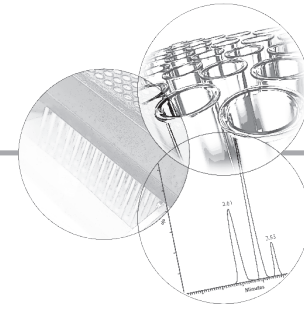


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Therapeutic drug monitoring in cancer chemotherapy

For a select number of drugs, proper management of patients includes monitoring serum or plasma concentrations of the drugs and adjusting the doses accordingly – this practice is referred to as therapeutic drug monitoring (TDM). The need for TDM arises when pharmacokinetic variability of drugs is not easily accounted for by common clinical parameters. Many chemotherapeutic drugs have large interindividual variability, yet TDM is not commonplace in chemotherapy management. This review will discuss pharmacokinetics in the context of chemotherapeutic drugs, examine the few instances where TDM is currently used in the field of oncology and propose other drugs where TDM might be useful for dose adjustments in the management of chemotherapy.

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Introduction & therapeutic drug monitoring basics

The number of treatment options available in cancer chemotherapy has rapidly increased in recent years, yet this field has historically relied on an antiquated approach to individualized drug dosing based on body size measurements (mg/m^2) or weight (kg) and continues to do so in today's clinical environment. This practice has largely developed out of the toxicology concept of species scaling, where pharmacological effects can be related to body size, often log-transformed [1]. Despite dose correction based on body size, the degree of interpatient variation in clinical outcomes following standard doses of chemotherapy is large and plasma concentration of cytotoxic drugs may vary up to 14-fold or even 100-fold in the case of 5-fluorouracil (5-FU) [1]. In order to decrease interindividual pharmacokinetic differences, drug doses should ideally be tailored to the individual patient not by body surface area (BSA), but by plasma concentrations of the drug (i.e., therapeutic drug monitoring [TDM]). If significant relationships between blood concentrations of a particular chemotherapeutic drug and clinical efficacy of a therapeutic regimen can be established, the development of TDM strategies will lead to the selection of an optimal regimen and dose for each individual patient based on drug pharmacokinetics.

Therapeutic drug monitoring has been utilized for the clinical management of patients since the early 1960s [2]. Currently, TDM has widespread application in many different drug classes, such as antiepileptics, cardioactive drugs, bronchodilators, antibiotics, antiretrovirals, antipsychotics, antimetabolites and immunosuppressants. TDM entails the measurement and interpretation of drug concentration in biological fluids for individualization of drug dosages or schedules to maximize therapeutic effects, minimize toxicities or both [2]. Theoretically, a drug should meet several criteria for TDM to be useful, some of which are listed in **Box 1**.

Compared with other drug classes, TDM has seen limited use for chemotherapeutic drugs, except for the routine monitoring of methotrexate (MTX), along with limited monitoring of busulfan and azothioprine. Based on criteria 1–3 listed in **Box 1**, many chemotherapeutic agents appear to be candidates for TDM. However, studies to investigate the utility of TDM in chemotherapy are challenging for many reasons, including the fact that assays for many of these agents are not widely available. The utility of TDM is also limited by the lack of established therapeutic concentration target ranges – there is often no established relationship between blood concentration or drug exposure and clinical efficacy. However, it should be noted that the relationship between drug exposure and toxicity is frequently well established because toxic effects are related to systemic exposure to a drug, as described by general PK parameters. Additionally, the routine use of combination therapy in oncology patients makes establishing relationships between drug concentration and therapeutic end points difficult.

This review will discuss chemotherapy pharmacokinetics by reviewing some of the current approaches to chemotherapy dosing and

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KEY TERMS

Therapeutic drug monitoring: Measuring the blood concentration of a drug and coordinating that concentration with a target concentration related to desired clinical effects

Pharmacokinetics: Study of the time–concentration relationship of drugs in the body (i.e., 'what the body does to the drug')

Box 1. Criteria for therapeutic drug monitoring.

- Significant inter- or intra-individual pharmacokinetics variability.
- Defined relationship between blood concentration and pharmacological effects.
- Narrow therapeutic index.
- Availability of a precise and accurate assay.

describing the sources of potential pharmacokinetic variability. In addition, this paper will investigate the current applications of TDM in cancer chemotherapy and explore potential future candidates for TDM based on current research.

Pharmacokinetics

■ Pharmacokinetics basics

The pharmacological effect of a drug is elicited by direct interaction of the drug with a receptor controlling a specific function, or by a drug-mediated alteration of the physiological process regulating the function [3]. Studies have shown that for many drugs a strong correlation exists between the serum drug concentration and the observed pharmacological effect [3]. In pharmacokinetic studies, it is assumed that changes in drug concentration in blood versus time are proportional to changes in local concentrations at the receptor site or body tissue. This assumption is called the property of kinetic homogeneity and is applicable to all pharmacokinetic models in post-absorptive and post-distributive phases of the time course [3]. The property of kinetic homogeneity is an important assumption in TDM because it is the basis on which all therapeutic and toxic concentration reference values are established.

Pharmacokinetics is the study of how the body processes and, ultimately, eliminates drugs and includes the analysis of absorption, distribution, metabolism and elimination. Below is a brief exploration of these major areas of pharmacokinetics in the context of cancer chemotherapy. A summary of the major aspects of pharmacokinetics and the potential impact of polypharmacy is shown in **FIGURE 1**.

■ Absorption

Most chemotherapeutic agents are administered intravenously or orally. The fraction of a drug that is absorbed into the systemic circulation after oral administration is referred to as the bioavailability. Intravenous administration of a drug directly introduces it into the systemic circulation and results in rapid and complete bioavailability.

For oral administration to be useful, the bioavailability of a drug must be great enough that the active component will quickly and efficiently pass from the gastrointestinal system into the systemic circulation. Bioavailability must typically be greater than 70% for oral administration of drugs to be considered useful [3]. The absorption process depends on the drug dissociating from its dosed form (liberation), dissolving into gastrointestinal fluids and diffusing across biological membranes into the bloodstream [3]. After entering the portal circulation, the drug is also subjected to first-pass metabolism by the liver. As a result, oral agents typically have a far greater pharmacokinetic variability than intravenous agents. A particularly good example of this is demonstrated in a study of etoposide, an epipodophyllotoxin used in testicular and lung cancers. The coefficient of variation for the AUC for oral versus intravenous administration of etoposide was shown to be 58 and 28%, respectively [4]. Due to the risk of subtherapeutic drug concentrations, etoposide is uniformly administered intravenously when used for testicular cancers.

■ Drug distribution

Once a drug enters the circulation, it interacts with carrier proteins, such as albumin and α1-acid glycoprotein (AAG), and is carried by various transport processes to different organs and tissues. The equilibrium that exists between protein-bound and free drug is one important factor in determining overall drug distribution. Only the free (unbound) drug fraction is able to cross cellular membranes and elicit a biological response. The protein-binding characteristics of a drug can have a profound influence on the distribution and elimination of that drug and on the manner in which steady state concentrations are interpreted. Acidic drugs are typically bound primarily to albumin and basic drugs are largely bound to α1-AAG [3].

Due to the influence that drug–protein binding can have on drug distribution, differences in plasma protein levels can result in pharmacokinetic variability. As an example, malnutrition and liver metastases can cause a significant decrease in serum albumin, which can lead to an increase in unbound drug. Conversely, inflammatory conditions may increase drug–protein binding due to increased levels of α1-AAG and other acute phase reactants [5].

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■ Metabolism

The liver is the principle organ responsible for drug metabolism and plays a crucial role in pharmacokinetic variability. Once a drug is distributed into the liver by transporter proteins, the drug can be biotransformed by phase I (chemical alteration) and/or phase II (conjugation) reactions before elimination. The polymorphic genes of transporter proteins as well as phase I and phase II enzymes are a major source of interindividual pharmacokinetic variability.

Hepatic uptake

Organic anion-transporting polypeptide (OATP) proteins are a family of transporter proteins that move drugs from the circulation into hepatocytes. A study by Nozawa *et al.* has shown that OATP1B1 is involved in the hepatic disposition of SN-38 (active metabolite of irinotecan; 7-ethyl-10 hydroxycamptothecin) and that genetic polymorphisms of OATP1B1 may contribute to the known interpatient variability in the disposition of irinotecan [6]. Among the variants examined by Nozawa, the OATP1B1*15 reported allele frequency of 10–15% and exhibited decreased SN-38 transport activities.

Phase I metabolism

Phase I 'nonsynthetic' metabolism modifies the drug's chemical structure through oxidation, reduction, hydrolysis, cyclization and decyclization reactions. Of particular importance is the cytochrome P (CYP) 450 isoenzyme family, which plays a dominant role in oxidative reactions in phase I metabolism. Important isoforms for anticancer drugs include CYP1A, CYP2B, CYP2C and CYP3A [7]. According to Baker *et al.*, CYP3A activities in patients with advanced cancer demonstrated a 14-fold variation in a population of 134 patients [7]. This study's multivariate analysis concluded that C-reactive protein, as well as concomitant use of other drugs, food constituents and complementary and alternative medicines had either inhibitory or inductive effects on CYP3A in their patient population. Another example of phase I metabolism is cytochrome CYP2C19, an enzyme involved in the metabolism of the chemotherapeutic agent cyclophosphamide. Cyclophosphamide is used to treat both lymphoma and breast cancer patients. One study has demonstrated that CYP2C19 reduced activity in patients with advanced metastatic cancer [8].

Phase II metabolism

Phase II conjugation reactions are usually detoxification reactions that involve conjugation of glucuronide, acetate, glutathione, methyl, glucose, sulfate or methionine to the polar functional groups of phase I metabolites. Products of conjugation reactions have increased molecular weight and are typically inactive. One of the most well-studied chemotherapy agents metabolized by phase II metabolism is irinotecan. Pharmacokinetic variations of UDP-glucuronosyltransferases (UGTs) involve polymorphic variants in which transcriptional efficiency correlated with TA repeats in the TATA box of UGT1A1 and could lead to variations in plasma concentrations of irinotecan [9,10].

■ Drug elimination

Excretion of chemotherapeutic agent occurs mainly through the biliary tract and kidney. Elimination though pathways such as lungs, skin, sweat and tears are negligible.

Biliary excretion

A source of biliary excretion of amphipathic and lipid soluble chemotherapeutic agents is through ATP-binding cassette (ABC) transporters

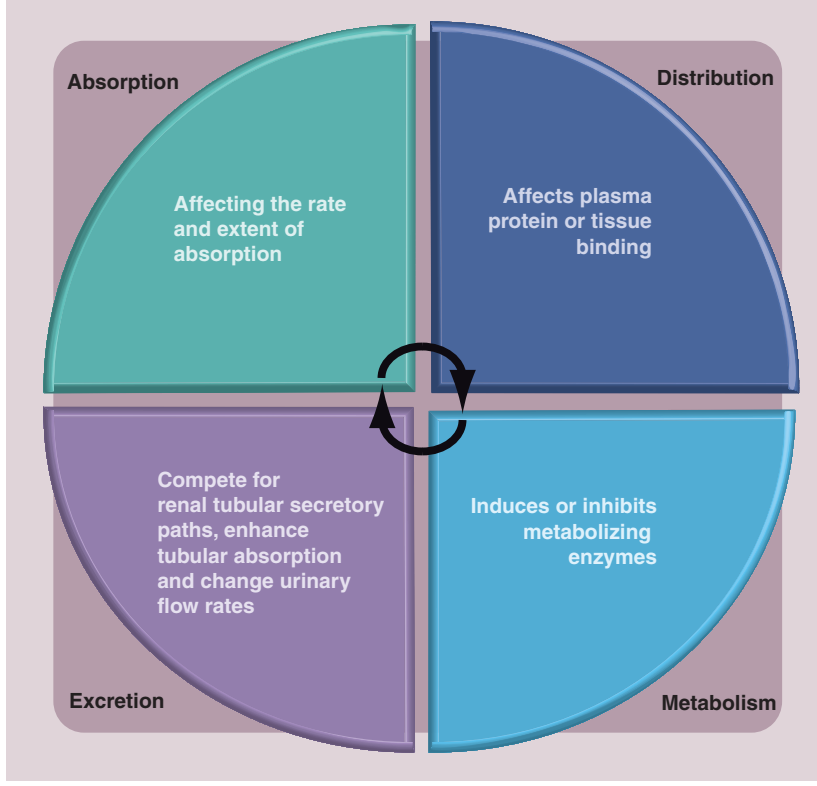


Figure 1. Potential impact of polypharmacy on pharmacokinetic parameters. Summary of aspects of absorption, distribution, metabolism and excretion.

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ABCB1 and ABCC2 [6]. ABCB1 is involved in the transport of amphipathic and lipid soluble drugs, whereas ABCC2 is mainly involved in the excretion of conjugate metabolites. Specific polymorphisms of ABCB1 and ABCC2 can influence disposition and tumor response to irinotecan by regulating transported activity, as shown in patients with advanced small-cell lung cancer [11]. Other causes of pharmacokinetic variability include disease processes affecting the liver and its synthetic abilities. For example, mechanical obstruction by hepatic metastasis has been associated with decreased excretion of chemotherapeutic agents [12,13].

Renal clearance

Renal clearance is another major route for drug elimination. Two examples of pharmacokinetic variability in drugs eliminated primarily by renal clearance are cisplatin and MTX. Cisplatin is a platinum agent used in bladder and testicular cancer. When co-administered with NSAIDS, the NSAIDS can cause renal insufficiency and have lethal effects [14]. MTX is an antifolate analogue used in leukemia and breast cancer treatment. Subtherapeutic levels of MTX have been identified when co-administered with bicarbonate, because bicarbonate increases urine pH, which leads to increased MTX clearance [15].

Other sources of pharmacokinetic variability

■ Age

All four components of drug disposition (absorption, distribution, metabolism and excretion) are affected by age. Since there is an 11-fold greater incidence of cancer in individuals of more than 65 years of age and more than 60% of all diagnosed cancers are found in individuals in this older age group, this makes age-dependent drug disposition of fundamental importance [16]. Age-related changes in chemotherapeutic drug absorption include reduced gastric acid secretion, reduced gastrointestinal motility and reduced splanchnic blood flow in patients with increased age [17–19]. Changes in distribution can also result from an increased proportionality of body fat, reduced intracellular water or reduced plasma albumin [20–22]. Metabolic changes with age include a reduction of 20–50% of liver mass in patients of 80 years of age or older and up to a 30% reduction of CYP450 activity in patients over the age of 70 when compared with patients in the 20–29-year age group [23,24]. Changes to drug excretion in older individuals include reduced hepatic blood flow and a glomerular filtration rate (GFR) reduction of 1 ml/min per year over the age of 40 [20–22]. Age-related changes drastically affect the pharmacokinetics of drug disposition and should be taken into account in chemotherapy treatments.

■ Polypharmacy

The co-administration of drugs can also affect the four major components of drug disposition. Polypharmacy effects are vast and include changes in absorption, distribution, metabolism and elimination. One example of polypharmacy influencing pharmacokinetics is with the study of tamoxifen, a breast cancer chemotherapy agent, when concomitantly used with selective serotonin reuptake inhibitor antidepressants. In a study of 80 breast cancer patients, the mean plasma concentration of endoxifen, an active metabolite of tamoxifen, was decreased by 56% with co-administration of the selective serotonin-reuptake inhibitor paroxetine [25].

Chemotherapy dosing

■ Body surface area

Cytotoxic chemotherapy is characterized by significant variability in toxic and therapeutic effects. In an attempt to reduce this variability due to interindividual pharmacokinetic differences, chemotherapy doses are individualized according to a patient's BSA. The concept was based on studies that established that the basal metabolic rate varied among species as a function of a power less than 1 (-0.075) of weight, which corresponds to the variation of BSA as a function of weight [26]. The original formula based on length and weight was developed by Du Bois and Du Bois in 1916 and was applied to clinical pharmacology in the 1950s to predict safe starting doses in Phase I clinical trials [26]. Shortly thereafter, the formula was adopted for dose calculation of cytotoxic drugs. Below is one of the most commonly used formulas for BSA:

$$BSA\left(m^2\right) = 0.007184 \times (height)^{0.725} \times (weight)^{0.425}$$

The advantages of using this method include a simple and easy-to-use formula and early studies have statistically correlated the BSA with pharmacokinetics (docetaxel and paclitaxel) [27,28]. It should be noted that another study by Rudek *et al.* has shown that BSA does not sufficiently correct for variability in docetaxel [29].

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Conversely, the pharmacokinetic parameters of many other chemotherapeutic agents do not correlate with BSA and BSA dosing has failed to produce consistent systemic drug exposure [1]. In clinical practice it is more common to reduce doses in response to adverse effects than to increase dose intensity when the therapy is well tolerated. This can be explained by the fact that it is easy to recognize toxic levels but it is difficult to detect subtherapeutic levels. For example, trials of adjuvant cyclophosphamide, doxorubicin and 5-flourouracil in breast cancer have shown that underdosing may lead to an almost 20% relative reduction in disease-free survival [1,30–36].

■ TDM & pharmacokinetics-based dosing

A priori method

A priori methods allow the estimation of the dose necessary to reach a target exposure in a given patient using morphological, biological and physiological data such as body weight, age, gender, serum creatinine level and GFR [37]. The dosing of carboplatin by Calvert's and Chatelut's formulas provides an important example of how individual dose estimation is determined using the *a priori* method. Calvert's formula calculates a target AUC between 3 and 8 mg/ml using GFR [38]. Conversely, Chatelut's formula was developed using pharmacokinetic modeling that requires age, gender, weight and serum creatinine levels [39]. Although *a priori* methods account for some interindividual pharmacokinetic variability, their performance is not as good as that of *a posteriori* methods because there is a typical variability of 20% [37].

Test-dose method

The test-dose method is composed of two steps [37]:

- The individual pharmacokinetic parameters are determined after a bolus injection of a moderate dose;
- The parameters are used to calculate the full dose necessary to achieve the target exposure.

This method has been used for MTX owing to the frequent dose adjustments necessary to accommodate the significant interindividual pharmacokinetic differences observed with this drug [40,41]. Use of this approach to chemotherapeutic dosing requires that the drugs have linear pharmacokinetics and may require additional blood samples to determine pharmacokinetic patterns before efficient treatments are begun.

A posteriori methods

Nomograms

A nomogram is a graphical calculating tool with a 2D diagram designed to allow the approximate graphical computation of a function. They have been routinely used for dose adjustments of 5-FU, with the x-axis representing the observed AUC (0–48 h) values and the y-axis representing the percentage of decrease in dose needed to reach the targeted AUC. In one study, 5-FU dose adjustments using the nomogram method were used in a population of patients with head and neck carcinoma. The study showed that by using nomograms for 5-FU, the patients were getting similar therapeutic response rates and reduced toxicities while being administered a lower mean dose [42].

Multilinear regression

Multilinear regression models describe the relationship between exposure-variable AUC values at different time points by using two to three blood samples collected at specified intervals. The exposure-variable AUC is a linear function of the concentration of drug measured at particular time points. This method has been proposed for monitoring AUCs of etoposide, doxorubincin, carboplatin, cyclophosphamide, topotecan, irinotecan and chemotherapeutic metabolites. However, it has not shown sufficient precision for use as a TDM approach [43–52] and is not considered viable with clinical practice owing to the requirement for precise blood sampling, which is difficult to achieve in routine clinical practice.

Bayesian method

The Bayesian estimation method for dose individualization requires a pharmacokinetic population model and a few concentration data points collected after or during administration of the drug. It is a one-stage method where data observed in all patients are considered as a whole, so that it is possible to scatter the different sampling times amongst several patients. The Bayesian method assumes that each patient is a member of a specific population defined by the values intrinsic to the population. This allows for the possibility of having only one blood sampling per patient, compared with multiple sampling ith other methods, and thus provides the flexibility to accommodate the few data points at suboptimal time points that may be seen in some clinical settings [37].

One of the most frequently used Bayesian methods is the nonlinear mixed effect model. With this model, interindividual pharmacokinetic variability can be partly explained by

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KEY TERM

Immunoassay: Method of analysis using antibodies to selectively bind and quantitate the analyte of interest

individual factors (covariates) such as age, gender or weight [37]. This method provides information on which covariates are used in determining the pharmacokinetics of the drug and how much variability can be explained by them. Bayesian estimation has been used in TDM for 30 years, but only a few of these models have been used in clinical trials or routine practice of chemotherapeutic agents. These agents include MTX [53,54], carboplatin [55–58] and suramin [59,60]. In addition, Bayesian estimators have been developed for cisplatin, etoposide, teniposide, doxorubicin, pirarubicin, irinotecan, paclitaxel, topotecan, vincristine and vinorelbine [37]. With the use of Bayesian dose adjustments, fewer toxic effects were reported. Major advantages and disadvantages of a Bayesian modeling approach are listed in **Box 2**.

Current & prospective use of TDM in chemotherapy agents

■ Current TDM

Methotrexate

Methotrexate is a folic acid antagonist that inhibits DNA, RNA and protein synthesis in proliferating cells and is widely used in the treatment of leukemias and lymphomas. Most notably, a relationship between MTX treatment and efficacy has been established in acute lymphoblastic leukemia (ALL). In 1998, Evans *et al.* reported that pediatric ALL patients that were treated with individually adjusted doses of MTX had significantly better outcomes than those given a fixed dose [61]. Before being adopted as an oncology treatment, MTX was used in low doses for the treatment of autoimmune diseases, including rheumatoid arthritis, psoriasis and Crohn's disease.

Box 2. Advantages and disadvantages of Bayesian modeling.

Advantages

- It is possible to visually check modeling efficiency by looking at the concordance between the measured concentration and the predicted concentration time curve.
- Estimation of several pharmacokinetic parameter values and exposure indices simultaneously.
- Flexibility with respect to sampling times, rendering this method more relevant to the constraints of clinical development.
- Inclusion of new data in the population database allows progressive refinement of the population pharmacokinetic mode.

Disadvantages

- Estimator is difficult to develop.

Therapeutic monitoring of MTX drug concentrations is a common practice due to the high interpatient variability of pharmacokinetics, efficacy and toxicity. Additionally, MTX blood concentrations are directly related to observed pharmacological effects. Both the mechanism of action and reported drug toxicities are dependent upon the MTX dosing regimen used (low- or high-dose). Monitoring high-dose therapies is particularly desired, due to the increased costs and toxicities associated with this dosing regimen. Efficacy of MTX dosing is widely variable due to differences in cancer cell sensitivities to the drug [62]. In fact, tumor cells are occasionally reported to demonstrate resistance to MTX, thus drug monitoring may help confirm whether therapeutic levels have been reached in these patients. Another unique feature of MTX that makes monitoring drug concentrations beneficial is the ability to reverse the action of the drug with folinic acid (leucovorin). Monitoring plasma concentrations allows for individualized leucovorin rescue therapies; when toxic MTX levels are reached, leucovorin treatment 'rescues' bone marrow and intestinal epithelial cells by allowing them to resume DNA replication.

As mentioned previously, the test-dose method is frequently employed for monitoring MTX therapies in oncology patients. Serum or plasma concentrations are commonly monitored every 24 h for a period of 3 days [63]; however, several different infusion protocols exist. Bayesian methods have also been used to not only monitor dosing strategies, but also to monitor leucovorin rescue therapy [64]. Immunoassay techniques have routinely been used for detecting MTX concentrations, although chromatographic assays have recently been developed [65].

Busulfan

Busulfan is an alkylating agent primarily used for the treatment of chronic myelogenous leukemia (CML), often in high doses as a conditioning agent prior to bone marrow or hematopoietic stem cell transplantation. Alkylation of DNA by busulfan specifically targets proliferating myeloid cells. For myeloablative purposes, busulfan is often co-administered with cyclophosphamide. Both oral and intravenous busulfan are commonly dosed by body weight (mg/kg). Plasma busulfan concentrations are routinely monitored in high-dose patients (16 mg/kg) due to

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the well-documented inter- and intra-individual pharmacokinetic variation, narrow therapeutic index and direct relationship between exposure and outcome [66–68]. Children in particular exhibit a high degree of busulfan pharmacokinetic variation, with differences documented in age, disease and dosing regimens [66,69–71]. Population pharmacokinetic modeling has also confirmed the wide interpatient variability that is observed in pediatric patients [72]. Toxicities associated with busulfan use include veno-occlusive disease, neurological disorders, pancytopenia and, more rarely, interstitial pulmonary fibrosis ('busulfan lung') [63,67]. Newer therapies commonly incorporate imatinib in place of busulfan in order to reduce these associated toxicities [73].

Therapeutic drug monitoring of busulfan concentrations is commonly achieved by reporting AUC or steady state concentrations (C_{ss}). Owing to differences in routes of administration and dosing intervals, several samples are needed to establish the pharmacokinetic pattern of busulfan therapies. Obtaining seven or 12 points of measurement throughout a preliminary drug course provides a concentration–dose curve that aids clinicians in patient-specific dose adjustments. Limited sampling models aim to use as few as two to four drug concentrations to monitor therapy, in order to decrease the time-consuming and complex nature of monitoring this drug [74–76]. However, although these approaches limit the number of blood draws required from each patient, the timing of each draw is critical to final calculations and the utility of these models in specific patient groups must still be evaluated. The use of Bayesian pharmacokinetic modeling has been reported to reduce toxicity and mortality in pediatric bone marrow and hematopoetic stem cell-transplant patients [74,77–79].

Busulfan concentrations can be monitored in serum, plasma and cerebrospinal fluid by chromatography with and without MS or by immunoassay methods [63,80]. ELISA formats have also recently been adapted to detect busulfan [81]. Newer LC and MS/MS methods to detect busulfan concentrations in oral fluids have recently been described [69]. Use of this noninvasive sample type aims to alleviate the risk and inconvenience of multiple blood draws for monitoring therapies.

Azathioprine

The thiopurine agent azathioprine has been used as an immunosuppressant for a wide range of diseases, including multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease and also for transplantation. Azathioprine, acting as a prodrug, and its main metabolite, 6-mercaptopurine (MP), are also used for the treatment of lymphomas and childhood ALL. Both drugs act by inhibiting purine metabolism and, thus, DNA synthesis, in proliferating lymphocytes. Azathioprine and 6-MP pharmacokinetics and the formation of cytotoxic metabolites can vary considerably between patients. Absorption of azathioprine is necessary to exert the effects of the inactive prodrug, and the inactivation of 6-MP by thiopurine methyltransferase is highly polymorphic between individuals [82]. Population studies of xanthine oxidase, an important metabolizer of MP, have shown a four- to ten-fold interindividual variation [83]. A decreased level of xanthine oxidase potentiates the cytotoxic effects of MP, as seen by the co-administration of the xanthine oxidase inhibitor allopurine. Due to these variations and the established relationship between 6-MP pharmacokinetics and efficacy, routine monitoring of azathioprine and 6-MP concentrations often help improve dosing strategies in patients [84].

6-mercaptopurine concentrations are quantified to monitor most azathioprine therapies. Alternatively, 6-MP eventually forms 6-thioguanine nucleotides that can also be followed for TDM. Thiopurine methyltransferase activity in red blood cells can also be determined to predict whether an individual patient is at risk for excessive myelosuppression after exposure to thiopurine agents. Drug concentrations can be measured in plasma, urine, red blood cells, neutrophils and lymphocytes using chromatographic methods [85]. Urine concentrations of 6-MP have been correlated with plasma AUC concentrations and may provide a less-invasive alternative to multiple phlebotomies for TDM [86].

Prospective chemotherapy candidates for TDM

■ mTOR inhibitors

Sirolimus, also known as rapamycin, is an immunosuppressant agent originally developed as an antifungal agent. However, this use was abandoned when it was discovered that the drug possessed significant antiproliferative and immunosuppressive properties. Sirolimus, along with the its derivative everolimus, comprise a class of drugs known as inhibitors of mTOR. These drugs have primarily been used to prevent rejection in organ transplantation and are now finding increased use in the treatment of some

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cancers [87]. Both sirolimus and everolimus are metabolized by CYP3A4, a family of enzymes involved in the metabolism of several immunosuppressive agents used in organ transplant and chemotherapy [88]. The genetic polymorphism of these enzymes and combination of drug therapy can affect plasma concentrations of mTOR and may lead to toxicities or treatment failure [89,90]. Cattaneo *et al.* analyzed 2658 sirolimus trough samples in 495 kidney transplant recipients and found that dose-normalized sirolimus trough concentrations were significantly higher in patients treated with cyclosporine and sirolimus, compared with patients treated with mycophenolate mofetil and sirolimus or with patients treated with steroids and sirolimus [91]. They also showed that mean intra- and interpatient variability was 19 and 47%, respectively. Their study suggests that different immunosuppressants significantly interfere with sirolimus pharmacokinetics and can affect clinical outcomes. Several studies with everolimus have demonstrated the same potential for interindividual variability and drug–drug interactions as seen with sirolimus in a review by Sanchez-Fructuoso [92].

In the context of cancer chemotherapy, the issue is more complicated. There are several studies that demonstrate a favorable response (overall survival, progression-free survival and median time to progression) when mTOR inhibitors are added to existing chemotherapy regimens [93–95]. However, as with many of the other chemotherapeutic agents, there is no definitive correlation between drug exposure and desirable clinical response. There is a well-defined relationship between drug exposure and toxic effects, but the conventional wisdom is that TDM is not necessary and toxic effects can be mitigated through early recognition and prompt intervention to provide supportive measures for the patient [96]. Although TDM of mTOR inhibitors is the standard of care in transplantation, it is not widely implemented – if at all – in the field of clinical oncology, with the exception of monitoring immunosuppression in bone marrow transplant patients [97].

■ 5-flourouracil

5-flourouracil has been used in the treatment of cancer for over 40 years. Its mechanism of action involves the inhibition of thymidylate synthase which blocks the synthesis of pyrimidine thymidine, a nucleotide required for DNA replication. 5-FU effects are seen in rapidly dividing cancer cells that rely heavily on nucleotide synthesis. It has principally been used in the treatment of colorectal cancer and pancreatic cancer since 1978 [98]. One source of variability in patient response is a functional variant of dihydropyrimidine dehydrogenase (DPD) resulting in deficiency of that enzyme; DPD is responsible for the degradation of 80–90% of 5-FU. A total of 19 DPD variant alleles have been identified with decreased enzyme activity, one of which is a point mutation resulting in a truncated protein with reduced activity [99]. In addition, pharmacokinetic variability can account for variation in patient response. Recent studies have shown that the current standard approach for calculating treatment doses of 5-FU is not effective in achieving optimal plasma concentration. The standard BSA-based dosing is associated with up to 100-fold plasma 5-FU variability and is a major contributor to treatment toxicity and treatment failure. Two studies exemplify the limited utility of 5-FU dosing based on BSA. The first is a study by Gamelin *et al.*, where the authors established a lack of association between BSA and 5-FU clearance in 81 patients with metastatic colorectal cancer [100]. The second study by Milano *et al.* showed a similar lack of association between BSA and 5-FU clearance in a study of 380 patients with head and neck cancer [101].

Clinical studies in the past 20 years have demonstrated reduced toxicity and improved clinical outcomes with pharmacokinetic dose management. These studies have identified an optimal target therapeutic range and formulated dose-adjustment algorithms for optimal plasma concentration ranges [98]. Direct monitoring of 5-FU may be a more rational approach compared with the standard approach and would aid in achieving optimal systemic drug exposure in a more personalized manner. One of the most recent studies of 208 patients with metastatic colorectal cancer by Gamelin *et al.* demonstrated a response rate of 18.3% and an overall survival of 16 months using BSA dosing, while a response rate of 33.7% and an overall survival of 22 months using pharmacokinetically guided dosing. They concluded that individual FU dose adjustment, based on pharmacokinetic monitoring, resulted in improved objective response rates, a higher survival rate and fewer toxicities [102].

■ Imatinib

Imatinib is a chemotherapeutic agent used in the treatment of CML and gastrointestinal stromal tumors (GISTs). It is a 2-phenylaminopyrimidine

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derivative that functions as a specific inhibitor of many tyrosine kinase enzymes by occupying the tyrosine kinase active site, leading to a decrease in enzyme activity. In patients with CML, a clonal myeloproliferative disorder of hematopoietic stem cells, a cytogenetic presence of the Philadelphia (Ph) chromosome t(9,22)(q34;q11) occurs in 90–95% of patients. The formation of the Ph chromosome results from the *ABL* protooncogene being translocated from chromosome 9 to the *BCR* gene located in chromosome 22. The resultant fusion gene, *BCR–ABL*, transcribes a chimeric 8.5 mRNA that translates into a protein denoted as p210. This protein increases tyrosine kinase activity and transforms normal hematopoietic cells into CML cells [103,104]. Imatinib acts by binding to the ATP binding pocket of the BCR–ABL fusion chimeric protein, causing a stabilization of the inactive form of BCR–ABL [105]. Treatment with imatinib results in a complete hematological remission rate of more than 95% and a major cytogenetic response rate of more than 80% in newly diagnosed CML patients [106].

Studies of imatinib's pharmacokinetic have shown high bioavailability of 98% when administered orally [106]. In the systemic circulation, it is 95% bound to plasma protein albumin and α-1 AAG. It is primarily metabolized in the liver by several isoenzymes of the CYP450 system, including CYP3A4 and, to a lesser extent, CYP1A2, CYP2D6, CYP2C9 and CYP2C19 [107]. It is eliminated predominately by fecal excretion (68%) and in urine (13%) [106]. Several factors have been proposed as determinants of suboptimal concentration of imatinib in patients undergoing oral imatinib therapy, including baseline presence of BCR–ABL mutations [108,109], interindividual variability of metabolizing enzymes (CYP system) [110,111] and variability in efflux and uptake transporter activities [112].

The standard recommended dose is 400 mg daily in the chronic phase and 600 mg in the accelerated phase or blast crisis [105]. The mean plasma trough C_{ss} 24 h after administration of 400 mg of imatinib is 1.46 μM/l [113]. However, despite adequate dosing, not all patients in the chronic phase of CML achieve cytogenetic responses (cytogenetic response is defined by the percentage of Ph chromosome identified during metaphase in bone marrow). A study by Larson *et al.* reported that the reason for poor therapeutic response in some patients was the interindividual variation in plasma levels of imatinib [107]. In addition, Druker *et al.* [113] and Peng *et al.* [114] reported that a complete hematological response was achieved when plasma imatinib levels were higher than 1 μM. A recent study by Singh *et al.* reported mean plasma levels of imatinib in responders were significantly higher than those in nonresponders (2.34 ± 0.52 vs 0.69 ± 0.15 μM) [106].

Gastrointestinal stromal tumor is the most common mesenchymal tumor of the GI tract with activating mutations of the *KIT* or *PDGRRA* genes [115,116]. Imatinib targets the ATP binding site of the tyrosine kinase region in KIT and PDGF receptor-interrupting proliferation and survival signaling pathways within the cell [117,118]. Constitutive activation of c-KIT, associated with various mutation profiles is observed in the majority of GISTs. The most common mutation of the KIT gene is located on exon 11 and is associated with a better response to imatinib when compared with another common mutation in exon 9 [119]. The exon 9 mutation occurs in 10–15% of patients and is a subset of GISTs that has an aggressive clinical behavior [120]. Approximately 10% of patients have undetectable mutations (wild-type [wt]) [121]. Widmer *et al.* recently showed that patients harboring a 'sensitive' c-KIT genotype (exon 11 mutation) are exposed to concentrations that are near the top of the concentration–response curve while patients with the 'resistant' genotype (exon 9 mutation) are lying in the steep part of the curve where the concentration–response relationship can be observed. Patients with exon 9 mutations would benefit from adjustment of their imatinib exposure levels owing to the ability to correlated concentration and therapeutic effect [122].

Additionally, interindividual plasma concentration could also be caused by: an increase in cellular efflux of imatinib by P-gp transporter [122], a decrease in cellular influx mediated by the uptake carrier organic cation transporter (hOCT1) [123], modulation of imatinib binding to α1-binding glycoprotein in plasma [119] and enhanced drug metabolism [124]. A given dose therefore yields very different circulating concentrations between patients, as shown in data by Larson *et al.* [107]. Demtri *et al.* also recently correlated that in patients with advanced GIST, IM trough levels at steady state were associated with clinical benefit and patients with imatinib C_{min} below 1100 ng/ml showed a shorter time to progression and lower rate of clinical benefit [119]. These findings suggest that the plasma levels of imatinib affect response to therapy and TDM should be used to check optimal therapeutic levels

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in routine clinical practice. Another interesting study demonstrated that everolimus administered in combination with imatinib shifts the equilibrium of imatinib between red blood cells and plasma toward the red blood cells, such that plasma availability is reduced [125].

■ EGF receptor inhibitors

The EGF receptor (EGFR) is a cell surface receptor for members of the EGF family of extracellular protein ligands. It acts as a tyrosine kinase receptor, triggering complex intracellular signaling cascades. Mutations involving EGFR often lead to constant activation, which then results in uncontrolled cell division and a predisposition for cancer [126]. Mutations that lead to EGFR overexpression, known as upregulation or overactivity, have been associated with a number of cancers, including lung, pancreatic, colorectal and head and neck cancers. Consequently, EGFR is the target of an expanding class of anticancer therapies. Some examples are gefitinib for lung cancer, erlotinib for lung and pancreatic cancer and cetuximab for colon, head and neck cancers. The pharmacokinetics of these three agents are discussed below.

Gefitinib is a drug that inhibits EGFR tyrosine kinase by binding to the ATP binding site of the enzyme, thus blocking the RAS signaling transduction cascade in the EGFR tyrosine kinase pathway. It is a lipophilic dibasic compound with two pKa values of 5.28 and 7.14 and exhibits a pH-dependent solubility in gastrointestinal fluid. The solubility of gefitinib in aspirated human gastric fluid at pH 5.0 is 4.98 gm/ml and in aspirated intestinal fluid at pH 7.0 is 0.085 mg/ml [127]. Due to its dibasic properties, studies have shown that changes in the gastric pH levels alter the oral bioavailability of gefitinib [128]. Gefitinib is extensively distributed in the body and is mainly eliminated as a metabolite [129]. A dose of 250 mg of gefitinib has a variable plasma half-life of 39.7 h with a range between 26.9–83.2 h [128]. A recent study by Wilson *et al.* reported that interindividual differences in plasma exposure to gefitnib is likely multifactorial and may include low jejunal pH, increased expression of enzymatic and transporter activity and rapid small intestine transit [130].

Erlotinib is an orally active EGFR tyrosine kinase inhibitor that blocks EGFR-mediated intracellular signaling and arrests cell-cycle progression in the G1 phase [131]. Erlotinib is indicated for the treatment of patients with locally advanced or metastatic non-small-cell lung cancer after failure of at least one prior chemotherapy regimen and is approved for use in combination with gemcitabine chemotherapy for treatment of locally advanced, inoperable or metastatic pancreatic cancer [132]. Erlotinib is extensively metabolized by hepatic enzymes CYP3A4 and CYP1A2 and to a lesser degree by the extrahepatic enzyme CYP3A4 in the intestine, CYP1A1 in the lung and CYP1B1 in tumor tissues [133]. It is 95% protein bound in plasma and has a clearance of 6.3 l/h and a volume of distribution of 136.4 l. It has a highly variable C_{ss} of 1200 ± 620 ng/ml and a mean half-life of 24.4 ± 14.6 h [134]. Lu *et al.* studied 1047 patients receiving erlotinib with non-small-cell lung cancer to assess the pharmacokinetics of erlotinib in a large patient population and reported total bilirubin, α1-AAG and smoking status as the most critical factors explaining interindividual variability for erlotinib clearance [134]. This population pharmacokinetic model predicts that patients with a high concentration (95th percentile) of AAG (2.3 g/l) or total bilirubin (1.1 mg/ml) would have erlotinib clearance decrease by 31 and 30%, respectively. Furthering Lu's paper, Thomas *et al.*, used population pharmacokinetics in patients with squamous cell carcinoma to reported patient age, hepatic function, ABCG2 genetic polymorphism and smoking status to contribute to the high interindividual variability in erlotinib pharmacokinetics [135].

■ Platinum-based agents

Cisplatin is a platinum-based chemotherapy drug widely used to treat various types of solid tumors, including sarcomas, small-cell carcinoma, ovarian cancer, lymphomas and germ cell tumors. Owing to the strong pharmacokinetic relationship between dose and therapeutic response, dose intensity is important to achieve optimal therapeutic response. Early studies have shown that free cisplatin clearance is related to patient's BSA, therefore dosage is mostly adjusted using the *a priori* method, with BSA as the single parameter [136,137]. Using this approach, later studies have shown mixed results for accurately predicting cisplatin exposure and toxicities based on BSA [138]. By using Bayesian adaptive dosing instead of BSA, Salas *et al.* were able to achieve C_{max} target levels with an overall precision of 6.6% despite high interpatient variability (54%) described with cisplatin pharmacokinetics [138]. Their method also allowed 20% higher doses than the ones commonly recommended in the literature and with decreased toxicities [138].

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Carboplatin is a platinum-based chemotherapy agent that was introduced for the treatment of patients with favorable prognosis testicular teratoma. Cisplatin-based chemotherapy such as bleomycin and vinblastine has over a 90% complete remission rate for patients with good prognosis, but patients are often plagued with acute toxicity, including nausea and vomiting. Of even greater concern is the chronic nephrotoxicity, neurotoxicty and ototoxicity associated with these chemotherapy agents [38].

Carboplatin's major route of elimination is via the kidney, thus inadequate renal function can lead to toxic or subtherapeutic concentrations. Due to the decrease in toxicities noted in carboplatin treatment in prior studies using *a priori* estimation, further studies of carboplatin by Harland *et al.* and Egorin *et al.* reported that dosing of carboplatin should not be based on BSA but rather on a simple formula that accounts for pharmacokinetic contribution of renal excretion (24 h creatinine clearance) [139,140]. Several formulas based on *a priori* determination have followed, including Calvert's, Chatelut, Sanaka, Schwartz and Cockcroft–Gault equations; the most widely used method to calculate an individual dose of carboplatin is a modified Crockcoft–Gault equation:

$$(140 - age) \times \left(\frac{actual\ weight}{72 \times serum\ creatinine}\right)$$

Implementation of these formulas for calculation of individualizing doses of carboplatin has reduced pharmacokinetic variability and has led to dosing of carboplatin being based on pharmacokinetics rather than BSA. Four other studies applying a Bayesian estimation method to individualized dosing have reported a significantly decreased variation in carboplatin exposure as result of TDM, compared with the standard dose [56,58,141,142].

■ Etoposide

Etoposide phosphate is a topoisomerase II inhibitor that is used in the treatment of malignancies such as Ewing's sarcoma, testicular cancer, lymphoma, nonlymphocytic leukemia, glioblastoma multiforme and small-cell carcinoma of the lung. Traditionally, dosing is based on BSA and administered over 3–5 days. However, Nguyen *et al.* studied the population pharamacokinetics of etoposide and concluded that interindividual variability was important in the treatment of small-cell lung cancer patients and that doses should be adjusted based on serum creatinine level [143]. Supporting Nguyen's findings, a study of 52 patients with small-cell lung cancer using population pharmacokinetics showed no relationship between BSA and etoposide clearance, but concluded that there was a significant relationship between etoposide clearance and kidney function (serum creatinine) [144]. In addition, the authors reported a significant reduction (median AUC: 260 vs 339 mgh/l) of systemic exposure of etoposide secondary to the 28% increase (median CL: 2.42 vs 1.89 l/h; $p < 0.0005$) in clearance when ifosfamide was coadministered.

■ Doxorubicin

Doxorubicin is an anthracycline antibiotic that is widely used in the treatment of a variety of cancers, including hematological malignancies, soft-tissue sarcomas and many types of carcinomas. It functions by intercalating with DNA and, thus, inducing apoptosis of cancer cells. There have been very few studies of the relationship between doxorubicin pharmacokinetics and clinical response. A recent study of doxorubicin by Elis *et al.* studied the association of doxorubicin pharmacokinetics with remission rates in Hodgkins lymphoma patients [145]. They demonstrated that successful remission was associated with a higher median AUC. Those with median serum concentration of 280 ng/ml obtained successful remission compared with those who failed remission with median concentration of 73 ng/ml [145]. The authors concluded that TDM of doxorubicin may be warranted in patients with Hodgkins lymphoma, due to the wide variability observed in interindividual pharmacokinetics.

■ Suramin

Suramin is an aromatic polysulfonated compound that has been evaluated as a chemotherapy agent in prostate cancer. Studies have shown that suramin has a concentration-dependent effect on prostate cancer **(Table 1)** [146]. In addition, studies using maximal tolerated dosing have demonstrated suramin plasma concentrations of 100–200 µM and half-lives ranging between

Table 1. Suramin concentration dependency†.

Concentration	Effect
<50 µM	Inhibits reverse transcriptase, protein kinase C, TGF-β, basic FGF and RNA polymerase
>50 µM	Inhibits IL-2, IGF 1, toposisomerase 2, EGF and TNF
>100 µM	Cytotoxicity

†The biological effects of suramin are dependent upon the concentration administered.

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30 and 50 days. The unusually long half-life of suramin increases the probability of drug accumulation and this, coupled with significant toxic effects, have led to population-based pharmacokinetic studies that recommended a fixed dosing schedule with plasma drug concentrations maintained between 70 and 200 µM [146].

Additional studies of suramin have reported that suramin concentrations between 10 and 50 µM significantly enhanced the therapeutic efficacy of chemotherapy doxorubicin, paclitaxel, docetaxel and mitomycin without increased toxicity when used to treat subcutaneous or metastatic human xenograft tumors of breast, prostate and bladder in immunodeficient mice [146]. A recent study of suramin dosing using nomograms based on population pharmacokinetics reported successful delivery to target concentrations of suramin in greater than 95% of the treatments [147].

Conclusion & future perspective

Advances in the field of oncology have led to the development of many new chemotherapy agents, but to effectively and safely use these drugs we must take into account the wide pharmacokinetic variability that exists in a diverse population. From the initial oral absorption of a drug to the final elimination from the body, there are many sources of pharmacokinetic variability that can cause underdosing and treatment failure to overdosing and toxic buildup. The current approach to chemotherapy treatments by using BSA-based dosing does not take into account the wide interpatient pharmacokinetic variability and is not optimum for the treatment of cancer, yet there has been no substantial change in clinical practice. Minimizing interpatient variability using pharmacokinetically guided dosage may reduce the probability of toxic effects and treatment failures. Treatment failure reduces the chances of long-term disease-free survival for cancer patients and successful first time chemotherapy treatment is of particular importance in childhood ALL. It has been shown that treatment failure and subsequent intensive second remission therapy carries the risk of secondary malignancies [148].

Any argument for the replacement of BSA-based dosing must propose a better alternative. While it is hoped that the design of new agents will lead to increased success in the treatment of cancers while minimizing toxicity, it is essential that we use the drugs currently available to their maximum potential. For drugs in clinical use, studies using population-based approaches to identify factors with predictive value for drug pharmacokinetic and therapeutic effect are clearly valuable, as shown in our discussion. The majority of prospective dose-adaptation studies for chemotherapeutic agents have shown positive results when pharmacokinetically guided dose adaptation have resulted in desired systemic exposure. These types of studies often report tight control of plasma concentration levels, especially when using the Bayesian approach. However, few studies have evaluated tumor response and toxicity profiles in the context of TDM by randomized comparative trials. The obstacles to these types of trials are significant. Funding these trials is difficult because Phase III randomized control trials are expensive and will be competing with other trials investigating newer 'blockbuster'-type treatments for resources. In addition, they require a large number of patients and the number of patients willing to enroll in clinical trials is limited – there will be competition with other trials for patient enrollment as well. Drug regimens are constantly changing as clinicians/scientists are investigating different combinations of therapeutic agents. Lastly, for most chemotherapeutic drugs, there is no widely available assay for monitoring patients. In most, if not all, the pharmacokinetic and TDM studies, drug measurements have been made using HPLC or LC–MS/MS methods. However, most clinical laboratories do not have access to these methods due to lack of capital resources, technical expertise or both. Automated immunoassays are a preferred method for clinical laboratories, but are costly to develop and present their own technical challenges in the form of crossreactivity with drug metabolites and structural analogues, as well as heterophilic antibody interferences. Development of commercial assays will be dependent on defining the clinical need for TDM assays in this field.

In the future, it will be important to find ways to gather data to investigate the potential benefits of TDM in oncology patients. It is equally important for investigators and/or *in vitro* diagnostics companies to develop robust, easily accessible assays for the measurement of chemotherapeutic agents in blood. Last and most importantly, it will take a large-scale collaborative effort between oncologists, pharmacists and laboratorians to move from empiric treatment of cancer to a more rational approach to drug delivery, based on drug concentrations and other clinical biomarkers.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Executive summary

Pharmacokinetics

- One significant source of interindividual variability in chemotherapy is due to differences in patient pharmacokinetics.
- Pharmacokinetic variability comes from a variety of sources, including drug absorption, distribution, metabolism and clearance.
- Patient pharmacokinetics also vary with age and the addition of other pharmaceutic agents.

Chemotherapy dosing

- The typical method for minimizing interindividual variability in pharmacokinetics is to normalize dosing to patient body surface area.
- In many cases, body surface area-based dosing does not acceptably reduce variability.
- Pharmacokinetics-based dosing includes both *a priori* and *a posteriori* methods for dose individualization.

Current therapeutic drug monitoring in cancer chemotherapy

- There are only three drugs that are monitored with any regularity in cancer chemotherapy: methotrexate, busulfan and azothioprine.

Prospective chemotherapy candidates for therapeutic drug monitoring

- There are several drugs that have demonstrated significant pharmacokinetic variability that may be candidates for therapeutic drug monitoring, including mTOR inhibitors, 5-fluorouracil, imatinib, EGF receptor inhibitors, platinum-based agents, etoposide, doxorubicin and suramin.
- Establishing therapeutic drug monitoring protocols for these agents, and others, is difficult because, for most chemotherapy agents, there is no defined relationship between blood concentration and clinical efficacy.

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