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Therapeutic Drug Monitoring of 5-Fluorouracil

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Abstract

Purpose—For over 50 years, 5-FU has played a critical role in the systemic chemotherapy of cancer patients. 5-FU serves as the main backbone of combination chemotherapy for patients with colorectal cancer (CRC) in both the adjuvant and metastatic disease settings. Herein, we review the current status of 5-FU therapeutic drug monitoring (TDM) and discuss its potential role in the clinical practice setting.

Method—PubMed and abstracts from the American Society of Clinical Oncology (ASCO) were searched up through September 2015 for clinical data relating to 5-FU TDM.

Results—5-FU dosing has been typically determined by using body surface area (BSA). However, it is now well-established that BSA-based 5-FU dosing is correlated with a wide variation of 5-FU systemic exposure. Pharmacokinetic (PK) studies of 5-FU systemic exposure have shown a wide range of interpatient variation of 5-FU plasma drug levels. Over the past 30 years, increasing efforts have been placed on optimizing 5-FU dosing with the main goals of increasing antitumor efficacy while reducing drug-associated toxicity. There is growing evidence to show that 5-FU dosing based on plasma 5-FU drug level is feasible and that 5-FU TDM can improve clinical outcomes by improving efficacy of 5-FU-based combination regimens and reducing toxicities.

Conclusion—Dose adjustment of 5-FU is feasible and PK-based dosing can significantly improve clinical outcomes by reducing toxicities and improving efficacy.

Introduction

Therapeutic drug monitoring (TDM) is the measurement of drug concentrations in biological samples to individualize the drug dosage for the improvement of drug efficacy and reduction of related toxicities [1–3]. This approach is widely used in everyday clinical practice for a number of different medications to treat non-cancer human diseases. In sharp contrast, TDM is only rarely used in cancer therapy. However, given the very narrow therapeutic index and substantial interpatient pharmacokinetic (PK) variability associated with cytotoxic agents and targeted agents, TDM should be viewed as a clinically relevant strategy to be incorporated in cancer therapy [3]. The main component of TDM is the determination of a

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significant correlation between the dose of administered drugs, systemic drug exposure, and pharmacodynamic (PD) response. The practical utility of TDM in cancer care has been investigated with several cytotoxic cancer drugs including 5-flurouracil (5-FU), methotrexate, and busulfan with new developments of therapeutic algorithms of individual dose adjustment to optimize the exposure-response relationships and improve clinical outcome.

Since the initial synthesis and development of the fluorinated pyrimidines as an important class of cytotoxic chemotherapy by Heidelberger and colleagues in the 1950's, 5-FU has been the backbone of systemic combination chemotherapy for the treatment of colorectal cancer (CRC) and other GI cancers, breast cancer, and head & neck cancer. For the past five decades, 5-FU administration schedules have evolved quite significantly for the treatment of mCRC cancer patients: bolus intravenous (IV), continuous IV infusion, intermittent IV infusion, hybrid schedule of bolus plus intermittent IV infusion; addition of the biochemical modulator leucovorin (LV); 5-FU monotherapy versus combination with other cytotoxic agents, including oxaliplatin or irinotecan for the treatment of CRC; and 5-FU monotherapy or 5-FU-based regimens in combination with various biological agents, including bevacizumab, cetuximab, panitumumab, ziv-aflibercept, and ramucirumab.

Until the early 1980's, 5-FU was administered primarily by IV bolus injection. However, the bolus approach was severely limited by 5-FU's extremely short half-life of 10–15 minutes. Moreover, 5-FU, as with other antimetabolites, is most active in the S-phase of the cell cycle. For this reason, IV bolus injection of 5-FU was found to have only a limited effect on the overall population of tumor cells. Since the mid-1970s, infusional regimens of 5-FU have gained increasing acceptance in everyday clinical practice due to improved efficacy and safety profile when compared to IV bolus regimens. Infusional IV administration of 5-FU over 46 hours has now become the standard fluoropyrimidine administration schedule that is used in modern combination chemotherapy regimens for CRC including FOLFOX or FOLFIRI.

5-FU dosing, as with most other cytotoxic agents, has been traditionally determined using body surface area (BSA). There is now a large body of evidence confirming the lack of scientific rationale for BSA-based dosing of 5-FU and most other cytotoxic agents. As has been previously demonstrated by several investigators, BSA-based dosing is associated with several limitations, perhaps the most important being wide interpatient variability in 5-FU drug levels when dosing is based on BSA [4, 5]. Gamelin and colleagues investigated the potential correlation between BSA and 5-FU plasma clearance in 81 patients with metastatic CRC (mCRC) receiving weekly 8-hour continuous infusion of 5-FU at a starting dose of 1,300 mg/m^2 [5]. A 10-fold interpatient variability in 5-FU plasma clearance (range, 0.49 to 4.93 L/h/m^2) was observed. Furthermore, no correlation was observed between BSA and 5-FU plasma clearance in all 81 patients during the first course of 5-FU infusion therapy.

Significant efforts have focused on optimizing PK models of 5-FU clearance. In the early days of 5-FU development when bolus administration schedules were in favor, it was difficult to optimize PK models of 5-FU clearance given 5-FU's nonlinear PK properties, which resulted in substantial interpatient variability [6–8]. In sharp contrast, with

intermittent or continuous infusion schedules of 5-FU, which have now become standard of care, plasma levels increase until steady-state is reached. As a result, 5-FU clearance and AUC can be accurately calculated when the infusion rate of 5-FU and plasma concentration at steady-state are known. A large number of clinical studies have documented wide variation in 5-FU systemic exposure and drug clearance [1, 3, 9]. PK analysis of 5-FU systemic exposure with infusional 5-FU regimens revealed significant interpatient variation of 5-FU plasma levels. There is a well-documented relationship between 5-FU plasma concentration and biological effects including toxicity [10, 11]. Severe toxicities induced by 5-FU based chemotherapy including febrile neutropenia, nausea/vomiting, and diarrhea cause significant clinical and financial burden on healthcare system through hospitalization and increased risk of morbidity and mortality [12, 13]. These toxicities are especially severe in patients with previously undiagnosed dihydropyrimidine dehydrogenase (DPD) deficiency on 5-FU exposure due to significant unpredictable toxicity generated by 5-FU [14, 15]. It has been shown that 5-FU TDM has beneficial impact on pharmacoeconomic side of 5-FU based chemotherapy [12, 13].

Over the past 30 years, increasing efforts have been placed on optimizing 5-FU dosing with the main goals of increasing clinical efficacy while reducing drug-associated toxicity [16, 17]. There is now convincing data showing that dose adjustment of 5-FU based on plasma 5-FU drug levels is feasible and that PK-based dosing can significantly improve clinical outcomes by reducing toxicities and improving efficacy. Herein, we review the current status of 5-FU TDM based on published data (Table 1).

Existence of PK-PD Relationships

In the 1970s, Hillcoat et al reported on the close correlation between 5-FU plasma drug concentrations and tumor response in patients with gastrointestinal tract (GI) cancers [18]. In this study, patients received 5-day continuous infusion of 5-FU at a dose of 1200 mg/m^2/day on days 1–5 in combination with the nitrosourea CCNU 150 mg/m^2 on day 1, and this combination regimen was administered every 6 weeks. Plasma 5-FU concentrations were measured and found to vary widely amongst the patients. Moreover, patients who had either a partial response (PR) or stable disease (SD) were noted to have significantly higher plasma 5-FU area under the plasma concentrations x time curve (AUC) than those without a tumor response. This was the first clinical evidence documenting an association between 5-FU plasma exposure and clinical activity.

The relationship between 5-FU PK and response to treatment was investigated by Seitz and colleagues in 13 patients with GI cancers receiving weekly IV bolus injection of 5-FU 15 mg/kg [19]. Plasma clearance of 5-FU after an IV bolus injection of 5-FU varied widely among patients (range, 0.39 to 2.55 L/min). The mean 5-FU clearance was significantly greater in patients without tumor response than that in patients with PR or SD (1.40 L/min *versus* 0.73 L/min; $p < 0.02$).

A similar PK/PD study analyzed the plasma concentration of 5-FU in 29 patients with head and neck cancer receiving combination chemotherapy of continuous 5-day infusion of 5-FU 1,000 mg/m^2/24 hr on days 2–6 and cisplatin at a dose of 100 mg/m^2 on day 1 [10]. In this

study, a close correlation was observed between increased systemic exposure to 5-FU (5-FU AUC > 30 mg•35;h/L) and incidence of toxicities including myelosuppression, mucositis, and diarrhea.

van Groeningen et al from the Free University in Amsterdam, the Netherlands analyzed 5-FU PK in 21 patients with advanced cancers [20]. 5-FU was administered as a weekly IV bolus at a starting dose of 500 mg/m^2 with 20% dose escalation every 4 weeks until dose-limiting toxicity was observed. The use of a logistic regression method revealed that the incidence of toxicities was closely correlated with 5-FU *versus* AUC.

Yoshida and colleagues analyzed the potential correlation between 5-FU dosing and the degree of toxicity in 19 patients with mCRC [11]. In this study, patients received continuous IV infusion of 5-FU at a dose of 190–600 mg/m^2/day for 7 consecutive days. Among all study patients, 9 patients (toxic group) experienced > grade 2 stomatitis, anorexia, nausea/vomiting, disorientation, and toxic dermatitis. Steady-state concentrations (C_{SS}) of 5-FU and AUC from 0 to 72 hours from the start of 5-FU infusion ($AUC_{0–72\ hours}$) were determined. There was an approximately 2-fold difference of 5-FU serum C_{SS} (0.198 *versus* 0.106 mg/L; $p < 0.05$), $AUC_{72\ hours}$ (12.53 *versus* 6.32 mg•35;h/L; $p < 0.05$), and total body clearance (40.6 *versus* 106.7 L/h/m^2) between the group of patients experiencing toxicity (N = 9) and the group that did not experience toxicity (N = 10).

The relationship between 5-FU dose, 5-FU C_{SS}, and toxicity was analyzed in a phase I study of 42 patients receiving a 3-day continuous IV infusion of varying doses of 5-FU (from 185 mg/m^2/day to 3,600 mg/m^2/day) [21]. 5-FU C_{SS} values varied significantly even within the same dose level of 5-FU administered, and the interpatient coefficient of variation of 5-FU C_{SS} in cycles of 5-FU alone was 15.6% (range, 8.6% to 24.9%). Of note, a significant correlation was observed between 5-FU C_{SS} and frequency of stomatitis: % frequency stomatitis = $100(1 - e^{-0.122Css})$, $r^2 = 0.80$. There was also a significant relationship between 5-FU C_{SS} and the extent of neutropenia: % reduction in WBC = $100(1 - e^{-0.060Css})$, $r^2 = 0.61$. These results provided a theoretical basis for developing a 5-FU dose adjustment algorithm to achieve a target 5-FU C_{SS} that may vary as informed by the risk and severity of toxicity deemed acceptable in a given therapeutic senario.

In a series of 380 patients with squamous head and neck cancer, Milano et al. investigated the influence of age and sex on 5-FU clearance where cisplatin was administered in combination with a 5-day continuous IV infusion of 5-FU at a dose of 1,000 mg/m^2/day [4]. They identified a wide variation in 5-FU clearance with 10–20 fold range in both men (median, 179 L/h/m^2; range, 29 to 739) and women (median, 155 L/h/m^2; range, 56 to 466) [4]. This same group then analyzed the prognostic value of 5-FU $AUC_{0–105}$ in 186 patients receiving 3 cycles of cisplatin 100 mg/m^2 plus continuous 5-day infusion of 5-FU 1,000 mg/m^2/day in the first-line therapy of head and neck cancer [22]. The averaged 5-FU $AUC_{0–105}$ was 27.9 mg•35;h/L. Tumor response was significantly linked to averaged 5-FU $AUC_{0–105}$ ($p = 0.05$), but not to averaged dose of administered 5-FU. Univariate analysis showed a significant correlation between averaged $AUC_{0–105\ hours}$ and OS ($p = 0.001$). Multivariate analysis revealed that averaged $AUC_{0–105}$ remained as a significant prognostic

variable (p = 0.025). Furthermore, patients with 5-FU $AUC_{0–105}$ > 29.0 mg•35;h/L were found to have significantly improved OS (p = 0.001).

A group of investigators from France identified a significant relationship between 5-FU plasma levels and toxicity in a prospective, randomized phase II trial [23]. Patients with mCRC (N = 40) received a weekly schedule of IV 5-FU that was administered over 8 hours. The dose of 5-FU was 1,000 mg/m^2 in the initial cycle of therapy, and it was then increased in individual patients every 3 weeks by 250 mg/m^2 up to 2,000 mg/m^2 or the first signs of toxicity. Blood samples were taken at the 4^{th} and 8^{th} hours of every 5-FU infusion, and 5-FU plasma levels were determined by high performance liquid chromatography (HPLC). Twenty-four cases of acute toxicities including diarrhea, stomatitis, nausea, hand-foot syndrome, and neutropenia were observed during the first 3 months of therapy. A highly significant correlation was observed between 5-FU plasma levels greater than 3,000 μg/L, which corresponds to a 5-FU AUC of 24 mg•35;h/L, and the incidence of acute toxicity (p = 0.0001). Eighteen patients with a documented response, either PR or CR, had plasma 5-FU levels reaching higher than 1,800–2,000 μg/L, which corresponds to a 5-FU AUC of 14.4–16.0 mg•35;h/L, as early as in the first or second cycle of therapy. However, patients who did not have a documented tumor response were found to have low initial 5-FU plasma levels of 600 μg/L. Of note, a close follow-up of the CEA tumor marker revealed a rise in this tumor marker as soon as the 5-FU drug levels fell below 2,000 μg/L. Based on these findings, Gamelin and colleagues proposed a 5-FU dose adjustment schema that was based on 5-FU plasma levels present in the previous course of 5-FU infusion, and this schema used a target range of 5-FU AUC values of 16–24 mg•35;h/L.

Retrospective Studies on PK-based Dose Adaptation

A 5-FU dose adjustment algorithm was proposed by Wihlm et al, which was guided by the half-cycle AUC ($AUC_{0–3\ days}$) based on toxicity data from 14 patients with head and neck cancer receiving cisplatin 100 mg/m^2 and continuous 5-day infusion of 5-FU 1,000 mg/m^2/day [24]. In a follow-up trial, 35 patients treated with the same chemotherapy underwent 5-FU dose adjustment based on this algorithm. The incidence of toxicity and tumor response were compared with a retrospectively selected cohort of 55 patients treated with the same protocol without 5-FU dose adjustment. Adjustment of the 5-FU dose based on $AUC_{0–3\ days}$ led to a significant reduction in hematologic and GI toxicity (33% *versus* 47%). Of note, 5-FU dose reduction did not have a negative impact on clinical efficacy as ORR (44% *versus* 42%) and median overall survival (OS) was 8.8 months in the control arm *versus* 14.1 months in the dose-adjusted arm.

Saam and colleagues reported on the first US experience of a PK-guided approach toward administering FOLFOX chemotherapy in the treatment of patients with early-stage and metastatic CRC using immunoassay of 5-FU level [25]. PK blood samples were collected at 2 hours after the start of the 5-FU infusion and before the end of the infusion from a total of 357 patients receiving infusional 5-FU 2,400 mg/m^2 as a part of FOLFIRI or FOLFOX6 chemotherapy for the treatment of CRC. A wide range of 5-FU AUC from 1 to 69 mg•35;h/L was observed with the PK samples from the first cycle, with a mean AUC of 20.4 mg•35;h/L. Only 21% of patients had plasma 5-FU concentrations within the 5-FU AUC

target range of 20–24 mg•35;h/L. Specifically, 51% of patients had AUC levels that were below 20 mg•35;h/L, and 27.7% of patients had AUC levels that were above 24 mg•35;h/L. Only 11.1% of patients receiving FOLFOX4 regimen with 5-FU dose at 1,200 mg/m^2 had 5-FU AUC levels within the target range of 20–24 mg•35;h/L, and more than 86% of patients were below the target range. The longitudinal distribution of 5-FU AUCs over 4 consecutive cycles was analyzed in a cohort of 64 patients receiving combination chemotherapy with continuous infusion of 5-FU 2,100 mg/m^2 over a period of 44–48 hours. Approximately 50% of patients were found to be wide outliers with the first PK sample, while only 14.5% of patients were wide outliers by the fourth cycle.

Kline et al reported on a small cohort of patients with CRC receiving PK-guided infusional 5-FU containing combination chemotherapy and 5-FU levels were measured with the 5-FU immunoassay [26]. A total of 21 CRC patients (9 with stage IV disease; 12 with stage III disease) as part of routine clinical care had 5-FU PK testing. For this study, the investigators selected a target 5-FU AUC value of 20–24 mg•35;h/L. Patients received several different infusional 5-FU combination regimens, including FOLFOX6/bevacizumab (N = 8), FOLFOX6 (N = 11), FOLFIRI (N = 1), and FOLFOX4 (N = 1). Only 4 patients achieved the target 5-FU AUC range without any subsequent dose titration. Approximately two-thirds of patients achieved therapeutic 5-FU AUC values with 1–2 dose adjustments while one-third of patients required > 3–4 dose increases. The observed heterogeneous patterns of 5-FU AUC values in cycle 1 and during dose optimization of 5-FU levels suggest significant variations in 5-FU metabolism among patients receiving infusional 5-FU containing combination chemotherapy.

Kaldate and colleagues conducted a retrospective analysis of the PK data from 589 CRC patients receiving FOLFOX6 chemotherapy, by utilizing an immunoassay [27]. Patients received infusional 5-FU at a dose of 1,600–3,600 mg/m^2 in cycle 1, and had at least two PK samples drawn on consecutive cycles. Regression modeling analysis of paired PK data in 307 patients showed a significant correlation between 5-FU AUC and change in the amount of 5-FU dose with $r^2 = 0.51$: change in AUC (mg•35;h/L) = 0.02063* dose change of 5-FU (mg/m^2). This model suggests that dose adjustments of 5-FU in the range of 145–727 mg/m^2 on subsequent cycles would be sufficient to bring 5-FU AUC to the target AUC range of 20–30 mg•35;h/L. Based on their analysis, a modified dose adjustment algorithm was proposed with a suggested optimal AUC range of 20–30 mg•35;h/L, which was broader than the AUC range of 20–24 mg•35;h/L previously developed by Gamelin [23, 28]. The target range of 5-FU AUC 20–24 mg•35;h/L was found to be too narrow for everyday clinical practice, and with this in mind, the upper limit of the target AUC range was increased to 30 mg•35;h/L. This modified target drug range is considered to be sufficiently large to realistically accommodate the intrapatient 5-FU PK variability, and is now considered the currently accepted 5-FU AUC target range.

Kline and colleagues retrospectively evaluated the effect of PK-guided dose adjustment of 5-FU in patients who received combination chemotherapy containing continuous infusion of 5-FU (mFOLFOX6 or mFOLFIRI) [29]. The original analysis included a total of 84 patients, of which 35 patients received adjuvant chemotherapy for stage II/III disease, while 49 patients had mCRC. Among the 84 patients, 46 patients received infusional 5-FU dosed by

the BSA method (BSA arm), and 38 patients received 5-FU dose with adjustment based on PK monitoring (PK arm). For patients in the PK arm, the first 5-FU dose was determined using the traditional BSA method, and the 5-FU dose on subsequent cycles was adjusted according to the results of the PK analysis, with a target AUC of 20–24 mg•35;h/L. Blood samples were collected at the 26th hour after the start of infusional 5-FU, and 5-FU plasma levels were measured by a 5-FU immunoassay test. All patients received 5-FU doses of 2,400 mg/m^2 in the first cycle of treatment. The median PFS of patients with mCRC was 14 months in the PK arm *versus* 10 months in the BSA arm (p = 0.16). In a subgroup of patients receiving adjuvant chemotherapy, there was a significant improvement in disease-free survival (p = 0.0429) and a significantly lower incidence of serious toxicities (32% *versus* 69%; p = 0.0437) in the PK arm in comparison to the BSA arm. In patients receiving adjuvant chemotherapy, PK-guided 5-FU dose adjustment significantly delayed the onset of grade 3/4 toxicities. Patients in the BSA arm experienced severe toxicities after receiving 2 doses of 5-FU, but those in the PK arm experienced toxicities after the sixth or seventh doses of 5-FU (p = 0.0144). Although this was a retrospective analysis in a small number of patients receiving adjuvant chemotherapy, this result warrants further examination of the role of 5-FU TDM in the adjuvant treatment of patients with early-stage CRC.

Braiteh and colleagues reported on their experience with PK-guided 5-FU dose adjustment in patients with CRC receiving chemotherapy in the adjuvant or metastatic settings [30]. This analysis was based on 1,000 PK samples collected from 380 patients with CRC at 24 ± 6 hours after the start of infusional 5-FU. The majority of AUC values (62%) in the first cycle of therapy were outside the target 5-FU AUC range of 20–30 mg•35;h/L irrespective of 5-FU dose or chemotherapy regimen, where 50% of patients were below the target range and 12% were above the target range. For patients (N = 254) on the same 5-FU dose based on BSA (2,400 mg/m^2), the plasma 5-FU AUC variability was greater than 12-fold. Four hundred and seventy-three patients had two consecutive cycles with AUC results (evaluable cycle pairs), and 67% of the initial AUC values from these evaluable cycle pairs were outside the target range. Based on the 5-FU dose adjustment algorithm that had been developed by Kaldate et al, the 5-FU doses were decreased in 43% (N = 28) of the 65 patients whose 5-FU AUC values were above target range, but 57% of these patients did not receive 5-FU dose reduction. 5-FU doses were increased in 52% (N = 130) of the 230 patients whose initial 5-FU AUC values were below the target range, but 48% of these patients did not receive 5-FU dose increase. In 158 evaluable cycle pairs where the initial AUC was out of target range and 5-FU dose adjustment was made, 52% moved into the target 5-FU AUC range in the following cycle as a result of the 5-FU dose adjustment. Consistent with earlier studies, some patients required 3–4 cycles of dose adjustment to reach the target 5-FU AUC range.

Prospective PK-based Dose Adjustment with Clinical Evaluation

Santini et al determined the 5-FU AUC in 170 patients with squamous cell carcinoma of head and neck receiving first-line chemotherapy with cisplatin and continuous 5-day infusion of 5-FU [31]. In 81 patients (arm 2), 5-FU AUC during the first 3 days ($AUC_{0–3\ days}$) was analyzed in real-time to decide whether to reduce the dose of 5-FU during the second half of the cycle. The dose in the second half of the course was reduced in

40% of patients treated on arm 2. There was a statistically significant improvement in overall response rates (ORR) (47% *versus* 31%; $p < 0.05$) in arm 2 relative to arm 1. Moreover, a statistically significant reduction in the incidence of toxicity > grade 2 (12.4% *versus* 20%; $p < 0.05$) was observed. This study showed that a PK-based approach could effectively enhance the clinical efficacy of 5-FU-based chemotherapy and reduce toxicities.

In a phase II study, Gamelin and colleagues investigated the use of 5-FU dose adjustment based on plasma drug levels in 152 patients with mCRC [28]. Patients received weekly 8-hour infusion of 5-FU 1,300 mg/m^2 at the initial dose with LV 400 mg/m^2, and 5-FU dose was adjusted weekly according to 5-FU plasma levels with the goal of reaching an optimal therapeutic 5-FU plasma range of 2,000 to 3,000 μg/L. The dose of 5-FU required to achieve the optimal therapeutic 5-FU plasma range differed greatly in the individual patients with the mean dose of 5-FU after 3 months of treatment being 1,803 mg/m^2 (range, 950 to 3,695 mg/m^2). The initial 5-FU dose of 1,300 mg/m^2 was the appropriate dose for only 6 patients among the entire group of 152 patients. The 5-FU dose was increased in 124 patients, and the incremental increase was at least 50% in 58 patients. In 14 patients, the initial 5-FU dose of 1,300 mg /m^2 was associated with 5-FU plasma levels greater than 3,000 μg/L, which then required an immediate reduction in the 5-FU dose to avoid acute toxicities. 5-FU drug levels greater than 3,000 μg/L were significantly associated with increased acute toxicity ($p = 0.0001$) as manifested mainly by diarrhea (39% all grade, and 5% grade 3) and hand-foot syndrome (30% all grade, and 2% grade 3). The ORR in the entire patient population was 43.4%. The median OS duration was 19 months, and the median recurrence-free survival was 11 months.

A similar clinical study was performed in head and neck cancer by Fety and colleagues who conducted a randomized multi-center study to evaluate the clinical significance of PK-guided 5-FU dose adjustment in head and neck cancer patients receiving cisplatin 100 mg/m^2 plus continuous 96-hour infusion of 5-FU 1,000 mg/m^2/day [32]. Patients were randomized to either standard dose of 5-FU (BSA arm) or 5-FU dose adjustment according to 5-FU $AUC_{0–48\ hours}$ (PK arm). In total, 106 patients were evaluable for toxicity and response. In the PK arm (N = 49), 5-FU doses and AUC were significantly reduced during cycle 2 and cycle 3 ($p < 0.001$) as compared with the BSA arm (N = 57). The incidence of grade 3/4 neutropenia and thrombocytopenia was significantly higher in patients treated on the BSA arm when compared to those treated on the PK arm (17.5% *versus* 7.6%; $p = 0.013$). Grade 3/4 mucositis was not observed in any patient treated on the PK arm, while in contrast, a 5.1% incidence of grade 3/4 mucositis was observed in the BSA arm ($p < 0.01$). ORR was comparable in the two treatment arms: 77.2% in the BSA arm *versus* 81.7% in the PK arm. This study was the first randomized trial to demonstrate the potential clinical benefit of PK-guided dosing of 5-FU.

In a phase II study, Ychou and colleagues demonstrated the feasibility of PK-guided 5-FU dose adjustment using the infusional LV5FU2 regimen in mCRC patients [33]. In cycle 1, patients received the standard LV5FU2 regimen (LV 200 mg/m^2/day followed by a bolus of 5-FU 400 mg/m^2/day and continuous 22-hour infusion of 5-FU 600 mg/m^2/day for 2 consecutive days, every 2 weeks), and 5-FU AUC values were determined. If no grade 3/4 toxicity was observed, the dose of infusional 5-FU in cycle 2 was increased according to a

pre-defined dose escalation algorithm based on 5-FU AUC in cycle 1. Among the 53 eligible patients, 46 patients received an increased dose of 5-FU in cycle 2. PK data were available for 46 patients in both cycles 1 and 2. Mean 5-FU AUC values for cycle 1 were significantly lower than those for cycles 2 (9.1 *versus* 14.7 mg•35;h/L·m^2; $p < 0.0001$). ORR was 47% in the first-line per-protocol population (N = 34). Progression-free survival (PFS) and OS in first-line per-protocol patients (N = 34) were 9.2 and 20 months, respectively. Of the 53 patients, 19% experienced 3/4 gastrointestinal toxicities and 30% hematologic grade 3/4 toxicities. This study showed for the first time that the dose of infusional 5-FU could be adjusted using a predefined algorithm based on 5-FU AUC in cycle 1.

Gamelin and colleagues conducted the first large, prospective, multicenter, phase III randomized trial in patients with mCRC receiving a weekly 8-hour infusion of 5-FU at a dose of 1,500 mg/m^2 [34]. Patients with mCRC were randomized to a control arm where they received a fixed dose of 5-FU based on BSA (BSA arm; N = 104) or to the experimental arm where they received the first dose of 5-FU based on BSA and all subsequent doses with PK-guided dose adjustment (PK arm; N = 104). In the PK arm, 5-FU doses were adjusted on a weekly basis, and the dose of 5-FU subsequently administered was based on a single-point measurement of 5-FU C_{SS} by HPLC analysis until the plasma 5-FU concentration reached 2,500 to 3,000 μg/L, which corresponds to AUC 20–24 mg•35;h/L. In the PK arm, the 5-FU AUC target concentration was achieved in 94% of patients within a mean of 4 cycles (range, 1–10 cycles). In patients randomized to the PK arm, the mean 5-FU dose after 3 months of treatment was 1,790 mg/m^2/week (range, 765 to 3,300 mg/m^2/week). In the BSA arm, a dose of 1,500 mg/m^2 was administered to all patients. Of note, plasma 5-FU levels were in the target therapeutic range in only 4 of 49 patients. Treatment in the BSA arm resulted in a reduction in 5-FU dose in 24 patients, delay in subsequent treatment in 30 patients (for a mean of 8 days, and a maximum of 1 month), and discontinuation in 1 patient. Five patients were found to be in the toxic range after the first dose, which mandated an immediate and marked reduction of the 5-FU dose to less than 1,000 mg/m^2 with the subsequent cycles of therapy. With no adjustment in 5-FU dose, these patients would have been at increased risk of toxicity.

The primary endpoint of this study was tumor response, and a significant correlation was observed between 5-FU plasma levels and ORR ($p = 0.004$). A significantly higher ORR was observed in the PK arm when compared to the BSA arm (ORR, 33.6% *versus* 18.3%; $p < 0.0004$). The survival rate after 1 year was 59.5% in the BSA arm and 70.5% in the PK arm. After 2 years, the survival rate was 29.6% in the BSA arm and 40.5% in the PK arm ($p = 0.08$). Median OS was 16 months in the BSA arm and 22 months in the PK arm. Although this difference of 6 months was not statistically significant, this study was not sufficiently powered to detect a difference in OS. Moreover, as this was a first-line study, the subsequent second-line and salvage treatments would presumably have been the same in both arms, which would then dilute out the potential beneficial effect of PK-guided dosing. With respect to toxicity, diarrhea and hand-foot syndrome were the two most frequent side effects experienced in this study, and they mainly occurred during the first 3 months of treatment. Toxicities were significantly more frequent and severe in patients treated on the BSA arm than in the PK arm ($p = 0.003$). Plasma 5-FU levels between 2,500 to 3,000 μg/L were correlated with the occurrence of grade 1/2 diarrhea and grade 1 hand-foot syndrome, while

a significant correlation was observed between plasma 5-FU levels > 3,000 μg/L and grade 3 diarrhea and hand-foot syndrome (p = 0.02). This study is important as it demonstrated for the first time that PK-guided 5-FU dose adjustment is feasible in clinical practice on an individual basis for the treatment of mCRC patients. The measurement of plasma 5-FU AUC values appears to be the optimal measure for ensuring appropriate dose-intensity for improved outcomes while minimizing toxicity, and this approach can be done more effectively and safely than by dose adjustment based on purely clinical evaluation.

Capitain and colleagues performed a prospective phase II study to examine the value of PK-guided 5-FU dose adjustment on the efficacy and tolerance of FOLFOX6 chemotherapy (oxaliplatin 85 mg/m^2, LV 200 mg/m^2, 5-FU 400 mg/m^2 IV bolus injection followed by continuous 46-hour IV infusion of 5-FU at the initial dose of 2,500 mg/m^2) in the first-line treatment of patients with mCRC [35]. A total of 118 patients with mCRC received first-line FOLFOX chemotherapy with 5-FU dose adjustment based on PK data. As a control population, a total of 39 patients received the same FOLFOX6 chemotherapy with 5-FU dosed being administered according to BSA. The 5-FU dose administered in the first cycle was 2,500 mg/m^2 in both patient groups, which was then tailored according to PK monitoring with target 5-FU plasma concentrations in the range of 2,500 to 3,000 μg/L, which corresponds to AUC 20–24 mg•35;h/L. In patients treated with PK-guided dosing, the actual 5-FU dosage at 3 months of treatment was 110.47% of the theoretic dosage (range, 60 to 140%). At 3 months, the 5-FU dose was increased by at least 10% in 64% of patients, with a mean increase of 20% (range, 10 to 40%). In 36% of the patients in the PK arm, the 5-FU dose increase was greater than 20%, with a mean increase of 26% (range, 20 to 40%). In 19% of patients in the PK arm, the 5-FU dosage was decreased by at least 10%. The incidence of grade 3/4 toxicity was generally lower in patients treated with PK-guided dosing when compared to patients treated with BSA dosing: diarrhea, 1.7% *versus* 12%; mucositis, 0.8% *versus* 15%; neutropenia 18% *versus* s 25%; and thrombocytopenia 12% *versus* 10%. ORR and median OS was higher with PK dosing when compared to BSA dosing: ORR at 3 months, 69.7% in the PK arm *versus* 46% in the BSA arm; mOS, 28 months *versus* 22 months; and mPFS, 16 months *versus* 10 months, respectively. Although this was not a randomized study to directly compare PK dosing with traditional BSA dosing of 5-FU, this study is important as it was the first to employ a PK-based approach to dose 5-FU within the context of a more modern FOLFOX chemotherapy regimen. This study also provides further support to the concept that PK-guided dosing of 5-FU results in improved clinical efficacy and reduced toxicity when compared to BSA dosing.

Patel et al. from UNC-Chapel Hill conducted an observational study of PK-guided dosing of 5-FU using an immunoassay [36, 37]. Seventy patients with mCRC received mFOLFOX6 with a 5-FU starting dose of 2,400 mg/m^2, with or without the addition of bevacizumab. Plasma 5-FU AUC values were determined from blood samples obtained at 2–44 hours after start of the 5-FU infusion. 5-FU doses in cycles 2–4 were adjusted based on the 5-FU AUC values obtained from cycle 1, and a 5-FU AUC target range of 20–25 mg•35;h/L was used as the basis for dose adjustment. The primary endpoint of this study was the percentage of patients reaching the target 5-FU AUC 20–25 mg•35;h/L by cycle 4, and the secondary endpoint was the incidence of 5-FU related toxicity compared to historical control. Only 30% of patients among the entire cohort were within the target 5-FU AUC in cycle 1, and

this number increased to 46% in cycle 4 (p = 0.05). For each subsequent cycle, the odds of a patient reaching the desired 5-FU AUC range increased by 28% (p = 0.04). In all, approximately 75% of patients required 5-FU dose adjustment during the first 4 cycles of chemotherapy. The median 5-FU dose required to achieve the target 5-FU AUC in cycle 4 was 2,613 mg/m^2 (range, 1,925 to 3,484 mg/m^2). PK-guided 5-FU dose adjustment with the 5-FU immunoassay was associated with a lower incidence of grade 3/4 mucositis (3% *versus* 15%) and diarrhea (5% *versus* 12%) when compared to historical controls, while no difference in grade 3/4 neutropenia was noted (30% *versus* 33%).

The clinical feasibility of PK-guided 5-FU dose adjustment and its impact on clinical outcome was studied by clinical investigators at the University of Pittsburgh Cancer Institute [38, 39]. They used this approach to treat patients with both early-stage and metastatic disease. A total of 58 patients (15 patients with stage 3 disease and the remaining 43 patients with mCRC) were treated with mFOLFOX6 (N = 45) or FOLFIRI (N = 13) chemotherapy. With the first cycle of therapy, only 36% (N = 21) patients had 5-FU AUC values within the target range of 20–30 mg•35;h/L. Fifty-five percent of patients were below the target AUC while 9% (N = 5) patients were above the target range. Of the 32 patients below the target AUC range, 22 patients received increased doses of 5-FU with the subsequent treatment cycles. The median number of dose modifications required to reach the target 5-FU AUC values in these patients was 1 (range, 1 to 2). The majority of these patients (86%, N = 19) tolerated the increased 5-FU dose well without an increased 5-FU toxicity.

Soh et al reported on the clinical experience with PK-guided dose adjustment of 5-FU in Asian patients (N = 50) with GI cancers (CRC in 76% and gastric cancer in 12%) receiving LV5FU2 (2%), FOLFIRI (36%), or mFOLFOX6 (62%) chemotherapy [40]. Plasma 5-FU AUC values were determined using an immunoassay, and 5-FU dose adjustments were recommended using a target 5-FU AUC of 18–28 mg•35;h/L. Median AUC was 24.2 in cycle 1 (N = 35), 23.7 in cycle 2 (N = 38), 20.6 in cycle 3 (N = 38) and 22.0 mg•35;h/L in cycle 4 (N = 35). Despite adjustment of 5-FU dose based on the 5-FU AUC level measured on prior cycles, the proportion of patients achieving the target AUC did not change significantly (54% in cycle 1, 32% in cycle 2, 40% in cycle 3 and 51% in cycle 4). A higher than expected rate of grade 3/4 neutropenia (52%) and less mucositis (0%) and diarrhea (8%) were observed. All grade other toxicities were in line with what has been previously reported. No correlation was observed between grade 3/4 toxicities and high AUC values. The findings from this Asian study are in sharp contrast to all other studies published as they suggest that PK-guided 5-FU dose adjustment did not lead to a greater number of patients achieving the target 5-FU AUC. Larger numbers are certainly needed to confirm the potential clinical utility and benefit of PK-guided 5-FU dose adjustment in Asian patients.

German investigators used an immunoassay to dose adjust 5-FU in mCRC patients (N = 75) who received up to 6 cycles of infusional 5-FU according to the AIO (N = 16), FOLFOX6 (N = 26), or FUFOX (N = 33) regimens [41]. BSA-dosing was used in all patients to administer the initial dose of 5-FU, but subsequent doses were adjusted based on the 5-FU AUC values obtained from the previous cycle, using a target 5-FU AUC of 20–30 mg•35;h/L. The average 5-FU AUC for cycle 1 was 20 mg•35;h/L, with only 32% of patients within the target range. The majority of patients were not within the target AUC

range, with 62% of patients below the target range and 6% above the target range. Regardless of the chemotherapy regimen, BSA-based 5-FU dosing in cycle 1 resulted in the majority of patients being under-dosed (62%). By cycle 4, the average 5-FU AUC was 25 mg•35;h/L, and a significantly higher proportion of patients were within the target 5-FU AUC range than at cycle 1 (32% *versus* 55%; p = 0.005). A lower number of 5-FU-related grade 3/4 toxicities was observed compared to historical data: diarrhea (5% *versus* 12%), nausea (3% *versus* 9%), fatigue (0% *versus* 12%), and mucositis (0% *versus* 15%), showing that the increased average 5-FU dose intensity guided by PK monitoring did not lead to more toxicities.

Quantitation of Plasma 5-FU Levels

The challenges to 5-FU TDM begin at the pre-analytical stage to a far greater extent than for most other anticancer drugs. 5-FU is extremely unstable in whole blood and plasma at room temperature, primarily due to the ubiquitous presence of the catabolic enzyme dihydropyrimidine dehydrogenase (DPD) that rapidly degrades 5-FU to the 5-FU metabolite dihydro 5-FU. In general, blood samples need to be placed on ice immediately, and plasma has to be isolated as quickly as possible to separate plasma from cells [42]. Inappropriate handling of samples could potentially result in rapid degradation of 5-FU, which would result in an underestimation of drug concentrations. This could then lead to subsequent overdosing of patients in the event that 5-FU blood levels are used to determine the next dose of 5-FU. Alternative approaches to ensure 5-FU stability include the addition of DPD inhibitors to the tube used for collecting blood.

Several analytical techniques have been developed for quantitating 5-FU and related compounds [43]. In the 1960’s, cell-based culture assays were developed to measure 5-FU levels, and these assays were subsequently replaced with gas chromatography in the mid-1970’s. Since the 1970’s, chromatographic separation of 5-FU has traditionally been performed using high performance liquid chromatography (HPLC). A number of different HPLC methods were developed, and they include reversed-phase, reversed-phase ion-pairing, and normal-phase chromatography. In general, the limits of detection for these various HPLC methods are in the range of 25–150 ng/mL (0.19–1.15 μM). Gas chromatography-mass spectrometry (GC-MS) has been another commonly used analytical method to measure 5-FU levels. The potential advantage of mass spectrometry is that it provides significantly greater sensitivity with limits of detection down to 0.5 ng/mL (3.85 nM) when compared to HPLC and requires a 1-mL plasma sample. A more sophisticated LC-MS/MS assay has been validated to FDA guidance, and the potential advantage of this methodology is that it requires only 100 μL for a lower limit of detection of 10 ng/mL (0.08 μM).

A nanoparticle immunoassay for quantitating 5-FU in human plasma has been developed based on a turbidimetric method and cross-validated against an LC-MS/MS assay [44]. This immunoassay was found to be precise, linear, and sensitive. A comparison was made between the immunoassay and a standard LC-MS/MS assay using 156 human plasma samples from patients receiving 5-FU based chemotherapy regimens, including 5-FU/LV, FOLFOX, and FOLFIRI. The 5-FU concentration range for the reference LS-MS/MS assay

was 79–1791 ng/mL (0.61–13.78 μM) with a mean of 410 ng/mL (3.15 μM), while the range obtained for the immunoassay was 93–1774 ng/mL (0.72–13.65 μM) with a mean of 389 ng/mL (2.99 μM). A remarkably high correlation coefficient of 0.986 was observed between the two different assays.

There are several potential advantages of the immunoassay over traditional HPLC and/or LC-MS assays, and these include (1) shorter time-to-result for clinical samples; (2) smaller amount of sample size required; and (3) automated quantitation using a well-established, validated clinical chemistry analyzer that can allow for testing of a large number of samples at a given time. Conventional chromatographic methods require sophisticated instrumentation as well as a higher degree of training of staff personnel to operate the machinery. The turnaround time for HPLC and/or LC-MS/MS methods is generally longer than the immunoassay given the increased need for sample preparation steps and the increased time to analyze samples and all calibrators before the sample result can be finalized. Historically, one of the potential weaknesses of immunoassays has been cross-reactivity of the antibody to analytes structurally related to the analyte of interest. With respect to the 5-FU immunoassay that is presently being used, this does not appear to be an issue as the cross-reactivity for dihydro-5-FU, uracil, capecitabine, and tegafur were determined to be <1%, 9.9%, 0.05%, and 0.23%, respectively [44].

5-FU TDM with Capecitabine

Capecitabine is an orally administered 5-FU prodrug that is inactive in its parent form and undergoes 3 different enzymatic steps for eventual conversion to 5-FU. The first 2 steps take place primarily in the liver, while the 3rd and final step is catalyzed by thymidine phosphorylase (TP) and uridine phosphorylase (UP) [45–48], and this occurs in both normal and tumor tissue. It has been shown that both TP and UP are expressed at higher levels within tumor tissue when compared to normal tissue, thereby leading to preferential conversion to 5-FU in tumor tissue with decreased systemic 5-FU exposure [49]. As a result, 5-FU concentrations in plasma after administration of capecitabine are an indirect reflection of the true exposure of tissues to 5-FU. Moreover, systemic exposure to capecitabine and capecitabine metabolites in plasma appears to be poorly predictive of safety and efficacy.

Over the past 10 years, there has been an increasing use of capecitabine monotherapy and capecitabine-based combination regimens in the treatment of mCRC, which has been due, in large part, to the convenience and increased patient preferences for oral chemotherapy [50–54]. Randomized studies have shown that capecitabine-based treatments are associated with equal clinical efficacy and reduced toxicities when compared to infusional 5-FU regimens [50–54]. Finally, several pharmacoeconomic studies have demonstrated that capecitabine-based chemotherapy is associated with significant cost savings when compared to infusional 5-FU chemotherapy, and these economic benefits are seen for the adjuvant therapy setting as well as for the treatment of metastatic disease [55–59].

Gieschke et al analyzed the potential relationship between systemic exposure to capecitabine metabolites and efficacy and safety in mCRC patients (N = 481) from two randomized phase 3 studies [60]. Systemic exposure based on plasma concentrations of capecitabine and its

metabolites including 5'-DFUR, 5-FU, and α-fluoro-β-alanine (FBAL) was determined at the time windows of 0.5–1.5 hours, 1.5–3.0 hours, and 3.0–5.0 hours after capecitabine administration (1,250 mg/m^2) on the first day of cycles 2 and 4, respectively. A positive association was observed between FBAL AUC and grade 3/4 diarrhea ($p = 0.035$) and between 5'-DFUR Cmax and survival (HR = 0.938; $p = 0.0048$). However, the association between FBAL AUC and grade 3/4 diarrhea was not consistent when analyzing the two phase III studies separately, raising the possibility that this association may not be clinically significant [60]. FBAL is one of the main metabolite of 5-FU. As a result, it is conceivable that the FBAL AUC might simply reflect the extent of systemic 5-FU exposure [60]. There was broad overlap in systemic drug exposure between patients regardless of the occurrence of treatment-related grade 3/4 adverse events or response to treatment, leading to weak relationships between systemic exposure to capecitabine metabolites and safety and efficacy parameters.

Since the publication of the Gieschke study, additional efforts have focused on developing improved assay methods to measure capecitabine and its metabolites. A rapid and selective liquid chromatography/tandem mass spectrometric method (HPLC-MS/MS) was developed by Vainchtein et al for the simultaneous determination of capecitabine and its metabolites 5'-deoxy-5-fluorocytidine (5'-DFCR), 5'-deoxy-5-fluorouridine (5-DFUR), 5-FU, and dihydro-5-FU (FUH_2) in human plasma. Using a plasma sample of 200 μL, the assay was able to quantify a range from 10–1000 ng/mL for capecitabine, from 10–5000 ng/mL for 5'-DFCR and 5'-DFUR, and from 50–5000 ng/mL for 5-FU and FUH_2 [61]. This Dutch group then updated the HPLC-MS/MS method to rapidly, accurately, and precisely quantitate capecitabine and its metabolites in human plasma, and this assay is now being used to support clinical studies with capecitabine or 5-FU [61].

Nakamura et al evaluated capecitabine PK and PD of capecitabine in 34 mCRC patients [62]. Capecitabine was administered at a dose of 2,000 mg/m^2/day from day 1 to day 14 twice a day every 3 weeks with or without oxaliplatin and bevacizumab. Blood samples were collected at 1 hour and 2 hours after capecitabine administration on cycle 1 day 8. Plasma 5-FU concentrations were measured using an immunoassay, and the concentrations of 5'-DFCR, 5'-DFUR, and parent capecitabine were measured using LC-MS. Wide variations were observed in plasma 5-FU, 5'-DFCR, 5'-DFUR, and parent capecitabine. A correlation was observed between parent capecitabine and the AUC of their metabolites, 5'-DFCR and 5'-DFUR. The strongest correlation was found to exist between 5-FU and 5'-DFUR (Spearmann correlation index: 0.82). There was no correlation between hand-foot syndrome and the concentration of parent capecitabine or the AUC of the metabolites. However, a significant correlation was observed between 5-FU AUC and GI toxicity (Wilcox $p = 0.0205$). The plasma concentration of 5-FU measured by the 5-FU immunoassay predicts the GI toxicity by receiver operating characteristic (ROC) curve (sensitivity = 0.80; specificity = 0.70).

Investigators at the University of Pittsburgh Cancer Institute and the Asan Medical Center in South Korea are currently investigating the potential relationship between systemic exposure to capecitabine metabolites and parameters of efficacy and safety in patients with early-stage gastric cancer enrolled in a phase 3 adjuvant capecitabine trial (Title: Adjuvant Capecitabine

Versus Observation Alone in Curatively Resected Stage IB Gastric Cancer [NCT01917552]). Blood samples are being collected from patients enrolled in this study on cycle 1 day 1 before the first dose of capecitabine as well as at 2 and 4 hours after the first dosing, respectively. 5-FU blood levels will be analyzed using an immunoassay. Systemic exposure to capecitabine metabolites will then be correlated with parameters of efficacy and safety.

Dihydropyrimidine Dehydrogenase (DPD)

Dihydropyrimidine dehydrogenase (DPD) is the key enzyme involved in the metabolic degradation of 5-FU, and up to 80–90% of an administered dose of 5-FU is degraded to the dihydro 5-FU metabolite (FUH_2). The presence of DPD deficiency results in a reduced ability to metabolize and clear 5-FU, and the half-life of the drug, which is normally in the range of 10–15 minutes, can be markedly prolonged [63–71]. A pharmacogenetic autosomal recessive syndrome has been identified in which partial and/or compete deficiency in the DPD enzyme has been observed in 3–5% and 0.1% of the general population, respectively [67, 68, 72–75]. In this setting, patients experience excessive, severe toxicity in the form of myelosuppression, diarrhea and mucositis, and neurotoxicity. To date, more than 30 sequence variations in the DPD gene have been identified, with the most well-established variant being DPD*2A (c.1905+1G>A; IVS14+11G.A;rs3918290). This is a single-nucleotide variant at the intron boundary of exon 14 that results in a splicing defect, skipping of the entire exon, and a completely inactive protein. The other *DPD* variants that are associated with reduced DPD enzyme activity and increased 5-FU toxicity include *DPD**5, *DPD**6, *DPD**9A, *DPD**13 (c.1679T>G; I560S; rs55886062), c.2846A>T (D949V; rs67376798), c.1236G>A (E412E; rs56038477) [76, 77].

DPD gene variants can present in heterozygous form, homozygous form, or double heterozygous form, which is also known as compound heterozygous. Capitain and colleagues analyzed the potential effect of genetic factors on 5-FU metabolism and the correlation with tolerance and efficacy in 76 patients with mCRC receiving PK-monitored infusional 5-FU chemotherapy [78]. In this patient cohort, 3.9% (3 patients) were heterozygous for *DPD* variants. Nine patients (11.8%) presented with abnormally low 5-FU clearance levels, 6 patients displayed heterozygous single-nucleotide polymorphisms (SNPs) of 2846 A>T, one for G>A SNP in the GT 5’-splice recognition site of intron 14 (IVS 14+1G>A), and 6 patients did not have any evidence of DPD SNPs. Despite PK-guided dose adjustment of 5-FU, a high level of early adverse events was observed in the 9 patients with low 5-FU clearance, with 33.3% grade 3/4 GI toxicity (and up to 66% for the three patients with DPD heterozygote). In patients with known DPD deficiency, very early toxicity of 5-FU could be avoided by using a reduced dose of 5-FU at the first cycle followed by 5-FU dose adjustment on subsequent cycles based on the PK data.

Genotype-directed dosing of the fluoropyrimidines has been shown to be a feasible strategy to increase safety for patients with the *DPD**2A variant [79]. To provide further support for this approach, Henricks et al developed a gene activity score to account for the different DPD enzyme activities of the main *DPD* variants and to translate these various genotypes into phenotype [77]. In particular, this tool allowed for a more precise differentiation

between the various *DPD* variants and their functionality. Four well-characterized variants were investigated, *DPD**2A, c.2846A>T, *DPD**13, and c.126G>A/HapB3, as they were previously found to be associated with low DPD enzyme activity and severe 5-FU toxicity. Based on the gene activity score, a schema for individualized dose adjustments in 5-FU has been developed.

Deenen et al. recently reported on 2,038 patients who were prospectively screened for DPD*2A variant, of whom 22 were found to be heterozygous [80]. The most common tumor type in this study was colorectal cancer, and 90% of patients were treated with the oral fluoropyrimidine capecitabine while the remaining 10% of patients were treated with intravenous 5-FU. These variant allele patients received an initial dose reduction of 50% followed by dose titration based on tolerance. The risk of >grade 3 toxicity was reduced significantly from 73% in historical controls to 28% by genotype-directed dosing. Moreover, the toxicity observed with genotype-guided dosing was relatively short in duration and well-controlled with standard supportive care measures. A base-case cost analysis was performed, which showed that the cost per patient in the screening strategy was $3,767 compared to $3,828 in the non-screening strategy, which results in a cost savings of $61 per patient. The importance of this work is that it is the first prospective study to document the safety, PK, and costs of *DPD**2A genotype-guided dosing in fluoropyrimidine-based chemotherapy. Moreover, this study highlights the feasibility of upfront genotyping of the *DPD**2A variant in daily practice to individualize fluoropyrimidine chemotherapy.

It is now well-appreciated that DPD mutations do not account for all of the observed cases of DPD deficiency. In fact, up to 50% of patients who experience 5-FU toxicity will have no documented alterations in the *DPD* gene. Moreover, individuals with normal DPD enzyme activity may be found to have high plasma levels of 5-FU and exhibit increased toxicity to 5-FU therapy. These findings suggest that there must be factors other than DPD status that contribute to 5-FU metabolism and eventual 5-FU toxicity. It has been suggested that fewer than 50% of individuals who experience grade 3–4 toxicity have mutations in the DPD gene and/or have diminished DPD activity that would identify individuals with low 5-FU clearance [68, 72, 74]. It is now known that 5-FU clearance is a function of several factors in addition to DPD. A recent report by Bocci et al described using a test dose of 5-FU to identify individuals at risk for developing toxic effects. This study identified 3 of 188 patients tested who displayed low drug clearance, and all three individuals exhibited normal DPD activity [75]. These patients would have been missed with DPD genomic analysis and/or assessment of DPD enzymatic activity, highlighting the importance of monitoring a more clinically relevant phenotype, such as 5-FU drug level.

Dihydrouracil/Uracil Ratio and Uracil Breath Test

Routine determination of DPD enzymatic activity in peripheral blood mononuclear cells is limited by time-consuming and labor-intensive methods. Moreover, the methods are not readily available in most outpatient treatment facilities, making their use in everyday clinical practice not feasible.

With this in mind, Gamelin et al established a liquid chromatography method to simultaneously measure plasma pyrimidines and their dehydrogenated metabolites, with a specific focus on uracil (U) and dihydrouracil (UH_2) [5]. They were able to determine the UH_2/U ratio in plasma and then used this ratio as an indicator of the potential risk of DPD deficiency. In the case of DPD deficiency, the ratio would be predicted to be low, with a large increase in U and a reduction in the uracil metabolite UH_2. They studied 81 patients with mCRC who were treated with a weekly 5-FU schedule of 1,300 mg/m^2 administered over an 8-hour period, and they determined that the UH_2/U ratios were normally distributed with a mean value of 2.82, and they were highly correlated to 5-FU plasma levels after the first course of therapy, 5-FU plasma clearance, and individual optimal therapeutic 5-FU dose. No side effects were observed in patients with UH_2/U ratios of >2.25. In contrast, increased toxicity was observed only in patients with initial UH_2/U ratios <1.8. This study is important as it was the first to suggest the use of the UH_2/U ratio to identify patients with DPD deficiency and those at increased risk for developing toxicity. In addition, based on the initial UH_2/U plasma ratios, an individual 5-FU dose adjustment algorithm was developed.

In contrast to this study is recent work conducted by investigators from Switzerland who found that the baseline UH_2/U plasma ratio was a relatively poor predictor of reduced DPD activity and associated 5-FU toxicity [81]. This group determined that the UH_2/U ratio was a reflection of first-order DPD kinetics where the enzyme was not saturated, which then explains their finding of overlap between ratios of carriers of *DPD* risk variants and non-carriers. However, a much stronger correlation was observed between UH_2/U ratio and 5-FU AUC exposure in the presence of 5-FU when compared at baseline. This group concluded that the UH_2/U plasma ratio may better predict DPD activity at increased substrate concentrations. Moreover, they suggested that a loading test approach with uracil, the normal DPD substrate, should be considered at some reasonable time frame prior to PK blood draws to then determine a UH_2/U ratio that would more accurately reflect systemic DPD activity and identify patients at high risk of 5-FU toxicity. With this in mind, van Staveren et al developed an oral uracil loading test to identify patients with reduced DPD activity [82]. In this study, DPD enzyme activity in PBMCs was determined, and a total of 47 patients were then divided into 2 groups, normal (N=28) and deficient (N=19), based on the enzyme activity results. Uracil was administered orally at a dose of 500 mg/m^2 to all patients, and blood samples were then taken using either a full sampling scheme or a limited sampling strategy to determine the concentrations of U and UH_2 and then calculate the U/UH_2 ratio. This study identified a sensitivity and specificity of 80% and 98%, respectively, for the U/UH_2 ratio at the 2-hour time interval. This study is important as it provides evidence that the oral uracil loading test can effectively identify patients with DPD deficiency. As the U/UH_2 ratio is a relatively easy biomarker to calculate, this oral uracil loading test with a limited sampling strategy could be implemented in daily clinical practice.

To discriminate between normal individuals and those with either partial or severe DPD deficiency, a relatively simple, non-invasive uracil breath test (UraBT) was developed by Diasio and colleagues [83–85]. This approach takes advantage of the fact that defective uracil catabolism is present in individuals with partial or severe DPD deficiency. Thus, patients with partial or severe DPD deficiency would be expected to expire reduced levels of $^{13}CO_2$ in their breath following ingestion of an aqueous solution of 2-^{13}C-uracil when

compared to normal individuals. It has now been well-demonstrated that the UraBT can be effectively used as a screening test to rapidly identify DPD-deficient patients prior to the initiation of 5-FU based chemotherapy. Of note, this test has a sensitivity of 100% and a specificity of 96%. One of the limitations of this approach, however, is that it is unable to aid in the appropriate dosing of 5-FU in patients with partial or severe DPD deficiency. In addition, recent studies have suggested that the UraBT has only moderate accuracy in discriminating between patients experiencing severe toxicity from those with only mild to no toxicity to 5-FU chemotherapy.

Pharmacoeconomics of 5-FU TDM

The pharmacoeconomics of cancer therapy is emerging as an ever more relevant issue that must be considered when making specific treatment decisions. The consequences of 5-FU associated toxicities, including febrile neutropenia, nausea/vomiting, and diarrhea, can result in significant clinical and financial burden on the patient as well as on the healthcare system [13]. Dose adjustment of 5-FU based on plasma 5-FU level is feasible, and it is clear that PK-based dosing can significantly improve clinical outcomes by reducing toxicities and improving efficacy. The potential economic benefit of PK-based *versus* BSA dosing of 5-FU was investigated in patients with mCRC in the United Kingdom (UK) who were treated with various 5-FU combination regimens [86]. A decision tree model was used to perform a counterfactual simulation of the cost-effectiveness of PK *versus* BSA dosing of 5-FU in various standard chemotherapy regimens in the UK population, and all patients were assumed to have received first-line therapy for 6 or 12 cycles or until disease progression. The costs for the model were estimated from the perspective of the national health system. The ICER per incremental quality adjusted life-year (QALY) for PK *versus* BSA dosing was £3,467 for FOLFOX4, £3,594 for FOLFOX6, £3,508 for FOLFOX6 plus bevacizumab, £23,428 for FOLFIRI, £21,874 for FOLFIRI plus bevacizumab, and £28,862 for 5-FU/LV. The average ICER across all chemotherapy regimens was £7,336 per incremental QALY gained. Thus, PK dose management of 5-FU based chemotherapy regimens for mCRC patients appears to be cost-effective from a UK national payer perspective.

A similar analysis was conducted to assess the pharmacoeconomic benefit of PK-guided 5-FU dose adjustment with FOLFOX *versus* BSA-based FOLFOX in U.S. patients with mCRC [12, 13]. As part of this analysis, a Markov model was developed, and the published PFS and OS curves were fitted with Weibull models. Progression risks and cause-specific mortality were extrapolated from the fitted survival models. The costs for administration of chemotherapy and management of toxicities were estimated based on Medicare reimbursement rates for hospital and physician services, and drug costs were based on the Medicare average sale prices according to 2013 US dollars. The main cost driver for 5-FU PK-guided FOLFOX chemotherapy related to the costs of the 5-FU immunoassay ($400) that was used for 4 cycles to establish the optimal dose. 5-FU PK-guided FOLFOX provided 2.03 quality adjusted life-year (QALY) gain at a cost of $50,205 compared to BSA-guided FOLFOX with 1.46 QALY gain at a cost of $37,173. The incremental cost per QALY was $22,694/QALY. In all univariate and multivariate sensitivity analyses conducted, the incremental cost-effectiveness ratio of 5-FU PK-guided FOLFOX therapy remained below $50,000/QALY, which has generally been considered to be the threshold value in the U.S.

for cost effective treatments and/or interventions. This study provides further evidence that 5-FU PK-guided FOLFOX therapy is cost-effective for patients with mCRC. However, further studies are required to confirm the true cost effectiveness of 5-FU TDM for other 5-FU-based treatment regimens for mCRC from a U.S. payer perspective.

Conclusion

For over 50 years, 5-FU has played a pivotal role in the systemic chemotherapy of cancer patients. In particular, 5-FU has served as the backbone of current combination regimens for patients with CRC in both the adjuvant and metastatic disease settings. Given its extremely short half-life and cell cycle-specific cytotoxic effects, the administration of 5-FU has been optimized as IV continuous infusion. Over the last four decades, 5-FU TDM has made significant progress, and there is now a validated algorithm of 5-FU dose adjustment based on plasma 5-FU levels to reduce toxicity and improve efficacy of 5-FU. Several methods have been developed to directly measure 5-FU drug levels in peripheral blood, including HPLC, GC-MS, and LC-MS/MS. More recently, an immunoassay has been developed that can accurately and sensitively measure 5-FU. This test has significant logistical advantages over traditional HPLC and LC-MS/MS methods. Evidence is also emerging to show that the use of 5-FU TDM results in significant cost savings with quality adjusted life-year (QALY) gain for FOLFOX chemotherapy in patients with mCRC. Finally, in this era of precision medicine, 5-FU TDM should be considered a clinically relevant and central element of personalized medicine in the everyday care of cancer patients.

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Table 1

Studies of 5-FU Therapeutic Drug Monitoring in Cancer Patients

Author	Cancer Type/No of Patients	Method	Chemotherapy	Key Findings
Hillcoat et al[18]	GI cancers (N = 27)	HPLC	5-day continuous IV infusion of 5-FU plus CCNU	Wide interpatient variation of plasma 5-FU levels; Tumor response is related with 5-FU systemic exposure
Seitz et al[19]	GI cancer (N = 13)	HPLC	Bolus IV 5-FU	Correlation between plasma clearance of 5-FU and tumor response
Thyss et al[10]	Head & neck cancer (N = 29)	HPLC	5-day continuous IV infusion of 5-FU plus cisplatin	Close correlation between 5-FU systemic exposure and frequency of toxicity
Van Groeningen et al[20]	Advanced cancer (N = 21)	HPLC	Weekly bolus IV 5-FU	Significant correlation between 5-FU plasma AUC and the incidence of toxicities
Santini et al[31]	Head & neck cancer (N = 170)	HPLC	5-day continuous IV infusion of 5-FU plus cisplatin	5-FU dose modification based on 5-FU $AUC_{0–3\ days}$; PK-guided 5-FU dose adjustment is related with significant reduction of toxicity and improved efficacy
Yoshida et al[11]	mCRC (N = 19)	HPLC	7-day continuous IV infusion of 5-FU	Significant correlation between toxicity and C_{SS} of 5-FU, $AUC_{0–3\ days}$, and total body clearance
Trump et al[21]		HPLC	3-day continuous IV infusions of 5-FU at varying doses	Interpatient variation of 5-FU C_{SS}; Significant correlation between 5-FU C_{SS} and the frequency of toxicity
Milano et al[4]	Head & neck cancer (N = 380)	HPLC	5-day continuous IV infusion of 5-FU plus cisplatin	Wide variation in 5-FU drug clearance with 10–20 fold interpatient difference
Wihlm et al[24]	Head & neck cancer (N = 14)	HPLC	5-day continuous IV infusion of 5-FU plus cisplatin	5-FU dose adjustment based on $AUC_{0–3\ days}$ significantly reduced toxicity
Milano et al[22]	Head & neck cancer (N = 186)	HPLC	5-day continuous IV infusion of 5-FU plus cisplatin	Significant correlation between 5-FU $AUC_{0–105\ hours}$ and efficacy of chemotherapy
Gamelin et al [23]	mCRC (N = 40)	HPLC	Weekly 8-hour continuous IV HPLC infusion of 5-FU	Significant correlation between the 5-FU plasma level and toxicity; 5-FU dose adjustment algorithm according to 5-FU plasma levels at previous cycle to reach the target range of 5-FU plasma levels at 2,000–3,000 μg/L
Gamelin et al[28]	mCRC (N = 152)	HPLC	Weekly 8-hour continuous IV infusion of 5-FU; 5-FU dose adjustment based on plasma 5-FU levels	Wide interpatient difference in the 5-FU dosage necessary to achieve the optimal therapeutic 5-FU plasma range; First demonstration of 5-FU therapeutic dose adjustment based on plasma 5-FU level
Fety et al[32]	Head & neck cancer (N = 122)	HPLC	96-hour continuous IV infusion of 5-FU plus cisplatin; 5-FU dose	Significantly less toxicity with PK-guided 5-FU dose adjustment

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Author	Cancer Type/No of Patients	Method	Chemotherapy	Key Findings
			adjustment based on plasma 5-FU level	
Ychou et al[33]	mCRC (N = 53)	HPLC	Standard LV5FU2 regimen	The dose of infusional 5-FU could be adjusted using a predefined algorithm based on 5-FU AUC in cycle 1
Gamelin et al[34]	mCRC (N = 208)	HPLC	Weekly 8-hour continuous IV infusion of 5-FU	Randomized phase 3 trial; Significant correlation between 5-FU plasma levels and ORR; Significant correlation between plasma 5-FU levels > 3,000 μg/L and severe toxicities
Capitain et al[35]	mCRC (N = 118)	HPLC	FOLFOX6	PK-guided 5-FU dosing was correlated with less occurrence of grade 3/4 toxicities, and higher ORR and survival
Kline et al[26]	CRC (N = 21)	Immunoassay	FOLFOX6/bevacizumab (N = 8), FOLFOX6 (N = 11), FOLFIRI (N = 1) and FOLFOX4 (N = 1)	Wide variation of 5-FU AUC value in cycle 1 and during dose optimization of 5-FU levels
Saam et al[25]	CRC (N = 357)	Immunoassay	FOLFIRI and FOLFOX6	Wide range of 5-FU AUC values on initial PK samples; More than 86% of patients were below the target range of plasma 5-FU AUC
Kaldate et al[27]	mCRC (N = 589)	Immunoassay	FOLFOX6	New target 5-FU AUC range as 20–30 mg•h/L
Patel et al[37]	mCRC (N = 58)	Immunoassay	FOLFOX6 ± bevacizumab	Only 31% of patients were within the target 5-FU AUC range on the initial PK samples
Patel et al[36]	mCRC (N = 70)	Immunoassay	FOLFOX6 ± bevacizumab	About 75% of patients required 5-FU dose adjustment during the first 4 cycles of chemotherapy; PK-guided 5-FU dose adjustment was associated less incidence of grade 3/4 mucositis and diarrhea
Kline et al[29]	Stage 2/3 CRC (N = 35), mCRC (N = 49)	Immunoassay	mFOLFOX6 and mFOLFIRI	In the adjuvant setting, PK-guided 5-FU dose adjustment significantly improved disease-free survival and incidence of severe toxicities; PK-guided 5-FU dose adjustment significantly delayed the onset of severe toxicities
Braiteh et al[30]	CRC (N = 380)	Immunoassay		62% of AUC results in the first PK samples were outside the target 5-FU AUC range of 20–30 mg•h/L; 50% of patients were below the target range and 12% were beyond the target range; Some patients required 3–4 cycles of dose adjustment to reach the target 5-FU AUC range
Goel et al[38, 39]	Stage 3 CRC (N = 15), mCRC (= 43)	Immunoassay	mFOLFOX6 (N = 45) and FOLFIRI (N = 13)	55% of the patients were below the target 5-FU AUC on the initial PK samples; 9% of patients were above the target range; The majority of these patients tolerated 5-FU dose adjustment without worsening of 5-FU related toxicity
Soh et al[40]	GI cancers (N = 50; CRC in 76% and gastric cancer in 12%)	Immunoassay	de Gramont (2%), FOLFIRI (36%) or mFOLFOX6 (62%)	In Asian patients with GI cancer, PK-guided 5-FU dose adjustment did not achieve the target 5-FU AUC

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Author	Cancer Type/No of Patients	Method	Chemotherapy	Key Findings
Kunzmann et al[41]	mCRC (N = 75)	Immunoassay	AIO (N = 16), FOLFOX6 (N = 26) or FUFOX (N = 33)	In German patients with mCRC; 62% of patients had below the target 5-FU AUC range in the initial PK samples; By cycle 4, significantly higher proportion of patients were within the target 5-FU AUC range than at cycle 1; With PK-guided 5-FU dose adjustment, fewer 5-FU-related grade 3/4 toxicities