Making TDM work to optimize cancer chemotherapy: a multidisciplinary team approach

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Several factors can limit the use of therapeutic drug monitoring (TDM) for cancer chemotherapeutic agents, including poorly defined concentration–effect relationships for many antineoplastic agents. This is further complicated by cancer being a highly heterogeneous group of diseases, each of which may have a unique concentration–effect relationship for any given drug or drug combination. Nonetheless, TDM clearly has the potential to improve the clinical use of antineoplastic agents, most of which have very narrow therapeutic indices and highly variable pharmacokinetics. A substantial body of literature accumulating during the past 15 years demonstrates relationships between systemic exposure to various anticancer drugs and their toxic or therapeutic effects. This review highlights selected studies that illustrate concentration–effect relationship for the antineoplastic effects of 5-fluorouracil, mercaptopurine, and methotrexate. A much larger number of pharmacodynamic studies have established the relationship between serum concentration and dose-limiting toxicities for anticancer agents, including epipodophyllotoxins, platinum compounds, camptothecin, anthracyclines, and antimetabolites. In this review we will focus on anticancer drugs for which the pharmacodynamics of antineoplastic effects have been elucidated. We will also address issues critical to the optimal use of TDM in a clinical setting, which requires effective participation by a multidisciplinary team of professionals.

Therapeutic drug monitoring (TDM) has been utilized for the clinical management of patients since the early 1960s. It entails the measurement and interpretation of drug concentrations in biological fluids and the individualization of drug dosages or schedules to maximize therapeutic effects, minimize toxicities, or both [1]. Theoretically, a drug should meet several criteria for TDM to be useful, including considerable inter- or intraindividual pharmacokinetic variability, a defined relationship between concentration and pharmacological effects, a narrow therapeutic index, and the availability of a precise and accurate drug assay [2, 3]. TDM is widely applied to a variety of medications in different therapeutic classes, including cardiovascular agents, antiepileptics, antibiotics, respiratory smooth muscle relaxants, antiinflammatory agents, some cancer chemotherapeutic agents, immunosuppressants, and antidepressants. Among all therapeutic classes of drugs, TDM has seen limited use with antineoplastic agents, methotrexate (MTX)1 being the only such drug routinely monitored in most institutions. The primary reason is the poorly defined concentration–effect relationships for most anticancer drugs.

Limitations of TDM for Cancer Chemotherapy

The clinical utility of TDM for antineoplastic agents is currently limited by several factors [4–6]. First, there is an incomplete understanding of the pharmacology and pharmacokinetics of most anticancer agents. This is further confounded by the plasma drug concentration being an indirect measure of the amount of drug in the target tissue, because the site of action is often remote from intravascular spaces (as may be the case for many classes of therapeutic agents) and because solid tumors may have a unique blood supply. Furthermore, there is typically a major time lag between the measurement of drug in plasma and assessment of the ultimate pharmacodynamic effect, cure. Using improvement of cure rate as the effect variable typically requires 5 years of follow-up to reliably assess outcome. Thus, the complexity and time required for such studies is substantially different from that usually required for drugs with more acute effects (e.g.,

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1 Nonstandard abbreviations: MTX, methotrexate; Cpss, steady-state plasma concentration; AUC, area under the concentration–time curve; EORTC, The European Organization for Research and Treatment of Cancer; MTD, maximum tolerated dose; MTSE, maximum tolerated systemic exposure; 5FU, 5-fluorouracil; MP, mercaptopurine; ALL, acute lymphoblastic leukemia; TG, thioguanine nucleotides; TPMT, thiopurine S-methyltransferase; HDMTX, high-dose methotrexate; MTXPG, methotrexate polyglutamates.
antibiotics), but more similar to that in assessing pharmacodynamics of long-term immunosuppression or seizure control. Defining concentration–effect relationships is also complicated by the fact that cancer is almost always treated with multiple drugs given in combination [7]; this makes it difficult to precisely define the pharmacodynamics of individual agents, especially during phase III clinical trials. Combination chemotherapy not only complicates assessment of therapeutic effects, it also often complicates the pharmacodynamics of drug toxicity. For example, myelosuppression and mucositis are common adverse effects of many antineoplastic agents, making it difficult or impossible to differentiate the pharmacodynamics of these overlapping effects during combination chemotherapy. Hence, it is difficult to establish reliable concentration–effect relationships and “therapeutic ranges,” which are essential elements for TDM.

Cancer is a heterogeneous group of diseases, and the intrinsic characteristics of tumors affect the concentration–effect relationship for antineoplastic agents. Tumors exhibit heterogeneity in blood supply and cellular characteristics, leading to differences in sensitivity toward and resistance to antineoplastic agents [8]. This is nicely exemplified with MTX, where cellular differences in the membrane transporter for MTX entry into cells (reduced folate carrier), the cellular enzyme that activates MTX (folylpolyglutamate synthetase), the enzyme that inactivates MTX (γ-glutamyl hydrolase), and the intracellular concentrations of target enzymes (e.g., dihydrofolate reductase) can each substantially modify tumor sensitivity to MTX. These intrinsic cellular characteristics contribute to variability in drug response among patients and limit the precision of “therapeutic ranges” for anticancer agents.

**Potential Benefit of TDM for Cancer Chemotherapy**

Despite the challenges of TDM for antineoplastic agents, there is great potential to improve cancer treatment if TDM can be properly applied in the oncology clinic. Anticancer drugs have the prerequisites for TDM: highly variable pharmacokinetics and narrow therapeutic indices (i.e., the concentration that elicits the therapeutic effect does not differ much from the concentration causing toxicity). Maximal efficacy of cancer chemotherapy is of prime importance because of the enormous consequences to cancer patients. Underdosing of patients, which may compromise the probability of cure for cancers that are curable with chemotherapy, is not acceptable. Likewise, certain cytotoxic side effects, such as myelosuppression, can be life-threatening. Hence, there is great clinical utility for a therapeutic range that defines the concentrations producing efficacy as well as those producing undesirable adverse effects. Many clinical trials show the benefits of maximum treatment intensity [7], suggesting that treating patients with dosages producing concentrations in the upper end of the nontoxic range could be advantageous. If plasma concentrations that produce unacceptable toxicity can be defined for each agent, then TDM could be useful in identifying which medication(s) is being overdosed in a combination regimen, and possibly which is being underdosed. Examples of potentially useful relationships between plasma drug concentration and therapeutic or toxic effects are discussed in the next section.

Although the ultimate use of TDM is to individualize chemotherapy to improve efficacy and avoid toxicity, other potential benefits include enhancement of compliance, minimization of pharmacokinetic variability among patients, dose adjustment in patients with hepatic and (or) renal dysfunction [8], and detection of drug interactions [3].

**Pharmacokinetics and Pharmacodynamics of Chemotherapy**

Drug disposition depends on the four basic pharmacokinetic processes in the body—absorption, distribution, metabolism, and elimination [9, 10]. These processes vary among patients and produce considerable differences in plasma concentrations for the same dosage given to all patients receiving treatment (Fig. 1). The concentration of drug in the blood may not directly reflect the concentrations at the cellular level, where most drugs exert their biological effects. Pharmacodynamics also exhibits wide inter- and intraindividual variation. The drug concentration at the site of action probably relates best with clinical responses; however, it is typically difficult or impossible to measure. From a practical standpoint, plasma drug concentrations usually provide an informative and feasible measurement for defining the pharmacodynamics of medications (Fig. 1). In this regard, several different measures of plasma drug concentrations have been used to define the pharmacodynamics of anticancer drugs, including pharmacokinetic parameters such as systemic clearance, steady-state plasma concentration ($C_{pss}$), and area under the concentration–time curve (AUC).

**CONCEPT OF DOSE-INTENSITY AND SYSTEMIC EXPOSURE**

The initial concept of dose-intensity was championed by Hryniuk and Bush [11], using data from clinical trials of breast cancer. Dose-intensity, the amount of drug given...
per unit time (e.g., mg/m² per week), is based on the assumption that dose scheduling or infusion time does not directly determine tumor cell kill—an assumption that may not be valid for many antineoplastic agents. Furthermore, calculation of dose-intensity gives equal importance to time delays and to dose reductions [12]. The concept of dose-intensity is important only for tumors that are responsive to the medications being evaluated. In such situations, where the dose–response curve is often quite steep, increasing the dose or dose-intensity could result in improved clinical effects [13].

The concept of systemic exposure as an alternative to dose-intensity for anticancer drugs has been reviewed previously [4, 14]. Systemic exposure has been proposed as a potentially more-informative measurement of dose-intensity [4], because measurement of systemic dose-intensity reflects sources of variability beyond simply the dose administered (Fig. 2). Cpss and AUC are parameters of systemic exposure in individual patients. Several different applications of systemic exposure have been used as an intermediate target endpoint for cancer treatment. Pharmacokinetically guided dose escalation in phase I clinical trials was proposed by The European Organization for Research and Treatment of Cancer (EORTC) Pharmacokinetics and Metabolism Group to facilitate determinations of the maximum tolerated dose (MTD) for investigational anticancer drugs [15, 16]. Later, the concept of maximum tolerated systemic exposure (MTSE) was suggested as a further extension of this concept for phase I and II clinical trials of antineoplastic agents [17, 18].

Over the past 15 years, studies have been done to determine the relationship between systemic exposure and clinical therapeutic outcomes. In this review, published results of pharmacodynamic studies for numerous cancer chemotherapeutic agents are summarized in Table 1 and reviewed in greater detail elsewhere [6, 19–24]. Representative examples are discussed in greater detail, including studies of 5-fluorouracil (5FU), MP, and MTX, to illustrate important concepts. Relationships between systemic exposure and both efficacy and toxicity have been elucidated for these three drugs in selected diseases.

**FLUOROURACIL**

5FU is widely used for a variety of solid tumors, including gastrointestinal malignancies, breast cancer, and head and neck cancer. Early studies of 5FU revealed a relationship between toxicity and systemic exposure. Au et al. [43] found that colorectal cancer patients receiving 5-day intravenous infusion of 5FU showed a correlation between hematological toxicity and Cpss; a higher proportion of patients developed leukopenia when the 120-h plasma concentration was >1.5 μmol/L. For 42 patients with advanced colorectal cancer given the same 5FU regimen, a similar relationship was observed between total AUC and toxicity (mucositis, diarrhea, leukopenia, anemia, and thrombocytopenia); a cycle AUC >30 000 μg/L·h was associated with a higher incidence of toxicity [44]. Likewise, in colorectal cancer patients with liver metastases, a higher AUC after intravenous and intrahepatic arterial bolus injection was correlated with decreased platelet and leukocyte counts [45]. Furthermore, efficacy was related to systemic exposure in patients with liver metastases receiving 5FU in continuous infusion; a lower plasma AUC was associated with an increase in hepatic tumor size measured 4 weeks later [46].

An initial study by Thyss et al. [47] showed that 5FU AUC was highly correlated with hematological and gastrointestinal toxicities in patients with head and neck cancer. An AUC threshold value of 30 000 μg/L·h (the same value as in the studies of colorectal cancer) was highly predictive of toxicity [47]. This study was subsequently extended to a larger population of 89 patients and similar results were obtained; the half-cycle and full-cycle AUC (AUC0–3days and AUC0–5days) were higher in toxic than in nontoxic cycles (Fig. 3) [48]. These data were further extended to a study of 186 patients with head and neck tumor who were receiving cisplatin and 5FU for 3 cycles as first-line chemotherapy. For each cycle, the 5FU dose was reduced to prevent toxicity...
Table 1. Summary of selected clinical pharmacodynamic studies with antineoplastic agents.

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<th>Drug</th>
<th>Type of study</th>
<th>Pharmacokinetic parameter</th>
<th>Drug effect(s) measured</th>
<th>Pharmacodynamic relationship</th>
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<td>Pharmacokinetic parameter</td>
<td>Drug effect(s) measured</td>
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Modified and updated with permission from Evans and Relling [4].
when the $AUC_{0–4.8\, \text{h}}$ was $>15\,000\, \mu\text{g/L\cdoth}$. Depending on the measured $AUC_{0–4.8\, \text{h}}$ and dosage adjustment, the total $AUC$ ($AUC_{0–105\, \text{h}}$) would be expected not to exceed $30\,000\, \mu\text{g/L\cdoth}$. Averaging the total $AUC$ for all 3 cycles, Thyss and colleagues demonstrated that patients with an average $AUC$ per cycle $>29\,000\, \mu\text{g/L\cdoth}$ exhibited longer survival. Because the optimal threshold $AUC$ for survival was close to the maximum tolerated $AUC$ for toxicity, they suggested that maximum tolerated $AUC$ should be attained to improve survival [49]. Fig. 4 illustrates the survival curves as a function of the averaged $AUC$ per cycle.

The precise mechanism of 5FU cytotoxicity remains uncertain. Two anabolites of 5FU, 5-fluorouridine and 5-fluoro-2'-deoxyuridine, are thought to be critical for cytotoxicity [50]. Barberi-Heyob et al. showed that $AUC$s of these two anabolites were correlated with toxicity; neutropenia was associated with a high 5-fluorouridine and low 5-fluoro-2'-deoxyuridine $AUC$, while mucositis was associated with high 5-fluoro-2'-deoxyuridine $AUC$ [51]. Further studies involving these anabolites may further elucidate the mechanism of SFU cytotoxicity and better define the optimal extent and measure of systemic exposure.

**Mercaptopurine**

MP is widely used in combination chemotherapy for childhood acute lymphoblastic leukemia (ALL) and in treatment of Hodgkin disease. An inactive prodrug, MP must be converted to its active intracellular nucleotide metabolites to exert cytotoxicity. To this end, intracellular thioguanine nucleotides (TGN) serve as the most informative measure of systemic exposure.

In the early 1980s, Lennard and her coworkers demonstrated that higher erythrocyte (RBC) concentrations of TGN were associated with development of neutropenia in children with ALL [56, 57] and in adults receiving the MP prodrug azathioprine. Subsequent studies demonstrated significantly better ($P < 0.01$) event-free survival in children with ALL who had RBC TGN concentrations above the population median (i.e., $275\, \text{pmol/8 \times 10^8 RBCs}$) in a treatment protocol that largely consisted of antimetabolites [58, 59]. Most studies have used RBC concentrations of TGN as the measure of systemic exposure to active metabolites, with no further differentiation of the mono-, di-, and triphosphate nucleotides of thioguanine. In nucleated cells (e.g., leukocytes, lymphoblasts), these thioguanine nucleotides are incorporated into DNA and RNA—which is considered an important mechanism of cytotoxicity. In erythrocytes, however, TGNs are not incorporated into DNA because mature erythrocytes do not have a nucleus; thus TGN accumulation in RBCs can serve as a measure of chronic systemic exposure to MP.

Thiopurine S-methyltransferase (TPMT) is a cytosolic enzyme that catalyzes the S-methylation of MP, an inactivation pathway that competes with the activation pathway to TGNs [60]. RBC TPMT activity correlates negatively with RBC TGN concentration; patients with high TPMT activity shunt more drugs to the inactivation pathway and less to TGN [59, 61]. TPMT exhibits genetic polymorphism, with $\sim 10\%$ of the population having intermediate activity and 1 in 300 individuals inheriting TPMT deficiency as an autosomal recessive trait. The genetic polymorphism of TPMT activity in RBCs is highly correlated with TPMT activity in all other tissues studied to date, including leukemic lymphoblasts [62]. Patients who inherit TPMT deficiency are at risk of severe hematopoietic toxicity if treated with routine doses of MP or azathioprine [63], and those with heterozygous phenotypes have an intermediate risk of toxicity. However, TPMT-deficient patients can be safely and effectively treated with MP or azathioprine if their dosage is reduced to $\sim 6$–10$\%$ of the conventional dosage [63, 64]; suitable patients can be identified by either measuring TPMT activity, monitoring RBC TGNs, or determining TPMT genotype [65].
Although RBC TGN is theoretically superior to measuring peak MP plasma concentrations (because the former reflects metabolism and TPMT activity in addition to compliance, absorption, and dosage), some studies have indicated a utility of measuring MP plasma concentrations. Hayder et al. found a correlation between the increase in mean MP peak plasma concentration and the decrease in leukocytes and erythrocytes in 20 children with ALL [66]. Koren et al. [67] found a relation between MP plasma AUC (normalized to MP mg/m² dosage) and the probability of relapse-free survival [67], although there are caveats of these findings that require further study [68].

**HIGH-DOSE MTX**

Most of the initial pharmacodynamic studies with MTX focused on the relation between MTX pharmacokinetics and the risk of severe hematopoietic and gastrointestinal toxicity after high-dose MTX (HDMTX) and leucovorin rescue. This has evolved to become the most common example of TDM for cancer chemotherapy. Over 20 years ago, Tattersall et al. showed that patients experiencing toxicity after HDMTX had a higher serum MTX concentration 48 h after bolus injection of MTX than did those without toxicity [69]. In a subsequent study of >100 patients with metastatic tumors and osteosarcoma, Stoller et al. showed that a 48-h plasma concentration >0.9 μmol/L was associated with a high frequency of toxicity [70]; numerous other investigators have reported similar findings [71, 72]. These studies established that if MTX plasma concentrations are too high, conventional low-dose leucovorin rescue will not adequately prevent toxicity. Most cancer centers now routinely measure MTX plasma concentrations at 24 and (or) 48 h after HDMTX, to identify patients who require more leucovorin [72]. This, along with better hydration and urinary alkalinization, has led to a marked decrease in severe toxicity after HDMTX.

Our group investigated the pharmacodynamics of HDMTX (1 g/m²) in children with ALL in first remission, and found that a higher systemic clearance and lower Cpss of MTX was associated with a higher risk of relapse [73, 74]. Children with a Cpss ≥16 μmol/L during a 24-h infusion of HDMTX had a significantly lower relapse rate (P = 0.046), which remained significant (P = 0.008) in a multivariate analysis with MTX Cpss, leukocyte count, hemoglobin, age, sex, race, and DNA index [74]. A similar prognostic value of MTX clearance was subsequently found by Borsi and Moe in 58 children with ALL [75] and in a multiinstitutional trial conducted by the Pediatric Oncology Group [76]. These data provided the basis, in part, for a prospective randomized study to determine whether treatment outcome of childhood ALL could be further improved by individualizing the dosage of HDMTX and other antileukemic agents [77].

More recent studies have also indicated a relation between MTX pharmacokinetics and its anticancer effects in osteosarcoma. Sæter et al. reported a tendency for better relapse-free survival in grade II responders among 98 patients with osteosarcoma who had higher 24- and 48-h serum MTX concentrations, although the difference was not statistically significant [78]. Graf et al. performed a retrospective analysis on three separate clinical studies [79], demonstrating that HDMTX pharmacokinetics was associated with disease prognosis in one of the studies (COS5-80). In this study of 86 osteosarcoma patients, a mean peak MTX concentration >1000 μmol/L was correlated with better tumor response and a longer disease-free survival than at lower concentrations. However, because of other possible confounding factors, these findings were not confirmed in the other two studies. Multivariate analysis of all patients (n = 198) confirmed that mean peak serum MTX concentration was the only significant prognostic factor in an analysis that also included tumor size, tumor site, patient sex, and type of surgery.

As depicted in Fig. 2, controlling treatment intensity by measuring plasma concentration can control for numerous treatment (e.g., compliance, dosage) and pharmacokinetic (e.g., absorption, metabolism, elimination) variables, which one would assume translates to greater treatment intensity with tumor cells. Recent studies of MTX intracellular disposition in ALL blasts of children demonstrate that this assumption has validity, but that characteristics of the leukemia (e.g., lineage, chromosome number) and the treatment (e.g., MTX dosage) influence the relation between intracellular and extracellular drug concentrations [80–83]. Patients with higher MTX Cpss had greater intracellular accumulations of active MTX polyglutamates (MTXPG), although patients with T-lineage ALL had significantly lower MTXPG at the MTX Cpss evaluated (P = 0.001 and 0.03) [82]. Furthermore, there was evidence that MTXPG accumulation may saturate at very high MTX plasma concentrations [80] and that the point at which this occurs differs by ALL lineage. There was also a relation between intracellular MTXPG concentrations in ALL blasts and acute antileukemic effects; complete disappearance of circulating blasts at day 4 was associated with higher values for MTXPG concentration, MTX AUC, and MTX Cpss [81]. Inhibition of de novo purine synthesis was significantly related to long-chain MTXPG concentrations in leukemic blasts, which was well characterized by an Emax model (P < 0.001) (Fig. 5). Pharmacokinetic analysis also showed a relation between MTX Cpss and stomatitis; a higher proportion of patients developed stomatitis as Cpss increased (Fig. 6) [81].

**Multidisciplinary Approach of TDM in Anticancer Drugs**

Establishing a pharmacodynamic relationship between drug concentration and drug effect is an essential foundation for use of TDM to optimize cancer chemotherapy. As progress is made in this area, the rationale for TDM will become increasingly compelling for selected antineoplastic agents. However, the successful implementation of TDM requires more than a “therapeutic range”; it requires a well-coordinated multidisciplinary effort. The proper execution of TDM includes the correct administration of drug, correct collection and processing of blood samples, precise and accurate...
measurement of drug and (or) metabolites, and appropriate interpretation of results [3]. The entire process involves a variety of professionals, including pharmacists, nurses, phlebotomists, technicians/technologists, clinical chemists, clinical pharmacists, pharmacokineticists, and physicians (Fig. 7). Each profession must clearly understand the limitations and difficulties of TDM and make efforts to fully optimize TDM at each step in the process; failure at any step can compromise the entire process.

NURSE/PHLEBOTOMIST
Accurate TDM requires correct administration of the drug being monitored, to ensure accuracy of the schedule and time of administration. Timing of drug administration and body fluid sampling is of particular importance for TDM and estimation of pharmacokinetic parameters. Optimal sampling times are commonly established by prior pharmacokinetic studies and computer simulation, and are usually chosen to provide the most reliable estimates of pharmacokinetic parameters (e.g., clearance, AUC, Cpss, peak) or of the parameter that is best correlated with clinical response. Unreliable pharmacokinetic parameters will be obtained if the reported sample collection time differs substantially from the actual sampling time, and this could adversely affect decisions about the optimal clinical management of the patient. Nurses and phlebotomists must therefore be educated and trained to ensure that the actual sampling time is recorded, and not the sampling time ordered, if the two differ. Adjusting the calculation of pharmacokinetic parameters when the actual sampling time differs from the scheduled time is usually straightforward, but this is possible only if the actual time is properly recorded. The importance of proper data collection by trained personnel was nicely exemplified by Charpiat et al. [88].

The method and site of sample collection are also important for accurate TDM [89]. Most often, blood is the biological fluid to be collected, but attention must be paid to the proper processing of blood samples once collected—some assays use plasma and others serum, and some require special collection tubes that contain additive to stabilize the sample. Given the numerous options that are unique to each drug, it is advisable to prepare a detailed summary sheet stipulating the proper collection and processing of samples for each drug, and make this readily available to all professional staff involved in the process.

CLINICAL CHEMIST/MEDICAL TECHNOLOGIST
Clinical chemists and medical technologists play a critical role in TDM, as precise and accurate quantitation of drugs or metabolites is a sine qua non of TDM. Because of advanced technology, quantification of many commonly monitored drugs or metabolites is automated with commercially available reagents. Except for MTX, however, this is not the case for anticancer drugs, most of which must be measured by HPLC- or gas-chromatographic-based methods. Whether automated or not, it is essential to utilize a well-characterized and fully validated analytical method to measure the drug(s) and metabolites used for TDM. A summary report of another conference, on analytical methods validation: bioavailability, bioequivalence, and pharmacokinetic studies, published in 1992 [90] contains useful discussions of several important issues related to drug analysis for TDM. Ideally, the selected assay should be accurate, precise, simple, rapid, and sensitive, demonstrating minimal matrix effects. Obviously, the method should be clinically and financially

![Fig. 5. Relation between the percent inhibition of DNPS and the concentration of long-chain MTXPG measured in ALL blasts from bone marrow 44 h after initiating MTX as a single agent in 11 consecutive patients with nonhyperdiploid B-lineage ALL who did not receive allopurinol. Each symbol depicts a patient, and the line depicts the best fit of an E_max pharmacodynamic model to the data. The statistical significance for k was determined by two-tailed Student’s test. Reproduced with permission from J Clin Invest 1996; 97:73–80, copyright by the American Society for Clinical Investigation [81].](image)

![Fig. 6. Relation between MTX Cpss and the proportion of patients who developed stomatitis, i.e., grade 2–4 (—) and grade 3–4 ( - - -) up to 10 d after MTX. P value is from univariate logistic regression of MTX Cpss as a determinant of grade 2 stomatitis. Reproduced with permission from J Clin Invest 1996;97: 73–80, copyright by the American Society for Clinical Investigation [81].](image)
feasible if it is to be performed on a routine basis and be cost-effective.

**PHYSICIAN/CLINICAL PHARMACIST**

The primary clinician, be it physician, clinical pharmacist, or other health professional, chooses the initial dosing regimen for the patient and makes subsequent changes of dosage depending on clinical response, TDM, and other clinical or biochemical parameters. Hence, clinicians and consultants must acquire enough knowledge in pharmacokinetics and pharmacodynamics to appreciate the potential and the limitations of TDM, and the sources and consequences of errors that can occur during the TDM process. Clinicians must carefully interpret results of drug concentrations, recognizing that drug measurements are only an intermediate endpoint, with clinical response being the ultimate measure of therapeutic success. Many institutions have established formal TDM consultation services to facilitate optimal integration of pharmacokinetics, drug monitoring, patient evaluation, and drug dosage adjustments.

**PHARMACOKINETICIST**

Well-designed clinical studies are essential to define the pharmacokinetics of medications and accurately elucidate their therapeutic range for TDM. One of the essential roles for the pharmacokineticist is to construct, evaluate, and select mathematically correct and physiologically appropriate models for determination of pharmacokinetic parameters. Their critical analysis is essential because this determines the accuracy of the calculated pharmacokinetic parameters [91] and subsequent dosage adjustments to achieve the target drug concentration. By establishing optimal sampling strategies, one can avoid excessive blood sampling of patients and to facilitate pharmacokinetic studies in large populations [92–96]. Likewise, it is critical to select the appropriate pharmacodynamic models for establishing robust concentration–effect relationships, which can eventually provide the foundation for establishing “therapeutic ranges” for TDM.

**Conclusions**

Numerous studies now establish significant relationships between the concentrations of anticancer drugs or their
metabolites in biological fluids (e.g., plasma) or cells (e.g., erythrocytes) and their pharmacological effects. The great majority of these relationships are for the toxic effects of anticancer agents, and therefore these data have the greatest clinical utility for establishing maximum treatment intensity within individual patients or diagnosing the pharmacological basis of toxicity when combination chemotherapy is being prescribed. Whereas maximum treatment intensity is generally considered most likely to produce anticancer effects, prospective, randomized studies are needed to unequivocally establish those medications and tumor types for which TDM enhances the efficacy of cancer chemotherapy. Once these studies have been completed, and as concentration–effect relationships are established for additional anticancer agents and for a larger number of drug-sensitive cancers, it should be possible to further improve the treatment of cancer patients by enhancing efficacy and minimizing toxicity of cancer chemotherapy.

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References

33. Campbell AB, Kalman SM, Jacobs C. Plasma platinum levels:


