

DOI:10.2478/jbcr-2020-0013
Review

TOXIC AND ADVERSE EFFECTS OF CHEMOTHERAPY WITH 5-FLUOROPYRIMIDINE DRUGS. COULD DIHYDROPYRIMIDINE DEHYDROGENASE ENZYME SCREENING SERVE AS A PREREQUISITE TO SUCCESSFUL CHEMOTHERAPY?

Velko T. Minchev

*Department of Medical Oncology,
SofiaMed University Multiprofile
Hospital for Active Treatment*

Corresponding author:

Velko T. Minchev
Department of Medical Oncology,
SofiaMed University Multiprofile Hospital
for Active Treatment,
16, G. M. Dimitrov Blvd,
1797 Sofia,
Bulgaria
e-mail: v_minchev@abv.bg

Received: August 17, 2020

Revision received: August 19, 2020

Accepted: December 11, 2020

Summary

The article presents a detailed survey of recent publications in the literature concerning clinical expertise, existing guidelines, and differing opinions on Fluoropyrimidine chemotherapy-related toxicity and the implication of Dihydropyrimidine dehydrogenase (DPD) screening aiming to prevent severe 5-Fluorouracil-induced adverse drug reactions. The first section provides information on the mechanism of action, clinical application, pharmacokinetics and pharmacodynamics, and toxicity and adverse reactions of 5-Fluorouracil, Capecitabine, Floxuridine, and Flucytosine.

The second section summarizes DPD phenol- and genotype data and provides reasons for determining a DPD life-threatening complete or partial enzyme deficiency. The pros and cons of the methodological approaches for DPD screening are analysed, and recommendations are made to introduce them into clinical practice.

The third section includes a brief economic analysis of expenses for DPD screening of patients scheduled for 5-Fluorouracil chemotherapy. The costs are compared to those related to the treatment of patients suffering from 5-Fluorouracil-induced toxicity and unwanted adverse effects.

Keywords: 5-Fluoropyrimidines chemotherapies, Dihydropyrimidine dehydrogenase screening, cost-effect analysis

Drugs for targeted and adjuvant chemotherapy

Modern chemotherapy is an essential part of the multimodal treatment of malignant diseases. It plays a significant role in the treatment of malignant tumours of the gastrointestinal tract (gastroesophageal, colorectal) and malignancies of the lung and breast. During the last decades, the strategies in designing new anti-tumour drugs have undergone dramatic changes, resulting from newly acquired knowledge on the molecular mechanisms of malignant transformations on a cellular level. Despite the outstanding results from applying drugs with a target mechanism of

action on a molecular level, the new therapeutic approaches are not likely to replace the classical cytotoxic drugs in the foreseeable future [1]. It is known that monoclonal antibodies or small target molecules are not highly effective when used for the monotherapy of solid tumors. However, when medical preparations like Tanstuzumab, Bevacizumab are administered in combination with cytotoxic drugs at an earlier stage of the disease, the anti-tumour therapy is much more effective [2]. Simultaneously, the toxicity of chemotherapeutics is suppressed by applying new anti-nausea and colony-stimulating medications.

Furthermore, resistance to cytotoxic preparations is prevented by bringing back to normal the functions of the cardiovascular system, initiating apoptosis, and suppressing processes that are induced in specific cascades of growth factors [3]. Drug resistance is an important barrier to achieving the desired effect of anti-tumour therapy. This resistance is determined by different pharmacokinetics and molecular mechanisms. It can also be provoked by mutations amplified or eliminated by target structures in the tumour cell, thus compromising many therapeutic schemes that are otherwise strongly supported in theory. Defects in recognizing DNA defects or over-expression of specific corrective enzyme systems also result in the occurrence of resistance to cytotoxic drugs [4]. Cytotoxic drugs should be administered at doses as close as possible to the maximum individual dose to suppress tumour growth. During each cytotoxic treatment cycle in patients with tumours larger than 1 cm or over 10⁹ tumour cells, less than 99% of all the tumour cells die. This necessitates multiple cycles to be carried out, with a carefully planned periodicity [3].

Cell-cycle as a target of cytotoxic agents

Slow-growing tumours, like carcinomas of the gastrointestinal tract and the lung, are more sensitive to agents that strongly affect DNA (alkalizing drugs) or maintain higher intracellular concentrations (fluoropyrimidines). All cells, irrespective of their origin, go through the same cell proliferation stages:

- stage G1 – preceding DNA synthesis;
- stage S - DNA synthesis;

- stage G2 – an interval after DNA synthesis is complete;
- stage M – mitosis, when the cell divides into two daughter G1 cells with duplicated DNA;
- stage G0 - quiescent stage that can last and would not pass into stage G1.

During all cell cycle stages, a few specific proteins - p53, and chk-1 and chk-2, monitor the DNA integrity. In the case of DNA damage, a recovery process begins. If the damage is severe, the cell cycle ceases, and apoptosis occurs. Some chemotherapeutic agents act in a specific stage of the cycle, mainly in Stage S and Stage M. In contrast, others are cytotoxic in all the stages and are non-specific cytotoxic agents. The transition to each stage requires the activation of cyclin-dependent kinases (CDK). When they are activated, they bind to regulatory proteins (cyclins). The CDK4-6 – the cyclin D1 complex phosphorylate Rb1, blocks the inhibitory action of E2F transcription factors on proliferation and makes it possible for the cell cycle to transfer from Stage G1 to Stage S, or cancer, respectively [5]. Inhibitor proteins like p16 block the action of CDK. Inhibitors of CDK4-6, which block the CDK 4 / 6 - cyclin D1 complex, prevent the phosphorylation of Rb1, thus block the transfer of the cell cycle from Stage G1 to Stage S [6]. The tumour cells exhibit changes in the cell cycle regulation, bringing about unrestrained proliferation (mutation or loss of p16, or other inhibitory components of the so-called retinoblastoma pathway, an increased cyclin or CDK activity). In a normal cell cycle, cytotoxic agents will cause apoptosis when the cell is in the stage G1/S or G2/M borderline zones. When the p53 gene or other regulator proteins have mutated or are absent, the damaged cell will not deviate to apoptosis but will proceed to stage M and mitosis. This population of cells will develop as mutated and potentially resistant to cytotoxic drugs. The lack of regulation of the cell cycle leads to uncontrolled cell proliferation that characterizes the neoplastic process. The moment in which the cell cycle passes from stage G1 (pre-DNA synthesis) to stage S (DNA synthesis) is crucial for preventing abnormal cell proliferation. The CDK 4 / 6 - cyclin D complex plays the role of a key regulator, inhibiting the CDK 4 cascade (INK 4)-retinoblastoma (Rb), which induces the proliferation process [7].

Fluoropyrimidine anti-tumor agents

This section presents four long-known and widely used Fluoropyrimidine anti-tumour medications: 5-Fluorouracil, Capecitabine, 5-Fluorodeoxyuridine (Floxuridine) and its active analogue 5-Fluorodeoxyuridine monophosphate, and 5-Fluorocytosine (Flucytosine), which is an antifungal drug for oral administration. The drugs belonging to this group act in a uniform manner by suppressing the synthesis of essential precursors of DNA. The DNA molecule is made up of four bases: two pyrimidines (thymine and cytosine) and two purine (guanine and adenine). In mammalian cells, the pyrimidine bases exist in an active form as nucleosides only coupled with ribose or deoxyribose sugars. After triple phosphorylation, these precursors transform into nucleotides. Mammals cannot utilize thymine, cytosine, and guanine as free bases, and they are found in blood only as nucleosides and in cells only as nucleosides and nucleotides. The different purine and pyrimidine triphosphates are intracellular depots of PNA precursors (ribose sugar) and of DNA (deoxyribose sugar). Besides, uracil is included in RNA as a base, instead of thymine.

The conception of suppressing DNA synthesis requires the creation of analogues of these precursors, which can easily enter the tumour cells and be activated by the intracellular enzymes [8].

5-Fluorouracil

5-Fluorouracil (5-FU) is a pyrimidine analogue that irreversibly activates the enzyme thymidylate synthase, causing thymine deficits in the cell, leading to suppression of DNA synthesis and cytotoxicity. RNA synthesis is also suppressed, though to a lesser extent. These effects are most expressed in fast-growing cells and can end up with cell death.

Pharmacokinetics and pharmacodynamics

After intravenous application of 5-FU, it leaves the blood in a very short time (elimination half-life varies from 8 to 20 min), quickly penetrating the tumour cells, the spinal cord, and the mucosa because of the loose binding to the plasma proteins. Four hours after injection, its concentration in these cells is 6 to 8 times smaller than that in normally growing cells. 5-FU penetrates the cerebrospinal, pleural, and abdominal cavities. As little as 5% to 10% of the injected 5-FU is excreted with the urine with an elimination half-life of 0.76 hr. After entering the cell, 5-FU is involved in two mutually exclusive processes: catabolic processes and inactivation, and anabolic processes of generating active metabolites. In the liver catabolic pathway, 80 to 85% of the injected 5-FU is metabolized. The first stage of inactivation is catalysed by dihydropyrimidine dehydrogenase and elapses quickly, generating 5,6-dihydro-5-fluorouracil (5-FUH₂). During the second inactivation stage, FUH₂ converts to α -fluoro- β -ureido-propionic acid. In the third stage of inactivation, the end metabolite - α -fluoro- β -alanine is generated to be eliminated in the urine. The basic parameters of the pharmacokinetics of 5-FU are presented in Table 1.

Only a small fraction (1% - 5%) of the 5-FU injected is transformed intracellularly into cytotoxic compounds in the anabolic pathway. The first stage is the transformation of 5-FU, occurring in three paralleling enzyme processes. The least important for the cytotoxic effect is that of 5-fluoro-2-deoxyuridine monophosphate, which in two consecutive reactions of phosphorylation converts to 5-fluoro-uridine-diphosphate and 5-fluoro-uridine triphosphate. The latter conjugates with ribose or deoxyribose into 5-FU nucleotides, which are substrates of DNA polymerases. Once included as defective,

Table 1. Pharmacokinetic characteristics of 5-Fluorouracil

<i>Data from Thummel, K. E., et al., 2011 [9]</i>							
BioA (%)	ExcrU (%)	BoundP (%)	Clearance (ml/min/kg)	VolD (L/kg)	$\frac{1}{2}$ L (min)	PeakT (hr)	PeakC
28 (0 - 80)	< 10	8 - 12	16 ± 7	0.25 ± 0.12	11 ± 4	-	12.2 μ M

BioA Bioavailability, ExcrU Urinary excretion, BoundP Bound in plasma, Clearance, VolD Volume of distribution, $\frac{1}{2}$ L Half-life, PeakT Peak-time, PeakC Peak concentration

these 5-FU nucleotides irreversibly damage the DNA molecule. The RNA molecule is less damaged. Besides, and parallel to these damages, the 5-FU nucleotides irreversibly inhibit the enzyme thymidylate synthase, which inhibition ceases thiamine synthesis.

For over 60 years, 5-FU has been one of the first-choice medicines used in systemic chemotherapy for some of the most common malignant tumours like those of the colon, stomach, oesophagus, pancreas, breast, head and neck, or topical treatment for some dermatoses such as actinic keratosis, eczemas, Bowen's disease, and some skin cancers. In cases of systemic application of 5-FU, it should be considered that, with 5-FU, there is a strong dose/toxicity correlation and that the therapeutic window is narrow. These circumstances necessitate 5-FU administration under therapeutic drug monitoring (TDM). The preferred treatment regimen is to apply 5-FU at 14-day intervals as i.v. bolus infusion, followed by slow 48-hour i.v. application [10], which makes it possible to achieve a better therapeutic result, lower overall toxicity, and a more prolonged progression-free survival (PFS). The response rate in monotherapy with 5-FU varies from 10% to 30% [11]. This range comes to explain why in new treatment schemes combinations of 5-FU with other cytotoxic agents. Below, some widely accepted schemes for the combined application of 5-FU are shown. Table 2.

Treatment with 5-FU is personalized, with individualized doses. The European Medicine Agency Updated Joint Assessment Report [12] states that the ECF scheme is most commonly applied in peri-operative chemotherapy for gastroesophageal carcinoma. In this one, 5-FU is infused at a dose of 200 mg / m² for GC and 200

mg / m² to 1000 mg / m² for EC. In the schemes for peri-operative chemotherapy in colorectal carcinoma cases, the 5-FU infusion dose is from 600 mg / m² to a maximum of 3600 mg / m². In pancreatic cancer, the 5-FU infusion dose from 200 mg / m² to 500 mg / m² is combined with folic acid or Gemcitabine. In the schemes for peri-operative chemotherapy for breast cancer, the 5-FU bolus infusion dose is 500 mg / m² to 600 mg / m².

Toxicity and adverse events

5-FU exerts influence on both tumour and healthy cells, which causes dose-dependent toxic changes and adverse drug reactions (ADR). Among these, the toxic effects on the spinal cord cells, gastrointestinal mucosa, and the myocardium are dominant. It should be taken into account that, in fact, 5-FU is always administered in combination with other cytotoxic agents, so it is not always possible to prove the aetiology of the toxic effects definitely. Like toxic diarrhoea, nausea, and vomiting, the most common ADR occurs during the first 5-7 days after therapy is initiated. They are seen in 50% to 80% of patients with colorectal cancer, and in 30% of them, 3rd to 4th toxicity levels are observed. The pathophysiology of this complication includes an inflammatory reaction in the epithelium and the mucosa of the entire gastrointestinal tract [13]. The hematotoxic ADRs rank second. They are seen in 61% of the patients treated with 5-FU and occur 7 to 10 days after initiating therapy. They manifest with neutropenia, leukopenia, thrombocytopenia, and, more rarely, with anaemia. The toxicity is of the 3rd to 4th level [14]. The frequency of cardiovascular ADRs associated with 5-FU administration

Table 2. Therapeutic schemes of 5-FU combined application

<i>Abbreviation</i>	<i>Drug combination</i>	<i>Tumor</i>
FOLFIRI / FLIRI	5-FU - Folinic acid - Irinotecan	CRC
FOLFOX 4	5-FU - Oxaliplatin	CRC
FOLFOX 6	5-FU - Oxaliplatin - Leucovorin	CRC
FOLFIRINOX	5-FU - Irinotecan - Oxaliplatin	CRC
CMF	5-FU - Cyclophosphamide - Methotrexate	BC
MFL	5-FU - Methotrexate - Leucovorin	BC
FEC	5-FU - Epirubicin - Cyclophosphamide	BC
ECF	Epirubicin - Cisplatin - 5-FU	GC, EC

CRC Colorectal cancer, BC Breast cancer, GC Stomach cancer, EC Esophageal cancer

ranges from 2% to 18%, and they manifest with angina pectoris, tachycardia, dyspnoea, and hypotension. In rare cases, myocardial infarction, arrhythmia, or cardiogenic shock have been reported [15]. The pathophysiological mechanisms of the cardiotoxic effects of 5-FU are not fully understood. It is assumed that blood vessel spasms and cardiomyopathy due to oxidative stress are the leading pathogenic factor [16]. Neurological and ophthalmological alterations and alopecia have also been reported as ADRs.

Capecitabine

Capecitabine (CCB), an oral fluoropyrimidine carbamate, is a precursor of 5-deoxy-5-fluorouridine, a prodrug of 5-FU. CCB has a safer toxicity profile and an easier oral route of administration. That is why CCB is preferred for treatment if the infusion of 5-FU is not possible or a product with a safer toxicity profile is to be administered.

Pharmacokinetics and pharmacodynamics

When taken by mouth, CCB is quickly absorbed, and extensive conversion occurs in the hepatic cells. A study on patients with colorectal cancer given CCC after meals at a dose of 1250 mg / m² for 14 days established that the peak plasma concentration was 4.67 µg/mL. The time to reach the peak was 1.5 hr, the level of plasma protein binding was 54%, and elimination half-time was 0.85 hr [17]. In the liver, CCB is metabolized by the enzyme carboxylesterase-2 into 5-deoxy-5-fluorocytidine. The latter is converted by cytidine deaminase into 5-deoxy-5-fluorouridine, which in turn converts into 5-FU by the enzyme thymidine phosphorylase. One unique feature of this enzyme is that its concentration in the tumour cells is significantly higher than in healthy cells. This feature explains why the administration of CCB leads to higher concentrations of 5-FU in

the tumour tissues. In patients with colorectal cancer, the concentrations established in the tumour are 3.2 times higher than those in the adjacent healthy tissues and 21.4 times higher than those in the plasma. The basic parameters of CCB pharmacokinetics are presented in Table 3.

Chemotherapy with CCB is indicated in postoperative adjuvant treatment for colorectal cancer, gastric cancer, and breast cancer after failed treatment with other cytotoxic anti-tumour drugs (regimens with taxanes or anthracyclines). According to today's guidelines, CCB is almost always applied in chemotherapy combined with other anti-tumour agents. It has been stated in The European Medicine Agency Updated Joint Assessment Report [12], that in principle, chemotherapy for advanced metastatic CCB is applied as monotherapy twice a day at a dose of 1250 mg / m² for 14 days, followed by a 7-day rest period in combination with intravenous infusion of docetaxel at a dose of 75 mg / m² every third week. For gastrointestinal carcinoma, the treatment is generally after the ECF scheme. CCB is applied at doses of 800 mg / m² to 1000 mg / m² twice a day for 14 days, followed by a 14-day rest period or 625 mg / m² twice a day, if applied for a longer time. If the initial dose is 800 mg / m² twice a day for 14 days, followed by a 7-day rest period, t, CCB is applied on the first day of treatment combined with 200 mg / m² irinotecan.

Toxicity and adverse events

The toxic effects and ADR associated with CCB are similar to those of 5-FU application, but their intensity is much lower. The most frequent manifestations of toxicity are diarrhoea and myelosuppression. A more characteristic manifestation of CCB toxicity is the so-called hand-foot syndrome marked by erythema, desquamation, pain, and paraesthesia on touching

Table 3. Pharmacokinetic characteristics of Capecitabine

<i>Data from Thummel, K. E., et al., 2011 [9]</i>							
BioA (%)	ExcrU (%)	BoundP (%)	Clearance (L / hr / m ²)	VolD (L / m ²)	½ L (hr)	PeakT (hr)	PeakC
---	3	<60	145 (34 %)	270	1.3	0.5-1-0	6.6±6.0 µM

BioA Bioavailability, ExcrU Urinary excretion, BoundP Bound in plasma, Clearance, VolD Volume of distribution, ½ L Half-life, PeakT Peak-time, PeakC Peak concentration

hands and feet. It is empirically assumed that diarrhoea, mucositis, and myelosuppression are more often seen in bolus injection cases than with infusion, while the hand-and-foot syndrome is more frequent in cases of infusion than in bolus injection [8].

5-Fluorodeoxyuridine (Floxuridine) and its active analogue 5-Fluorodeoxyuridine monophosphate

5-Fluorodeoxyuridine is applied for a long-term hepatic arterial infusion in patients with metastatic colorectal carcinoma or patients undergone hepatic resection for liver metastases. When these agents are introduced via hepatic arterial infusion for 14 to 21 days, a desired therapeutic effect is achieved in 40% to 50% of the patients. These medical products are currently administered to a limited number of patients because of the high risk of life-threatening biliary sclerosis/ cirrhosis. That is why such therapy should be discontinued at the first sign of ADR [18].

5-Fluorocytosine

5-Fluorocytosine (Flucytosine, FC) was synthesized more than 60 years ago as an antifungal agent to be administered per os. This product has no antifungal effect per se. However, when taken by mouth, it is easily absorbed in the gastrointestinal tract and transferred to bodily fluids in patients with a fungal infection. From the bodily fluids, Flucytosine is transferred through cytosine permease into the fungal cells, where it deaminates into 5-FU. Severe 5-FU toxicity symptoms can occur in patients when treatment is administered against the background of a likely dihydropyrimidine dehydrogenase deficiency. This is why FC is included in this section.

Pharmacokinetics and pharmacodynamics

5-Fluorocytosine is almost entirely (76%-89%) absorbed through the mucosa of the gastrointestinal tract. The distribution volume is approximately equal to the total body fluid at a minimal binding to the plasma proteins. When a dose of 37.5 mg/kg is taken, the plasma peak concentration level is between 70 µg/mL до 80 µg/mL, achieved within 1-2 hours in half-life

time ranging from 3 to 6 hours. 5-Fluorocytosine has low molecular weight and is highly soluble in water. It rapidly enters all body cavities and fluids. Approximately 80% of the FC taken is excreted in the urine unchanged. The renal clearance of FC is almost equal to the renal creatinine clearance. The plasma levels of FC have to be monitored twice a week, especially in patients with kidney filtration failure. The antifungal action of FC is determined by the enzyme cytosine deaminase, which converts inactive Flucytosine to active 5-Fluorouracil [19]. The popular assumption that cytosine deaminase has a lower activity in mammalian cells is currently under revision. It has been proved that bacteria normally found in the human digestive tract can deaminate FC, which in turn determines the development of severe toxic effects of 5-FU. One specific indication for treatment with Flucytosine is severe systemic fungal infections, such as cryptococcoses, including meningitis and candidiasis, including candidemia and candiduria disseminated candidiasis, chromomycosis, including chromoblastomycosis, and rare forms of aspergillosis. Dosage varies from 100 mg/kg to 200 mg/kg, divided into 3 - 4 intakes. Regular twice a week, monitoring the FC serum concentration is recommended, the optimal therapeutic values ranging from 35 µg/mL to 70 µg/mL.

Toxicity and adverse drug reactions

Myelosuppression with leucopenia and thrombocytopenia, hepatotoxicity with liver transaminases elevation has been reported as toxic effects and ADR in FC application. The toxic complications in FC and 5-FU therapy are similar. Nausea, vomiting, and diarrhea have been reported. Concerning the fact that antifungal treatment is lengthy, regular monitoring of the FC blood levels is required.

Dihydropyrimidine dehydrogenase

The metabolic inactivation of 5-FU takes place in many tissues, but degradation in the liver pathway predominates. It occurs by reducing the pyrimidine ring in the 5-FU molecule, which generates 5-Fluoro-5,6 dihydrouracil – a metabolite ranking first in the metabolic cascade of 5-FU. The process is catalysed by

dihydropyrimidine dehydrogenase (DPD). Most of this enzyme is located in hepatocytes, and smaller quantities are present in the intestinal epithelium, tumour cells, and other tissues [8].

Complete/partial deficiency of DPD enzyme activity

The antimetabolite effect of 5-FU is seen in tumour cells and healthy, rapidly dividing cells. This ubiquitous activity leads to the dose-dependence of cytotoxic effects that vary in degree and manifestations. DPD plays a crucial role in the development of therapeutic and/or cytotoxic effects of 5-FU. After the i.v. introduction of 5-FU, a small quantity (5%- to 20%) of the agent is excreted unchanged with the urine for 6 hours. The remainder of the 5-FU injected is metabolized mainly in the liver. It is assumed that DPD metabolically processes 80%-85% of the injected 5-FU. The enzyme's decreased activity and deficiency is the primary modulator of the 5-FU plasma levels and the ensuing toxic effects. The deficiency/lack of DPD enzyme activity and ADRs related to 5-FU are characterized by rapid onset (less than 20 days after the first infusion) and a severe clinical presentation. The scheme of anabolism and catabolism of 5-FU is presented in Fig. 1.

The high toxicity of 5-FU in DPD deficiency was first reported more than 30 years ago [20,21,22]. However, efficient approaches for minimizing the high 5-FU toxicity in DPD deficiency states have not been suggested yet. A debate has been started only recently for adopting criteria and designing protocols when therapeutic schemes imply the administration of

pyrimidine analogues. One characteristic feature of DPD is the wide variability of its activity. The latter varies from relative deficiency to almost complete lack of activity, mainly attributed to the DPYD gene's polymorphism, which codes for the synthesis of DPD. The DPYD gene comprises 23 exons, located on 950kb on chromosome 1p22. Today, over 160 variants of nucleotide polymorphism have been described, resulting in the reduced or completely lacking activity of the enzyme [23]. It is known that some homozygote or heterozygote mutations in certain loci of the DPYD gene can produce a complete or nearly complete lack of the enzyme's activity. There are four such loci identified: c.1905+1G>A, known as DPYD*2A, c.1679T>G, known as DPYD*13, c.2846 A>T and c.1236G>A / HarpB3 [24, 25, 26]. There is reliable data that patients with the above-cited heterozygotic mutations of DPYD are at high risk for severe toxicity when treated with fluoropyrimidines [27, 28,29,30]. In Caucasians, the frequency of DPYD*2A is 1%, for c.2846 A>T - 2.6% - 6.3%, for c.1236G>A/HarpB3 it is 0.07%, and for DPYD*13 - 0.07% - 0.1% [31]. Empiric data show that DPD activity deficiency is more expressed when the mutations are in DPYD*2A и DPYD*13 compared to the other two DPYD mutations. However, it had been proved that the number of patients carrying DPYD mutations, which are responsible for decreased DPD activity, and respectively, 5-FU cytotoxicity, is significantly smaller than the number of patients with toxic reactions.

On the other hand, only about 50% - 80% of

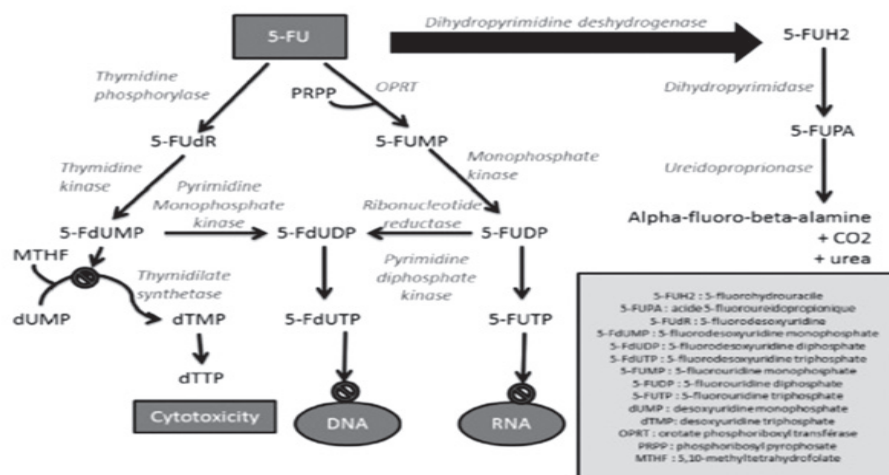


Figure 1. Metabolism and anabolism of 5-FluoroUracil

the carriers of DPYD mutations, which cause deficiency or lack of DPD activity, experience cytotoxicity due to fluoropyrimidines. That may suggest that other alleles in DPYD also contribute to toxicity and the possibility of compensation through other mutations of the gene. It is assumed that other genetic variants [32, 33, 34] and epigenetic regulators [35] can contribute to toxic adverse reactions in fluoropyrimidine therapy. DPD activity demonstrates a significant circadian rhythm and is also related to age, gender, ethnicity, dose, and administration route, among other not yet well-studied factors [36,37]. The frequency of the main DPYD variants in the population and the cohort of Caucasian patients undergoing fluoropyrimidine therapy is presented in Table 4.

Verification screening of subsisted partial /complete deficiency of DPD enzyme activity

During the last couple of years, the problem of designing and proposing generally applicable guidelines for clinical practice has been approached [39]. In April 2018, the Group of Clinical Pharmacology on Oncology (GCPO)

recommended that the following steps be taken before initiating 5-FU therapy: 1. Screening for DPD deficiency before applying treatment with 5-FU or Capecitabine; 2. Phenotype DPD screening, investigating the plasma uracil, and, if possible, quantifying the dihydrouracil/uracil ratio and genotyping of DPYD by the *2A, *13, p.D949V и HapB3 mutations; 3. reducing the initial dose of 5-FU depending on the DPD status determined [38]. A scale was suggested for evaluating the DPD activity and determining the dosage of fluoropyrimidine agents [40]. Data is presented in Table 5.

Phenotyping for DPD activity can be done by summing up the coefficients of two DPYD variants plus the coefficient of a variant with the lowest activity [41]. The data summarized are presented in Table 6.

In 2018, Institut national du Cancer carried out a large scale phenotyping study, including 38863 patients [31]. The results showed that 3 500 patients (9.4%) had partial DPD enzyme activity deficiency. A complete deficiency was found in 31 patients (0.08%). The Directive of The Royal Dutch Pharmacists Association recommends therapeutic behaviours when chemotherapy with

Table 4. The frequency of major DPYD variants in the Caucasian race

Data from Loriot, M. A., et al., 2018 [38]

DPYD variants	Frequency in the population	Proportion of Carriers		Number of carriers per 100 000 patients	
		xt-3	xm-3	xt-3	xm-3
DPYD* 2A	0.8 %	1.5 %	0.01 %	1500	10
DPYD* 13	0.1 %	0.2 %	0.0001 %	200	0,1
c.2846A>T	0.6 %	1.0 %	0.004 %	1000	4
HapB3	2.4 %	4.6 %	0.06 %	4600	60
<i>xm-3</i>	<i>homozygous</i>	<i>xm-3</i>	<i>heterosygous</i>	-	-

Table 5. Quantitative indices of DPD enzyme activity

DPD activity	Normal	Deficient	None
Coefficient	1.0	0.5	0

Table 6. DPD probability phenotyping determined by PDYD genotyping

DPD phenotype	DPYD normal metabolizer	DPYD moderate metabolizer	DPYD poor metabolizer
Coefficient	2.0	1.0 or 1.5	0 or 0.5
5-FU dose	Not reduced	Reduced by 25 % to 50 %	Not applicable

fluoropyrimidines is initiated. In cases of zero DPD activity, there is a very high risk for severe, potentially fatal toxicity. A standard dosage of 5-FU could prove to be an overdose with a coefficient of 100, and alternative chemotherapy should be recommended. In case the DPD activity is 0.5, there is a high risk for severe or potentially fatal toxicity. A standard 5-FU dosage can be an overdose. In such cases, initial therapy with a 25% standard dose or alternative chemotherapy is recommendable. In cases of 1.0DPD activity, there is a high level of significant or potentially fatal toxicity. A standard dose of 5-FU could prove to be an overdose. So initial therapy with 50% of the standard dose is recommended. When the DPD activity is 1.5, there is a high level of risk for significant or potentially fatal toxicity, and a standard dose of 5-FU could prove to be an overdose. Then the initial dose should be 75% of the standard dose. EMA has pointed out that there is an opinion on the level of DPD activity related to chemotherapy with fluoropyrimidines in Bulgaria, but it is quite conditional and optional.

Similarly, defined directives for investigating the DPD activity level before chemotherapy with fluoropyrimidine agents have a conditional and not necessarily mandatory significance in Germany, the Netherlands, and Norway. In France, these directives have been mandatory since 2017 [12]. The main reason for this diversity of behaviour is that the so-far published data from clinical trials are not sufficient to set precise criteria and design guidelines to be generally approved and accepted in oncology practice.

Methodology for verification of subsisted partial/complete deficiency of DPD enzyme activity

Two methods are used to establish the DPD status of patients before the initiation of therapy with fluoropyrimidine agents:

A) genotype analysis, which proves the presence of DPYD variants, provoking partial / complete deficiency of DPD enzyme activity;

B) phenotype analysis directly determines the DPD enzyme activity by measuring the plasma concentration of uracil [42] and/or the dihydrouracil/uracil ratio [43].

A simplified scale for analysing the

dihydrouracil/uracil ratio in a clinical setting has been proposed [44]. According to this scale, if the ratio is higher than 6, the dosage of 5-FU should be standard. When the ratio ranges from 6 to 3, the 5-FU dose should be 50% of the standard dose, and an individual plan for pharmacokinetic control is made. If the ratio is between 3 and 1.5, the 5-FU dose is decreased by 70%, with an individual pharmacokinetic control plan to be made. When the ratio is under 1.5, applying 5-FU is not recommended, and if it is unavoidable, the dosage is reduced by 80%. There is no uniform view of which of the two approaches is more appropriate for the time being. Under these circumstances, the French Institut National du Cancer started a three-year clinical research program (FUSAFE, 2015-2017 in 2014 for developing recommendations for establishing subsisted partial/complete deficiency of DPD enzyme activity. The standpoint is that both genotyping and phenotyping should be recommended given their specificity, sensitivity, and applicability in clinical practice. The final version of the recommendations was published in 2018, with the following conclusions:

Phenotyping is a gold-standard method to avoid early severe toxicity.

Because of technological advances in this field, phenotyping is the method of choice for clinical practice.

Genotyping is easier to apply in clinical practice, yet it is difficult to apply it routinely for verifying subsisted partial/complete deficiency of DPD enzyme activity.

Phenotyping is a more sensitive method for verifying subsisted partial/complete deficiency of DPD enzyme activity [31, 38].

The best solution would be combining these two methods, but this is hardly applicable in everyday practice. The determination of uracil plasma levels higher than 150 ng / mL indicates a complete DPD deficiency, associated with a high risk for very severe fluoropyrimidine toxicity. Plasma levels over 16 ng / mL and under 150 ng / mL indicate partial DPD deficiency and a higher risk for fluoropyrimidine toxicity. Since plasma levels of uracil vary, the threshold accepted as defining an absolute contraindication for treatment with fluoropyrimidine agents is 150 ng / ml [45].

One alternative method for verifying subsisted

partial/complete deficiency of DPD enzyme activity is defining the DPD enzyme activity in mononuclear cells (lymphocytes, neutrophils) in peripheral blood [45,47]. Although this method is recognized as a golden standard, it is not applied routinely because of its costs, time, and labour input. The calculated area under the curve (AUC) is considered a highly informative pharmacokinetic indicator that can most adequately correlate with the 5-FU effectiveness and toxicity. Continuous pharmacokinetic control of dosage and therapeutic monitoring of the agent are recommended in cases of long-time i.v. infusion of 5-FU. Clinical experience has shown that the target values of AUC that would verify that the efficacy of 5-FU therapy ranged from 20 mg x h / L to 30 mg x h / L. An exemplary scale demonstrating AUC changes and corresponding dosage changes is presented in Table 7. (EMA November 22, 2019). Dosages of 5-FU ranging from 291 mg / m² to 727 mg / m² provide AUC optimal values of the therapeutic scope of 20 mg x h / L to 30 mg x h / L.

Economic analysis of reactive vs. prospective screening of partial/complete deficiency of DPD enzyme activity

Therapy with fluoropyrimidine agents is applied to millions of patients with malignant tumours. Complete deficiency of DPD enzyme activity is found in 0.01% - 0.5% of the Caucasian population. There is a high risk of life-threatening or fatal toxic events from initiating fluoropyrimidine therapy in patients with such a

deficiency. Partial deficiency of DPD activity is established in 3% - 8% of this population. There is an increased risk for severe or potentially life-threatening events in patients with such deficiency if fluoropyrimidine treatment is started. Under such conditions, thousands of patients could be at risk for severe to fatal toxicity. Therefore, genotyping and phenotyping are necessitated in patients if treatment with fluoropyrimidine agents is to be applied. A study was carried out in the USA in 2017 to establish a correlation between the DPYD* 2A mutations (approximately 1%) and the probability of 5th-degree, i.e., fatal toxicity to 5-FU. To prevent one fatal outcome from toxic reactions to 5-FU in the studied patient cohort, 1000 patients had to undergo genotyping for DPYD* 2A mutations. Given the cost (USD 82) for genotyping for DPYD* for one patient, the expenses for preventing one fatal outcome due to toxicity to 5-FU would amount to USD 82 000, making the cost acceptable against the background of other medical procedures and tests.

Currently, prospective testing for DPYD mutations is not routine for economic reasons and the lack of definite guidelines regarding dosage schemes in patients with proven DPD deficiency. In 2018, an economic analysis was made in Ireland on the treatment cost-effectiveness in 134 patients given first-line therapy with 5-FU for three years. In this group, 30 patients (23%) developed 3rd- to 4th-degree toxicity, and in 17% of them (5 patients), DPYD mutations were proved. The cost of hospital stay for the patients with DPYD mutations amounted

Table 7. The changes of 5-FU dosing specified by AUC

AUC (mg x h / L)	Dose (mg / m ²)
> 40	727 ↓
37 - 39	582 ↓
34 - 36	436 ↓
31 - 33	291 ↓
20 - 30	Standard
17 - 19	291 ↑
14 - 16	436 ↑
11 - 13	582 ↑
0 - 10	727 ↑
↑ <i>increment</i>	↓ <i>decrement</i>

to 232 061 EUR, while the costs for prospective testing for DPYD mutations would have been 23 718 EUR. The conclusion from this clinical-economic analysis is that prospective testing has a multifold better coefficient for cost and effect. Provided that treatment with 5-FU is associated with the development of high toxicity and deleterious adverse drug reaction, applying pharmaceutical monitoring is justifiable from both medical and financial-economical points of view, and therefore should be undoubtedly encouraged.

Acknowledgment

This work was supported by the Council of Medical Science of MU-Sofia under Contract D-98/24.06.2020.

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