Pharmacokinetically Guided Dose Adjustment of 5-Fluorouracil: A Rational Approach to Improving Therapeutic Outcomes

M. Wasif Saif, Adrienne Choma, Salvatore J. Salamone, Edward Chu

Chemotherapy dosing of the fluoropyrimidine 5-fluorouracil (5-FU) is currently based on body surface area. However, body surface area–based dosing has been associated with clinically significant pharmacokinetic variability, and as such, dosing based on body surface area may be of limited use. The clinical activity of 5-FU is modest at standard doses, and in general, dosing is limited by the safety profile, with myelosuppression and gastrointestinal toxicity being the most commonly observed side effects. Various strategies have been developed to enhance the clinical activity of 5-FU, such as biochemical modulation, alterations in scheduling of administration, and the use of oral chemotherapy. Studies that have shown an association between plasma concentration with toxicity and clinical efficacy have shown that pharmacokinetically guided dose adjustments can substantially improve the therapeutic index of 5-FU treatment. These studies have shown that only 20%–30% of patients treated with a 5-FU–based regimen have 5-FU levels that are in the appropriate therapeutic range—approximately 40%–60% of patients are underdosed and 10%–20% of patients are overdosed. To date, 5-FU drug testing has not been widely used because of the lack of a simple, fast, and inexpensive method. Recent advances in testing based on liquid chromatography–mass spectrometry and a nanoparticle antibody–based immunoassay for 5-FU may now allow for routine monitoring of 5-FU in clinical practice. We review the data on pharmacokinetically guided dose adjustment of 5-FU and discuss the potential of this approach to advance therapeutic outcomes.

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The fluoropyrimidine 5-fluorouracil (5-FU) has been used in daily clinical oncology practice for nearly 50 years, and it has been well established that a good correlation exists between 5-FU plasma levels and the biological effects of 5-FU treatment, both in terms of clinical efficacy and toxicity (1–6). Although 5-FU pharmacokinetic studies using cell-based and physical detection methods have been conducted since the mid-1960s (7–14), the application of 5-FU pharmacokinetic monitoring to clinical practice has become more realistic and practical since 5-FU administration via infusion schedules evolved to become the standard of care over the past 5–8 years (15,16).

Clinical studies that were conducted during the past 20 years have demonstrated reduced toxicity and improved clinical outcomes with pharmacokinetic dose management. These pharmacokinetically guided studies have identified an optimal target therapeutic range for 5-FU and have recommended dose-adjustment algorithms to bring plasma concentrations into the optimal range (17–29). Recent work has shown that many patients who are currently being treated with 5-FU are not being given the appropriate doses to achieve optimal plasma concentration. Of note, only 20%–30% of patients are treated in the appropriate dose range, approximately 40%–60% of patients are being underdosed, and 10%–20% of patients are overdosed. Studies that have shown associations between 5-FU plasma concentration with toxicity and clinical efficacy have demonstrated that pharmacokinetically guided dose adjustments substantially improve these biological effects, which are associated with 5-FU therapy. However, 5-FU monitoring has not been widely used, at least not in the United States, and certainly not outside the clinical research setting, given the absence of simple, fast, and inexpensive testing methods for 5-FU monitoring. Recent developments with testing based on liquid chromatography–mass spectrometry (LC-MS/MS) and a nanoparticle antibody–based immunoassay for 5-FU may now allow for routine monitoring of 5-FU in clinical practice. We review the data on pharmacokinetically guided dose adjustment of 5-FU and discuss the potential of this approach to advance therapeutic outcomes.

The rationale for pharmacokinetically guided 5-FU monitoring

Current Dosing of 5-FU

The standard approach for calculating 5-FU drug dosage, as with many anticancer agents, has been to use body surface area (mg/m²). Unfortunately, there is no rigorous scientific basis for this strategy, and the body surface area–based dose for 5-FU and the majority of other anticancer agents is generally recommended according to the maximum dose tolerated that had been established in early-phase clinical trials. Dosing based on body surface area is associated

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with considerable variability in plasma 5-FU levels by as much as 100-fold (29–31), and such interpatient and intrapatient pharmacokinetic variability is a major contributor to toxicity and treatment failure. Several potential sources of interindividual pharmacokinetic variation exist, including pharmacogenetic differences in absorption, distribution, metabolism, and excretion of anticancer drugs (31–35). Other factors that must also be considered include performance status, age, sex, weight, and circadian diurnal variation. Certain drug-specific factors can lead to pharmacokinetic variability. These factors include mode and schedule of drug administration; dietary status and interactions with food; interactions with prescription and nonprescription drugs, nutritional supplements, and herbal medicines; and compliance with oral agents (36).

The potential relationship between body surface area and 5-FU pharmacokinetics has been investigated by two main groups in Europe. In a series of 81 patients with metastatic colorectal cancer (mCRC), Gamelin et al. (37) documented a complete lack of association between body surface area and 5-FU clearance (Figure 1). Milano et al. (38) observed a similar lack of association between body surface area and 5-FU clearance in a study of 380 patients with head and neck cancer who were administered 5-FU plus cisplatin chemotherapy. They also showed that 5-FU clearance followed a Gaussian distribution and that the interindividual variability for clearance could vary as much as 100-fold. In addition, they found that 5-FU clearance was lower in women than in men but did not vary with age.

Taken together, these data support the view that dosing of 5-FU according to body surface area is of only limited use. Direct monitoring of 5-FU blood levels with appropriate dose adjustments may represent a more rational approach for 5-FU dosing. Using a pharmacokinetically based dosing approach would aid the oncologist in “personalizing” the 5-FU dose so as to obtain optimal systemic drug exposure at, in the end, would be more effective and less toxic for the individual patient. In a review of the role of clinical pharmacokinetics and drug monitoring in cancer therapy, Chabner (39) provided further support for this approach when he suggested that cancer patients would benefit from pharmacokinetic dose management.

Pharmacokinetic Variability of 5-FU and Association With Biological Effect

5-FU was initially synthesized in the 1950s, and it continues to be the cornerstone of all major CRC treatment regimens for adjuvant therapy and for advanced metastatic disease. When it was first developed in the United States, 5-FU monotherapy was usually administered via a bolus schedule. However, during the past 5 years, many bolus schedules have been replaced by infusional regimens based on the work of de Gramont et al. (15,16) in France and Europe. The main treatment regimens used in the United States in the first-, second-, and third-line treatment settings are presented in Table 1 (40,41). The many treatment options now available to patients have transformed mCRC from an acute disease resulting in near-certain short-term mortality into a chronic illness, with median overall survival in the range of 24–28 months. As a result, individual patient characteristics are increasingly being considered in developing treatment strategies to ensure patient safety, allow longer-term systemic therapy, and improve patient quality of life while maintaining treatment intensity to maximize clinical efficacy.

One of the potential limitations of 5-FU therapy is the considerable pharmacokinetic variability that has been documented for both bolus and infusional schedules of administration. Despite this variability, a strong association has been identified between 5-FU plasma levels and biological effect, as it relates to toxicity and efficacy (Table 2). The pharmacokinetic parameter that has been most closely associated with biological effect is total drug exposure or area under the curve (AUC) drug concentration (1–6,17–28). Determination of AUC for bolus 5-FU schedules is somewhat difficult because of the number of samples that must be collected in a short period. In contrast, determination of AUC levels with infusional schedules of 5-FU is considerably simpler because only one sample, which is usually collected at steady state (2 hours into the infusion and throughout the infusion until the end), is required. With infusional regimens, the AUC can be simply calculated from the steady-state concentration (Css) by the relationship with time of continuous infusion in hours (T:

\[
\text{AUC}/T_{\text{ci}} = \text{Css}
\]

or

\[
\text{Css} \times T_{\text{ci}} = \text{AUC}.
\]

The studies presented in Table 2 highlight the wide variability of 5-FU drug levels with infusional-based 5-FU regimens, whether it be 2-, 3-, or 5-day continuous infusion schedules, and they document the association between AUC levels and toxicity and response. The testing method most often used to measure 5-FU plasma levels has been high-performance liquid chromatography (HPLC) (11–13). In general, AUC levels greater than 25 mg·h/L were associated with an increased risk for developing toxicity in patients with CRC (1–6,23,24). Of note, other studies focusing on patients with head and neck cancer (42–44) found that AUC levels greater than 30 mg·h/L were associated with the development of toxicity, suggesting that target AUCs for 5-FU may differ based on the particular solid tumor type. With respect to CRC, the hematologic toxic effect most often associated with AUC was neutropenia, and the nonhematologic toxic effects associated with AUC included diarrhea, stomatitis, and hand–foot syndrome. In terms

Figure 1. Distribution of 5-fluorouracil (5-FU) plasma clearance vs body surface area (BSA) during the first treatment course of 81 colorectal cancer patients (37). Reprinted with permission. Copyright 2008, American Society of Clinical Oncology. All rights reserved.
of clinical activity, an early study by Hillcoat et al. (1) showed a close association between AUC and response rate and stable disease, whereas a more recent study by DiPaolo et al. (28) investigating a bolus schedule of 5-FU/leucovorin (LV) as adjuvant therapy of early-stage colon cancer identified a strong association between AUC and disease-free interval.

**Practical Considerations for Pharmacokinetically Guided 5-FU Dose Management**

**Testing Methods**

In the mid-1960s, cell-based assays were used for conducting pharmacokinetic studies of 5-FU (7,8). By the mid-1970s, this semiquantitative method was replaced with gas–liquid chromatography and gas chromatography–mass spectrometry (10). HPLC subsequently replaced gas–liquid chromatography, and it is currently the most commonly used method to measure 5-FU drug levels in the clinical setting (9–12). Liquid chromatography–mass spectrometry (LC-MS/MS) has become increasingly popular, given its higher sensitivity (13). Although these methods are highly sensitive, they require sophisticated instrumentation and as such are expensive, labor intensive, and not readily amenable for widespread clinical use.

To further address the issue of 5-FU testing, an immunoassay for 5-FU has been recently developed (14). Novel, highly selective monoclonal antibodies for 5-FU were developed and then covalently attached to 200-nm nanoparticles, which serve as a label in a homogeneous competitive immunoassay format. The specificity of these antibodies to 5-FU was confirmed when it was shown that cross-reactivity was less than 1% for dihydro-5-FU, one of the main catabolites for 5-FU, 0.05% for capecitabine, and 0.23% for tegafur. This immunoassay requires only a small amount of plasma (<10 µL); takes only about 11 minutes to perform; and when using an Olympus AU400 ImmunoAnalyzer, up to 400 patient samples can be analyzed per hour. Compared with chromatography, the instrumentation required for this test is inexpensive and requires minimal technical training. Moreover, Beumer et al. (45) measured 5-FU drug levels in patients with colorectal cancer and head and neck cancer who were being treated with 5-FU–based regimens and found that the immunoassay results were consistent with those generated by other validated methods, including HPLC and LC-MS/MS. Thus, this novel technology has important advantages in

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**Table 1. Use of chemotherapy regimens for metastatic colorectal cancer in the United States**

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Use as first-line therapy, %</th>
<th>Use as second-line therapy, %</th>
<th>Use as third-line therapy, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOLFOX or FOLFIRI plus bevacizumab</td>
<td>44.3</td>
<td>23.8</td>
<td>3.3</td>
</tr>
<tr>
<td>FOLFOX</td>
<td>24.0</td>
<td>5.9</td>
<td>0.6</td>
</tr>
<tr>
<td>Capecitabine based</td>
<td>13.8</td>
<td>11.3</td>
<td>13.2</td>
</tr>
<tr>
<td>Infusional 5-FU/LV</td>
<td>4.7</td>
<td>2.2</td>
<td>0.0</td>
</tr>
<tr>
<td>FOLFIRI</td>
<td>3.5</td>
<td>9.5</td>
<td>0.2</td>
</tr>
</tbody>
</table>

* Source: Data Monitor, 2006.
† FOLFOX4 = 5-FU 400 mg/m² followed by 600 mg/m² infusion for 22 hours on days 1 and 2; LV 200 mg/m² IV on days 1 and 2; oxaliplatin 85 mg/m² IV on day 1. Each cycle is repeated every 2 weeks. FOLFOX6 = 5-FU 400 mg/m² on day 1, followed by 2400 mg/m² infusion for 46 hours; LV 400 mg/m² IV on day 1; oxaliplatin 100 mg/m² IV on day 1. Each cycle is repeated every 2 weeks. FOLFOX7 = 5-FU 2400 mg/m² infusion for 46 hours; LV 200 mg/m² IV on day 1; oxaliplatin 130 mg/m² IV on day 1. Each cycle is repeated every 2 weeks. FOLFOX8 = 5-FU 3800 mg/m² infusion for 46 hours; LV 200 mg/m² IV on day 1; oxaliplatin 100 mg/m² IV on day 1. Each cycle is repeated every 2 weeks. LV = leucovorin; IV = intravenous.
‡ FOLFIRI = 5-FU 400 mg/m² on day 1, followed by 2400 mg/m² infusion for 46 hours; LV 200 mg/m² IV on day 1; irinotecan 180 mg/m² IV on day 1. Each cycle is repeated every 2 weeks.
§ Bevacizumab = 5 mg/kg every 2 weeks or 7.5 mg/kg every 3 weeks. Each cycle is repeated every 2 weeks.
¶ Capecitabine monotherapy = 1000–1250 mg/m² orally twice a day on days 1–14. Each cycle is repeated every 3 weeks. Capecitabine combination therapy = 850–1000 mg/m² orally twice a day on days 1–14. Each cycle is repeated every 3 weeks.

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**Table 2. Clinical studies highlighting 5-FU variability and relationship of AUC to biological effect in colorectal carcinoma**

<table>
<thead>
<tr>
<th>Author</th>
<th>Country</th>
<th>Year</th>
<th>Biological effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hillcoat et al. (1)</td>
<td>Canada</td>
<td>1978</td>
<td>Association between AUC and clinical efficacy (RR and SD)</td>
</tr>
<tr>
<td>Au et al. (3)</td>
<td>United States</td>
<td>1982</td>
<td>Association between AUC and toxicity (myelosuppression)</td>
</tr>
<tr>
<td>van Groeningen et al. (4)</td>
<td>Netherlands</td>
<td>1988</td>
<td>Association between AUC and toxicity</td>
</tr>
<tr>
<td>Yoshida et al. (5)</td>
<td>Japan</td>
<td>1990</td>
<td>Association between AUC and toxicity</td>
</tr>
<tr>
<td>Trump et al. (6)</td>
<td>United States</td>
<td>1991</td>
<td>Association between AUC and toxicity (stomatitis and myelosuppression)</td>
</tr>
<tr>
<td>Gamelin et al. (19)</td>
<td>France</td>
<td>1998</td>
<td>Association between AUC and toxicity (diarrhea, hand–foot syndrome) and clinical efficacy (RR and OS)</td>
</tr>
<tr>
<td>Ychou et al. (23,24)</td>
<td>France</td>
<td>1999, 2003</td>
<td>Association between AUC and toxicity (myelosuppression, diarrhea, hand–foot syndrome)</td>
</tr>
<tr>
<td>Gamelin et al. (22)</td>
<td>France</td>
<td>2008</td>
<td>Association between AUC and clinical efficacy (response rate, PFS, and OS)</td>
</tr>
<tr>
<td>DiPaolo et al. (28)</td>
<td>Italy</td>
<td>2008</td>
<td>Association between AUC and clinical efficacy (DFS)</td>
</tr>
</tbody>
</table>

* 5-FU = 5-fluorouracil; AUC = area under the drug concentration curve; DFS = disease-free survival; OS = overall survival; PFS = progression-free survival; RR = response rate; SD = stable disease.
Establishing Target AUCs With the Newer Infusion Regimens

The development of an optimal target therapeutic range for cytotoxic agents, and for 5-FU specifically, is based on a balancing of toxic effects that result from higher levels of drug concentrations and compromised clinical efficacy resulting from suboptimal dosing. The main goal was to establish a target drug concentration that will be just below that which will induce grade 3–4 toxicity in the majority of treated patients, thereby ensuring a balance between the positive and the negative biological effects of 5-FU therapy.

Much of the work to establish the appropriate target 5-FU range was based on identifying an association between AUC levels and toxicity. Gamelin et al. (17–22,25–27) in France have been the leaders in conducting pharmacokinetically guided clinical studies with 5-FU infusion-based regimens for mCRC because the de Gramont leucovorin LV5FU2 regimen was first adopted as the standard of care in Europe and the United States. Recently, Di Paolo et al. (28) reported on the results of a phase II pharmacokinetic study of patients who were treated with a bolus 5-day 5-FU/LV regimen in the adjuvant therapy of patients with stage II/III colon cancer. They observed a strong association between 5-FU AUC levels and disease-free survival, with a relative improvement in 10-year disease-free survival of 50% in the group with an AUC value greater than 8.4 mg·h/L. This AUC value of 8.4 mg·h/L, when multiplied for the number of treatment days per month, corresponds to the same monthly target exposure value that Gamelin et al. have identified in studies using infusion-based regimens. In the United States, where infusion-based regimens have become the standard of care in the past 5–8 years, monitoring studies have been less common, mainly because of the absence of a simple, fast, and reliable testing method.

Evidence exists to suggest that the therapeutic AUC range of 5-FU may differ depending on the specific regimen that is being used and the particular cytotoxic agent that is being administered with 5-FU (46). For example, when the reduced folate LV is combined with 5-FU, as in the case of infusional LV5FU2, the optimal range for 5-FU AUC is 20–25 mg·h/L. In contrast, when 5-FU is combined with cisplatin, a regimen that is widely used for head and neck cancer, the target range for 5-FU AUC is higher, on the order of 25–30 mg·h/L. However, given the unavailability of suitable drug assay methods, therapeutic drug monitoring with dose modification is used only rarely in cancer treatment and usually only in the context of a clinical trial setting. Currently, the only example where drug monitoring is being used in clinical practice is with high-dose methotrexate therapy, in which methotrexate drug levels are routinely monitored to determine the subsequent dose and scheduling of LV rescue.

A consistent target range of AUC for all of the newer 5-FU/LV infusion–based regimens has been established as 20–25 mg·h/L, despite different administration modes (bolus vs infusion) and schedules (several hours to several days) (21–24,27,28). The LV5FU2 regimen on which these studies were based is the foundation for the FOLFOX and FOLFIRI combination regimens. Established target steady-state levels for LV5FU2 have provided guidance for studies evaluating the target range of 5-FU in the FOLFOX and FOLFIRI regimens. To date, it appears that the target AUC is consistent across all treatment regimens as long as 5-FU is administered via an infusion schedule.

Clinical Value of Pharmacokinetically Guided 5-FU Dose Management

Dose Monitoring in Clinical Practice

Gamelin et al. (21) at the Paul Papin Cancer Center in Angers, France, routinely monitor patients who are treated with 5-FU–based chemotherapy for CRC. This group treats approximately 5000 CRC patients each year with the various 5-FU–based regimens (LV5FU2, FOLFOX, FOLFIRI), and 5-FU drug levels are measured in all patients using standard HPLC methods. The dose of 5-FU administered with the first cycle is based on body surface area, with all subsequent doses based on pharmacokinetic-guided dose adjustment. Their testing protocol is simple in that only one patient sample is taken, usually toward the end of the 5-FU infusion. The 5-FU plasma levels are then used to recommend a 5-FU for the next dose of 5-FU, and this treatment algorithm is presented in Table 3. In a subset of 802 patients with CRC treated with this approach, they found that 345 (42.7%) patients had 5-FU levels that were at the target level and did not require further dose adjustment, 373 (46.3%) patients had 5-FU levels that were below the target level and needed dose adjustment upward, and 88 (11%) patients had 5-FU levels that were above the target level and required dose reduction (21). Using this pharmacokinetically guided approach, they were able to substantially reduce the incidence of grade 3 or 4 toxic effects resulting from 5-FU therapy, the need for hospitalization and supportive care, and the need for treatment interruption and dose delays. One of the potential limitations, however, is that the HPLC method used for 5-FU testing is labor intensive, which limits turnaround time and the number of patients that can be monitored.

Dose Management to Improve Clinical Efficacy and Safety Profile

A randomized, phase III multicenter clinical trial was conducted using an infusion regimen of 5-FU/LV and compared standard dosing with therapeutic dose monitoring. Each arm consisted of 104 patients with mCRC, and patients were treated with 5-FU at a somewhat unconventional dosing schedule of 1500 mg/m² given as an 8-hour infusion on a weekly basis with 400 mg/m² LV (22). In group A, patients were dosed according to body surface area, and in group B, patients were dosed based on body surface area in the first cycle with subsequent doses adjusted to a target level of 20–24 mg·h/L based on drug levels, as determined by HPLC-based monitoring in the previous cycle. To achieve the prescribed target concentration levels, 18 (17.3%) patients in group B had their dose adjusted downward, 15 (14.4%) patients were at target level, and 71 (68%) patients in group B required an increase in dose to achieve the target levels. Thus, 85% of the patients who were randomly assigned to the dose-adjusted group did not receive an optimal dose of 5-FU. On average, four cycles of therapy were administered.
required to achieve the target concentration, and impressively, the therapeutic range for 5-FU was eventually achieved in 94% of patients.

With respect to safety profile, the overall incidence of grade 3–4 toxicity was substantially reduced (Table 4). In particular, grade 3–4 diarrhea was markedly reduced in patients receiving pharmacokinetically guided 5-FU when compared with body surface area–dosed 5-FU (4% vs 18%). Of note, the 58% of patients who were below target concentration and needed dose adjustment upwards did not experience a clinically significant increase in grade 3–4 toxicity over the entire treatment period, except for hand-foot syndrome, which was higher in group B. Given the reduced toxicity in group B, patients were treated for longer periods (791 months) when compared with patients who were treated in group A with standard dosing (680 months). In terms of clinical efficacy, patients who received 5-FU with pharmacokinetically guided dosing had an overall response rate of 34%, which was twofold higher than the 17% response rate seen in patients dosed by body surface area (Table 4). This difference was highly statistically significant ($P = .004$). An improvement in median overall survival was observed from 16 months for the body surface area–dosed patients to 22 months for the pharmacokinetically guided group. Although this difference did not reach statistical significance ($P = .08$), it should be noted that overall survival was not an endpoint of the study nor was the trial sufficiently powered for overall survival. Moreover, one would predict that the subsequent salvage therapies would have been well balanced in both arms, thereby diluting out any potential effects of pharmacokinetic dose adjustment on survival.

Gamelin et al. (26) recently presented data of two patient cohorts treated in parallel for mCRC in the front-line setting. In this study, patients were treated with a modified FOLFOX 4 (mFOLFOX4) regimen (oxaliplatin dose: 85 mg/m$^2$ every 2 weeks, 5-FU: 400 mg/m$^2$ bolus + 2500 mg/m$^2$ over 44 hours every 2 weeks and folinic acid 200 mg/m$^2$ every 2 weeks). Group A consisted of 39 patients whose 5-FU dose was calculated based on body surface area. Group B consisted of 118 patients, and although their initial 5-FU dose was determined by body surface area, all subsequent 5-FU doses were adjusted based on 5-FU drug levels, which were determined by HPLC. In group A, the dose of 5-FU remained unchanged, except in patients who experienced grade 3–4 toxicity. In contrast, for patients treated in group B, the dose was adjusted individually to achieve a target concentration of 600 µg/L based on a dose-adjustment algorithm using the 5-FU plasma concentration measured during 5-FU administration. In group B, dose adjustment was required in greater than 80% of the patients treated to achieve the 5-FU target concentration, and of this group, nearly 50% required dose adjustments of greater than 20%.

Patients treated with 5-FU in the dose-monitoring group (group B) experienced much less overall grade 3–4 toxicity than those in group A who were treated with standard dosing of mFOLFOX4. Specifically, the incidence of grade 3–4 diarrhea, mucositis, and neutropenia were 1.7%, 0.8%, and 18%, respectively, which is much lower than what was observed in patients in group A, whose incidence was 12%, 15%, and 25%, respectively. In terms of clinical efficacy, overall response rate was increased in the group B patient cohort (69.5% vs 46%), and median progression-free survival (16 vs 10 months) and overall survival (28 vs 22 months) were also higher for patients who were treated with 5-FU dose adjustment. Although this study was not randomized and the patient numbers are not well balanced, the findings from this study provide further evidence that therapeutic drug monitoring of 5-FU represents a controlled and safe approach to dose adjustment with potential benefits both in terms of efficacy and toxicity.

### Table 3. Dose-adjustment algorithm for 5-fluorouracil (5-FU) with FOLFOX6*

<table>
<thead>
<tr>
<th>5-FU plasma concentration, µg/L</th>
<th>AUC, mg h/L</th>
<th>Dose adjustment (± percent of previous dose)</th>
<th>Toxicity grade ≥2</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;110</td>
<td>AUC&lt;5</td>
<td>+150</td>
<td>Grade 2: dose reduction of 200 mg</td>
</tr>
<tr>
<td>110–220</td>
<td>5&lt;AUC&lt;10</td>
<td>+100</td>
<td></td>
</tr>
<tr>
<td>220–330</td>
<td>10&lt;AUC&lt;15</td>
<td>+25</td>
<td></td>
</tr>
<tr>
<td>330–450</td>
<td>15&lt;AUC&lt;20</td>
<td>+15</td>
<td></td>
</tr>
<tr>
<td>450–550</td>
<td>20&lt;AUC&lt;25</td>
<td>Unchanged</td>
<td></td>
</tr>
<tr>
<td>550–650</td>
<td>25&lt;AUC&lt;30</td>
<td>−10</td>
<td></td>
</tr>
<tr>
<td>650–750</td>
<td>30&lt;AUC&lt;35</td>
<td>−15</td>
<td></td>
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<tr>
<td>750–850</td>
<td>35&lt;AUC&lt;40</td>
<td>−20</td>
<td></td>
</tr>
</tbody>
</table>

* AUC = area under the drug concentration curve. Reprinted with permission. Copyright 2008, American Society of Clinical Oncology. All rights reserved (22).

### Table 4. Gamelin phase III study: summary of toxicity and efficacy*

<table>
<thead>
<tr>
<th>Dosing</th>
<th>Diarrhea</th>
<th>Mucositis</th>
<th>Hematologic</th>
<th>n</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA</td>
<td>18</td>
<td>2</td>
<td>2</td>
<td>104</td>
<td>.003</td>
</tr>
<tr>
<td>Pharmacokinetically guided</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>104</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Dosing</th>
<th>Diarrhea</th>
<th>Mucositis</th>
<th>Hematologic</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA</td>
<td>18.3</td>
<td>33.6</td>
<td></td>
<td>.004</td>
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<tr>
<td>Pharmacokinetically guided</td>
<td>16</td>
<td>22</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* BSA = body surface area; OS = overall survival. P values (two-sided) were calculated using Fisher exact test.
Dihydropyrimidine Dehydrogenase (DPD) Deficiency

DPD is the rate-limiting enzyme involved in the catabolism of 5-FU. Up to 80%–85% of an administered dose of 5-FU is broken down by this enzyme to inactive metabolites. 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 approximately 10–15 minutes, can be markedly prolonged to up to 159 minutes (46–54). A pharmacogenetic syndrome has been identified in which partial and complete deficiency in the DPD enzyme has been observed in 3%–5% and 0.1% of the general population, respectively (50,51,55,56). 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, and these complex genotypes are inherited in an autosomal codominant fashion.

Of note, even in patients who express normal DPD activity, there is wide interindividual variability in activity of up to 20-fold (52). The association between low DPD activity and high 5-FU plasma levels is strong in individuals who exhibit profound deficiency. However, it is now increasingly appreciated that DPD mutations are unable to account for all of the observed cases of DPD deficiency because as many as 50% of patients who experience increased 5-FU toxicity have no documented alterations in the DPD gene. Moreover, individuals who have normal enzyme activity may also be diagnosed with high plasma levels of 5-FU, resulting in increased toxicity. It is clear, therefore, that factors other than DPD status may contribute to 5-FU metabolism and eventual 5-FU toxicity.

Several cancer centers and reference laboratories in the United States provide testing services to detect the major DPD genotype associated with the deficiency. Unfortunately, there is no approved test for measuring DPD enzyme activity and/or protein expression, although such testing is being performed at certain select centers in the United States. Although identification of a deficient genotype identifies a person at potential risk for toxic effects, it is clearly not the only risk factor. 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 (51,55,57). 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. (58) described using a test dose of 5-FU to identify individuals at risk for developing toxic effects. This study identified three of 188 patients tested who displayed low drug clearance. Interestingly, all three individuals exhibited normal DPD activity, and as such, they would have been missed with a genotype and/or enzyme activity assay for DPD, highlighting the importance of monitoring the actual phenotype, which in this case would be the 5-FU drug level.

Role of Thymidylate Synthase (TS)

TS catalyzes the enzymatic reaction that provides the sole de novo intracellular source of thymidylate, an essential precursor for DNA synthesis. As such, this enzyme has been an important target for cancer chemotherapy for more than 40 years, and specifically for 5-FU, capecitabine, and other antifolate-based TS inhibitor compounds, such as pemetrexed, raltitrexed, and pralatrexate. Several studies (59–61) have shown that TS levels in tumor tissue may be associated with response to 5-FU–based chemotherapy, in that low levels of TS expression in the tumors of mCRC patients were associated with clinical response and improved survival. It has also been well documented that the promoter enhancer region of the TS gene, TYMS, is polymorphic (62,63). The promoter region usually contains a double-tandem repeat (2R) or a triple-tandem repeat (3R), and the importance of these polymorphisms is that homozygous 3R/3R tumors express higher levels of TS mRNA and TS protein compared with homozygous 2R/2R tumors. A study conducted by Lecomte et al. (64) showed that patients with the 2R/2R genotype experienced statistically significantly higher grade 3–4 toxicity than patients with the 3R/3R genotype (43% vs 3%). This study suggested that TS genotyping may help identify patients at increased risk for developing toxicity to 5-FU chemotherapy. Schwab et al. (65) recently provided further evidence supporting the association between the 2R/2R genotype and the 5-FU toxicity. However, the increased risk for toxicity was 1.6-fold, which was much less pronounced than that reported in the study by Lecomte et al. In fact, this study concluded that TS genotyping had only a limited role in 5-FU–related toxicity and that other nongenetic factors, such as sex, mode of administration, and the use of LV might be more relevant.

Role of Circadian Variation

Circadian rhythm has been shown to be a potentially important factor that determines 5-FU pharmacokinetic variability (66). Several circadian mechanisms have been identified that include alterations in the enzyme activities of several key enzymes involved in 5-FU metabolism and in the proliferative activity of bone marrow and intestinal mucosa. An inverse relationship between DPD enzyme activity in peripheral blood mononuclear cells and 5-FU plasma concentrations, and a circadian rhythm in 5-FU drug levels and DPD activity was identified (48–54). Based on this and other clinical data, it was postulated that circadian timing or chronomodulation of drug delivery might allow for an increase in 5-FU dosage.

Levi et al. (67) conducted a randomized phase II study comparing fixed-infusion-rate delivery of oxaliplatin, 5-FU, and LV chemotherapy with chronomodulated delivery of the same chemotherapy regimen in patients with mCRC. In patients who were randomly assigned to the fixed-delivery arm, drug delivery was kept constant during a 5-day period, and in the chronomodulated group, patients received a maximum delivery of oxaliplatin at 4:00 pm and a maximum delivery of 5-FU and LV at 4:00 am. Chronomodulated delivery of chemotherapy resulted in a marked reduction in toxicity, especially in the form of grade 3–4 mucositis, and a statistically significant improvement in clinical efficacy in terms of overall response rate, progression-free survival, and median overall survival. The results of this study highlight the potential importance of chronomodulation. However, at the same time, this study underscores the complexities involved with using such a dosing strategy. In particular, one issue to consider is how best to identify the right peak time for optimal drug delivery. In contrast to the study by Levi et al., other investigators (68,69) have failed to identify consistent circadian patterns in 5-FU pharmacokinetics in cancer patients receiving prolonged drug infusion. As a result, chronomodulated administration of 5-FU, although interesting, would not appear to be a practical approach that can be used for daily clinical oncology practice.
Economic Importance of Pharmacokinetically Based 5-FU Dosing

Pharmacokinetically guided dose adjustment of 5-FU may result in substantial savings, given the reduced incidence of toxic effects and improved clinical outcomes. In a multicenter randomized study of head and neck patients who were treated with 5-FU and cisplatin, Fety et al. (29) demonstrated that the costs associated with toxicity were considerably reduced for patients receiving 5-FU by a dose-managed approach when compared with those treated with standard 5-FU dosing. For the 5-FU dose-adjusted group, the total cost for treatment of toxicity was $6803, whereas the costs of toxicity for the standard treatment group were $21758, which represents a substantial reduction in medical costs of nearly 70%.

It has now been shown that the use of pharmacokinetically guided dose adjustment with infusional LV5FU2 regimen may achieve the same level of clinical efficacy, in terms of response rate and overall survival, when compared with what has been reported for the more complex FOLFOX and FOLFIRI combination regimens using standard dosing of 5-FU (17,18,22,23). It should be noted that these comparisons involve phase II studies with the pharmacokinetically guided approach vs larger phase III studies with the standard dosing approach. However, these findings may have important pharmacoeconomic implications. If only chemotherapy drug costs are considered, the cost of the infusional LV5FU2 regimen is relatively inexpensive, on the order of less than $500 for a 6-month treatment course. In contrast, the drug costs for either the FOLFOX or the FOLFIRI combination regimens would be substantially more expensive, in the range of $22000–$30000 during this same 6-month period, when 5-FU is dosed using the conventional body surface area–based approach (70). The cost that would be required for 5-FU testing with the new 5-FU immunoassay is projected at $3000 during this 6-month period. When this cost is combined with the cost of LV5FU2 chemotherapy, the projected overall cost would still be substantially lower than what is projected for FOLFOX/FOLFIRI chemotherapy. As a result, dose monitoring has the potential to yield substantial savings in overall health-care costs by reducing the costs associated with toxicity and complications and by making less expensive regimens more effective.

In a recent study of dose adjustment with FOLFOX4, Gamelin et al. (26) observed that 5-FU dose adjustment resulted in a significant reduction in overall incidence of grade 3 or 4 toxicity when compared with standard dosing of 5-FU. Although an economic analysis was not conducted as part of this study, the savings from the reduction of treatment of complications would, in theory, have been considerable. In terms of clinical efficacy, dose-adjusted patients achieved a response rate of 70% and median progression-free survival of 16 months, which are impressively high for a cytotoxic chemotherapy regimen alone. As a comparison, in the randomized phase III NO16966 study (71), the combination of FOLFOX plus bevacizumab yielded a median progression-free survival of 9.4 months compared with 8.0 months in patients receiving FOLFOX chemotherapy alone. Meropol and Schulman (70) have estimated that, on average, the cost of 12 months of FOLFOX4 chemotherapy without dose adjustment would be $59978, whereas the cost of FOLFOX4 without dose adjustment plus bevacizumab would be $107175. In contrast, the cost of FOLFOX4 without bevacizumab but with 5-FU dose adjustment is projected to be $65278, which includes the $6000 laboratory cost for 5-FU monitoring. Such a cost analysis suggests that optimizing drug concentrations of conventional cytotoxic chemotherapy regimens may lead to improvements in clinical efficacy such that the newer and more expensive biological agents may not be required, at least upfront. Clearly, rigorous pharmacoeconomic studies are required to more fully elucidate the economic value of 5-FU pharmacokinetic dose management.

Future Developments

A simple, cost-effective, and rapid testing method for 5-FU levels would further help replace body surface area dosing with pharmacokinetically guided 5-FU dose management. Moreover, this approach would add an important and complementary tool for physicians to identify and treat patients who experience 5-FU toxicity and who might have DP D deficiency and/or any other genetic defect that results in alterations in 5-FU metabolism. The advantages for such a test include the following:

1. The 5-FU monitoring test will determine the phenotype, which is the sum of all the factors that contribute to 5-FU drug levels, and it can therefore identify those individuals who may have normal DPD activity but are still unable to metabolize and excrete the drug rapidly.

2. The genotype test for DPD can identify individuals who are DPD deficient, but it does not provide guidance on dose adjustment. When used in conjunction with a genotype test, the 5-FU monitoring assay would further aid therapy by fine-tuning the dosing to achieve optimal drug levels.

3. The 5-FU monitoring test can be used in conjunction with a reduced test dose to identify patients at risk before treatment.

4. 5-FU monitoring can be used within a few hours of the start of infusional 5-FU therapy to quickly identify individuals at increased risk for developing toxicity, such as those with poor drug clearance, so that early adjustments could be made.

To further address the role of therapeutic drug monitoring as it relates to 5-FU chemotherapy, a prospective randomized clinical study is being planned in the United States using the mFOLFOX7 regimen plus the anti-vascular endothelial growth factor antibody bevacizumab in the frontline treatment of mCRC. Patients will be randomly assigned to receive 5-FU using standard body surface area–based dosing or to receive 5-FU using pharmacokinetically guided dose adjustment. The primary endpoints of this study will be time to tumor progression and safety profile. In this study, 5-FU drug levels will be measured using the newly developed nanoparticle antibody–based immunoassay. The goal of this study will be to provide further support for the use of pharmacokinetically guided dose adjustment of 5-FU in everyday clinical practice for patients with mCRC.

Conclusions

There is now a large body of clinical data showing that body surface area–based 5-FU dosing is unable to achieve optimal drug concentrations in a high percentage of patients. In fact, studies...
that have been done over the past 30 years support the concept of pharmacokinetically guided 5-FU dose management. There is an unmet clinical need for a simple, fast, and reliable test that can be used on a routine basis to manage 5-FU dosing. The association between toxicity and high 5-FU plasma levels has been reported since the late 1970s, and plasma drug levels that result in grade 3–4 toxicity have been identified. Published studies document the ability to reduce toxicity associated with newer infusion 5-FU treatment regimens through pharmacokinetically guided dose adjustment. Of note, approximately 40%–50% of patients today are underdosed, and based on the recent clinical data, it is becoming increasingly clear that dose escalation can improve response rates and overall survival without increasing toxic side effects. Despite different administration modes (bolus vs infusion), administration schedules (several hours to several days), and various 5-FU–based combination regimens, a therapeutic dose range of 20–25 mg·hr/L has been consistently shown to yield optimal results in terms of clinical efficacy and safety. A dose-adjustment algorithm has been developed to guide practical implementation of 5-FU dose management.

The current standard of practice is that drugs with common patterns of toxicity used in combination therapy regimens are all adjusted downward when toxicity is observed because there is no reliable means of identifying which component is the primary contributor to the toxicity. Monitoring 5-FU plasma levels in 5-FU–based chemotherapy regimens, such as FOLFOX and FOLFIRI, would provide a rational approach for identifying the toxicity-inducing component of the combination regimen and would allow specific dose adjustment of 5-FU without having to modify the doses of other cytotoxic partners. DPD genotype detection testing is currently being offered by a number of reference laboratories in the United States. However, it is now well established that this test is unable to fully address the medical need and identifies only at most 40%–50% of patients at risk for increased 5-FU toxicity. A 5-FU monitoring test that directly measures drug levels would provide a more accurate assessment of 5-FU clearance. Finally, although nearly all of the studies focusing on pharmacokinetic dose adjustment have been done in the context of CRC, the findings would appear to have an even broader application for therapeutic drug monitoring of any treatment regimen that incorporates a fluoropyrimidine.

References


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