

## Review Article

# Individualization of 5-Fluorouracil in the Treatment of Colorectal Cancer

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Chemotherapeutic agents are generally characterized by a large inter-individual pharmacokinetic variability. The balance of efficacy and toxicity is critical and the imbalance can have devastating effects on patients. Standard dosing methods are inadequate in optimizing systemic exposure. Therapeutic drug monitoring (TDM) has the potential to improve the clinical use of chemotherapy agents. TDM has been successfully applied to optimize a few anticancer treatments including carboplatin, methotrexate, and 6-mercaptopurine. 5-Fluorouracil (5-FU) is considered the backbone in treatments of advanced CRC. Toxic side effects remain a significant problem despite increased safety of newer regimens. Molecular mechanisms that control DPD-activity are complex and have not been fully elucidated and cannot be used effectively to individualize Fluorouracil dosing as there is no established standard genetic test for DPD deficiency. Current phenotypic methods to measure DPD-activity are cumbersome. A reliable relationship between DPD genotype and 5-FU toxicity phenotype has not been established. Standard dosing of 5FU is by body surface area (BSA). Low correlation exists between exposure and BSA. A better predictor of total exposure is the area under the curve (AUC). Toxicity and efficacy have been correlated to AUC with target of 24-30 (mg,h/l). The therapeutic window is very narrow and difficult to attain by clinical follow-up alone. TDM has been shown effective in adjusting the dose based on AUC.

## 1. Introduction

An important component of improving the outcome in patients with cancer is increasing safety without reducing the efficacy of chemotherapy and optimizing efficacy without increasing toxicity.

Chemotherapeutic agents are generally characterized by a large interindividual pharmacokinetic variability, with relationships linking toxicity to systemic exposure, and toxic effects better related to exposure than to dose or dose intensity [1–5].

Individualizing doses and application schedules should add to the efficacy and safety of chemotherapeutic agents, because of their narrow therapeutic index.

Currently, the majority of chemotherapeutic agents are administered at a dose adjusted to body surface area (BSA) or to bodyweight. However, for most chemotherapeutic agents, there is no correlation between plasma clearance, exposure and BSA, and subsequently therapeutic outcome and toxicity.

Therapeutic drug monitoring (TDM) involves the measurement and interpretation of drug concentrations in

biological fluids and the individualization of drug dosages or schedules to maximize therapeutic outcomes, minimize toxicities, or both [6].

Plasma drug concentrations are assumed to correlate to the concentration at the site of action, which should correlate to the drug effect. The TDM approach can only be chosen after the drug has been administered, that is, *a posteriori*.

The prototype drug would not only have a narrow therapeutic index, but would have efficacy or toxicity that is clearly and robustly correlated with drug concentrations in plasma, serum, or another easily accessible body fluid. A timely method for assaying drug concentrations that would lend itself to implementation in the standard clinical lab setting is also needed. Finally, the drug should demonstrate variability in toxicity or efficacy to justify the use of resources for performing TDM.

Another approach is *a priori* individualization. It refers to the process of predicating drug effect and toxicity before the drug is given. An example is pharmacogenetic studies, which relate specific genetic factors to drug exposure and response. Genotyping is the direct analysis of genetic variation in an individual that gives rise to a specific drug metabolism

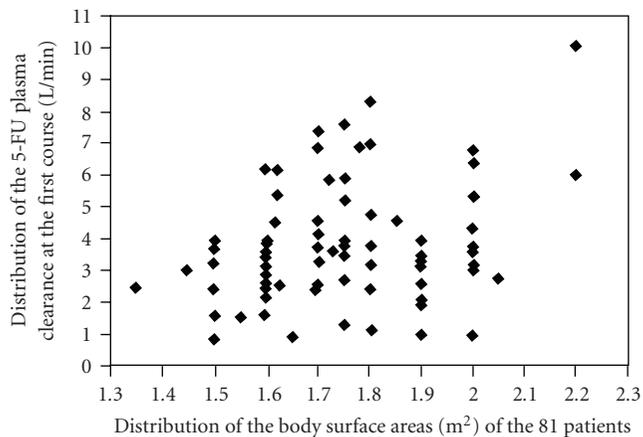


FIGURE 1: Absence of correlation between 5-FU plasma clearances of 81 patients and their body surface areas [9].

phenotype. Genetic polymorphism in drug metabolizing enzymes can introduce variability in its pharmacokinetic and pharmacodynamics.

Phenotyping of a drug metabolizing enzymes means the combined analysis of genetic and other factors for determining individual metabolic activity [7, 8].

5-Fluorouracil (5-FU) is the most frequent chemotherapy drug used in combination therapy to treat a wide variety of malignancies of the gastrointestinal tract, breast, and head and neck. Traditionally, 5-FU is administered by continuous infusion along with biomodulators and other chemotherapeutic agents. Toxic side effects, including diarrhoea, hand and foot syndrome, and mucositis, remain a significant problem despite somewhat increase safety of prolonged infusions and increased effectiveness of newer regimens.

Currently dosing of 5-FU by body surface area (BSA) is the standard. However, there is no correlation between plasma clearance, exposure and BSA [9]. (See Figure 1)

With equal BSA dosing, a wide variability in systemic exposure, is noted, resulting in one extreme of toxicity due to overdosing, or another extreme of suboptimal effectiveness because of underdosing.

Toxicity and efficacy of 5-FU have now been correlated to exposure, quantified by the Area under the curve (AUC) or 5-FU levels at steady state. The window between toxic and effective levels of 5-FU is very narrow and difficult to attain by clinical follow-up alone.

The monitoring of fluorouracil plasma concentrations seems principally useful for individual *a posteriori* dose adjustment.

To individualize fluorouracil administration before the first dose, assessment of the individual dihydropyrimidine dehydrogenases (DPD) activity may be useful, because this genetically highly polymorphic enzyme controls approximately 80% of fluorouracil elimination. Some patient have partial to absolute loss of DPD enzyme activity. Patients who are genetically deficient in this enzyme are at particular risk of life-threatening toxicity [10].

Several methods to assess DPD activity in patients have been proposed (genotyping, various phenotyping methods), but each of them has limitations.

This review will look at different methods adopted to individualize fluorouracil dosing including adjusting the dose to the results of DPD activity tests before 5-fluorouracil administration *a priori*, and/or the adaptation of doses after 5-fluorouracil exposure *a posteriori* using TDM and test dose methods. It will also try to deduce if these are reasonable approaches to better prevent toxicity and increase efficacy and are feasible and practical to be adopted as standard of care.

## 2. Background

**2.1. Colorectal Cancer.** Colorectal cancer involves the colon, rectum, and the anal canal. It is one of the three most common cancers occurring in adult men and women in the United States, and accounts for about 1 in 9 cancer diagnoses [11]. For both adult men and women, colorectal cancer is the third leading cause of cancer-related deaths in the United States [1]. Colorectal cancer is the second most common cancer after lung cancer, in terms of both incidence and mortality, in England and Wales. Although prostate cancer is more common in men and breast cancer more common in women, colorectal cancer affects both sexes [12].

Mortality and incidence rates associated with colorectal cancer in the United States have decreased over the past decades. These contrast with an increasing rate of incidence in countries where overall risk was relatively low (e.g., Japan and parts of Asia) and a stabilizing rate of incidence in high-risk countries in Northern and Western Europe [2]. Colorectal cancer mortality rates are comparable between the United States, Western Europe, and Japan [13].

Survival rates (relative to age-matched groups without colorectal cancer) are now around 45% at five years after diagnosis; beyond five years, relative survival rates decline only slightly: most of those who live this long are cured [12].

Multiple factors are associated with the development of colorectal cancer, including acquired and inherited genetic susceptibility, environmental elements, and lifestyle choices. Lifestyle factors, such as obesity, physical inactivity, chronic hyperinsulinemia, and alcohol and tobacco use, increase risk of colorectal cancer. Observational studies report associations between high-dietary intake of processed and red meats and fat, and a diet low in fibre, folate, fruit, and vegetables with increased risk of colorectal cancer. Regular aspirin and NSAID use and postmenopausal hormone replacement therapy decrease risk, but recommendations have not been made because of unresolved issues regarding risk-to-benefit considerations. Inherited genetic susceptibilities and clinical risk factors, such as inflammatory bowel disease, are well-known risks for colon cancer [14–22].

Approximately 20% of patients with colorectal cancer present with metastatic disease [23]. The most common site of metastasis is the liver, often the only site of metastatic disease in 40% of patients, followed by the lungs, and then bones, specifically the sacrum, coccyx, pelvis, and lumbar

vertebrae. Liver metastases are present in 5% to 10% of patients at presentation.

**2.2. Treatment.** Treatment modalities for colorectal cancer include surgery, XRT, chemotherapy, and other targeted molecular therapies (e.g., angiogenesis inhibitors, epidermal growth factor receptor inhibitors). Surgery is the important and definitive procedure associated with cure; XRT can improve curability following surgical resection in rectal cancer and may reduce symptoms and complications associated with advanced disease. Chemotherapy is used in adjuvant treatment regimens as well as in treatment for advanced stages of disease. Much progress has been made in the treatment of advanced disease, in the ability to identify candidates for potentially curative surgical procedures, and the availability of active drug regimens that can improve patients' survival.

For patients for whom treatment intent is curative, surgical resection of the primary tumour is the most important component of therapy, further adjuvant chemotherapy or chemotherapy plus XRT may be appropriate. For selected patients with resectable metastases, surgical resection may be an option. However, for most patients with metastases, systemic chemotherapy is the mainstay of treatment [24, 25].

For more than 40 years, fluorouracil has been the most widely used chemotherapeutic agent for the adjuvant treatment of colorectal cancer, both as a single agent and in combination with other agents. Newer agents such as oxaliplatin and capecitabine have been incorporated into combination chemotherapy regimens for the adjuvant treatment of colon cancer.

Fluorouracil was investigated as single-agent chemotherapy agents for use after surgery. In 1988, a meta-analysis was published that evaluated phase III trials that compared adjuvant fluorouracil to surgery alone [26]. A small, statistically insignificant improvement in survival was noted with fluorouracil-based regimens. Since then, most trials focused on improving the efficacy of fluorouracil in the adjuvant colon cancer.

Concurrently, it was discovered that the pharmacology of fluorouracil provides several opportunities to increase its antitumor activity. The addition of leucovorin increases the binding affinity of the active fluorouracil metabolite to a target enzyme called TS, thus enhancing its cytotoxic activity. The combination of fluorouracil plus leucovorin has undergone extensive study in the adjuvant setting. Several large randomized trials have evaluated the efficacy of fluorouracil plus leucovorin as adjuvant therapy for patients with stage II or III colon cancer [27]. In the United States, the Roswell Park regimen and the Mayo Clinic regimen were most commonly used, while in Europe, treatments such as the de Gramont regimen favour a continuous intravenous schedule of fluorouracil [28–30].

The schedule of fluorouracil and leucovorin administration varies in the different regimens. Clinical studies comparing the efficacy of bolus and continuous infusion schedules generally favour continuous infusion of fluorouracil, which is probably related to its short plasma half-life and S-phase specificity for optimal TS inhibition.

Continuous IV infusions also permit increased fluorouracil dose intensity, which may account for the higher response rates observed with prolonged infusions of fluorouracil. In most of the commonly used combination regimens, fluorouracil is administered by both IV bolus injection and continuous IV infusion.

Clinically significant differences in toxicity also differ based on the dose, route, and schedule of fluorouracil administration. Leukopenia is the primary dose-limiting toxicity of IV bolus fluorouracil, although diarrhoea, stomatitis, and nausea and vomiting can also occur [31].

A distinct toxicity, palmar–plantar erythrodysesthesia (“hand-foot syndrome”), and stomatitis occur most frequently with continuous IV infusion [31].

Regardless of the method of administration, toxicity is related to its catabolism and pharmacogenomic factors. Dihydropyrimidine dehydrogenase (DPD) is the main enzyme responsible for the catabolism of fluorouracil to inactive metabolites. A rare pharmacogenetic disorder characterized by complete or near-complete deficiency of this enzyme has been identified in cancer patients. Patients with this enzyme deficiency develop severe toxicity, including death, after fluorouracil administration. Molecular studies have identified a relationship between allelic variants in the *DPYD* gene (the gene that encodes DPD) and a deficiency in DPD activity. The gene for DPD has recently been localized to chromosome 1p22, and at least one mutation has been identified [32–35]. The most frequent mutation in patients with partial or complete DPD deficiency is allele DPD\*2A. Patients heterozygous for this polymorphism have low DPD activity and toxicity to 5-FU. Approximately 3% of patients may be genotypically heterozygous for a mutant *DPYD* allele, although differences between sex and races are unknown at this time.

Despite the activity of fluorouracil-based adjuvant chemotherapy, the results obtained thus far indicate need for continued improvement. New chemotherapy agents and chemotherapy regimens are constantly being investigated in an attempt to improve on the response and safety of fluorouracil plus leucovorin in the adjuvant setting. Most attempts to improve the adjuvant therapy for colon cancer add a third active chemotherapy agent to a fluoropyrimidine-based regimen.

Accepted initial chemotherapy regimens for colorectal cancer include oxaliplatin plus fluorouracil and leucovorin, capecitabine alone, or fluorouracil plus leucovorin alone. While for metastatic disease, other options include irinotecan plus fluorouracil and leucovorin, bevacizumab plus a fluorouracil-based regimen, capecitabine alone, or fluorouracil plus leucovorin alone [36].

### 3. Individualization Approaches

**3.1. Prediction of Toxicity. (A Priori Methods).** The approach to the prediction of severe toxicities due to 5-FU has been a matter of debate for many years and still remains a hot topic in oncology. The efforts in this field are almost entirely focused on the analysis of dihydropyrimidine dehydrogenase (DPD) gene mutations and of peripheral blood mononuclear

cell (PBMC) DPD activity. Other approaches look at drug targets as interindividual differences in the expression of drug targets could lead to resistance or toxicity towards standard chemotherapy regimens. The main targets for fluorouracil are thymidylate synthase (TS) and its promoter, thymidylate synthase enzyme (TYMS). Genetic polymorphisms in the TS have been shown to influence response [37, 38] and toxicity of fluorouracil-based therapies. Since they are drug targets, they would be valuable in identifying patients likely to respond to treatment rather than toxicity. They may become useful in characterizing patients in whom 5-FU is not an effective treatment option.

**3.2. Test Dose Approach.** This method entails the administration of an intravenous (I.V.) low dose of 5-FU (250 mg/m<sup>2</sup>, administered as I.V. bolus without folic acid) in candidates for 5-FU chemotherapy to determine clearance of the drug. This is considered a phenotypic approach to individualization.

Administration of a 5-FU test dose to calculate the pharmacokinetic parameters, such as 5-FU clearance and *half life*, that could be profoundly altered in the presence of an impaired systemic clearance [39], has been proposed as a screening procedure to prevent full-dose administration in patients with impaired metabolic clearance of fluorouracil [40] at the same time allowing the choice of complementary chemotherapeutic 5-FU-free protocols.

One hundred and eighty eight patients with colorectal cancer (CRC) able to receive 5-FU-based adjuvant chemotherapy were enrolled [39]. The reduced test dose was administered 2 weeks before standard treatment, and a complete pharmacokinetic analysis was performed. After the first cycle of chemotherapy, drug-induced toxicities were recorded in order to evaluate any possible relationship between pharmacokinetics and treatment tolerability.

It was demonstrated that C<sub>max</sub> and AUC values of 5-FU metabolite were related to DPD activity measured in human peripheral blood mononuclear cells, suggesting that pharmacokinetic analysis should be considered predictive of that enzyme activity. Although all patients tolerated the reduced test dose, confirming the safety of such a dose, 3 of 188 enrolled patients (1.6%) revealed marked impaired 5-FU and metabolite kinetics, consistent with previously reported rates of severe toxicities [40].

The advantage of the fluorouracil test dose approach lies in the fact that the substrate of interest, is given in a low dose. Moreover, it can overcome problems with assessment of DPD activity which have not shown consistent results. Additionally, the 5-FU test dose can be easily performed in a hospital setting with a clinical pharmacology unit such as those usually present in medium- or large-sized university hospitals. The analysis can be available in a few days and the patients can be ready to start the 5-FU therapy.

The disadvantage of this method is that nonlinear pharmacokinetics severely limits its usefulness as a tool for prediction of full-dose pharmacokinetics.

Validation of the process of the test dose and the proposed cut-off values of pharmacokinetic parameters of 5-FU metabolite in a larger population of patients is needed

to confirm the results and test the generalizability of the conclusion. Although interest in this approach is not new and may be established for other drug, available evidence is very limited and comes from the same group of researches which makes formulating a conclusion on the value of this approach very difficult.

**3.3. Dihydropyrimidine Dehydrogenase Deficiency.** The activity of DPD may be an important determinant for predicting the efficacy and toxicity of Fluorouracil. Individuals can be screened for alterations in DPD activity by phenotyping and/or genotyping before the first administration of fluorouracil. Numerous genetic and phenotypic (i.e., DPD activity, breath test or plasma dihydrouracil/uracil ratio) approaches have been proposed to prevent life-threatening toxicities. However, both the genotype and phenotype options have some advantages but also some limitations.

**3.3.1. Genotyping Strategy.** 5-Fluorouracil is a prodrug that requires activation through a series of anabolic steps. Most of the dose is metabolized to 5-fluoro-deoxyuridine monophosphate (FdUMP), the active drug, by thymidine kinase [41]. In most patients, more than 85% of a 5-FU dose is inactivated by dihydropyrimidine dehydrogenase (DPD) [42]. Polymorphisms in genes that encode drug-metabolizing enzymes such as DPD might be clinically important if they have a reliable effect on enzyme activity and drug disposition (i.e., genotype can affect phenotype). Three patterns of inherited alleles are possible for autosomal co-dominant genes: (1) two normal (“wild type”) alleles which give normal levels of DPD activity (2) one variant allele and one normal allele which results in 50% reduction in activity (3) two variant alleles where DPD activity is completely lacking. These correspond to high, intermediate, and low (i.e., deficient) enzyme activity.

Deficiency in DPD activity may reflect allelic variation in the *DPYD* (DihydroPYrimidine Dehydrogenase) gene that encodes the DPD enzyme [42]. However, there are 23 known allelic variants with varied relationships to DPD activity.

Considering the common use of fluorouracil in cancer patients and the relatively high prevalence of the *DPYD* mutations associated with a decrease or lack of DPD activity in the normal population, patients who are to receive fluorouracil should benefit from genetic screening.

It has been suggested to use a polymerase chain reaction (PCR)-based genotyping genetic method for the examination of these mutations, and identify those patients who can receive full doses, those who need dose reduction, and those who should not receive 5-FU.

However the presence of *DPYD*\*2A does not always explain patients who are phenotypically deficient in DPD activity and those with fluorouracil toxicity [43].

Studies of patients phenotypically deficient in DPD activity and of patients with fluorouracil toxicity detected *DPYD*\*2A in only 14 of 22 (64%) [44] and in 6 of 25 (24%) [45] subjects, respectively.

Thus, the complexity of the *DPYD* gene, and the mostly unclear clinical relevance of the majority of mutations

reported to date, limits the usefulness of single mutation genotyping tests [46].

Low DPD activity in a patient receiving 5-FU can increase plasma concentrations of the active drug (FdUMP) and lead to toxicity. In a study of 57 patients with head or neck cancer who received 5-day continuous intravenous infusions of 5-FU 1000 mg/m<sup>2</sup>/day, there was a linear correlation between DPD activity and 5-FU clearance [47]. Patients with the lowest DPD activity had the lowest 5-FU clearance and highest incidence of severe hematologic and gastrointestinal toxicities [42].

In a prospective study of 48 patients with colorectal cancer who were receiving 5-FU and leucovorin as adjuvant therapy after surgery, DPD activity in lymphocytes correlated with 5-FU-related toxicity [48]. Nine (82%) of the 11 patients with low DPD activity experienced 5-FU-related side effects (e.g., mucositis, diarrhoea, myelotoxicity, angina pectoris, hypertension). Symptoms were reversed by reducing the dosage in three patients, and interruption of 5-FU therapy was required in another three patients. Five (17%) of the 29 patients with medium DPD activity experienced mild toxicity (e.g., diarrhoea, transient hypertension), with no need for 5-FU dosage reduction or interruption in therapy. One (13%) of eight patients with high DPD activity experienced toxicity, but no dosage reduction or interruption in therapy was required.

The molecular mechanisms that control DPD activity are complex and have not yet been fully elucidated and cannot be used effectively to individualize Fluorouracil dosing and currently there is no established standard genetic test for DPD deficiency.

**3.3.2. Phenotyping Strategies.** These strategies include by the assessment of DPD activity directly in PBMCs or indirectly using metabolic ratios of pyrimidines in plasma or urine, or using a uracil breath test.

*(a) Assessment of DPD Activity in Peripheral Blood Mononuclear Cells.* PBMCs are used as a surrogate for the determination of DPD activity. Radio-assays with <sup>14</sup>C-fluorouracil have been developed [49, 50]. The mean value of DPD activity in cancer patients was estimated to be (189–222 pmol/min/mg) [49, 51].

Uniform threshold of DPD activity in PBMCs, below which patients could be at higher risk, was not identified because DPD activity was measured by several laboratories using different techniques. But majority of DPD deficient patients have exhibited a DPD value that was ≤30% of the normal mean.

Difficulties with this strategy are related to several factors. Firstly DPD values follow a circadian rhythm which makes sampling time important. Peaks usually occur during the night and trough around noon, with large interindividual variation [53]. secondly, the measurement of PBMC-DPD activity is time consuming and labour intensive making the test difficult to be used in clinical practice. Most importantly, is the equivocal association between PBMC-DPD activity and 5-fluorouracil clearance. Studies on this relationship have reported good ( $r_2 = 0.51$ ;  $P < 0.0001$ ) [47],

weak ( $r_2 = 0.10$ ,  $P 0.002$ ) [51] and no correlation [54]. Additionally, no association between PBMC-DPD activity and the fluorouracil toxicity has been observed in some patients [51]. These findings suggest that measuring DPD activity in PBMC is not sufficient to predict the severity of fluorouracil toxicity or to suggest dose modifications to prevent toxicity.

*(b) Assessment of DPD Activity with Endogenous Substrates: Pyrimidines.* In pyrimidine metabolism, uracil (U) and thymine (THY) are firstly converted to dihydropyrimidines (dihydrouracil [UH2] and dihydrothymine [THYH2]) by DPD. In healthy subjects, the UH2:U ratio in biological fluids follows a circadian rhythm which was consistent with the circadian rhythm of PBMC-DPD activity. In cancer patients, the circadian rhythm is disturbed and plasma UH2:U ratios decreased with increasing fluorouracil concentrations and returned to baseline value when fluorouracil was not detectable any more, suggesting a competitive inhibition of DPD by fluorouracil [55].

Since fluorouracil, U and THY are metabolised by the same pathway, and high levels of the naturally occurring pyrimidines are present in urine and/or plasma. In patients with deficiency, [9] both ratios (UH2:U and THYH2:THY) are reduced and could theoretically be used for the diagnosis of DPD deficiency before fluorouracil treatment. The concentrations of these pyrimidines can be measured in plasma and urine by HPLC [56].

The proposed urinary pyrimidine tests alone are imperfect to predict adverse effects caused by DPD deficiency, because they are not sensitive enough for detecting partial DPD deficiencies. Additionally, it is not understood if other enzyme deficiencies related to pyrimidine metabolism have an impact on urinary pyrimidine tests.

Since U accumulates in plasma when systemic DPD is inhibited, UH2:U ratios in plasma measured by HPLC have also been proposed as a gauge for DPD activity. Gamelin et al. showed that in 152 patients a low UH2:U plasma ratio (<1.8), as expected in DPD deficiency, is associated with higher fluorouracil plasma concentrations (>3 mg/L) and toxic effects after the first weekly course fluorouracil treatment. Toxic adverse effects were observed only in patients with UH2:U ratios of <1.8. The investigators proposed to individualise the fluorouracil dose according to plasma UH determined before the first treatment cycle [9].

Problems with this approach include, firstly, that for urinary U, the reliability is affected by sampling time and by U from dietary sources. Secondly, a low DPD activity, as it is present in heterozygotes, is still sufficient to maintain U and THY homeostasis under normal conditions. Hence, normal plasma U concentrations may be present in individuals with a partially decreased DPD activity, who are the same patients most likely to develop unanticipated toxicity to fluorouracil administration [57].

Current phenotypic assays that measure DPD activity are cumbersome.

A reliable relationship between DPD genotype and 5-FU toxicity phenotype has not been established. Prospective studies are needed that demonstrate that adjusting 5-FU

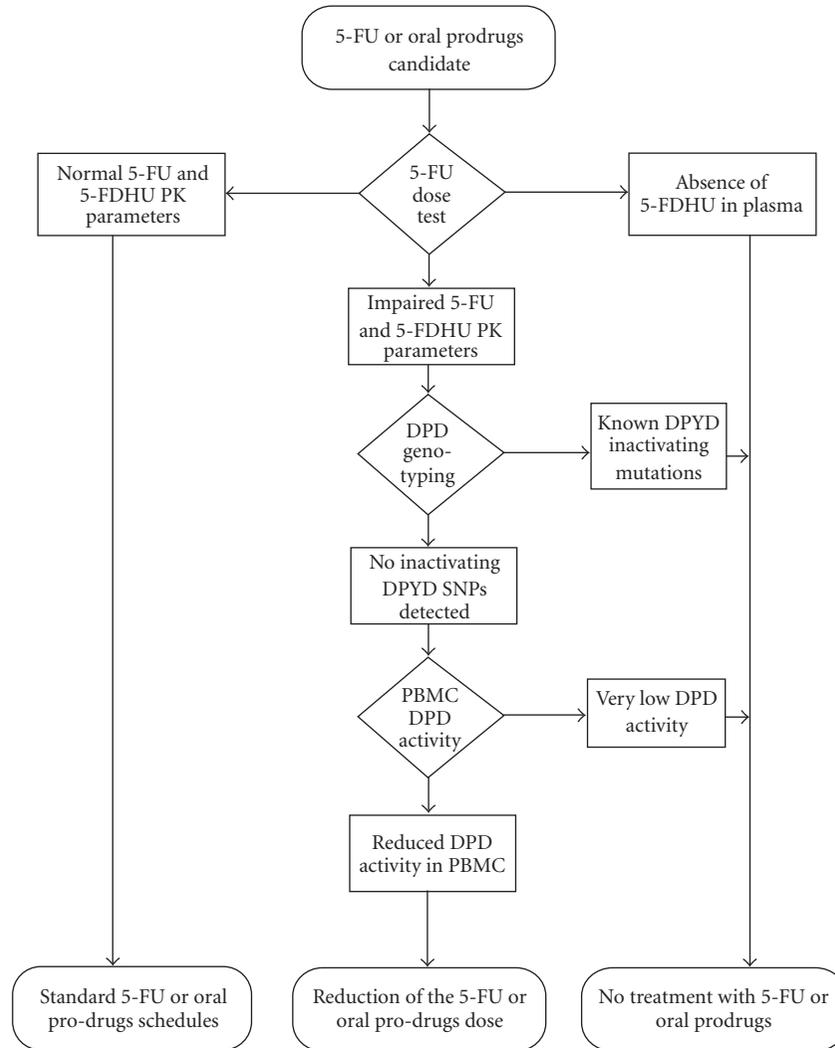


FIGURE 2: Suggested diagnostic flow chart for the detection of patients with impaired fluoropyrimidine metabolism. 5-FU, 5-fluorouracil; 5-FDHU, 5-fluoro-5, 6-dihydrouracil; DPD, dihydropyrimidine dehydrogenase; PBMC, peripheral blood mononuclear cell; PK, pharmacokinetics; SNP, single nucleotide polymorphism [52].

dosage based on pre-treatment DPD activity can decrease the incidence of toxicity.

(c) *Integrated Approach.* An integrated approach has been proposed that combines the test dose approach with the genotyping/or phenotyping approach. Bocci et al. propose a diagnostic algorithm (Figure 2) to screen candidate patients before 5-FU therapy. The 5-FU test dose could be regarded as a triage test, allowing detection of the fraction of patients with normal, impaired or absent 5-FU metabolism. Other analyses, such as DPD genotyping or even DPD PBMC activity, could be used later as add-on tests and, limited to the still undiagnosed subgroup, to detect those degrees of enzyme activity impairment suitable for possible reduction of 5-FU dose or different treatments. The 5-FU test dose can be regarded as a triage test, for detection of the fraction of patients with normal, impaired or absent 5-FU metabolism. DPD genotyping or DPD PBMC activity, can be

used later additionally in undiagnosed subgroup, to detect those degrees of enzyme activity impairment suitable for possible reduction of 5-FU dose or different treatments. This approach can be more economic as more expensive analysis (genotyping) is only performed in select patients. It can be a safer strategy because it can overcome the deficiency in one approach, and patients at risk can be correctly identified early [52].

Another combined approach that was tested was the two-step strategy, combining firstly single nucleotide polymorphism (SNP) detection and uracil plasma measurement, followed, in cases where metabolic deficiency was suspected, by dihydrouracil/uracil ratio determination to confirm deficiency and to determine the optimum 5-FU dosage, appeared the best approach, with 83% and 82% sensitivity and specificity, respectively. This approach was developed after comparing 4 different methods in a population of two hundred and fifty two French Caucasian patients treated by 5-FU infusion [64].

TABLE 1: Relationship between treatment efficacy and pharmacokinetic parameters.

| Study                    | Pharmacokinetic variable, threshold | Administration schedule; tumor type |
|--------------------------|-------------------------------------|-------------------------------------|
| Seitz et al. [58]        | AUC                                 | Weekly IVB; CRC                     |
| E.C. Gamelin et al. [59] | AUC $\geq$ 25 mg·h/L                | Weekly 8-hour CVI; CRC              |
| E. Gamelin et al. [60]   | AUC $\geq$ 25 mg·h/L                | Weekly 8-hour CVI; CRC              |
| Ychou et al. [61]        | AUC $\geq$ 25 mg·h/L                | 2-Day/2 weeks CVI; CRC              |
| Milano et al. [62]       | Mean AUC, 30 mg·h/L                 | 5-Day CVI; HNC                      |
| Fety et al. [63]         | Mean AUC, 30 mg·h/L                 | 5-Day CVI; HNC                      |

TABLE 2: Relationship between toxicity and pharmacokinetic parameters.

| Study                      | Pharmacokinetic variable, threshold     | Toxicity                         |
|----------------------------|---|----------------------------------|
| Thyss et al. [65]          | AUC > 30 mg·h/L                         | Stomatitis, diarrhea, Leukopenia |
| Van Groeningen et al. [66] | AUC                                     | stomatitis, Leukopenia           |
| Trump et al. [67]          | C <sub>ss</sub>                         | stomatitis, Leukopenia           |
| Ychou et al. [61]          | AUC > 25 mg·h/LC <sub>ss</sub> > 3 mg/L | Diarrhea, HFS, Leukopenia        |
| Gamelin et al. [59]        | AUC > 25 mg·h/LC <sub>ss</sub> > 3 mg/L | Diarrhea, HFS                    |
| Gamelin et al. [60]        | AUC > 25 mg·h/LC <sub>ss</sub> > 3 mg/L | Diarrhea, HFS                    |

Because early detection of patients with cancer who are at risk of developing life-threatening toxicity to FU might allow dose reductions or selection of an alternative treatment regimen, various genotypic and phenotypic methods have been developed to predict toxicity and/or response. None, however, has proven to be reliable in prospective clinical studies, demonstrating a great margin of uncertainty.

Routine detection of DPD deficiency in patients with cancer likely to undergo FU therapy has been examined. However, DPD deficiency has been observed in a relatively small percentage of patients with grade 3 to 4 FU toxicity, leaving a large number of patients with an unexplained molecular basis of toxicity.

In summary, currently, no reliable markers of sensitivity or resistance to fluorouracil (FU) have been validated to permit their use as a standard of care for the management of patients with cancer, despite the large number of studies attempting to identify useful genotypic or phenotypic predictors of response to treatment. This may be attributed to the complexity of the involved molecular events and/or incomplete understanding of the process involved.

**3.4. Pharmacokinetic Adjustment. (A Posteriori Methods).** 5-fluorouracil pharmacokinetics are characterized by a wide interpatient and intra-patient variability and different clearance values when equal doses calculated by body surface area are administered to different patients, leading to marked differences in systemic exposures (see Figure 1) [9].

Measures of systemic exposure towards fluorouracil (AUC, steady-state plasma concentration [C<sub>ss</sub>], peak plasma concentration [C<sub>max</sub>]) have been correlated with the incidence of toxicity, tumour response and survival [59, 60, 65, 68, 69]. Most studies suggest that improved responses occurred in patients with a mean fluorouracil AUC be in the range of 24–30 AUC units (mg·h/L; Table 1) [58, 59, 61–63]. A number of studies have demonstrated that the target AUC for 5-FU is the same regardless of tumour type,

administration schedule, and drug combination. Patients with 5-FU concentrations below this level are receiving suboptimal therapeutic effect, whereas patients above this level are at high risk of toxicity (Table 2) [59–61, 65–67, 70]. The therapeutic index between toxicity and efficacy of 5-FU is very narrow and therefore is difficult to reach by clinical follow-up alone. Interestingly, toxicity because of 5-FU exposure is not increased by the addition of other drugs in the regimen.

Dose-adjustment strategies based on fluorouracil AUC have been reported for some fluorouracil-containing regimens, such as weekly 8-hour continuous intravenous infusion with intravenous bolus folinic acid 200 mg/m<sup>2</sup>, [60] long-term 5-fluorouracil continuous intravenous infusion with or without cisplatin [69] and a bimonthly regimen consisting of intravenous folinic acid 200 mg/m<sup>2</sup>/day over 2 hours followed by a fluorouracil intravenous bolus 400 mg/m<sup>2</sup>/day and a continuous fluorouracil intravenous infusion 600 mg/m<sup>2</sup>/day over the next 22 hours on two consecutive days (2000 mg/m<sup>2</sup> per cycle, called LV5FU2-regimen) [61].

Effective 5-FU dose management in CRC has been demonstrated in phase II/III studies. In a study of 152 patients with colorectal cancers treated weekly with fluorouracil 8-hour infusions plus folinic acid 400 mg/m<sup>2</sup>, the initial dose of fluorouracil (1300 mg/m<sup>2</sup>) was adapted every week to the steady-state concentrations measured the week before by means of a dose adjustment chart to reach a predefined therapeutic plasma concentration range of 2–3 mg/L (AUC<sub>8</sub> 16–24 mg·h/L). Fluorouracil plasma concentrations were measured 3 and 7 hours after the start of the fluorouracil infusion. This method provided a high survival rate (median OS 19 months), with good tolerance [60]. This dose-individualised schedule was compared with a conventional 5-fluorouracil regimen (1300 mg/m<sup>2</sup>, 8-hour weekly infusion, plus folinic acid 400 mg/m<sup>2</sup>) in a phase III randomised trial of 208 patients with colorectal cancer. There

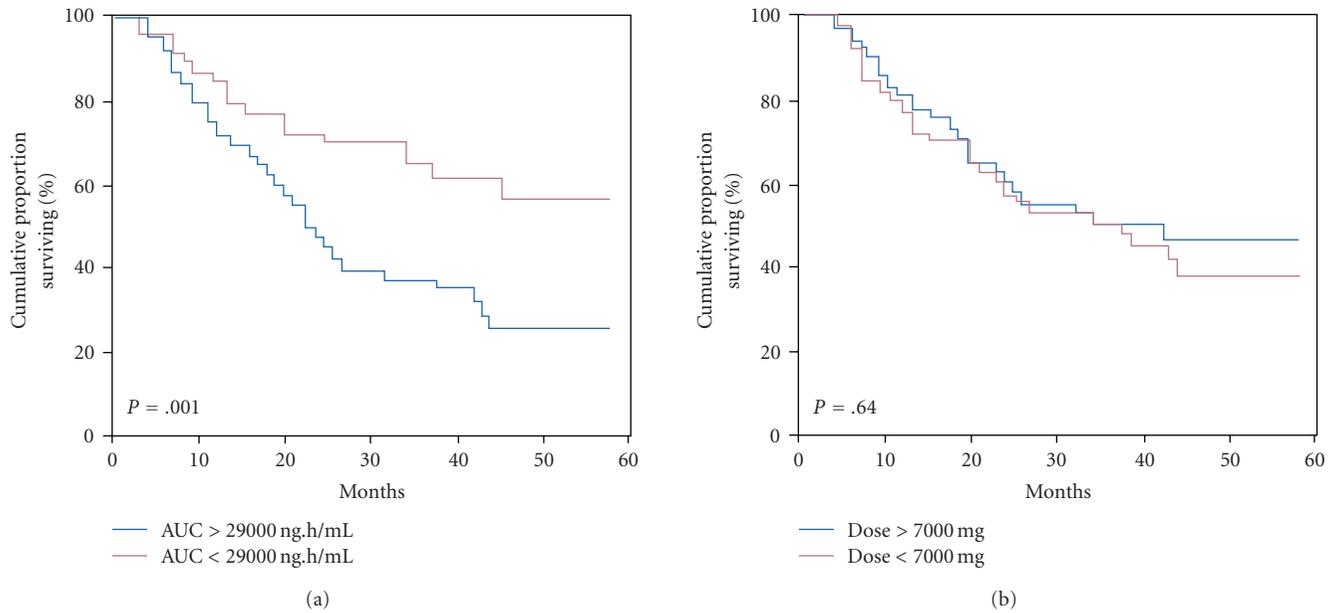


FIGURE 3: AUC Versus Dose: survival [62]. (a) Survival curves as a function of the average AUC per cycle. (b) Survival curves as a function of the average total dose per cycle.

was more severe diarrhoea in the dose adapted arm (15% versus 5%), but also a higher rate in complete and partial responses (39% versus 19%,  $P = .0004$ ) [71].

Ychou et al. [61] adapted the individual fluorouracil dose for a bimonthly LV5FU2 regimen (on 2 consecutive days folinic acid  $200 \text{ mg/m}^2$ , fluorouracil  $400 \text{ mg/m}^2$  intravenous bolus, followed by a 22-hour intravenous infusion of fluorouracil  $600 \text{ mg/m}^2$ ) to the AUC attained during the first cycle. Pharmacokinetic parameters were determined during the first and second cycle. The dose given in the second cycle was to be increased when the AUC during the first cycle was below  $20 \text{ mg}\cdot\text{h/L}$ . The dose was reduced by 25% in the presence of grade 3/4 toxicities. This pharmacokinetically adapted regimen represented a compromise between efficacy (CR = PR in 47% [ $n = 19$ ]; OS: 18.6 months) and tolerability (19% [ $n = 10$ ] gastrointestinal and 30% [ $n = 16$ ] haematological grade 3 toxicities).

More recently, a nonrandomized, retrospective, pharmacokinetic study looked at the possible correlations of 5-FU, and its active metabolite, 5-FDHU pharmacokinetics with DFS in colorectal cancer patients given 5-FU-based adjuvant chemotherapy, it also attempted to identify cut-off values of pharmacokinetic variables related to better prognosis in terms of DFS. Individual plasma concentrations of 5-FU and 5-FDHU were determined on day 1 of the first cycle with a validated high performance liquid chromatography (HPLC) method, and the main pharmacokinetic variables were determined. No dose adjustment was made based on these variables obtained. The value of this work lies in that these patients were followed for a long time (5 years) and their DFS rates at that point were correlated with the AUC of 5-FU. In the 58 subjects who had cancer recurrence, the AUC was significantly lower than the group who did not have

recurrence, confirming that 5-FU levels have a role in clinical settings. As a matter of fact, univariate analysis showed that 5-FU pharmacokinetics is predictive of the risk of relapse. This major conclusion was in accord with previous studies although the cut-off values for AUC suggested ( $>8.4 \text{ h}\cdot\text{mg/L}$ ) was very different from earlier evidence [72].

In the same year, in a randomized study Gamelan et al., found that around 70% of patients who had their blood levels measured required dose adjustment to achieve optimal therapeutic response while minimizing excessive toxicity. More than 58% of patients required increased dosing to reach target therapeutics levels, whereas 10%–20% of patients required dose reduction to reduce toxicity while maintaining therapeutic effectiveness. The objective response rate was 18.3% for patients receiving traditional BSA-based therapy and 33.7% for those receiving PK-targeted therapy ( $P = .004$ ). Median OS was 16 months for the BSA-based and 22 months for PK-targeted patients ( $P = .08$ ). Toxicity was seen in more patients receiving BSA-based therapy ( $P = .003$ ), with diarrhoea and hand-foot syndrome as the most common adverse effects. Successful FU dose adjustment to the target AUC was achieved in 94% of the patients requiring a mean of four cycles to determine the necessary individualized dose. Also notable, the mean dosage to achieve the target AUC was nearly four-fold from original dose. Only 8% of patients receiving BSA-based therapy were found to have an AUC within the target range [73].

A direct comparison between these studies is difficult, because not only regimens, and co-medication, varied but also the intensity of monitoring and the time span that elapsed between monitoring and the adaptation of the dosage. Randomised studies comparing outcome and toxicity in patients who get either standard doses or individualised

by a TDM strategy should be carried out for those regimens not yet examined and considered more standard of care at the present time such as 5-FU with oxaliplatin or irinotecan; FOLFOX or FOLFIRI.

These authors were successful in demonstrating that individualized therapy led to a statistically significant improvement in objective response rate in the presence of less toxicity, with a trend toward improved median overall survival (OS).

5-fluorouracil dose management based upon individual pharmacokinetics requires a comprehensive team approach by physicians, nurses, pharmacists, and laboratory personnel for effective quality implementation. Of primary importance, dose adjustment charts have been established for many of the major infusion regimens, allowing for a practical and easily interpretable approach to 5-FU dose management.

In order to be relevant in a clinical setting such approaches usually follow a limited sampling strategy. *The advantage of this approach is that it reduces severe toxicity, hospitalizations, supportive care, and treatment interruption and eventually results in overall reduced cost of care.* Few studies have been published that developed limited sampling models for determining the optimal AUC fluorouracil pharmacokinetics [74, 75]. In these studies; fluorouracil was given as a short-term infusion at variable doses. The optimal single time point for the estimation of the AUC was estimated to be two time points relative to dosing (5 and 45 minutes). For continuous infusions, no limited sampling strategies based on predictions of AUC values have been published, but nomograms and charts for dose adjustments based on steady-state fluorouracil concentrations were developed. Plasma sampling was done at 8:00 am and 5:00 pm every day during a 3-day infusion, [69] or every 4th and 8th hour during a 6-day infusion [59, 62]. The nomograms were presented as graphs or tables, and related dose decrements or increments to plasma concentration estimates made during the preceding cycle of therapy.

**3.5. Head and Neck Cancers (HNC).** 5-fluorouracil treatment remains a standard in the management of HNC. Previous retrospective data have established a link between 5-FU AUC and treatment-related toxicity [69] and that systemic exposure to 5-FU, rather than dose, is related to OS rates (Figure 3) [62]. Dose adjustments based on an individual pharmacokinetic survey is a validated and valuable approach to optimize treatment of patients with HNC. survival rate was significantly higher for individuals whose systemic exposure/AUCs  $> 29 \text{ mg}\cdot\text{h/L}$  [62]. in a study of patients with HNC receiving 5-FU modulated with LV [76] and also in another study based upon a combination of 5-FU/cisplatin with irradiation [77]. Reinforced the findings that systemic exposure to 5-FU is correlated to risk of toxicity. In a prospective randomized study of 106 patients, reduced incidence of toxicity without reduced efficacy was demonstrated [63]. Dose adjustment based on pharmacokinetic follow-up showed a significant decrease in neutropenia and mucositis in the pharmacokinetic-adjusted arm versus the standard arm in which no adjustment was made. The objective RR was comparable in the two treatment arms.

Target levels for HNC treatment have been established in clinical trials, and a simple dose adjustment chart has been developed, enabling easy adoption of the practice in clinical settings.

Is this clinical data strong enough to mandate the use of TDM and integrate it into standard practice? The answer may be yes considering the results of improved response rates and a trend towards improved survival. However, in most of these studies, the FU regimen used in this study is one rarely used in current practice in most countries. Now, the addition of oxaliplatin, irinotecan, or bevacizumab to the FU foundation has demonstrated improved response rates and overall survival for metastatic colorectal cancer, rendering it difficult to change prescribing practices to revert back to single agent FU. It may be more valuable now to generate new data on the impact of PK-guided FU in the context of oxaliplatin or other commonly used combination therapy regimens.

**3.6. Challenges.** Firstly, multiple disciplines are needed to ensure the correct administration of the drug, collection and processing of blood samples for analysis, assay performance, and interpretation of the raw drug concentrations into a meaningful direction for dose adjustment. Most oncology practices lack access to the laboratory medicine and pharmacy expertise needed for TDM. In addition to the technical infrastructure to adequately process samples for clinical pharmacokinetic analysis, What makes this process more complicated is that needs to happen in a relatively short time frame to prepare a recommended dose before the next scheduled treatment.

TDM is more difficult to apply than standard dosing approaches, so there must be a net benefit both clinical and economical to make it meaningful.

The use of limited sampling methods with dose adjustment algorithms, such as the one developed by Gamelin et al., can make the process more practical. In addition, considering that at this time, most drug regimens utilize every two weeks, or every three weeks dosing schedules, allowing more time to complete the necessary steps of the process.

**3.7. Conclusion.** A prospective comprehensive pharmacogenetic approach would be more suitable for the currently used combination chemotherapy regimens. This could potentially improve treatment outcome by permitting more rational selection of patients likely to benefit from chemotherapy and at the same time identification of those at risk of developing life-threatening toxicity. However, this is still not available for FU or its combination with other agents. Additional well-designed studies are still needed before the use of predictive molecular markers for FU toxicity can be recommended as the standard of care.

These studies underline the importance of systemic exposure of 5-FU in treating cancer. In order to achieve optimal exposure levels, a reliable pharmacokinetic guided dose-adjusted protocol is beneficial. Routine clinical use is made possible by the existing body of data, providing practical guidelines for target concentration levels and dose adjustment charts and identifying ideal candidates for TDM.

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