Pharmacogenomic progress in individualized dosing of key drugs for cancer patients

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SUMMARY
Determining the correct dosage for the majority of traditional chemotherapeutic agents presents a challenge because most drugs have a narrow therapeutic index, which results in a fine balance between doses that cause significant drug toxicity and loss of efficacy. Dosing calculations for most agents use the patient’s body surface area, a method that correlates poorly with drug pharmacokinetics. Genetic differences in drug-metabolizing enzymes are being evaluated in an effort to explain the pharmacokinetic and pharmacodynamic variability seen with many chemotherapeutic agents. Elucidation of the underlying reasons for this variability will enable individualization of therapy to minimize toxicity and maximize efficacy, and thus improve control over the narrow therapeutic index of these agents. Such investigations have led to Clinical Pharmacology FDA Subcommittee recommendations for changes to drug package instructions. This Review discusses the current limitations of body-surface-area-based dosing, examples of successful pharmacogenomic investigations that have used drug-metabolizing enzymes to decrease drug toxicity and/or improve efficacy, and the future promises of pharmacogenomic-directed pharmacotherapy.

KEYWORDS anticancer drugs, drug metabolizing enzymes, pharmacodynamics, pharmacogenomics, pharmacokinetics

REVIEW CRITERIA
Articles were identified by searching the PubMed database for relevant publications written in English from 1966 until 1 September 2008. Abstracts from ASCO published until June 2008 were also reviewed. The search terms included “anticancer drugs”, “pharmacogenomics”, “pharmacokinetics”, and “pharmacodynamics”. Studies from the identified literature were chosen based on the best clinical evidence that addressed this article’s objectives. Results of data analysis performed by the authors are also included.

INTRODUCTION
Chemotherapeutic agents are some of the most dangerous drugs in our current medical arsenal. Traditionally, these drugs have been administered at or near their maximum tolerated doses in an effort to maximize the efficacy of the agents. This practice comes at the cost of significant, yet often unpredictable, toxicity as a result of interindividual variability in drug pharmacokinetics. At present, drug dosages are only individualized on the basis of a patient’s body surface area (BSA). BSA has been demonstrated to be proportional to blood volume and glomerular filtration rate (GFR). These factors, however, are less likely to contribute to a drug’s efficacy and toxicity than liver function or variation in drug metabolism, transporters or target receptors (Box 1). The BSA dosing strategy was initially introduced as a mathematical way to estimate a tolerable starting dose for phase I human trials based on preclinical animal data.

The BSA-derived maximum tolerated dose represents an average dose across all patients (and subsequently, their genotypes) enrolled in the phase I dose-finding study. The correlation between drug pharmacokinetics and BSA has been investigated for many common chemotherapeutic agents, but the majority of studies have shown little or no clear relationship. The importance of this lack of correlation is underscored by the knowledge that giving the same drug at the same BSA-adjusted dose to two individuals will not always produce the same effect in both. Indeed, a 5–20-fold variation in pharmacokinetics is routinely observed among patients receiving BSA-adjusted doses. To correct this inaccuracy, we must find reliable markers for predicting not only a drug’s pharmacokinetics, but more importantly its toxicity and efficacy.

For most drugs, several metabolic pathways are responsible for the activation and deactivation of the agent. Genetic differences between individuals in the genes that encode enzymes involved in drug metabolism can result in varying efficiency of these pathways, which can

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drastically alter the degree of toxicity and/or efficacy an individual experiences from any given agent. For a drug with a complex metabolism, numerous factors need to be considered in predictions of the effects of the given drug. Our current understanding of metabolic pathways and the differences in the genes that regulate these pathways provide us with the first tools to begin the individualization of drug therapy.

The process of translating these findings into clinical practice begins with identifying the pathways of a drug’s metabolism that are most likely to affect the efficacy and/or toxicity of the agent. Polymorphisms in the genes involved in control of these pathways are then investigated, especially when phenotyping assays (e.g. blood levels, imaging, erythromycin breath test, etc) are not feasible. Once a correlation is found between polymorphisms and clinically relevant pharmacokinetic or pharmacodynamic variables, retrospective trials are conducted to determine these variables’ clinical relevance in terms of the drug efficacy or toxicity. Finally, a prospective interventional trial can be undertaken to determine how treatment can be varied for patients with differing genetic polymorphisms to optimize its efficacy and minimize toxicity.

Over the past 3 years, the FDA Subcommittee on Clinical Pharmacology has made four recommendations for changes to drug package instructions that were based on the influence of genetics on drug metabolism. These examples of thiopurine S-methyltransferase (TPMT), UDP glucuronosyltransferase (UGT1A1), cytochrome P450, family 2, subfamily D, polypeptide 6 (CYP2D6), cytochrome P450, family 2, subfamily C, polypeptide 9 (CYP2C9) and vitamin K_2,3-epoxide reductase complex, subunit 1 (VKORC1) provide lessons for the use of drug metabolism to improve clinical therapeutics.

**THE EFFECTS OF PHARMACOGENOMICS ON TOXICITY**

Mercaptopurine, thioguanine and azathioprine are thiopurine antimetabolites that inhibit DNA synthesis by incorporation of incorrect bases (thioguanine nucleotides). These immuno-suppressive agents are used in the treatment of leukemias and autoimmune disorders. The drugs are inactivated by the enzyme thiopurine S-methyltransferase (TPMT). Three alleles in the TPMP gene encoding this enzyme account for 95% of the mutant alleles seen in the Caucasian, Asian, and African-American populations. The TPMT*3A, TPMT*3C and TPMT*2 alleles all result in rapid proteolysis of TPMT, which causes deficiency in the enzyme. Approximately 10% of Caucasians and African-Americans are heterozygous for one of these alleles, which results in intermediate TPMT activity, and 0.3% are homozygous and nearly completely deficient in TPMT. When patients who are homozygous for one of these alleles are administered standard doses of thiopurines, all develop severe hematologic toxicity. The concentration of thioguanine nucleotides in erythrocytes was approximately 10-fold higher in homozygous deficient patients compared with the concentration observed in wild-type patients. Commercial tests are available to assess the patient’s blood for TPMT activity or to perform genotype analysis. In patients who lack TPMT activity, thiopurines can still be administered at 10–15% of the standard dose. Dosing recommendations are less clear for heterozygous patients who have intermediate enzyme activity and prospective studies would be required to determine the optimal dose for this patient population. Retrospective data, however, indicate that a reduction to approximately 65% of the planned dosage is required. In August 2003, these findings were used to guide the first package labeling changes for thiopurine antimetabolite drugs, in acknowledgement of the importance of pharmacogenetics on these drugs’ toxicity.
IRINOTECAN: DOSE INFLUENCES AND GENETIC RISK OF TOXICITY

Irinotecan is another example of a drug that is metabolized differently in some patients; these differences can predispose a subset of patients to increased drug toxicity. The addition of irinotecan to the combination of fluorouracil and leucovorin in 1996 represented the first major advance in the treatment of colorectal cancer since the late 1950s, when fluorouracil originally became the standard of care. Regimens that contained irinotecan resulted in a near-doubling of response rates, from around 20% to 40%, and prolongation of overall survival by up to 3 months.15,16 These advances, however, came with the risk of significant and unpredictable toxicity. The most common toxicities of irinotecan are acute and delayed diarrhea and neutropenia.17 The drug-associated mortality from bolus fluorouracil and leucovorin regimens that contained irinotecan was threefold higher than that of regimens that did not contain the drug.18 Death often resulted from drug-related gastrointestinal and vascular syndromes.18 Differences between individuals with respect to the drug’s metabolism were a rational route of exploration because of the noted correlation between active drug concentration and toxicity.19

The metabolic fate of irinotecan is complex and involves numerous activation and inactivation pathways (Figure 1). Irinotecan itself is a prodrug that must be activated to SN-38 by carboxylesterase. Once activated, SN-38 can exert its cytotoxic effects via binding to the target enzyme DNA topoisomerase I or can undergo cellular efflux. The addition of a glucuronide moiety results in formation of SN-38G, an inactive compound that is primarily eliminated through biliary excretion. In Asian population; only 1–4% of individuals are homozygous and heterozygous, respectively. In African-Americans, 17–33% and 38–50% are homozygous and heterozygous, respectively. This variant allele is seen to a lesser extent in the Asian population; only 1–4% of individuals are homozygous and 15–31% are heterozygous for the UGT1A1*28 allele.
who received irinotecan has been documented in retrospective and prospective reports.\textsuperscript{25–27} A retrospective study examined this relationship in 118 patients who received different doses and schedules of irinotecan. Patients who were either homozygous or heterozygous for the UGT1A1*28 allele had a significantly greater incidence of grade 4 leukopenia and/or grade 3 or 4 diarrhea (odds ratio 5.21; \( P < 0.001 \)).\textsuperscript{27} A small prospective pilot study that involved 20 patients who received irinotecan 300 mg/m\(^2\) intravenously over 90 min every 3 weeks supported these findings. Of these 20 patients, 7 had the 6/7 genotype and 4 had the 7/7 genotype. Grade 3 or 4 diarrhea and/or neutropenia were only reported in patients with either the 6/7 or 7/7 genotype. Additionally, these patients also experienced lower absolute neutrophil count (ANC) nadirs (\( P = 0.04 \)); the 7/7 patients had an ANC nadir 2.5-fold lower than 6/6 patients.\textsuperscript{26} These results were confirmed by a larger prospective study of 65 patients who were administered irinotecan 350 mg/m\(^2\) intravenously over 90 min every 3 weeks. In total, 30, 25 and 6 patients had the 6/6, 6/7 and 7/7 genotypes, respectively. The other four patients had either a 6/8, 5/6 or 7/8 genotype. Grade 4 neutropenia was experienced by 50% of the 7/7 patients, corresponding to a 9.3-fold relative risk increase in developing this toxicity compared to 6/6 genotype (\( P = 0.001 \); Table 1). Genotype SN-38 glucuronidation ratio is 3.9-fold lower in 6/6 genotype (\( P = 0.001 \)), 2.5-fold lower in 7/7 genotype (\( P = 0.04 \)), 2.8-fold lower in 6/7 genotype (\( P = 0.02 \)) and 2.5-fold lower in 6/7 genotype (\( P = 0.001 \)).

### Table 1: The effect of polymorphisms in glucuronidation enzymes\textsuperscript{a} on risk of irinotecan toxicity.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Irinotecan dosage</th>
<th>Study design</th>
<th>Sample size (n)</th>
<th>Pharmacokinetic relationship</th>
<th>Toxicity relationship</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ando et al. (2000)\textsuperscript{27}</td>
<td>Various doses and schedules</td>
<td>Retrospective</td>
<td>118</td>
<td>NE</td>
<td>7/7 Genotype had 5.2-fold risk of grade 4 leukopenia and/or grade 3 or 4 diarrhea (( P &lt; 0.001 ))</td>
</tr>
<tr>
<td>Iyer et al. (2002)\textsuperscript{26}</td>
<td>300 mg/m(^2) over 90 min</td>
<td>Prospective</td>
<td>20</td>
<td>7/7 Genotype SN-38 glucuronidation ratio is 3.9-fold lower than in 6/6 genotype (( P = 0.001 ))</td>
<td>7/7 Genotype had 2.5-fold lower ANC nadir than 6/6 genotype (( P = 0.04 ))</td>
</tr>
<tr>
<td>Marcuello et al. (2004)\textsuperscript{57}</td>
<td>Various doses and schedules</td>
<td>Retrospective</td>
<td>95</td>
<td>NE</td>
<td>7/7 Genotype had a 7.4-fold increased occurrence of grade 3 or 4 hematologic toxicity compared with 6/6 genotype (( P = 0.001 ))</td>
</tr>
<tr>
<td>Innocenti, et al. (2004)\textsuperscript{25}</td>
<td>350 mg/m(^2) over 90 min</td>
<td>Prospective</td>
<td>66</td>
<td>7/7 Genotype SN-38 glucuronidation ratio is 1.8-fold lower than in 6/6 genotype (( P = 0.03 ))</td>
<td>7/7 Genotype had 9.3-fold risk of grade 4 leukopenia (( P = 0.001 ))</td>
</tr>
<tr>
<td>Rouits et al. (2004)\textsuperscript{58}</td>
<td>85 or 180 mg/m(^2) over 90 min + fluorouracil + leucovorin (FOLFIRI)</td>
<td>Retrospective</td>
<td>75</td>
<td>NE</td>
<td>7/7 Genotype had a 7.4-fold increased occurrence of grade 3 or 4 hematologic toxicity compared with 6/6 genotype (( P = 0.001 ))</td>
</tr>
<tr>
<td>Toffoli et al. (2006)\textsuperscript{59}</td>
<td>180 mg/m(^2) over 120 min + fluorouracil + leucovorin (FOLFIRI or modified FOLFIRI)</td>
<td>Prospective</td>
<td>250 (PK in 71)</td>
<td>7/7 Genotype SN-38 glucuronidation ratio is 2.0-fold lower than in 6/6 patients (( P = 0.01 ))</td>
<td>7/7 Genotype had 8.6-fold risk of grade 3 or 4 hematologic toxicity compared to 6/6 genotype (( P = 0.02 ))</td>
</tr>
<tr>
<td>Pillot et al. (2006)\textsuperscript{60}</td>
<td>200 mg/m(^2) over 90 min + carboplatin AUC 5</td>
<td>Prospective</td>
<td>42</td>
<td>NE</td>
<td>7/7 Genotype had a 2.8-fold increased occurrence of grade 4 neutropenia compared with 6/6 genotype (( P = 0.09 ))</td>
</tr>
</tbody>
</table>

\textsuperscript{a}UGT1A1*28 is the most common variant of the UGT1A1 gene, with seven TA repeats in the promoter region. Carriers of this variant have enzymes with a lower glucuronidation activity than those who have wild-type UGT1A1*1 alleles that have six TA repeats in their promoter region. Abbreviations: 6/6, UGT1A1*28 wild-type allele; 7/7, UGT1A1*28 homozygous variant alleles that confer a reduced-glucuronidation phenotype; ANC, absolute neutrophil count; AUC, area under the curve; FOLFIRI, a chemotherapy regimen consisting of irinotecan plus 5-fluorouracil and leucovorin; NE not evaluated; PK, pharmacokinetic; UGT1A1*28 and the 6/6, 7/7 genotypes.
with the 7/7 genotype and subsequently recommended that reduced initial dose be used in these patients. The FDA also approved a genetic test (Invader UGT1A1 Molecular Assay; Third Wave Technologies Inc, Madison, WI) that used peripheral blood samples to identify individuals with the UGT1A1*28 allele.

UGT1A1*28 testing does seem to be of value in patients receiving higher doses of irinotecan (i.e. greater than 200 mg/m²). Last year, an analysis of published data, however, reported less utility at lower doses, including the regimens of 125 mg/m² weekly or 180 mg/m² every 21 days. This observation is surprising, and highlights that this gene–environment (drug) interaction cannot be viewed in a one-dimensional manner.

### TAMOXIFEN: AN EFFICACY EXAMPLE

Tamoxifen is a selective estrogen-receptor (ER) modulator that was initially approved by the FDA for the treatment of advanced breast cancer in 1977 and is included in the current standard of care for the adjuvant treatment of premenopausal women with endocrine-responsive localized breast cancer. In women aged 50 years or younger, 5 years of tamoxifen therapy reduced the risk of breast cancer recurrence by 45% and the risk of mortality by 32%. Response to tamoxifen is clearly related to the presence of ER on tumor cells; however, approximately 35% of patients with ER-positive disease do not respond to tamoxifen. Individual alterations in the drug’s metabolism were again sought to explain this lack of efficacy.

Tamoxifen undergoes extensive metabolism to several primary and secondary metabolites that have varying pharmacologic activity compared with the parent drug (Figure 2). Initially, 4-hydroxytamoxifen was believed to be the primary metabolite responsible for the drug’s activity because it inhibited the proliferation of estrogen-dependent breast cancer cells with 100-fold greater potency compared with the parent drug. The activity of another metabolite, 4-hydroxy-N-desmethyltamoxifen (endoxifen), was characterized in 2003 and found to be comparable to 4-hydroxytamoxifen with respect to growth suppression but was present in concentrations six-fold higher than the latter metabolite. Cytochrome P450 (CYP) 2D6 is the enzyme primarily responsible for the production of both 4-hydroxytamoxifen and endoxifen.

CYP2D6 is highly polymorphic and has been associated with 46 reported allelic variants, many of which result in the loss of enzyme function. Loss of CYP2D6 activity has been estimated to be present in up to 10% of the Caucasian population; CYP2D6*4 is the most important allele associated with loss of enzymatic activity. Patients who are homozygous and heterozygous for this variant allele had statistically lower endoxifen concentrations compared with patients who are homozygous for wild-type alleles. Additionally, patients homozygous for wild-type alleles who were taking drugs that inhibited CYP2D6 had endoxifen concentrations 58% lower than those not taking these agents.

The clinical relevance of these findings was assessed using paraffin-embedded tumor samples from 223 tamoxifen-treated patients. Buccal samples from 15 women were also collected to compare the genotype agreement between tumor and germline DNA. The primary goal was to assess the relationship between genotype and disease outcome. Patients with the CYP2D6*4/*4 genotype had a shorter time to relapse ($P = 0.023$) and shorter disease-free survival compared with patients who has a wild-type genotype ($P = 0.012$; Figure 3). No change in overall survival was observed; however, given the early nature of disease in this population of patients and the variety of treatment options available for disease recurrence, a difference in this end point would not be expected.

The genotype obtained from tumor samples was in 100% concordance with that found in buccal
samples. The presence of the CYP2D6*4 allele also affected the toxicity profile of tamoxifen. Moderate to severe hot flashes were experienced by 20% of the women who were either heterozygous for CYP2D6*4 and wild-type alleles, or homozygous for wild-type alleles compared with none of the 13 women with the CYP2D6*4/*4 genotype. Limitations of this study, however, include the predominantly Caucasian population (92%) and the fact that the only CYP2D6 allele found and discussed was CYP2D6*4.38

In Asians, Africans and African-Americans, up to 50% can have reduced activity of or non-functional CYP2D6 activity that can result from alleles other than CYP2D6*4.36,39 Co-administration of drugs known to inhibit CYP2D6 was also investigated in the above populations of patients. Patients were classified as extensive, intermediate, or poor metabolizers based on their genotype and whether or not they were prescribed a medication known to inhibit CYP2D6. Extensive metabolizers (n = 115) were patients who lacked a CYP2D6*4 allele and were not prescribed a CYP2D6 inhibitor. Intermediate metabolizers (n = 40) were patients who were either heterozygous for the CYP2D6*4 allele and were not prescribed a CYP2D6 inhibitor, or patients with wild-type alleles who were prescribed a weak or moderate CYP2D6 inhibitor. Poor metabolizers (n = 16) were patients who were either homozygous for the CYP2D6*4 allele, heterozygous for this allele and prescribed a moderate or potent CYP2D6 inhibitor, or homozygous for the wild-type allele and prescribed a potent CYP2D6 inhibitor. Potent inhibitors included fluoxetine and paroxetine. Moderate inhibitors included sertraline, cimetadine, amiodarone, doxepin, ticlopidine or haloperidol. Compared with extensive metabolizers, those classified as poor metabolizers had a shorter time to breast cancer recurrence (P = 0.007), shorter relapse-free survival (P = 0.005) and disease-free survival (P = 0.008). Intermediate metabolizers had lower relapse-free survival (P = 0.075) and disease-free survival compared with extensive metabolizers (P = 0.097). The 2-year rates of relapse-free survival were 98%, 92% and 68% for extensive metabolizers, intermediate metabolizers and poor metabolizers, respectively. As discussed in the prior study, no statistical difference in overall survival exists between the groups.40

A similar study examined the effect of additional genotypes and concomitant CYP2D6 inhibitors on endoxifen plasma concentrations. Medication histories and 33 CYP2D6 alleles were evaluated in 158 patients with breast cancer who were receiving tamoxifen. Endoxifen levels were analyzed during the fourth month of tamoxifen treatment. Extensive metabolizers were defined as patients with any two CYP2D6 alleles that resulted in an active enzyme (including, but not limited to *4), intermediate metabolizers were patients with one such allele, and poor metabolizers were defined as patients who lacked any functional allele. An additional classification of ultra-rapid metabolizer was added and defined as patients with multiple copies of any functional allele. Endoxifen levels were lowest in the poor metabolizers and highest in the extensive metabolizers and ultra-rapid metabolizers. CYP2D6 inhibitors also influenced endoxifen levels as noted in the prior study. The potent inhibitors paroxetine and fluoxetine significantly reduced endoxifen levels in extensive metabolizers to a similar extent to the concentrations found in poor metabolizers. Sertraline and citalopram decreased endoxifen levels moderately; however, venlafaxine did not cause any significant decrease in endoxifen levels.41 This information also supports the finding that other CYP2D6 alleles, including CYP2D6*5, CYP2D6*10 and CYP2D6*41, have also been associated with increased recurrence rates and reduced event-free survival.42

![Figure 3](image-url)
Avoidance of concomitant medications known to decrease CYP2D6 activity might improve outcomes for patients with the wild-type genotype, but we are left with the question of what the best treatment might be for premenopausal patients with ER-positive tumors who have impaired CYP2D6 activity as a result of their genotype. Whether increasing the dose of tamoxifen would improve efficacy is not known, nor whether using another form of endocrine therapy such as ovarian suppression with or without an aromatase inhibitor would optimize outcomes. The curable nature of ER-positive localized breast cancer highlights the importance of individualized therapy in affected individuals. Prospective, genotype-guided studies in populations of diverse ethnicity are needed to answer this question.

**WARFARIN: LOOKING BEYOND DRUG METABOLISM**

Warfarin has been used clinically as an anticoagulant since the late 1950s and continues to be one of the most commonly used agents for the prevention and treatment of venous and arterial thromboembolism. Numerous drug and patient characteristics complicate the ability to maximize the efficacy and tolerability of warfarin, including unpredictable variability between dose and response across populations and several potential interactions with common foods and drugs. The importance of these issues is underscored by the narrow therapeutic range of warfarin and the potential severity of complications for patients who cannot be treated by doses within this therapeutic range. Increased risk for bleeding exists when the international normalized ratio (INR) is above the usual accepted range of 2.0–3.0, although this risk is dependent upon specific indications. Embolic complications are more likely to occur when the INR is below this range. Though several environmental factors affect the efficacy of warfarin, some variability can be explained by individual differences in pharmacokinetics and pharmacodynamics.

Warfarin itself is commercially supplied as a racemic mixture of R-warfarin and S-warfarin. S-warfarin is responsible for 70% of the drug’s activity because it has up to five-fold higher potency than R-warfarin. S-warfarin is metabolized primarily by CYP2C9 to 7-hydroxywarfarin (Figure 4). Alterations in the activity of this enzyme were investigated to explain some of the variability in responses to warfarin. Numerous alleles of CYP2C9 have been reported that vary with ethnicity; however, the majority of clinical studies has involved two variant alleles, CYP2C9*2 and CYP2C9*3. The *2 allele is found in 11% of the Caucasian population and results in a 30% decrease in CYP2C9 activity compared with the *1 wild-type allele. The *3 allele is less common than the *2 allele, with an incidence of 7% in Caucasians; this variant results in an 80% decrease in enzyme activity. Both alleles are rare in African-Americans, found in approximately 2–3% of the population.

The effects of the *2 and *3 polymorphisms on warfarin anticoagulation status and serious bleeding events were evaluated in a retrospective cohort study of 185 European-American patients receiving long-term warfarin. The primary outcome of anticoagulation status was assessed according to the patients’ INR values, and was reported as the time elapsed until patients reached a therapeutic INR, the rate of above-range INRs, and the time needed to achieve stable warfarin dosing. The target INR range was 2.0–3.0. Nearly 70% of the patients were homozygous

**Figure 4** Metabolism of warfarin and vitamin K inhibition. Warfarin is supplied as a racemic mixture of R-warfarin and S-warfarin. S-warfarin is the more active of the two at inhibiting vitamin K reductase. The R isomer is metabolized by several CYP450 isozymes and the S isomer is metabolized primarily by CYP2C9. Abbreviations: CYP1A1, cytochrome P450, family 1, subfamily A, polypeptide 1; CYP1A2 cytochrome P450 1A2; CYP3A4, cytochrome P450 3A4; CYP2C9, cytochrome P450 2C9.
for the wild-type CYP2C9 allele and the other 31.4% had at least one variant allele. The presence of at least one variant allele resulted in a longer time needed to reach an INR in the therapeutic range, with a median difference of 95 days compared with patients who were homozygous for the wild-type allele. Additionally, those with variant alleles had a 1.4-fold higher risk of an above-range INR value and a 2.39-fold higher risk of a serious or life-threatening bleeding event. Finally, the maintenance dose of warfarin also varied with genotype; patients who were homozygous for wild-type alleles required higher doses than heterozygous patients or those who were homozygous for variant alleles. Although such observations are encouraging, differences in warfarin metabolism only seem to explain approximately 17% of the variability in warfarin dosing requirements, so other potential sources of variation, such as in the drug’s target, needed to be investigated.

The procoagulant activity of the vitamin K-dependent clotting factors (II, VII, IX and X) is maintained by a cyclic interconversion of vitamin K and vitamin K epoxide. VKORC1 is responsible for the regeneration of reduced vitamin K. The presence of warfarin disrupts VKORC1, which results in the production of clotting factors with impaired coagulation ability (Figure 3).

A retrospective study conducted in the same population of patients as the CYP2C9 study discussed above aimed to determine how haplotypes of the warfarin target, VKORC1, affected warfarin dosing. Ten single nucleotide polymorphisms (SNPs) in noncoding regions of the VKORC1 gene were found in more than 5% of the population and used to describe five major haplotypes. These haplotype frequencies were also described in a second, ethnically-diverse population of patients that included Americans of African, European, and Asian origin. Patients were grouped by haplotype according to the warfarin dose they required, into either low-dose (A) or high-dose (B) groups. Patients in the A/A haplotype group required 2.7 mg of warfarin per day as a maintenance dose, A/B patients required 4.9 mg per day, and B/B patients required the highest dose at 6.2 mg per day (P < 0.001). The A haplotype was found more commonly in Asian-American patients while the B haplotype was seen more often in African-Americans. This stratification explained an additional 25% of the variation in warfarin dosages.

The contributions of VKORC1 genotype, CYP2C9 genotype and other factors such as age, body size, and drug interactions known to affect warfarin dose requirements were combined with the goal of producing an algorithm to be used in clinical practice, an example of which can be seen online. These four variables were included in a multivariate regression model that accounted for 54.2% of the variation in warfarin dosing. This algorithm provides an example that can be used by practitioners to improve individualized warfarin dosing in patients, although close INR monitoring is still recommended.

Models that consider both genetic and environmental sources of variation are likely to be able to optimize the efficacy and toxicity of warfarin therapy to a greater extent than models based on either component alone.

**FUTURE DIRECTIONS**

These promising advances lead us to speculate on how oncology clinics might look in the future. In the same way that a patient’s left ventricular ejection fraction is evaluated before treatment with anthracyclines, or their serum creatinine level is measured before starting cisplatin, blood or tissue will probably be collected for genetic analysis to determine the best dose or treatment for patients. Perhaps after being weighed on admission, patients about to begin tamoxifen therapy will also have a quick swab of their cheek to collect buccal cells for CYP2D6 genotype analysis. To make this vision become a reality, we need validated tests that assess substances we can easily collect from patients during a clinic visit. Genotyping of UGT1A1, CYP2C9, and VKORC1 can be performed using blood, saliva, or any other source of DNA; however, ‘real-time’ functional assays are of even greater value. Biomarkers of enzyme function are gradually being applied to clinical studies. For example, the uracil breath test has also been developed; results of this test correlate with a patient’s level of activity of dihydropyrimidine dehydrogenase, the primary enzyme responsible for the metabolism of fluorouracil. Development of antibodies against commonly used anticancer drugs is also renewing the concept of therapeutic drug monitoring as a tool for improved individualization of drug therapy. Novel imaging probes are also undergoing development. In time, a great breadth of tools will become available for assessing pharmacokinetics and dynamics in a prospective manner.
CONCLUSIONS
The case for an individualized approach to dosing of anticancer agents is clear: many modern therapies are associated with unacceptable adverse events, insufficient therapeutic success, and rising costs. Pharmacokinetic and pharmacodynamic variations are an important source of those clinical problems, although such variation presents a practical way to try to improve treatment outcomes for cancer patients. We must begin to take the issue of optimal drug usage a bit more seriously. If we apply the same level of enthusiasm and rigor we currently have for novel agent development to the improvement of existing drugs, we will make dramatic improvements to the care of patient.

KEY POINTS
- The majority of chemotherapeutic agents are dosed according to the patient’s body surface area, which correlates poorly with drug pharmacokinetics.
- Genetic differences in drug-metabolizing enzymes partially explain the interindividual pharmacokinetic variability of many anticancer agents.
- Patients with thiopurine S-methyltransferase deficiency require 5–10% of the standard dose of thiopurine immunosuppressive agents to avoid severe hematologic toxicity.
- The UGT1A1*28 allele is associated with increased neutropenia and diarrhea in patients receiving irinotecan, especially at doses greater than 200mg/m²; the FDA has recommended empirical dose reduction in patients who are homozygous for this allele.
- Patients receiving tamoxifen who are classified as CYP2D6 poor metabolizers, on the basis of either genotype or concurrent drug therapy, are likely to have a shorter time to relapse and lower disease-free survival than extensive metabolizers or patients who are homozygous for the wild-type CYP2D6 allele.
- An algorithm that uses patient’s height, age and both VKORC1 and CYP2C9 genotypes has demonstrated the ability to account for 54.2% of the variability in warfarin dose requirements and could potentially improve the quality of care provided for patients receiving this anticoagulant.

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