vouches for the accuracy and completeness of the data reported.

3. Patients

Eligible adults ($\geq$ 18 years of age) had a World Health Organization (WHO) performance status of 0 to 2, had not previously received administration of fluoropyrimidines, and had histologically confirmed adenocarcinoma of the colon or rectum. Their estimated life expectancy had to be at least 3 months.

Written informed consent was obtained from all patients. The study was approved by the Committee on Human-Related Research of Angers and by the 10 local institutional review boards. An independent data and safety monitoring committee evaluated all serious adverse events (no. AFSSAP 050832).

Patients had to have adequate hematologic, hepatic, and renal function (including an absolute neutrophil count of $\geq 1.5 \times 10^9$/L, a hemoglobin level of $\geq$ 9 g/dL, and a bilirubin level at or below the upper limit of the normal range, according to the standards at the local laboratories).

4. Study design and treatment

This was a non-randomized multicenter cohort study with two groups of patients treated for colorectal cancer with 5-FU–based chemotherapy. Different standard protocols were allowed to be used, ie, FOLFOX4 [29], FOLFIRI [2], LV5FU2, and FUFOL [2] combined or not with bevacizumab or EGFR monoclonal antibody [30].

Patients were distributed in two arms: arm A—assessment of DPD activity was performed before treatment; arm B—no assessment of DPD activity was done before treatment. Every investigator site had to decide for the whole study, which arm they would enroll their patients in, based on institutional clinical practice.

In arm A, after the patient had signed the informed consent form, a blood sample was withdrawn to perform assess DPD activity before 5-FU administration: both phenotypic and genotypic assessments using a multiparametric approach were performed [26].

In the case of complete DPD deficiency detected pretherapeutically, 5-FU was considered contraindicated and therefore could not be administered. An alternative drug, a thymidylate synthetase

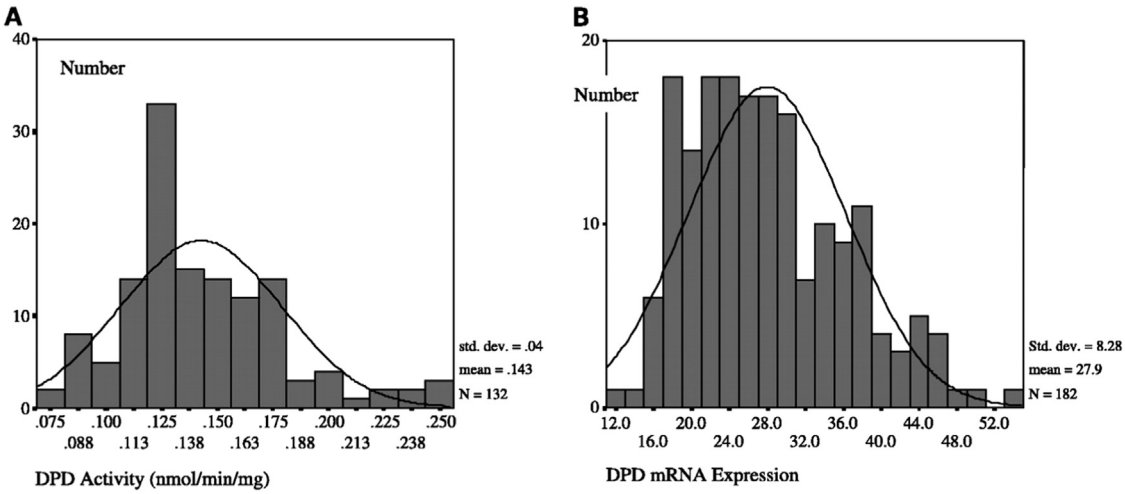

Fig. 2. Distribution of DPD enzyme activity in a healthy population. Distribution of DPD enzyme activity in the 132 caucasian individuals tested in this study. The distribution differs significantly from Gaussian Normal and has significant positive skew and kurtosis [7].

Table 2
DPD deficiency cases linked to mutant alleles of the *DPYD* gene.

Topic	*DPYD* mutations	Description of the mutation	Consequences	Reference
Patient who had previously developed neurotoxicity after 5-FU		Partial DPD deficiency in father and children consistent with an AR pattern of inheritance	Complete DPD deficiency in mononuclear cells with prolonged 5-FU half-life and no 5-FU catabolites in plasma or CSF; 89.7% excreted unchanged in urine	Diasio, 1988 [8]
Cancer patient with grade 4 toxicity 10 days after 5-FU treatment	IVS14 + 1G > A (DPYD*2A)	G→A point mutation at the 5'-splicing site consensus sequence (GT→AT) leads to skipping of the entire exon preceding the mutation during pre-RNA transcription	Proposed genotyping test for the G→A splicing point mutation could be useful in predicting cancer patients prone to toxicity after 5-FU	Wei X et al, 1996 [9]
Blood samples from 16 members of a British family; proband found with partial DPD deficiency after 5FU toxicity	Point mutations identified in 3 racial groups as G1601A (exon 13, Ser534Asn), A1627G (exon 13, Ile543Val), and G2194A (exon 18, Val732Ile)			Wei X et al, 1998 [10]
Toxic death after capecitabine in breast cancer patient with "deficient phenotype" and heterozygous for IVS14 + 1G > A	IVS14 + 1G > A (DPYD*2A)	Changes invariant splice donor site from GT→AT, skipping exon 14 immediately upstream of the mutated splice donor site as the DPD pre-mRNA undergoes splicing. DPD mRNA lacks 165-bp encoding aa 581–635; mutant DPD protein has no residual enzymatic activity	IVS14 + 1G > A in 40–50% of subjects with partial or complete DPD deficiency; heterozygous patients have substantially reduced enzyme activity; homozygous subjects lack detectable activity	van Kuilenburg ABP et al, 2002 [15]; van Kuilenburg ABP et al, 1999 [55]; Wei X et al, 1996 [9]
Patient with complete DPD deficiency	2846A > T variant			Gamelin E et al, 1999 [56]; van Kuilenburg ABP et al, 2000 [5]
Patients suffering from severe 5-FU associated toxicites	Eleven mutations identified: 1 splice-site mutation (IVS14 + 1G→A), 1 nonsense mutation (E386X), 4 missense mutations (M166V, V335L, I560S, D949V) and 5 SNPs(C29R, R21Q, S534N, I543V, V732I).			van Kuilenburg ABP et al, 2003 [11]
14 patients with a reduced DPD activity experiencing early severe toxicities	Mutations in 11/14; five with multiple mutations; four patients 85 T > C, six IVS14 + 1G > A; one homozygous for the 2194G > A; three heterozygous for 1627A > G; two homozygous for the 496A > G and 2846A > T			van Kuilenburg ABP et al, 2003 [11]
Four individuals with symptoms of 5-FU–related toxicity compared with 157 healthy individuals	Six sequence variants in DPD. 775A > G in 1 patient, 4 missense mutations 85 T > C, 496A > G, 1601G > A, and 1627A > G in another. 496A > G and 1601G > A in another; 85 T > C and the silent mutation 1896 T > C	1601G > A polymorphism, result in 5-FU related toxicity only in combination with other genetic variants		Gross E et al, 2003 [57]
Respective frequencies of 22 DPD mutations and correlation with severe acute toxicity	Four SNPs in 187 patients: D949V—rs67376798 - exon 22 IVS14+1 G > A—*DPYD**2—rs3918290, intron 14 I560S-*DPYD**13 - rs55886062-exon 13 Del TCAT-*DPYD**7 - exon 4	60% to 100% of patients with DPYD mutation experienced early grade 3 to 4 toxicity		Morel A et al, 2006 [12]
Toxic death after LV5FU2 in colon cancer;	Novel mutation 464 T > A identified in DPYD gene exon 5, detected in 2 other members of the family	Polymorphism led to replacement of leucine 155 by a stop codon in the protein	Total DPD deficiency	Morel A et al, 2007 [47]
Patient with complete DPD and *UTG1A1* deficiencies died from 5-FU and irinotecan-related toxicities,	IVS14+1G > A homozygote mutation in *DPYD* gene associated with TA7/7 homozygote mutation in *UTG1A1* gene promotor.			Mounier-Boutoille H et al, 2010 [58]

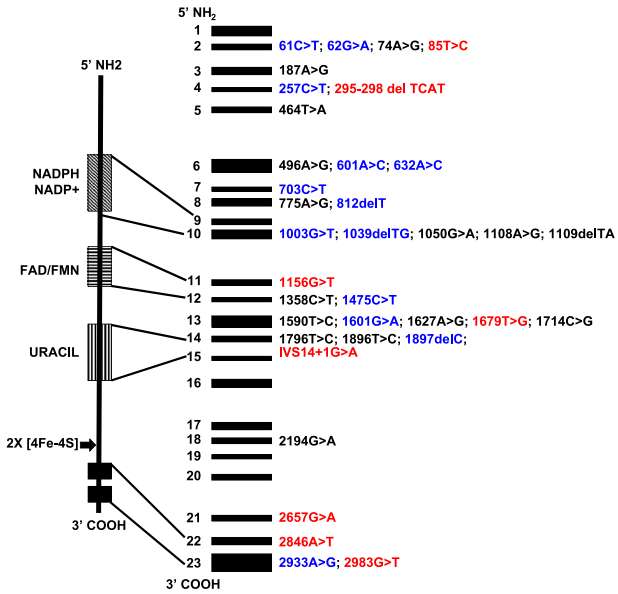

Fig. 3. Different SNPs in the hot spots of the *DPYD* gene. In red are shown the SNPs systematically screened in clinical practice.

inhibitor, not from the fluoropyrimidine class, was suggested after discussion with the investigator.

In the case of partial deficiency, the DPD activity was assessed and a dose of 5-FU adjusted to the metabolic capacities of the patient was proposed and discussed with the investigator, in order to allow the treatment with no serious 5-FU–induced adverse events. 5-FU PK monitoring, with a close follow-up, could be performed to further individually tailor the 5-FU dosing to the metabolism of the patient (5-FU$^{\text{ODPM Protocol}}$; ODPM, Angers, France).

In arm B, with no pretherapeutic assessment, after the patient had signed the informed consent form, 5-FU was administered at the standard dose in the selected regimen. A blood sample was obtained and kept in order to be able to assess DPD activity retrospectively when the study was finished, or in case of early severe emerging clinical events suspected to be related to DPD deficiency. In cases requiring hospitalization, DPD activity was assessed based on the multiparametric approach.

In case of toxic death deemed attributable to 5-FU by the investigator and/or the sponsor, an external and independent expert committee would review the data and decide if the death was due to a proven DPD deficiency, and the study would have to be definitively stopped.

Table 4
SNPs associated with severe DPD deficiency.

Exon	Nucleotide	Protein	Reference	NCBI SNP reference
22	A2846T	D949V	DPYD*9B	rs67376798
Intron 14	IVS14+1G > A	Exon 14 skipping	DPYD*2A	rs3918290
13	T1679G	1560S	DPYD*13	rs55886062
4	delTCAT 295-298	Frameshift	DPYD*7	rs72549309

5. Methods

5.1. Genotyping

The *DPYD* gene was genotyped as previously reported [12]. As previously described, the analysis of *DPYD* polymorphisms were based on pyrosequencing technology. Four SNPs associated with severe DPD deficiency were systematically investigated (Table 4) [12].

5.2. 5-FU catabolism index

As previously described, the catabolism index was determined by the quantification of endogenous uracil (U) and dihydrouracil (UH_2)[24] using HPLC and by determining the ratio UH2/U.[16,23]

5.3. Multiparametric approach

DPD screening was accomplished using a comprehensive approach including: genotyping (*DPYD* SNPs), phenotyping (UH_2/U ratio, uracil and dihydrouracil concentrations in plasma) and demographic parameters such as age and gender.[16] This multiparametric approach was performed using the calculator 5-FU$^{\text{ODPM Tox}™}$.[31] The results were sent to the investigator within 5 working days and, in case of DPD deficiency with a high risk of toxicity, the situation was discussed with the investigator. Based on the assessed DPD activity, 5-FU could be deemed contraindicated or a reduced initial dosing suggested based on 5-FU dose algorithm with individual dose adjustment and pharmacokinetic monitoring performed using the pharmacokinetics (PK)-monitoring solution 5-FU$^{\text{ODPM Protocol}}$ [27,31].

5.4. Safety monitoring

Safety monitoring relied on investigator assessments of treatment-related adverse events and serious adverse events,

Table 3
Frequency of different SNPs in a population of 487 patients, and frequency of IVS14 + 1G > A, A2846T, and T1679G in an extended population of 1,200 patients, and correlation with toxicity, any grade, and grade 3 to 4.

SNPs	Patients heterozygous / homozygous (N = 487)	*n* (%) patients with grade 1 to 4 toxicity	*n* (%) patients with grade 3/4 toxicity	Patients heterozygous / homozygous frequency (N = 1,200)	*n* (%) patients with grade 3/4 toxicity
IVS14 + 1G > A	9/1	7 (70)	6 (60)	16/1 (1.3/0.1)	11 (70)
2846A > T	10/0	7 (70)	6 (60)	19/0 (1.6)	13 (69)
85T > C	150/15	20 (12)	11 (6.6)	ND	8 (5.5)
−1590T > C	7/0	1 (14)	0	ND	0
1679T > G	1/0	1 (100)	1 (100)	2/0 (0.16)	2 (100)
2983G > T	0/0	X	X	ND	NA
295-298 del TCAT	0/0	X	X	ND	NA
1156G > T	0/0	X	X	ND	NA
2657G > A	0/0	X	X	ND	NA
0 SNP found	300	33 (11)	20 (6.6)	ND	NA

ND = not determined; NA = not applicable.

and the rates of dose modifications, dose delays, and premature discontinuations of the study drugs.

Every 2 weeks, patients were examined and adverse toxic events were evaluated and graded. Treatment-related adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0 [32] and were coded and summarized according to the preferred terms in the *Medical Dictionary for Regulatory Activities*, version 12.0. The worst grading was considered and reported.

Early acute adverse events deemed related to 5-FU were specifically reported. Early severe toxic side effects considered potentially related to 5-FU included diarrhea, neutropenia, thrombocytopenia, skin toxicity, and mucositis/stomatitis. Cardiac toxicity, eg, myocardial ischemia, cardiac arrhythmias, hyper- and hypotension, left ventricular dysfunction, and cardiac arrest, was not considered in this list as these events are not linked to DPD deficiency and due to different mechanisms, involving 5-FU catabolites [33]. Doses of each agent were reduced following adverse events as specified in the study protocol.

6. Study end points

The primary end point was severe toxicity (grade 4–5), potentially related to 5-FU occurring within the first two complete cycles. The secondary end points were early toxic deaths related to 5-FU, early grade 3, 5-FU potentially related toxic events, and time to occurrence of severe toxic events potentially related to 5-FU .

7. Statistical analysis

The primary objective of the study was to significantly reduce the percentage of early grade 4–5 serious adverse events, potentially related to 5-FU occurring during the first two cycles. A frequency of about 3% was expected in arm B with no assessment and about 1% in arm A with DPD assessment. A complete prevention of early severe neutropenia, thrombocytopenia and/or diarrhea was not expected, as oxaliplatin and irinotecan can provoke these adverse events as well.

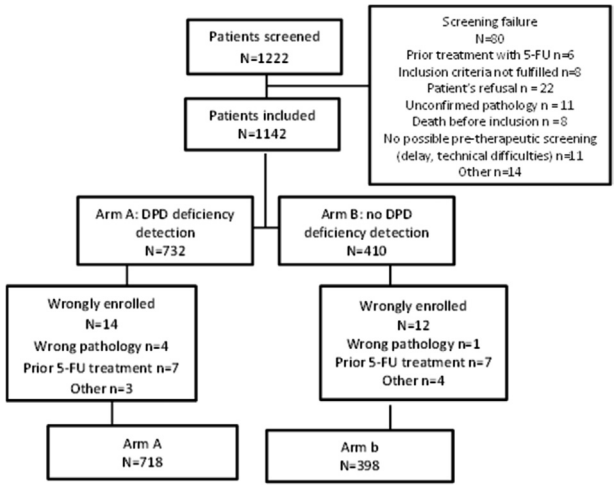

Fig. 4. Study design.

The secondary objectives were (1) to avoid any toxic death related to 5-FU, considering reports in the literature estimate early toxic deaths occur in about 0.3% of patients treated with 5-FU [11,16], and (2) to demonstrate a significant reduction of severe adverse events, grade 3–4 regardless of the timing of occurrence. All analyses were performed in the intention-to-treat population.

Considering a 5% lost to follow-up rate, the total number of patients to be included was 1,148 allowing for 1% α risk of error in order to conclude a difference that does not exist and 5% β risk of error to conclude to the absence of difference.

8. Results

A total of 1,142 patients were enrolled in the study from June 1, 2008 to July 31, 2012. Seven hundred thirty-two patients were

Table 5
Patients demographics.

	Arm A Detection (n = 718)	Arm B No detection (n = 398)	*P*
Age (range)	64.9 ± 11.0 (24–88)	63.7 ± 10.9 (24–88)	.0398
Performance status			.0789
0	397 (55.3)	221 (55.5)	
1	179 (24.9)	139 (34.8)	
2	28 (3.9)	24 (6.0)	
3	1 (0.1)	1 (0.2)	
Not reported	113 (15.7)	14 (3.5)	
Primary tumor location			
Colon	476 (66.3)	251 (63.1)	
Rectosigmoid	80 (11.1)	74 (19.6)	
Rectum	148 (20.6)	67 (16.8)	
Other	14 (2.0)	5 (1.3)	
Unknown	0	1 (0.2)	.004
Type of treatment			
Adjuvant	366 (51.0)	203 (51.0)	
Neo-adjuvant	55 (7.7)	3 (0.8)	
First-line metastatic	297 (41.3)	191 (48.0)	
Second-line metastatic	0	1 (0.2)	< .001
Treatment regimens			.086
LV5-FU2	50 (7.2)	19 (4.8)	
FUFOL	11 (1.5)	0	
FOLFIRI	23 (3.3)	13 (3.3)	
FOLFIRI + targeted therapy	113 (15.7))	41 (10.3)	
FOLFOX	404 (56.3)	247 (62.1)	
FOLFOX + targeted therapy	50 (7.0)	56 (14.1)	
Radiotherapy and 5-FU	44 (6.1)	0	
Other	23 (3.2)	22 (5.5)	

Table 6
DPYD genotyping and DPD phenotyping data.

	Whole population		Arm A: Pretherapeutic detection		Arm B: No detection	
	n	%	n	%	n	%
	1,116		718		398	
Genotype						
Ho *DPYD**2	1	0.09	1	0.14	0	0
h *DPYD**2	12	1.07	7	0.97	5	1.26
h D949V	11	0.98	6	0.84	5	1.26
h *DPYD**13	2	0.18	1	0.14	1	0.25
UH_2/U						
Median ± SD	11.6 ± 3.85		12.18 ± 3.82		10.34 ± 3.64	
Range	0.007 – 44.13		0.007 – 44.13		0.961 – 29.52	
Complete deficiency (genetic homozygous)	1	0.09	1	0.14	0	0
Complete deficiency (multiparametric)	2	0.18	1	0.14	1	0.25
Partial deficiency (genetic heterozygous)	25	2.24	14	1.95	11	2.76
Partial deficiency UH_2/U	32	2.87	10	1.39	22	5.53
Partial deficiency multiparametric determination	58	5.19	24	3.34	34	8.54

included in arm A and 410 in arm B. Fourteen patients in arm A and 12 in arm B were incorrectly enrolled. The demographics of the patients enrolled are displayed in Table 5 and the study design is shown in Fig. 4.

One hundred thirty-six (18.9%) patients in arm A and 54 (13.5%) in arm B received a treatment based on a combination of irinotecan and 5-FU (FOLFIRI regimen). Four hundred fifty-four patients (63.2%) in arm A and 303 (76.1%) in arm B received a combination of oxaliplatin and 5-FU (FOLFOX regimen). Approximately half of the patients were treated in an adjuvant setting in both arms: 366 patients (51.0%) in arm A and 203 patients (51.0%) in arm B.

Among the 718 evaluable patients in arm A, 15 (2.12%) were found to have a *DPYD* SNP: IVS14+1 G > A—*DPYD**2—rs3918290 (seven patients heterozygote and one homozygote), D949V—rs67376798 (six patients heterozygote) and I560S—*DPYD**13—rs55886062 (one patient heterozygote). Ten patients had a phenotype of DPD deficiency, ie, a significantly reduced UH_2/U ratio. Based on the multiparametric approach, 24 patients were found to have a partial DPD deficiency and one patient, the one IVS14+1 G > A—*DPYD**2 homozygote, had a complete DPD deficiency (Table 6).

According to the protocol, each of the 24 patients deemed to have partial DPD deficiency were initially treated with reduced 5-FU doses based on DPD activity assessment and 5-FU dose algorithm. This was then followed by individual dose adjustment with a PK follow-up. The patient with complete DPD deficiency had 5-FU replaced with another thymidylate synthetase inhibitor.

Nine grade 4 adverse events compatible with 5-FU–induced toxicity were reported prior to cycle 3 in nine patients in arm A (1.2%) (Tables 3 and 5). No grade 5 toxicities were observed (Tables 3 and 6). Among these nine patients, only one, who experienced grade 4 diarrhea, had partial DPD deficiency and was found to be homozygous for Gilbert's syndrome: the patient was UGT1A1 7/7 with an unchanged irinotecan dosing despite the UGT1A1 deficiency. Two other patients treated with FOLFIRI also had a UGT1A1 SNP: UGT 6/7. Based on these results, the pretherapeutic detection was efficient as the patients with DPD deficiency did not experience grade 4–5 toxic events potentially related to 5-FU.

The assessment of *DPYD* SNPs, UH_2/U using the multiparametric approach was retrospectively performed in the entire arm B population. Eleven (2.7%) patients had a *DPYD* relevant SNP, none of them was homozygous: IVS14+1 G > A—*DPYD**2—rs3918290 (five patients), D949V—rs67376798 (five patients), and I560S—*DPYD**13—rs55886062 (one patient). Twenty-two (5.4%) had a phenotype of partial DPD deficiency. Based on the multiparametric approach 34 patients (8.5%) were found to have a partial DPD deficiency and one patient had a complete DPD deficiency (Table 2).

Seventeen (4.2%) grade 4–5 toxic events compatible with 5-FU-induced toxicity (16 grade 4 toxicity and one grade 5 toxicity) were reported before cycle 3 in 12 of 398 evaluable patients (3.0%), meaning some patients experienced more than one severe adverse event (Tables 3 and 5). Among these 12 patients, four had a DPD deficiency (33%); thus the detection would have avoided these severe toxicities.

A grade 5 multivisceral toxicity occurred in a 65-year-old woman treated with FOLFOX 4 in an adjuvant setting for T3N1 colon cancer. The patient received one cycle with a total 5-FU dose of 4,536 mg (2,400 mg/m² 5-FU over 48 hours). On day 4 of cycle 1, the patient was hospitalized due to grade 3 nausea and vomiting and grade 2 mucositis, which quickly worsened to grade 3. On day 6 of cycle 1, the patient experienced febrile grade 4 neutropenia, documented septicemia, grade 5 diarrhea, and mucositis. She was transferred to the intensive care unit. Renal failure secondary to dehydration induced by severe diarrhea was diagnosed on day 13; the patient had to be ventilated and rehydrated but despite these measures died on day 22 of multivisceral failure. The grade 5 serious adverse event was declared by the investigator imputable to 5-FU. *DPYD* genotyping and DPD phenotyping were retrospectively performed for this patient and complete DPD deficiency was confirmed. The patient was heterozygote for D949V—rs67376798.

An independent committee of four international experts was established as planned in the study protocol. The experts unanimously acknowledged the imputability of 5-FU treatment-related

Table 7
Grade ≥4 toxic side effects, potentially related to 5-FU occurring before C3.

Arm A: Detection	Arm B: No detection	*P*
Neutropenia (5)	Neutropenia (11)	
Diarrhea (1)	Thrombocytopenia (3)	
Mucositis/stomatitis (1)	Diarrhea (1)	
Leucopenia (1)	Multivisceral toxicity (1)	
Anorexia (1)	Febrile aplasia (1)	
9	17	.0019

Table 8
Number of grade ≥3 toxic side effects potentially related to 5-FU, per group and total before C3.

Grade	Total	Arm A: Detection (%)	Arm B: No detection (%)	*P*
3	122	69 (9.6)	53 (13.3)	.0497
4	25	9 (1.2)	16 (4.0)	
5	1	0	1 (0.25)	

Table 9
Number of patients who experienced grade 4-5 before C3 related to 5-FU.

	Total	Arm A: Detection	Arm B: No detection	*P*
Patients	21 (1.9)	9 (1.2)	12 (3.0)	.0406

toxicity provoked by the DPD deficiency in the multivisceral failure and recommended to prematurely close the enrollment of patients in the study citing ethical concerns, in agreement with the rules defined in the protocol.

The primary objective of the study was to significantly reduce the percentage of early grade 4 toxicity potentially related to 5-FU, occurring before cycle 3 and to avoid the risk of premature toxic death. The percentage of grade 4/5 toxic events observed in arm A was significantly lower than in arm B ($P = .0019$) (Table 7) as was the percentage of grade ≥ 3 (Table 8). Likewise, the percent of patients who experienced grade 4/5 toxic events was lower in arm A than in arm B (1.2% ν 3.0%); this difference was statistically significant ($P = .0406$) (Table 9). The percent in arm B is consistent with the theoretical value of 3% reported in the literature and both percentages are compatible with the potential contribution of oxaliplatin or irinotecan in the occurrence of early severe toxicity.

A secondary objective was to demonstrate a significant reduction of the incidence of severe toxicity, grade 3 or higher within the first two cycles. The percent of patients with a toxicity grade 3 or higher observed in arm A was 10.8% (n = 78) compared to 17.55% (n = 69) reported in arm B ($P = .0497$). Another secondary objective was to reduce the occurrence of toxic death from 3/1,000 to 0. The percentage of death was reduced from 2.5/1,000 in arm B to 0 in arm A.

The time to occurrence of all grade ≥ 3 toxicities was determined in both arms. At 30 days, the percent of patients with no grade > 2 toxicity was 96.7% (95.1%; 97.8%) in arm A compared to 87.3% (83.5%; 90.2%) in arm B; at 60 days it was 87.7% (84.9%; 90.0%) in arm A compared to 79.3% (74.8%; 83.0%) in arm B and at 90 days it was 79.6% (76.2% ; 82.6%) in arm A compared to 73.7% (68.8%; 77.9%) in arm B. The difference between the two arms was significant ($P = .047$) (Fig. 5).

Based on a previous medico-economic study, the cost of a pretreatment screening test combining genetic and phenotyping was €190. The avoided cost per patient screened was €313 for two cycles of treatment and a savings of €2,780 per toxicity avoided. The incremental net benefit (INB) per patient screened was €426. The screening strategy allowed for avoiding toxicities and saving money [26,34].

9. Discussion

Early severe hematological, mucosal, cutaneous, and/or digestive toxic side effects, including death, encountered with 5-fluorouracil, have been ascribed to complete or partial deficiency of the enzyme DPD for many years. It is clearly a problem of public healthcare that cannot be underestimated. The present prospective study has assessed and demonstrated the importance of pre-therapeutic detection of DPD deficiency in patients planned to receive 5-FU–based chemotherapy. The activity of DPD, the key enzyme in the catabolism of fluoropyrimidines follows a Gaussian curve in the general population (Fig. 2), and some mutations in the *DPYD* gene have been reported to cause early severe 5-FU–related adverse events but the detection of *DPYD* SNPs alone has never provided an accurate assessment of the risk of early severe 5-FU–induced poly-visceral toxicity [16,26,27]. We determined that the combination of two complementary techniques, *DPYD* genotyping and DPD enzyme phenotyping, suitable for routine clinical application, is crucial for reliably detecting DPD deficiency. A multiparametric approach has been set up and an algorithm established, combining high-throughput *DPYD* genotyping, DPD phenotypic method, measuring the ratio UH_2/U in blood and patient's demographic parameters.

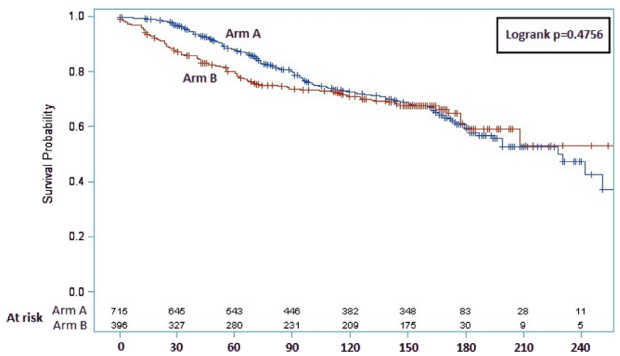

Fig. 5. Survival probability of the two populations of patients without grade ≥ 3 toxic events (arm A: blue, arm B: red).

With the patients in arm A of this study, we confirmed that 3.3% of patients had partial DPD deficiency, a percent similar to that previously reported [11,16]. One patient with metastatic colorectal cancer was found to have complete DPD deficiency and therefore at high risk of lethal toxicity. 5-FU was contraindicated and was replaced with another thymidylate synthetase inhibitor, with a different metabolic pathway. Of course, capecitabine could not be proposed, since it is a prodrug of 5-FU and multivisceral lethal toxic side effects have been reported with this drug as well [35]. This patient was able to receive the full planned treatment with no major toxicity. The other 24 patients with partial deficiency had a reduced initial dose of 5-FU and then could benefit from a PK-monitored 5-FU dose adjustments. In the entire arm A population of patients with and without DPD deficiency, only nine early grade 4 toxic events, ie, diarrhea, neutropenia, thrombocytopenia, were observed (1.3%) in nine patients treated with a combination regimen. We have to keep in mind that of the 718 patients, 136 (18.9%) have been treated by a combination of irinotecan and 5-FU in the (FOLFIRI regimen), and irinotecan itself can provoke early severe diarrhea, neutropenia, or thrombocytopenia, even more so than in the case of Gilbert's syndrome, which occurs in about 10% of the general population [36]. Likewise, 454 patients (63.2%) received a combination of oxaliplatin and 5-FU (FOLFOX regimen), and oxaliplatin can induce severe hematoxicity as well [37].

In arm B, with no pretherapeutic assessment, 17 grade 4/5 toxic events compatible with 5-FU–induced toxicity (16 grade 4 toxicity and one grade 5 toxicity) were observed before cycle 3 in 12 of 398 evaluable patients (3.0%). Again, the frequency was consistent with what was expected based on the literature [11,16]. When comparing the two arms, the incidence of early severe toxicity was significantly higher in arm B ($P = .0019$), confirming the positive impact of pretherapeutic DPD assessment. More than half of the patients, 51.0%, in each arm, were treated in an adjuvant setting, ie, because they were at risk of metastatic recurrence. As such, one patient (0.25%) received 5-FU in a LV5FU2 regimen in an adjuvant setting for a PT3N1 disease and experienced early polyvisceral toxicity, with a pattern of toxic events characteristic of 5-FU leading to her death. This patient was perhaps already cured of her disease after surgery but she died 13 days after administration of 5-FU, from grievous complications. This patient was retrospectively confirmed to have a complete DPD deficiency, although not homozygote but heterozygote for D949V—rs67376798 and the multivisceral toxicity followed by her death could have been easily avoided with pretherapeutic assessment of DPD deficiency. Furthermore, this result shows that there is no total correlation between homozygous status and complete DPD deficiency and a

complete DPD deficiency can be encountered both in heterozygous patients and in patients without mutations.

As planned in the protocol, an independent expert committee was convened to consider the toxic death due to 5-FU in a patient with complete DPD deficiency in arm B, in contrast to a patient in arm A who was pretherapeutically detected with complete DPD deficiency, who did not receive 5-FU, and therefore was able to be treated without incident. The committee unanimously recommended stopping the enrollment for ethical reasons.

Beside the two patients with complete DPD deficiency, 24 patients in arm A had a partial deficiency and could receive 5-FU–based chemotherapy with no severe toxicity. Clearly, a partial deficiency of DPD activity does not imply that 5-FU is contraindicated, provided that a dose adjusted to the DPD activity is administered. Using the multiparametric approach we have been able to determine the level of DPD activity for these patients and individually adjust the dose. Then a close PK follow-up could be performed for each patient. Interestingly, the percentage of patients retrospectively diagnosed with DPD deficiency in arm B was a little higher than in arm A, but that may be due to the fact that it was a smaller population.

Different approaches have been considered, attempting to prevent or manage early severe polyvisceral 5-FU–induced toxic effects. Clearly *DPYD* genotyping is not sensitive enough to cover most of the DPD deficiencies as we found evidence of DPD deficiencies with no detectable *DPYD* SNPs. Moreover, in practice, genotyping is most often performed retrospectively after polyvisceral toxicity and/or a toxic death and usually only IVS14+1 G > A—*DPYD**2 is tested, which does not cover the most frequent relevant SNPs [13,14,16,38–40]. In addition, it is usually considered that being heterozygous for a SNP leads to a partial deficiency, whereas being homozygous leads to a complete deficiency. This is clearly inaccurate as a patient in arm B who had complete DPD deficiency was heterozygote for D949V—rs67376798.

Likewise, we were able to show that DPD activity assessment with UH_2/U determination alone does not provide a fully reliable method [16,25,26]. The other approaches previously reported such as 2-^{13}C-uracil breath test or promoter methylation and large intragenic rearrangements of DPYD seem less interesting and have not been pursued in clinical practice [41–43]. Some authors recommend administering uridine triacetate, which received US Food and Drug Administration approval as an antidote for overexposure to 5-FU in March 2016 [44]. This approach can be useful in case of accidental overdosing, but is clearly insufficient in the case of DPD deficiency. Bypassing DNA synthesis blockade may only help to solve the problem of the hematopoietic toxicity but central neurotoxicity, ie, leukoencephalopathy induced by 5-FU, involves totally different mechanisms of toxicity, including uracil and fluoroacetate [45–47]. Fluoroacetate inhibits the Krebs cycle through the inhibition of the aconitase-catalase conversion of citrate to isocitrate, leads to impairment of the urea cycle and accumulation of ammonia, and, therefore, encephalopathy, and is accompanied by hyperammonemia and lactic acidosis [48,49]. 5-FU can also exacerbate thiamine deficiency [50,51]. Consequently, unlike G3 carboxypeptidase, which is the true antidote of methotrexate cleaving it in non-toxic and clearable compounds, giving downstream pyrimidines such as uridine or thymidine may solve only one of the issues.

The current study showed that the pretherapeutic detection of DPD deficiency was cost-effective, avoiding early severe life-threatening toxic events. Several studies assessed the cost-effectiveness of PK-guided 5-FU in patients receiving fluorouracil chemotherapy by continuous infusion and showed its economic interest, but they did not analyze the detection of the DPD deficiency [52,53].

In conclusion, the pretherapeutic DPD assessment reduced the incidence of early severe toxicities associated with 5-FU and avoided early toxic death due to this drug. The pretherapeutic detection of DPD deficiency requires combining *DPYD* genotype and DPD phenotype assessment using a multiparametric approach. Based on the reduction in toxicities experienced in those patients who were prescreened for DPD deficiency, as well as the cost savings associated with the lower toxicity rate, pretherapeutic evaluation for DPD deficiency should be considered for all patients who will receive a 5-FU–containing regimen.

Conflicts of interest

Erick Gamelin, Alain Morel, and Michèle Boisdon-Celle are cofounders of ODPM SAS.

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