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Therapeutic Drug Monitoring in Oncology: IATDMCT Recommendations for 5-Fluorouracil Therapy

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1 Introduction

5-fluorouracil (5-FU) is dosed by body surface area, a practice unable to reduce the inter-individual variability in exposure. Endorsed by the International Association of Therapeutic Drug Monitoring and Clinical Toxicology (IATDMCT), we evaluated clinical evidence and

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Conflict of Interest

Chu, Saladax Scientific Advisory Board and Saladax equity. Zhang, Saladax Scientific Advisory Board and Saladax equity. Joerger, Saladax Research Funding.

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strongly recommend TDM for the management of 5-FU therapy in patients with colorectal or head-and-neck cancer receiving common 5-FU regimens. Our systematic methodology provides a framework to evaluate published evidence in support of TDM recommendations in oncology.

2 Background

In 2015, the newly formed Therapeutic Drug Monitoring (TDM) in Oncology Scientific Committee of the IATDMCT convened a meeting in Rotterdam, The Netherlands, to discuss the state of TDM in oncology. One of the conclusions of that meeting was to identify those anticancer agents with the best data available to allow for evaluation of the potential utility of TDM, construct a framework to guide evaluation of the available literature relevant to TDM, and provide recommendations to guide optimal use in clinical practice, based on in-depth literature review and discussions with leading experts in the field.

A working group was organized by reaching out to clinician-scientists with experience in therapeutic areas utilizing 5-FU, with an earlier version of the following introduction and framework as a proposed approach to accomplish our task. This document is intended for all healthcare professionals involved in the clinical management of patients being treated with 5-FU, and aims to improve both standards of practice and patient care. Although the oral fluoropyrimidines capecitabine, UFT (tegafur/uracil), S-1 (tegafur (5-FU prodrug)/gimeracil/oteracil), and 5-FU share the same metabolic pathways for activation to the active cytotoxic metabolites and degradation, this document will focus only on the role of TDM for intravenous infusion of 5-FU and not address the potential role of TDM for these other fluoropyrimidine analogs given limited available data.

2.1 Dosing in Oncology

The standard approach for personalizing a chemotherapy dose has traditionally been based on body surface area (BSA). More recently, a flat dosing approach has been used in the dosing of most oral agents such as tyrosine kinase inhibitors, and dose-banding is used for capecitabine in some countries. The BSA-based dosing method was developed in 1916, using a very limited number of patients, as a means of converting drug doses in animals to doses for first-in-human trials. Without rigorous scientific evaluation, BSA-based dosing was then applied to determining individual patient dosing of chemotherapy drugs in the 1950’s, and it has remained a default approach for chemotherapy dosing ever since. Numerous studies have demonstrated that both BSA-based dosing as well as flat dosing results in significant differences in individual exposure based on wide variability in pharmacokinetic (PK) parameters such as clearance(1). Thus, neither flat dosing nor dose-adjustment based on BSA is an optimal approach for obtaining the maximum tolerated exposure (MTE) at the personalized maximum tolerated dose (MTD) (2).

While TDM is an important tool in guiding dosing for other areas of clinical medicine (i.e. infectious diseases, cardiology, psychiatry, neurology, transplant medicine, etc.), to date, it has not gained widespread acceptance in oncology. There are several reasons to explain the lack of uptake for TDM in cancer therapy, which include a limited number of studies identifying the optimal target ranges for drug exposure, the absence of widely available

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routine laboratory tests to measure various anticancer drugs in plasma, lack of TDM training for oncologists, economic/competitive considerations, and regulatory barriers. There are also several important logistical issues in implementing TDM: sample collection, processing, and collection times are clinically relevant challenges to the successful implementation of TDM programs in clinical practice. In the absence of TDM, however, patients will often have their dose decreased in the setting of severe toxicity, but will rarely have the drug dose increased in the absence of toxicity.

2.2 Oncology Drugs and TDM

There are currently three chemotherapeutic agents, carboplatin, busulfan, and methotrexate, where exposure is commonly individualized by means other than BSA (Table S 1). For these drugs, TDM became part of the standard of care with relatively small single-arm studies. The pathway to acceptance was a clear relationship between exposure and pharmacological effect. This was accomplished by observational studies where a statistically significant relationship was observed between a critical pharmacokinetic parameter and either toxicity or efficacy. A single-arm follow-up study using TDM to validate the proposed levels was then performed. Methotrexate is the one exception where a randomized phase 3 TDM study was conducted, although guidelines for the use of methotrexate TDM were established long before the results of the phase 3 study were reported in 1998 (3).

In oncology, new drugs or combinations are compared to current drug regimens by conducting randomized phase 3 studies. The two treatments are then compared to determine statistically significant superiority in clinical activity, with typical primary endpoints such as progression-free survival or overall survival. This is a well-established approach that has become the benchmark in drug development. Given that approximately 60% of the results coming from phase 2 studies are not reproduced in prospective phase 3 studies, this is an appropriate approach for drugs, particularly when comparing an unapproved novel agent to established therapy. A lack of resources has limited the conduct of randomized TDM studies in oncology to only three: for methotrexate (primary endpoint 5-year remission rate) and 5-FU (primary endpoints response rate and toxicity) (3–5). Instead of testing an investigational agent against established therapy, the goal of TDM is to enhance the clinical efficacy and/or decrease the toxicity of agents that have already been approved and established as active. Given this perspective, perhaps the burden of proof for TDM need not be a traditional phase III trial, and might be compared to how biosimilar drugs are now being approved without having to undergo testing via the traditional phase III trial route.

With TDM, the statistical significance of the relationship between exposure and pharmacological effect is commonly assessed during an observational phase 2 study, where all patients are given the same dose, resulting in a range of systemic drug exposures. In this manner, patients with low exposure serve as the control for patients with high exposure, and a maximum tolerated exposure (MTE) can be established. After any bias is excluded as a contributing factor (e.g. tumor-mediated clearance could contribute to low concentration-poor response association), and if a statistically significant relationship is shown, a follow-up phase 2 study may be conducted to validate that the dose adjustment algorithm is effective in controlling the variability of drug exposure, thereby successfully reaching the

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PK target range. Interestingly, the phrasing in package inserts of FDA-approved TDM assays, such as for methotrexate (MTX), suggest some flexibility in terms of the evidence required to incorporate TDM results in the clinical decision process (7, 8). Intended uses are described as “monitoring levels of MTX to ensure appropriate therapy” and “… quantitative measurement of MPA in human plasma … as an aid in the management of …therapy...”. For MTX, expected values are described as “No precise relationship between MTX serum levels and antineoplastic efficacy has been established, although levels below approximately 0.02 μmol/L were seen as necessary for resumption of DNA synthesis.”, and “The correlation between serum … concentration … in predicting MTX toxicity has been demonstrated. …a patient with a 24-hour serum concentration of greater than 5–10 μmol/L… is at an increased risk of toxicity …”.

3 Framework for Evaluating Evidence Supporting TDM

To help the working group focus on a uniform approach to evaluate the evidence for TDM, we modified the AGNP (Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie) consensus guidelines that have been established for TDM of psychiatric drugs (9), resulting in a framework to evaluate evidence supporting TDM (see Table S 2). The main modification that we have made for its application in oncology is an extra, higher, level of recommendation. In the field of psychiatry, clinical efficacy is often captured by a change in score on a symptom-scale, whereas in oncology, there is a clear distinction between evidence of activity as expressed by response rate (tumor shrinkage) and a survival benefit often required for FDA approval. Lack of prediction of survival benefit in phase III trials by response rate documented on phase II trials is a well-known issue in oncology. It should be noted that the AGNP recommends the randomized double-blind study as an optimum study design, but acknowledges that this has only rarely been done because of significant logistical challenges. Moreover, there is a preference for fixed-dose studies to define the correlation between exposure and outcome (9).

The main goal of the TDM in Oncology Scientific Committee was to identify oncology drugs that have sufficient pharmacological evidence to benefit from TDM and to evaluate these agents according to the framework outlined in Table S 2.

To better structure the information in the literature, our working group also posed specific questions regarding the clinical pharmacology of the drug, as outlined in Table S 3, that would address characteristics important to evaluation of TDM, based on earlier reports (10).

4 Pharmacokinetics (PK)

In reviewing 5-FU pharmacokinetic (PK) data, one needs to consider a number of technical and pharmacological issues as to how the various studies were performed. Variability in infusion pump speed will translate in variability in steady-state plasma concentrations of 5-FU, especially with the use of elastomeric pump balloons, which are sensitive to pressure, temperature, season, and patient activity (11, 12), but also with portable pumps delivering in essence a series of boluses (13). 5-FU may also be unstable after collection as it will continue to be metabolized by even small amounts of dihydropyrimidine dehydrogenase

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(DPD) present in blood and especially the buffy coat (14–17). Proper separation of plasma and/or addition of DPD inhibitors is critically important. Biologically, the elimination of 5-FU seems to change upon dosing, so that a sampling time of 5 half-lives after start of infusion does not yet correspond to a sample at true steady-state. In fact, it may take several hours to achieve stable steady-state 5-FU levels (18–20). In addition, variation in the timing of samples may contribute to variability as there is some degree of circadian rhythm in the activity of DPD and perhaps other 5-FU metabolizing enzymes (21–25). As such, the inter-individual and intra-individual/inter-occasion variability reported in especially the historical literature may be a somewhat inflated estimate of the true variabilities.

4.1 Is there significant inter-individual variability in plasma concentrations using current dosing methods based on body surface area (BSA)?

5-FU clearance has a large inter-patient variability, which is not reduced when drug dosing is based on BSA (26). An 8-h continuous infusion at 1,300 mg/m^2 was associated with a mean clearance of 134 L/h/m^2 (SD, 62) with a 10-fold range of 29 to 296 L/h/m^2. As seen in Table 1, 5-FU plasma clearance is higher with a long continuous infusion as compared with intravenous bolus injections. This observation can be explained by the saturation of 5-FU catabolism by DPD as plasma concentrations approach the K_m of DPD, reported to be approximately 4.6 mg/L (27), which then results in a more than proportional increase of 5-FU plasma concentrations with dose (18).. One of the potential covariates to explain inter-patient variability is sex with men reportedly having a 26% higher elimination than females (20, 28).

Although the various trials presented in Table 1 differ in many ways, studies with larger numbers of patients studied report more than a 40% CV. Because the reported inter-patient variability in drug clearance directly translates into variability in exposure at a given dose, taken together, these studies suggest that there is significant inter-individual variability in plasma concentrations using current 5-FU dosing methods, which are all based on BSA.

4.2 Is there limited intra-individual variability in plasma concentrations?

There is much less data on intra-individual variability with respect to 5-FU clearance and exposure (Table 2). If TDM is applied consistently, i.e. samples are drawn on different occasions/cycles of therapy at around the same time of day and at the same time after start of infusion, with appropriate technical handling of samples, the intra-individual variability should not be affected by circadian rhythm or reaching a steady-state in clearance during each infusion. Unfortunately, the various clinical studies reported in the literature do not provide specific information as to how consistent samples were collected. Overall, the intra-individual variability of 5-FU exposure and/or clearance appears to be approximately 20% (associated with a 2.3-fold range), and is therefore substantially lower than the inter-individual variability of more than 40% (associated with an 8.3-fold range).

In a cohort of 18 patients receiving 1 g/m^2/day × 5 days with 5-FU clearance determined for more than one cycle, the intra-patient variability in 5-FU clearance between therapy cycles was 1.10- to 2.75-fold, which corresponds to a 3%-24% CV (35). This difference in drug clearance within patients was often paralleled by a corresponding change in peripheral blood

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mononuclear cell (PBMC) DPD activity. Nutritional status has been shown to affect 5-FU clearance in *in vivo* experimental models, and nutrition may also contribute to the variability seen within and between humans (35, 47).

Kline et al. reported on their experience of 5-FU TDM, with repeat PK sampling as the dose was adjusted towards a target exposure (48). In general, the intra-patient variability appeared to be modest, yet intra-patient variability can translate into a significant increase in systemic drug exposure despite a decrease in dose based on previous PK sampling.

5 Pharmacodynamics (PD)

5.1 Is there a narrow therapeutic window?

As will be discussed in sections 6 and 8.4, and detailed in Table 3, 5-FU is similar to many cytotoxic anticancer agents, in that it is associated with a relatively narrow therapeutic window where toxicity and efficacy occur in overlapping levels of systemic drug exposure.

5.2 Are there easy and clinically relevant biomarkers to predict response and/or toxicity at a given dose?

DPD is encoded by the *DPYD* gene, and it is the enzyme responsible for 80%–90% of 5-FU clearance via metabolism to dihydro-5-FU (FUH_2). DPD deficiency results in a significantly reduced ability to clear 5-FU, and in the setting of partial or complete deficiency, the 5-FU half-life can be markedly prolonged from 10–15 min to 160 min or even higher (50, 51). A pharmacogenetic autosomal recessive syndrome associated with partial or complete deficiency in the DPD enzyme has been observed in 3%–5% and 0.1% of the general population, respectively, resulting in severe myelosuppression, GI toxicity in the form of diarrhea and mucositis, and neurotoxicity in the context of 5-FU therapy (50).

Several approaches have been developed over the years to predict toxicity to 5-FU therapy based on markers obtained prior to the start of therapy, and these are briefly discussed below. Although these approaches may allow for reasonably efficient detection of the extremely DPD-deficient phenotypes to prevent toxicity, they are not particularly easy, do not support selection of the appropriate dose for the majority of patients, and do not specifically identify patients who need large dose increases to achieve 5-FU exposures within the therapeutic window. Therefore, the tests described below are best suited for the identification of patients with severe DPD deficiency who should not receive even a single dose of 5-FU.

5.2.1 DPYD genotype—To date, more than 500 missense *DPYD* variants have been reported in NCBI dbSNP (Database of Single Nucleotide Polymorphisms (dbSNP). Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine. (dbSNP Build ID: 138). https://www.ncbi.nlm.nih.gov/projects/SNP/), the most well-established variant is c.1905+1G>A (rs3918290, also known as *DPYD**2A, *DPYD*:IVS14 + 1G>A), which is associated with completely inactive protein. There are other DPYD gene variants associated with loss of DPD function, and they include c. 1679T>G (rs55886062, *DPYD* *13, p.I560S), c.2846A>T (rs67376798, p.D949V), and c. 1129–5923C>G (rs75017182, HapB3) (50, 52). In patients with partial DPD deficiency detected prior to therapy, 5-FU toxicity can be avoided and/or reduced by using a reduced

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first dose of 5-FU (53). The positive and negative predictive value of *DPYD**2A to predict development of grade 3 toxicity are ~50% and ~95%, respectively (52). Deenen et al. recently reported on a study prospectively screening for *DPYD**2A. Heterozygous patients (n=22 out of a total of 2038 screened) received an initial dose reduction of 50%. The risk of grade ≥3 toxicity was significantly reduced from 73% in historical controls to 28%, and this approach was shown to be cost-effective (54).

Genotype-directed dosing of fluoropyrimidines has been proposed by developing a gene activity score to account for the different DPD enzyme activities of main DPYD variants allowing for a more differential dose adjustment. Unfortunately, some variants have conflicting or insufficient data with respect to their impact on DPD functionality, which then makes it difficult to assign a good score (55). It should also be noted that up to 50% of patients with 5-FU toxicity have no documented alterations in the DPYD gene, and individuals with normal DPD enzyme activity may have elevated plasma levels of 5-FU with increased toxicity associated with 5-FU therapy (50). There are also reports of DPYD mutations being associated with increased DPD enzymatic activity (56). Clearly, factors other than DPYD genotype contribute to 5-FU drug clearance (50).

5.2.2 DPD in PBMCs—DPD activity in PBMCs has been explored as an easily accessible read-out of an individual's ability to catabolize 5-FU.

In 68 head and neck cancer patients, DPD activity, while statistically significantly correlated, only explained 10% of the 5-FU systemic clearance (R=0.31; P=0.002). DPD activity did not significantly differ between patients who required a dose reduction and those who did not (23, 24, 57). In 26 CRC patients, DPD activity did not statistically differ between patients with grade ≤2 and those with grade ≥3 toxicity. None of the pharmacokinetic parameters of 5-FU or 5-FDHU correlated with PBMC DPD activity (58). In 188 GI cancer patients, no correlation was observed between PBMC DPD activity and 5-FU PK parameters such as systemic clearance (R = 0.00096, P =0.99) or area-under-the-concentration *versus* time curve (AUC) (R = 0.091, P = 0.50), nor was there a difference in PBMC DPD activity and patients with or without grade ≥2 toxicity (44).

Determination of PBMC DPD enzymatic activity is time-consuming and labor-intensive (50). Moreover, there are several reports that suggest it is only weakly correlated with systemic exposure or toxicity to 5-FU therapy.

5.2.3 DPD phenotype by uracil/dihydrouracil ratio—As a phenotypic read-out of DPD activity, Gamelin et al. measured the dihydrouracil to uracil (UH_2/U) ratio in plasma as an endogenous surrogate marker for 5-FUH_2/5-FU, and used this ratio as a potential indicator of the potential risk of DPD deficiency associated 5-FU toxicity (26). In 81 patients with metastatic colorectal cancer (mCRC), UH_2/U ratios and 5-FU plasma clearance had a correlation coefficient of R=0.639 (P< 0.001), and no toxicity was observed in patients with UH_2/U ratios >2.25, while toxicity was observed only in patients with initial UH_2/U ratios <1.8. In contrast, a study in 28 patients showed that baseline UH_2/U plasma ratios in most individuals reflects the non-saturated state of DPD and is, in fact, not predictive of decreased DPD activity or toxicity under the closer to saturable conditions of a 5-FU

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infusion. This finding then prompted the development of a uracil loading test approach (59). In 47 cancer patients identified by grade ≥4 toxicity after the first or second cycle of 5-FU or capecitabine treatment (19 DPD deficient and 28 DPD normal by PBMC), the U/UH_2 ratio two hours after an oral uracil dose of 500 mg/m^2 uracil could discriminate between patients with normal and deficient DPD activity with a sensitivity and specificity of 80% and 98%, respectively (60). A more recent report suggested pre-therapeutic screening of DPD deficiency with the U/UH_2 ratio as a predictor to identify completely and partially deficient individuals as it correlated with various haplotypes in 22 subjects (61).

5.2.4 DPD phenotype by uracil breath test—Diasio et al developed a non-invasive uracil breath test to detect partial or severe DPD deficiency by measuring $^{13}CO_2$ with IR spectroscopy (50). Patients with DPD deficiency would expire reduced levels of $^{13}CO_2$ in their breath after ingestion of 2-[^{13}C]-uracil, and indeed, this test was shown to correlate with uracil and dihydrouracil plasma PK parameters, and could identify PBMC DPD deficiency with 96% specificity and 100% sensitivity in 58 patients (62–64). In a larger population of 255 cancer-free patients, specificity was 99% and sensitivity 86% (63). Performance was worse in discriminating patients with grade 3–4 vs grade 0–1 toxicity (85% specificity; 62% sensitivity) and DPD-deficiency vs non-DPD-deficiency (85% specificity; 75% sensitivity) in 33 GI cancer patients treated with 5-FU (65). The uracil breath test does not help in determining the correct dose and is not recommended for clinical use.

5.2.5 DPD phenotype by 5-FU test dose—A recent approach to identify individuals at risk for toxicity uses a 5-FU test dose, which identified 3 of 188 patients with low drug clearance, in the presence of normal DPD activity (44). These patients would have been missed with DPD genomic analysis and/or assessment of DPD enzymatic activity, highlighting the importance of monitoring the ultimate phenotype of 5-FU catabolism, a 5-FU plasma concentration (50). An obvious disadvantage to this approach is the possibility of generating severe toxic reactions following a 5-FU test dose in severely DPD deficient patients (66).

5.2.6 Uridine triacetate—Although not a biomarker, uridine triacetate may be used to prevent and/or overcome 5-FU toxicity. This 5-FU antidote received FDA approval for the emergency treatment of adult and pediatric patients following 5-FU or capecitabine overdose regardless of the presence of symptoms, and of those who exhibit early-onset, severe, or life-threatening toxicity affecting the cardiac or central nervous system, and/or early onset, unusually severe adverse reactions (e.g., gastrointestinal toxicity and/or neutropenia) within 96 hours following the end of 5-FU or capecitabine administration. Uridine triacetate produces excess circulating uridine, which is then taken up into cells and converted to uridine triphosphate, which competes with 5-fluoro-uridine triphosphate for incorporation into RNA. In this way, uridine triacetate is able to prevent 5-FU associated cell damage and death (67). Severe toxicity generally develops immediately in predisposed patients, and might be noted clinically within 96 h of dosing (68). However, if TDM becomes readily available and efficiently implemented, a TDM level showing dangerously high 5-FU drug

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concentrations may prompt timely dosing of the now available antidote to prevent severe morbidity and mortality.

6 Exposure-Response (PK-PD)

It is well-established that every drug has an exposure-response relationship when assessed at the level of drug concentration at the target site *versus* proximal biochemical effects due to target modulation. Whether that exposure response relationship is discernable when assessed at the level of plasma concentration *versus* clinically relevant outcomes such as toxicity and efficacy depends on the various processes separating these levels. It is, therefore, important to evaluate the exposure- response relationship before attempting to modify dose based on measurements of such exposure.

Table 3 lists single-arm studies that do not aim to adjust 5-FU dose based on exposure, and it highlights the relationship between 5-FU exposure and outcome.

6.1 Is there an accepted and clinically relevant metric for systemic exposure to 5-FU?

There are different metrics of exposure that may correlate with outcome, including C_{max}, AUC, and time above a threshold concentration. The different administration schedules of 5-FU have evolved over time. 5-FU was initially administered as a bolus injection and then either as an infusional schedule for 22 or 46 hours, or as a hybrid of bolus immediately followed by an infusional schedule. The non-linear PK of 5-FU as a bolus means that determining C_{max} or back-extrapolation to concentration at time=0 (C_0), assuming instantaneous distribution of the bolus dose, is relatively cumbersome and requires multiple time points. Because of the short plasma half-life, infusions quickly approach steady-state concentrations (C_{ss}) and, with the prolonged continuous infusions, the concentration *versus* time profile approaches a rectangular shape, and C_{ss} easily converts to AUC by multiplication of C_{ss} by infusion duration. The vast majority of studies report AUC as a metric of exposure; C_{ss} is only occasionally reported in earlier studies.

6.2 Is there evidence for the relationship between 5-FU AUC and toxicity?

Because toxicity can be observed quickly (often in cycle 1) as a change from physiological baseline values, it is more easily obtained than response or survival metrics (see next section), which require longer observation periods. In virtually all of the clinical studies highlighted in Table 3, toxicity is studied as a dependent of 5-FU exposure, and statistically significant relationships are observed in all but two clinical trials. The first is the study by Jodrell et al., which is notable for its uncommon administration of 5-FU as a protracted venous infusion of up to 26 weeks (39). The second study is by Bocci et al., which only reported significant correlations between toxicity and T_{max} and half-life of the inactive metabolite FUH_2 after bolus administration of 5-FU (44). No 5-FU PK values by toxicity level or corresponding P-values were reported, although the discussion suggests that “interesting and significant relationships were found between 5-FUH_2 and 5-FU pharmacokinetic parameters and the toxicities that occurred after the first cycle of chemotherapy”, and it is unclear why no such correlation could be reported in this reasonably sized study of 185 patients.

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It should be noted that statistically significant correlations between 5-FU exposure and toxicity have been observed across several disease types (squamous cell carcinoma of the head and neck (SCCHN), nasopharyngeal cancer, and CRC), disease settings (metastatic, locally advanced), and dosing types (bolus, infusion).

6.3 Is there evidence for the relationship between 5-FU AUC and clinical activity?

As noted previously, clinical efficacy endpoints, such as tumor response and overall survival, require more time and larger study size, as well as a homogeneous population. Nevertheless, several clinical studies presented in Table 3 have found statistically significant correlations between 5-FU exposure and clinical outcome, mostly with response rates being the metric (69–72), but also as indicated by overall survival (42, 69). Moreover, while some studies were unable to document statistical significance based on the conventional $P<0.05$ cutoff value, the direction of the trend was always the same, with responses or survival associated with higher exposure. The relationship between 5-FU exposure and response documented in these retrospective, mostly observational studies, has been the foundation of interventional and more comparative designs listed and discussed in section 7.

6.4 Is the exposure-response relationship dependent on schedule or infusion duration?

Because of the non-linear PK of 5-FU, the exposure-response relationship may be partly dependent on whether 5-FU is administered as a bolus or as a continuous infusion. For infusions administered every 2 weeks, the transition to toxic exposures is somewhere in the range of 25–30 mg·h/L, whereas this transition occurs between 40 and 60 mg·h/L when summing the AUC of the 5 daily bolus administrations as applied in the less frequently used Mayo regimen, which is given every month, see Table 3. As will be discussed in section 8.4, the recommended AUC target range is 20–30 mg·h/L. This wider range will also accommodate small differences in the exposure-response relationships of 5-FU within the context of different combination regimens that might exist. As can be seen from Table 4, multiple regimens, single agent and combinations, and even different infusion durations are compared. From these differing starting points, applying a similar AUC target range consistently resulted in improvements of clinical activity and/or toxicity.

7 Evaluation of TDM

TDM is the measurement of drug concentrations in biological samples – typically plasma – to guide dose adjustment, in order to improve the benefit-risk ratio of a drug. TDM is appealing for drugs with a small therapeutic window such as with cytotoxic chemotherapy, a large inter-individual PK variability with small or moderate intra-individual PK variability, a reasonable correlation between systemic drug exposure (PK) and PD response, an established PK target range and repeated dosing (10). The PK characteristics of 5-FU along with the correlation between 5-FU systemic exposure and PD response, suggests a potential benefit from TDM as outlined in Sections 4–6.

In evaluating the study results discussed in this section, there are two aspects that may have diluted the impact of 5-FU TDM. The first is the at times conservative dose adjustment steps, which may have resulted in delayed achievement of exposure within the optimal

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therapeutic range and associated therapeutic benefits (32). The second is the fact that treating physicians will not always have followed actual dose-recommendations. This may be due to considerations of other clinical factors, due to reluctance to adopt this non-standard type of information in the decision-making process, and due to the belief on the part of treating physicians that they inherently know how to dose 5-FU, especially in the face of increased toxicity (16, 18).

Only two prospective randomized clinical trials have carefully investigated the value of 5-FU TDM relative to BSA-based dosing. One trial was in patients with advanced SCCHN (5) and another trial focused on patients with advanced CRC (4). A clinical trial in 105 patients with advanced SCCHN showed a clear benefit of 5-FU TDM (on a 96-h infusion) with regards to a reduction of severe neutropenia, thrombocytopenia, and mucositis (5). The trial by Gamelin et al. was conducted at various centers in France, and in 208 patients with advanced CRC (treated with an 8-h infusion). This study showed a significant reduction in the incidence of grade 3/4 5-FU-related toxicity as well as a significant improvement in clinical efficacy as determined by the primary endpoint overall response rates (4).

Several non-randomized clinical trials have applied 5-FU TDM in patients with mainly SCCHN or CRC as seen in Table 4. Several of these studies assessed 5-FU TDM with modern chemotherapy regimens such as FOLFOX6 (16, 30–32, 48, 81) or FOLFIRI (48, 81) in patients with advanced CRC. The following subsections will challenge the clinical value of 5-FU TDM with respect to 3 key features concerning PK variability, toxicity, and clinical activity. One meta-analysis pooled data from five trials and 654 patients suffering from advanced colorectal or HNSCC (82). The authors found PK-monitored 5-FU to be associated with a significantly improved radiological response rate (OR= 2.04, 95%CI 1.41–2.95, P=0.0002) compared with traditional BSA-based dosing. There was no evidence of improved tolerability: grade 3 to 4 diarrhea, neutropenia, and hand-foot syndrome were found not to be significantly different except that mucositis was less prominent for PK-monitored 5-FU (OR= 0.16, 95%CI 0.04–0.63, P=0.009) (82).

Although an in-depth discussion is beyond the scope of this document, cost-effectiveness has now become an ever more important consideration when patients are often unable to receive therapy due to significant “financial toxicity”. In this regard, 5-FU TDM has been reported to be cost-effective in the management of both mCRC and SCCHN (83, 84). Obviously, within every national healthcare system, the cost-effectiveness may be somewhat different. More importantly, individual healthcare actors may be incentivized in different ways. For example, in the United States, the current model for healthcare delivery in medical oncology does not foster individualization of anticancer therapy through TDM. While each system will have its own barriers and incentives, it is likely that inclusion of TDM in professional guidelines and placing a value on TDM such that logistical costs are covered, will increase adoption. In countries such as France and the Netherlands, TDM is currently being integrated into oncology clinical practice.

7.1 Is there evidence that TDM reduces variability in 5-FU exposure?

Several clinical trials have assessed improvement of inter-individual variability in 5-FU exposure as summarized in Table 4. Although there is only one randomized controlled

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clinical trial (showing a substantial improvement of the proportion of patients being 'within target' from 8% to 94% (4)), there is an overwhelming amount of longitudinal data within cohorts of patients that shows consistent reduction of variability in exposure upon applying TDM. Only a single conference abstract failed to show such effects from TDM of 5-FU (85).

7.2 Is there evidence that TDM reduces toxicity in patients receiving 5-FU?

Several clinical trials have assessed potential improvement of toxicity by using 5-FU TDM mainly in patients with advanced SCCHN or CRC (Table 4). There is compelling evidence with the randomized controlled clinical trial of Gamelin et al. in mCRC showing that TDM significantly reduces 5-FU-related diarrhea and hand-foot syndrome (HFS) (4), while in the randomized trial of Fety et al. in SCCHNC, neutropenia/thrombocytopenia and mucositis was significantly reduced (5). A reduction of toxicity is also consistently documented in many of the clinical trials that are non-randomized, and in the single-arm trials when compared with historical controls. The variation in the specific organ toxicities, for which the rates can significantly be reduced through TDM, may be a function of the context in each specific trial, including chemotherapies in the combination, and available supportive care.

7.3 Is there evidence that TDM improves clinical activity in patients receiving 5-FU?

Several clinical trials have assessed potential improvement of clinical activity by using 5-FU TDM mainly in patients with advanced SCCHN or CRC (Table 4). A randomized clinical trial in patients with advanced SCCHN did not report an improved response outcome by using 5-FU TDM (5).

The randomized trial of Gamelin et al. showed 5-FU TDM to significantly improve overall response rates from 18% with BSA-based dosing of 5-FU to 33% with PK-guided dosing of 5-FU using a somewhat unusual dosing schedule of weekly 8-hour 5-FU 1500 mg/m^2 single agent dosing regimen (4). Surgical resection of residual metastases occurred similarly in both arms (8 in the BSA arm, 11 in the TDM arm), and was reported to occur after treatment evaluation. 5-FU TDM was also associated with an improvement in overall survival, although the difference in overall survival did not reach statistical significance. However, it should be noted that this study was not sufficiently powered to look at the effect of 5-FU TDM on survival (4).

In summary, the use of TDM has been shown to improve clinical efficacy, as determined by response rates, as well as associated with a reduced risk of overall grade 3/4 toxicities. Taken together, TDM provides a positive benefit-to-risk ratio.

8 Implementation

8.1 Is the number of dose occasions in 5-FU treatment adequate to justify TDM?

The impact of TDM is limited to drug dosing occasions after the first dose is administered by conventional BSA-based dosing. The first dose provides the opportunity for the first drug measurement, and is itself by definition uninformed by TDM. In general, the more dosing occasions, the larger the theoretical impact of TDM. In mCRC, the pivotal clinical studies

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that established modern systemic triple combinations using a cytotoxic chemotherapy backbone plus a biologic agent, either bevacizumab (87) or cetuximab (OPUS, CRYSTAL) (88, 89) administered 5-FU in combination with oxaliplatin or irinotecan until disease progression or unmanageable toxicity. The median duration of 5-FU treatment in the OPUS study was 24 weeks, corresponding to 12 administrations of a 48 h 5-FU infusion (88). In the adjuvant therapy of early-stage colon cancer following potentially curative surgery, 5-FU systemic treatment is classically given for 6 months (90, 91), corresponding to 12 administrations of a 46-h 5-FU infusion. For SCCHN, patients with advanced or metastatic disease are recommended first-line combination treatment including cetuximab, a platinum salt (cisplatin, carboplatin) plus 5-FU according to results of the EXTREME trial (92). In the latter study, patients received 5-FU and cisplatin/carboplatin for six 3-weekly treatment cycles. Overall, repeated dosing of 5FU-based systemic therapy in both patients with CRC and SCCHN allows repeated dose adaptations.

8.2 Are reliable assays available?

Several academic institutions have established bioanalytical assays for 5-FU using either high-performance liquid chromatography (HPLC) (93) or liquid chromatography followed by tandem mass spectrometry (LC-MS/MS) (94, 95). Some of these assays quantitate the main metabolite of 5-FU, 5-fluoro-5,6-dihydrouracil (5-FUH_2), in addition to 5-FU (96). A commercial nanoparticle-based immunoassay for 5-FU has now been developed, and it has been validated in a multicenter setting. Moreover, this assay is easily implemented on widely available clinical chemistry analysis platforms. While all assays are reliable, the potential advantage of immunoassay speed, and application on automated instrumentation has been discussed previously (97).

8.3 Is the proper sampling timing and handling established?

Several important aspects need to be considered when performing 5-FU TDM. According to the short half-life of 5-FU of roughly 10–15 minutes, steady-state conditions would be expected approximately one hour after starting 5-FU infusion. In fact, clinical trials suggest that TDM samples are ideally taken at least 18 h after the start of 5-FU infusion (18–20, 30). Accordingly, most current protocols for 5-FU TDM recommend sampling to take place on day 2 of a 48-hours 5-FU infusion. With respect to the latter, blood sampling must not be done in case the infusion pump is empty or when the pump is considered to be in the final 30 minutes of emptying. If blood sampling for TDM is planned at the end of the drug infusions, patients should be recalled to the center approximately 4 h before the calculated end of infusion to avoid a substantial proportion of TDM failures as a consequence of empty drug pumps. Electric pumps are preferable compared to elastomeric pumps for patients in which 5-FU TDM is performed as they have a higher precision with regards to infusion times, while elastomeric pump balloons are sensitive to pressure, temperature, season, and patient activity (11, 12).

While the time of day of the sampling may have an impact on measured concentration through the known circadian variation of 5-FU metabolism, this impact is variable and appears to be relatively small with a difference between 3 PM to 6 AM mean of +20% (95% CI = 12–28%), comparable to even the residual variation within infusion (CV = 21%) (36).

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Any such variability should be accommodated by the AUC target range of 20–30 mg·h/L, and the sample timing could be standardized for a given patient whenever possible.

To avoid contamination and excessive 5-FU blood concentrations, blood sampling for TDM must be done from a peripheral vein at a distance from the central port where the patient typically receives the 5-FU infusion. 5-FU is unstable in whole blood and plasma at room temperature, primarily due *ex-vivo* catabolism of 5-FU by DPD (14–17, 50, 98). Blood samples should be placed immediately on ice, and plasma should be isolated as quickly as possible to separate plasma from cells. Inappropriate handling results in 5-FU degradation, which would then result in overdosing of patients based on falsely low 5-FU plasma concentrations. The addition of a DPD inhibitor such as gimeracil to the sample stabilizes 5-FU, allowing centrifugation within 24 h for collection of plasma (99).

8.4 Is there a recommended therapeutic exposure range based on the clinical evidence?

Specific target PK ranges have been proposed for 5-FU, and 5-FU AUC has been used in more recent clinical trials, as it can be easily calculated from steady-state plasma concentrations. The quantitative target range for 5-FU exposure, as expressed by AUC, is calculated from the measured concentration of 5-FU and the infusion duration. Gamelin et al. initially proposed a 5-FU AUC target range of 20–24 mg·h/L for an 8-h continuous infusion (4). However, this AUC target has only an approximately 20% range, which is rather small, especially given the significant intra-patient variability and reported commercial testing experience, potentially resulting in frequent unnecessary dose adjustments (30). Based on subsequent observational clinical studies and a review of the PK-PD data, the target range was subsequently widened to 20–30 mg·h/L, and applied to 46-hour infusion schedules of 5-FU as is now typically used with modern chemotherapy regimens, such as FOLFOX6 or FOLFIRI (16, 30, 33, 85, 100) (see also Figure 1). The recommended exposure target range of 20–30 mg·h/L is not appropriate for 5-FU bolus dosing and infusions of 120 h and longer.

8.5 Is there a dose-adaptation strategy (e.g. step-size of dose adjustments)?

Two major 5-FU dose adjustment algorithms have been published in the literature, one for the 5-FU target AUC of 20–25 mg·h/L (4) and one for the more recently updated 5-FU target AUC of 20–30 mg·h/L (30). The algorithm by Gamelin et al. recommends 5-FU dose adjustments over the range of 5-FU AUC of <4 mg·h/L to >31 mg·h/L (4), while the Kaldate algorithm recommends 5-FU dose adjustments over the range of 5-FU AUC of 8–10 mg·h/L to ≥40 mg·h/L (30). The dosing algorithm by Gamelin et al. has been validated in the only prospective randomized TDM study, and an increased proportion of patients experienced therapeutic 5-FU exposure by treatment cycle 4 (4). The dosing algorithm by Kaldate et al. has been validated in the recent single-cohort clinical trial of Wilhelm et al., and it was shown to be effective in increasing the proportion of patients with therapeutic 5-FU plasma exposure over time (16). Dose adaptation strategies have, been defined and explored in CRC patients receiving an outdated weekly 8-hour 5-FU 1500 mg/m^2 dosing regimen (4) and the more conventional and popular FOLFOX6, AIO, or FUFOX dosing regimens (16). Dose adjustment of a drug with non-linear PK, such as 5-FU is not as straightforward as that of a drug with linear PK. The infusion durations of 46 h currently employed result in

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concentrations that should not suffer much from this non-linearity. Still, the dose adaptation is relatively conservative in the dose step size. In the algorithm by Kaldate et al. an AUC of 8–10 mg·h/L would calculate to a tripling of the initial dose of on average 2200 mg/m^2 (30). Instead, the recommendation is an increase of 727 mg/m^2. Some conservatism with dose increases also minimizes the risk of significant overdosing based on a faulty single sample determination of 5-FU. As a result, more than one dose adaptation may be necessary to achieve exposure within the target AUC (see Figure 2). In addition, this example shows that for practical purposes, a sufficiently wide target concentration range needs to be defined to accommodate the within subject between occasion variability. Based on the more modern 46-h regimens described with Kaldate et al., we recommend utilizing this algorithm for dose adjustments (30). Obviously, the concentration value forms part of the information available to the treating physician, and the ultimate goal is to optimize patient treatment, not merely to optimize the exposure. Clinical toxicities observed should take precedence over 5-FU plasma values, and they may often be complementary. Indeed, physicians have been reported to ignore the guidance provided by TDM (potentially because of toxicity), or take small dose adjustment steps out of conservatism, which may reduce the clinical efficacy of TDM (32).

9 Conclusions and Recommendations on TDM of 5-FU in Cancer Patients

Based on the extensive literature review performed by our working group, we have been able to address the various pharmacological questions outlined in Table S 3, and this has resulted in the conclusions highlighted in Table 5.

We have summarized data on TDM of 5-FU therapy in patients with early or advanced CRC and patients with SCCHN. Based on careful review of all the available literature data and the framework to evaluate evidence supporting TDM based on published guidance used in psychiatry (see Table S 2), there is sufficient evidence to *strongly recommend* TDM for the management of 5-FU therapy in patients with early or advanced CRC and patients with SCCHN receiving common 5-FU dosing regimens. The clinical regimens where 5-FU TDM should be applied include FOLFOX4, FOLFOX6, FOLFOX7, FOLFIRI, LV5FU, FUFOX, AIO, weekly 1.5 g/m^2/8 hours for CRC and 1.0 g/m^2/day D1-4 or 1.0 g/m^2/day D1-5 for SCCHN as outlined in Table 4. The major criteria justifying TDM are fulfilled by 5-FU therapy, including a larger inter-individual variability than intra-individual variability, a narrow therapeutic window, established exposure-toxicity and activity relationships, the availability of established and validated bioanalytical assays, and algorithms for PK sampling and dose modifications. There is extensive data showing that TDM lowers variability in 5-FU exposure and lowers toxicity rates, data on the clinical benefit of 5-FU TDM in patients with CRC and SCCHN is more limited, with response rates being improved by TDM. While there is presently an absence of evidence that TDM improves survival in a randomized trial, TDM confers modest benefit with respect to clinical efficacy. However, such survival data would be needed for our working group to give TDM for 5-FU our highest recommendation of *unequivocally recommended*. The only prospective randomized study in SCCHN showed improved toxicity (powered primary endpoint), and response rate (secondary endpoint), but not survival benefit. The only prospective randomized study in CRC used a 5-FU dosing regimen that is no longer used in current treatment of CRC, and showed an improved response rate (powered primary endpoint) (4).

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An important conclusion from our in-depth review is that treatment should not rely solely on a concentration value, and optimal TDM must integrate the breadth of clinical factors and patient-specific information, as well as the drug concentration value. With this perspective, 5-FU TDM is not another option when compared to genotyping or phenotyping approaches, they are complementary. If available, only upfront DPD-oriented phenotyping (26, 60, 62, 63) or DPYD-genotyping (52, 54) approaches can completely prevent 5-FU exposure of many, but not all, severely DPD deficient patients. Beyond that point, 5-FU TDM is the best tool, currently available, to appropriately guide therapy. Although genotyping has a high specificity to identify patients with an increased risk of 5-FU associated toxicity, it has a rather poor sensitivity, with roughly 50% of patients prone to (severe) 5-FU associated toxicity remaining unrecognized. If not used in conjunction with TDM, DPYD genotyping will also not identify the significant number of patients with sub-therapeutic 5-FU exposure.

The different approaches of phenotyping the 5-FU catabolic enzyme DPD have been discussed earlier in this document, and each has specific issues ranging from the need for logistically challenging test-doses to lack of prediction of 5-FU clearance or toxicity, and DPD phenotyping has not been studied prospectively at present. While multiple tools to optimize 5-FU therapy (TDM, DPYD genotyping, DPD phenotyping) may be considered in any specific patient, TDM of 5-FU is considered the most integral tool to optimize the risk-benefit ratio of 5-FU therapy and in capturing the exposure in the individual patient, which is the metric most proximal to treatment outcome. In addition, if TDM becomes readily available and is efficiently implemented, a TDM level showing dangerously high concentrations may prompt timely dosing of the now available uridine triacetate antidote to prevent severe morbidity and mortality.

While our manuscript focuses on 5-FU, our systematic methodology provides a generic framework to evaluate published evidence in support of TDM recommendations for any drug in oncology. In applying our framework, gaps in our knowledgebase can be readily identified and targeted for further study and reporting.

The content of this position paper and its main conclusions was presented to, and endorsed by, the TDM in Oncology Scientific Committee of the IATDMCT at the 15th annual meeting in Kyoto, Japan, 2017, and the main conclusions were presented at the IATDMCT 2017 annual meeting.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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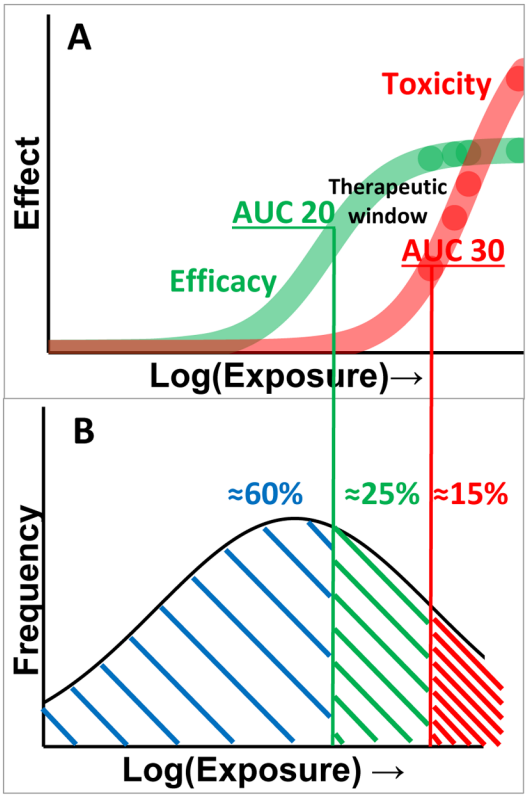

Figure 1.

Schematic depiction of (A) the exposure-response (green, AUC≈20 mg·h/L) and exposure-toxicity (red, AUC≈30 mg·h/L) relationships of 5-FU, defining the therapeutic window of 20–30 mg·h/L. The commonly practiced BSA-based dosing results in (B) a population distribution of exposure (solid line) and associated cumulative frequency distribution (dotted line) that shows that ≈60% of patients are under-dosed, ≈15% of patients are over-dosed, and only ≈25% of patients will experience exposure within the therapeutic window.

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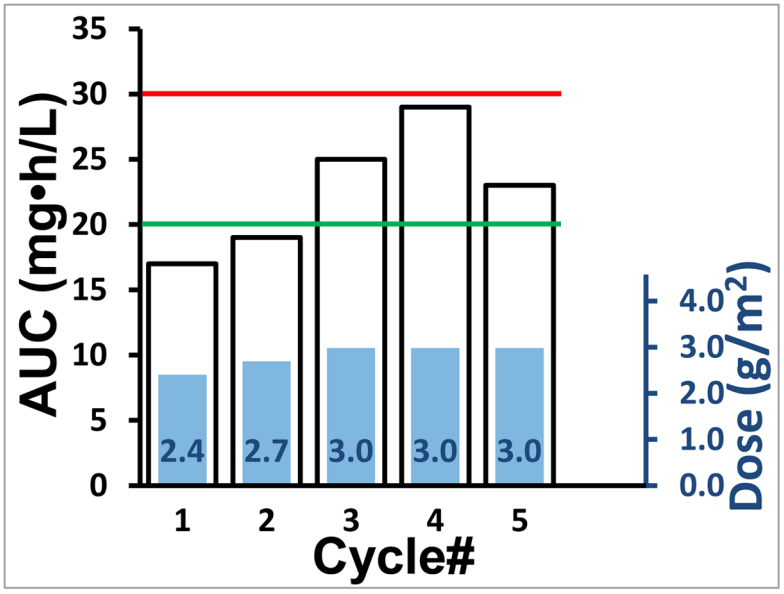

Figure 2.

Example of the implementation of the 5-FU dose adjustment algorithm in a single patient initiated on 2.4 g/m^2, followed by a dose increase to 2.7 g/m^2, and 3.0 g/m^2 to achieve an AUC within the target range of 20–30 mg·h/L. No dose adjustment was needed in cycle 4 or 5, and the variability in exposure experienced between cycles 3, 4, and 5 is a reflection of the within subject variability in measured AUC. A sufficiently wide target range reduces the likelihood of constant dose-adjustment, which would be chasing within-subject inter-occasion variability rather than adjusting to true and meaningful changes in exposure.

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Table 1

Average Values and Inter-Subject Variability of 5-FU Clearance or Exposure with Different Dosing Schemes.

Dose	Duration	N	Parameter	Mean (SD)	Range	CV%	Yr	Ref
4 g/m^2	120 h	119	CL (L/h/m^2)	109 (15)	39–267	41	2016	(29)
4 g/m^2	96 h	53	CL (L/h/m^2)	128 (51)	-	40	1998	(5)
2.4 g/m^2	46 h	589	CL (L/h/m^2)	117 (53)	-	45	2012	(30)
2.5 g/m^2	46 h	118	CL (L/h/m^2)	131 (38)	60–246	29	2012	(31)
2.4 g/m^2	46 h	356	CL (L/h/m^2)	118 (57)	35–2400	49	2011	(32)
2.4 g/m^2	46 h	48	CL (L/h/m^2)	104[a]	71–240		2016	(33)
2.4 g/m^2	46 h	47	CL (L/h/m^2)	126[a]	-	31	2014	(34)
1 g/m^2/day	CI	82	CL (L/h/m^2)	151 (41)	63–242	27	1992	(35)
1 g/m^2/day	CI	61	CL (L/h/m^2)	-	-	30	2015	(36)
1 g/m^2/day	Bolus+CI	38	AUC (mg/L·h/m^2)	9.1 (3.1)	3.9–16.4	34	2003	(37)
0.37–1.22 g/m^2/day	CI	380	CL (L/h/m^2)	181	29–739	-	1992	(28)
0.65–1 g/m^2/day	CI	30	CL (L/h)	237		44	2007	(38)
0.3 g/m^2/day	CI	58	$FU_{300}C_{ss}$ (ng/mL)	94 (25)	-	27	2001	(39)
0.3 g/m^2/day	CI	26	CL (L/h/m^2)	149 (75)	-	50	2002	(40)
1.5 g/m^2	8 h	90	Dose to AUC 20–25 mg·h/L (mg/m^2)	1790 (386)	900–3300	22	2008	(4)
1.3 g/m^2	8 h	81	CL (L/h/m^2)	134 (62)	29–296	46	1999	(26)
1.3 g/m^2	8 h	117	Dose to 2–3 mg/L (mg/m^2)	1803	950–3396	-	1998	(18)
0.5 g/m^2	20 min	14	AUC (mg/L·h)	-	-	52	1996	(41)
0.5 g/m^2	Bolus	14	AUC (mg/L·h)	-	-	19	1996	(41)
0.37 g/m^2	Bolus	115	CL (L/h/m^2)	51.5 (24.8)	-	48	2008	(42)
0.425 g/m^2	Bolus	181	CL (L/h)	79.2 (35.4)	-	45	2006	(43)
0.25 g/m^2	Bolus	185[b]	CL (L/h/m^2)	65.7 (31.9)	-	49	2006	(44)
0.37 g/m^2	Bolus	80	CL (L/h/m^2)	56.3 (33.8)	-	60	2002	(45)
0.25 g/m^2	Bolus	20	CL (L/h/m^2)	54.6 (15.8)	-	29	2000	(46)

AUC values were converted to clearance values wherever possible.

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CI, continuous infusion.

When interpreting a coefficient of variation (CV%), it is important to remember that a CV of 40% equates to a 95% confidence interval of mean · (1±1.96·0.4) and thus spans an 8.3-fold range; similarly, with a CV of 20%, the 95% confidence interval spans a 2.3-fold range.

[a] median

[b] after exclusion of 3 patients with CL <1 L/h/m^2

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Table 2

Intra-Subject Variability of 5-FU Clearance or Exposure.

Dose	Duration	N	Parameter	CV%	Yr	Comment	Ref
1.5–2.6 g/m^2/day	CI	4	Css	14–22	2012	≥7 courses/subject	(11)
0.75–2.875 g/m^2/day	CI	9	CL	6.8	1991	+dipyridamole	(49)
1 g/m^2/day	CI	82	CL	3–24	1992		(35)
1 g/m^2/day	CI	61	CL	19	2015		(36)
0.3 g/m^2/day	CI	58	$FU_{300}C_{ss}$	20	2001		(39)
0.5 g/m^2	20 min	14	AUC	32	1996	N=3/subject	(41)
0.5 g/m^2	Bolus	14	AUC	26	1996	N=3/subject	(41)

CI, continuous infusion.

Css, steady state concentration.

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Table 3

Single-Arm Studies Evaluating the Relationship between 5-FU Exposure, Toxicity, and Outcome.

5-FU regimen	Duration	N Disease	PK parameter	Exposure – Toxicity	Relation	Exposure – Activity	Relation	Year	Ref
6 g/m^2	120 h	29 SCCHN	AUC	T NT	>30 <30 P<0.001	-	-	1986	(73)
6 g/m^2 +cisplatin	120 h	77 SCCHN	$AUC_{0\text{-}5d}$	T NT	Median: 34 Median: 26 P<0.001	R NR	AUC0-3d: 11.0 AUC0-3d: 8.8 P=NS	1989	(74)
5.0 g/m^2 + cisplatin	120 h	14 SCCHN	$AUC_{0\text{-}5d}$	T NT WHO G≥2	45.3 35.4 P=0.011	-	-	1993	(75)
5.0 g/m^2 + cisplatin	120 h	68 SCCHN	$AUC_{0\text{-}105h}$*	T NT G≥2	33.7 25.3 P=0.0003	-	-	1994	(57)
5.0 g/m^2 + cisplatin	120h	186 SCCHN	$AUC_{0\text{-}5d}$*	-	-	R NR WHO Survival	28.7 27.2 P=0.01 P=0.025	1994	(69)
3.2 g/m^2 + cisplatin	120 h	89 SCCHN	$AUC_{0\text{-}5d}$	Leukopenia Mucositis	Ln(nadir) vs AUC P=0.04 grade vs AUC P=0.04	CR Non-CR	Median 31.4 Median 24.0 P=0.02	1996	(70)
4.0 g/m^2 +cisplatin	120 h	119/108 NPC	AUC	T NT CTCAE4 G≥3	50 (15) 31 (11) P<0.0001	R NR RECIST1 AUC: <25 vs 25–35 vs >35 <25 vs >35	39 (15) 33 (14) P=0.07 RR: (P-value) 50.0 (0.18) 67.5 (0.38) 76.1 (0.031)	2016	(29)
6 g/m^2	120 h	27 CRC	AUC	T NT	Median: 14.5 Median: 17.7 P=NS	PR+SD PD	Median: 29.9 Median: 15.8 P=0.05	1978	(71)
7.5 mg/kg/day	120 h	24 CRC	Cl (L/kg/day)	T NT	32.0 (16.8) 72.0 (37.3) P<0.001	-	-	1982	(76)
0.5 g/m^2	bolus	21 CRC	AUC	T NT WHO Gr≥1	Logistic regression No stats	-	-	1988	(77)
3.25–6.5 g/m^2	120 h	26 CRC	AUC	T NT WHO	Spearman rank P<0.05 Threshold AUC=30	-	-	1988	(78)

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5-FU regimen	Duration	N Disease	PK parameter	Exposure – Toxicity	Relation	Exposure – Activity	Relation	Year	Ref
0.19–0.60 g/m^2	72 h	19 CRC	$AUC_{0\text{-}3d}$/Cl	T NT WHO G≥2	12.5/41 6.3/107 P<0.05/0.01	R NR WHO	10.1/90 8.3/60 NS/NS	1990	(79)
1.11–10.8 g/m^2	72 h	35 advanced	C_{ss}	WHO G≥1 stomatitis %WBC↓	Hill R^2=0.88 Hill R^2=0.61	-	-	1991	(49)
1+ g/m^2	8 h	40 CRC	High vs low C_{ss}**	-	-	CR+PR MR+NR+PD OS_{1year}	High 82%-Low 14% P<0.01 High 71%-Low 45% P=0.2	1996	(72)
0.37 g/m2	Bolus x5	89 CRC	AUC	T NT GI G≥3/G≥2	18.8/13.6 7.9/7.9 -/P<0.001	-	-	2001	(80)
0.3 g/m^2/day	Continuous 26 weeks	58 CRC	C_{ss}	T/NT Diarrhoea Hand-foot Stomatitis Dose reduction	 P=0.164 P=0.410 P=0.949 P=0.941	R/NR	P=0.182	2001	(39)
0.37 g/m^2	Bolus x5	26 CRC	AUC/C_{max}	T NT CTC G≥3	25.8/34.6 8.5/18.8 P=0.002/0.016	-	-	2002	(58)
0.37 g/m^2	Bolus x5	185 GI	AUC	T NT WHO G≥2 -5-FDHU $t_{1/2}$ Stomatitis -5-FDHU t_{max} Neutropenia -5-FDHU t_{max} Diarrhea	5-FU NS P=0.008 P=0.032 P=0.014	-	-	2006	(44)
0.425 g/m^2	Bolus x5	181 CRC	$AUC/t_{1/2}$	G=0 G=1 G=2 G=3 G=4 WHO	7.4/8.4 8.4/8.9 10.5/10.9 12.1/11.1 14.3/13.5 P<0.0001/0.0001	-	-	2006	(43)
0.37 g/m^2	Bolus x5	115 CRC	AUC			DFS_{5year} vs Recurrence TTP (N=58) ROC AUC=8.4	9.3 (4.1) 7.5 (2.9) P<0.05 P<0.05 NS	2008	(42)

*mid-cycle dose adaptation based on exposure

**cycle-to-cycle within patient dose (de)escalation

AUC: area under the plasma concentration versus time curve (mg·h/L)

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Cl: clearance (L/h/m^2), unless stated otherwise)

C_{max}: maximum plasma concentration

CR: complete response

CRC: colorectal cancer

Css: steady state concentration

DFS: disease free survival

G: grade

GI: advanced gastrointestinal cancer

MR: minor response

NPC: nasopharyngeal carcinoma

NR: no response

NS: non-significant

NT: no toxicity

ORR: objective (radiological) response rate

OS: overall survival

PD: progressive disease

PR: partial response

R: response

ROC: receiver operating curve analysis, multivariate

RR: response rate (%)

SCCHN: squamous-cell cancer of the head & neck

SD: stable disease/disease stabilization

T: toxicity

t1/2: half-life (min)

TTP: time to progression

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Table 4

Improvement of PK Variability, Toxicity and Clinical Activity with 5-FU TDM.

Disease	5-FU regimen	N (type)	PK parameter	TDM effect	Toxicity	TDM effect	Activity	TDM effect	Ref
SCCHN	1.0 g/m^2/day D1-4 + cisplatin	106 (R)	Cycle 3 AUC CV%	40→31% *	G≥3 neutropenia/thrombocytopenia G≥3 mucositis	18→8% * 5→0% *	ORR	77→82% (NI)	(5)
SCCHN	1.0 g/m^2/day D1-5 + cisplatin	170 (NR)	NA only dose reductions	NA	G≥3 overall	20→12% *	ORR	31→47% *	(74)
SCCHN	1.0 g/m^2/day D1-5 + cisplatin	90 (NR)			G≥2 overall G≥3 overall	47→33% * 15→10%	Response OS	42→44% 9→14 months	(75)
mCRC	1.5 g/m^2/8 h QW	208 (R)	Within AUC 20–24 Dose targeting AUC 20–24	8→94% 1.8 (3.9) g/m^2 range 0.77–3.3	G≥3 diarrhea G≥3 mucositis G≥3 HFS G≥3 leukopenia	18→4% 2→2% 7→11% 2→0%	ORR 2-Y OS OS	18→34% * 30→41% 16→22 months	(4)
mCRC	FOLFOX6 (2.5 g/m^2)	157 (NR)	Dose targeting AUC 20–24	2.8 (0.5) g/m^2 range 1.5–3.5	G≥3 diarrhea G≥3 mucositis G≥3 neutropenia	12→1.7% 15→0.8% 25→18%	ORR PFS OS	46→70% 10→16 months 22→28 months	(31)
CRC	FOLFOX6/FOLFIRI	49 st.IV 35 St. II/III (NR)	Dose targeting AUC 20–24	NA	G≥3 overall (IV) G≥3 overall (II/III) G≥3 diarrhea (II/III)	37→37% 69→32% * 50→16%	PFS (IV) PFS (II/III)	10→14 months 18→NA months *	(81)
mCRC	1.3 g/m^2/8 h QW	152 (SA)	Dose targeting AUC 16–24	1.8 (3.9) g/m^2 range 0.95–3.7	G≥3 diarrhea G≥3 mucositis G≥3 HFS G≥3 leukopenia	7% 2.6% 5% 2%	ORR PFS OS	43% 11 months 19 months	(18)
mCRC	1.3 g/m^2/8 h QW	81 (SA)	Cycle 1→TDM AUC CV%	63→7%	NA	-	NA	-	(26)
CRC	FOLFOX6/FOLFIRI	356 (SA)	Within AUC 20–24 Cycle 1→TDM AUC CV%	21→37% 53→30% *	NA	-	NA	-	(32)
mCRC	FOLFOX6 ±bevacizumab	70 (SA)	Within AUC 20–25	28→47% *	G≥3 diarrhea G≥3 mucositis G≥3 neutropenia	6% 2% 33%	NA	-	(34)
GI	LV5FU, FOLFOX6, FOLFIRI	50 (SA)	Within AUC 18–28	53→51%	G≥3 diarrhea G≥3 mucositis G≥3 neutropenia	8% 0% 52%	NA	-	(85)
mCRC	AIO, FOLFOX6, FUFOX	75 (SA)	Within AUC 20–30 Cycle 1→TDM AUC CV%	34→57% * 33→25%	G≥3 diarrhea G≥3 mucositis G≥3 nausea G≥3 fatigue	5% 0.2% 3.4% 0%	NA	-	(86)

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Disease	5-FU regimen	N (type)	PK parameter	TDM effect	Toxicity	TDM effect	Activity	TDM effect	Ref
mCRC	FOLFOX7 +bevacizumab	48 (SA)	Within AUC 20–30	60→100%	G≥3 overall G≥3 diarrhea G≥3 mucositis G≥3 neutropenia	38% 2% 2% 27%	ORR PFS OS	48% 11 months 24 months	(33)

*Statistically significant (P<0.05)

AIO: combines folinic acid 500 mg/m^2 over 1 hour followed by 5FU 2600 mg/m^2 over 24 hours, given at weekly intervals.

AUC: area under the plasma concentration versus time curve (mg·h/L)

D: days

FOLFIRI: combines irinotecan 180 mg/m^2 over 90 minutes with folinic acid 30 mg iv-push, 5-FU 400 mg/m^2 iv-push followed by 5FU 2400 mg/m^2 over 46 hours. Cycles are repeated every 2 weeks.

FOLFOX6: combines oxaliplatin 100 mg/m^2 over 2 hours with folinic acid 30 mg iv-push, 5-FU 400 mg/m^2 iv-push followed by 5FU 2400 mg/m^2 over 46 hours. Cycles are repeated every 2 weeks.

FOLFOX7: combines oxaliplatin 130 mg/m^2 over 2 hours with folinic acid 30 mg iv-push followed by 5-FU 2400 mg/m^2 over 46 hours. Cycles are repeated every 2 weeks.

FUFOX: combines oxaliplatin 50 mg/m^2 over 2 hours with folinic acid 500 mg over 2 hours followed by 5-FU 2000 mg/m^2 over 24 hours, given on days 1, 8, 15 and 22 of a 5-week cycle.

G: grade

GI: advanced gastrointestinal cancer

HFS: hand-foot syndrome

LV5FU: leucovorin 200 mg/m^2/day followed by 5-FU bolus 500 mg/m^2/day and continuous 22-h infusion of 5-FU 600 mg/m^2/day for 2 consecutive days, Q2W.

mCRC: metastatic colorectal cancer

NA: not available

NI: not statistically improved

NR: non-randomized

ORR: objective (radiological) response rate

OS: overall survival

QW: every week

R: randomized

SA: single arm

SCCHN: squamous-cell cancer of the head & neck

St.: stage

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Table 5

Answers to Pharmacological Questions to Assess the Suitability of Applying TDM to 5-FU Therapy.

Pharmacokinetics (PK)
The inter-individual variability in 5-FU plasma concentrations using current dosing methods based on body surface area (BSA) is approximately 40%.
The intra-individual variability in 5-FU plasma concentrations is approximately 20%.
Pharmacodynamics (PD)
5-FU has a narrow therapeutic window, and for this reason, a simple test to measure 5-FU drug levels would be important to more precisely assess drug exposure and risk of toxicity.
There are no easy and clinically relevant biomarkers to predict response and/or toxicity, except for the use of DPD testing to identify many but not all of the rare patients with severe DPD deficiency.
Exposure-Response (PK-PD)
AUC is the accepted and clinically relevant metric for systemic exposure to 5-FU.
A relationship between 5-FU AUC and toxicity exists.
A relationship between 5-FU AUC and clinical activity exists.
The exposure-response relationship is somewhat dependent on infusion duration and this is documented.
Evaluation of TDM
TDM reduces variability in 5-FU exposure.
TDM reduces toxicity in patients receiving 5FU.
TDM improves response rates in patients receiving 5FU.
Implementation
5-FU infusional treatment offers a sufficient number of occasions to derive benefit from TDM
Reliable assays are currently available to measure 5-FU exposure.
Proper sampling includes ensuring ex vivo stability and sampling at least 18 h after start of infusion
The recommended therapeutic exposure range is AUC 20–30 mg·h/L for currently used 46 h infusions.
Dose-adaptation strategies are currently available.