# CANCER GENETICS '98
# Pharmacogenetics of Cancer Therapy: Getting Personal

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The term "pharmacogenetics" was coined in the 1950s to describe inherited differences in the disposition and effects of medications and other xenobiotics. Pharmacogenetic studies have focused largely on inherited variations in drug metabolism and polymorphisms of the genes encoding drug-metabolizing enzymes, to elucidate the molecular basis of these variations (Nebert 1997). Although genetic polymorphism in drug metabolism remains a primary focus, inherited differences in drug targets (e.g., receptors, genetic subsets of diseases, etc.) represent another facet of pharmacogenetics that can be of equal importance in determining the effects of some medications. Theoretically, these two determinants of drug disposition and effects can be used to select the most appropriate medication for a given disease (e.g., pharmacogenetics of receptors) and the optimal dose of medication for each individual patient (e.g., pharmacogenetics of metabolism and disposition). This minireview focuses on inherited differences in the metabolism of selected anticancer agents (for a discussion of the genetic determinants of molecular oncogenesis, see Hartsough and Steeg 1998 [in this issue]; Narod 1998 [in this issue]).

To date, several dozen genetic polymorphisms of drug-metabolizing enzymes have been identified and characterized (Nebert et al. 1996), some of which are known to affect anticancer drugs (table 1). The list includes practically all major classes of drug-metabolizing enzymes, including cytochromes P450, N-acetyltransferases, glutathione and uridine diphosphate glucuronosyltransferases, and methylases. Of the two principal mechanisms leading to differences in drug metabolism, discoveries of inactivating mutations in the coding regions or exon/intron boundaries of genes for specific drug-metabolizing enzymes are far more common than reports of mutations affecting regulatory mechanisms (up- or downregulation of the enzymatic activity) of these enzymes. Polymorphisms of drug-metabolizing–enzyme genes also may influence cancer susceptibility, through metabolic activation or inactivation of xenobiotics and pollutants (Nebert et al. 1996). Extensive reviews on specific polymorphisms can be found elsewhere (Grant 1993; Gonzalez and Fernandez-Salguero 1995; Krynetski et al. 1996; Meyer and Zanger 1997); here we discuss the thiopurine S-methyltransferase gene (*TPMT*), to illustrate the potential importance of polymorphisms in drug-metabolizing enzymes for cancer chemotherapy.

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## Thiopurine Therapy and the *TPMT* Polymorphism

Mercaptopurine (MP) and thioguanine (TG) are among the most widely used antileukemic agents, and azathioprine (an MP prodrug) is a widely prescribed immunosuppressant. MP and TG are inactive prodrugs, requiring metabolism to thiopurine nucleotides via the purine-salvage pathway (e.g., HPRT), to exert cytotoxicity (Elion 1989).

Alternatively, these two drugs can undergo S-methylation catalyzed by TPMT or oxidation to thiouric acid by xanthine oxidase. However, hematopoietic tissues do not have measurable xanthine oxidase activity, leaving S-methylation to inactive S-methylated bases as the major competing metabolic pathway in these cells. Clinical studies have established that cellular accumulation of TG nucleotides (TGNs) is inversely related to TPMT activity (fig. 1) (Lennard et al. 1990), because high TPMT activity shunts more drug down the methylation pathway, leaving less for activation to thioguanosine mono-, di-, and triphosphates (TGNs). Furthermore, several studies have documented that TPMT-deficient patients accumulate very high TGN concentrations in erythrocytes and presumably other hematopoietic tissues, leading to severe hematopoietic toxicity, unless their dosage is reduced (Evans et al. 1991). TPMT activity typically is measured in erythrocytes, because this correlates with TPMT activity in other normal (Van Loon et al. 1992) and neoplastic (McLeod et al. 1995) tissues. In erythrocytes of TPMT-deficient patients (fig. 1), TGN accumulates to levels 10–20 × higher than the median values of other patients (Lennard et al. 1986).

**Table 1**

**Cancer Chemotherapy Metabolized by Polymorphic Enzymes**

| Anticancer Agent | Metabolic Pathway/ Enzyme | Interindividual Variability | Polymorphism | Reference |
|---|---|---|---|---|
| 5-fluorouracil | Inactivation bydihydropyrimidine dehydrogenase | 10-fold | Inherited | Diasio et al. 1988; Wei et al. 1996 |
| MP, TG, azathioprine | Inactivation by TPMT | >30-fold | Inherited | Krynetski et al. 1996 |
| Amonafide | Activation by N-acetyltransferase | >3-fold | Inherited | Ratain et al. 1991 |
| Busulfan | Inactivation by glutathione S-transferase | 10-fold | ? | Czerwinski et al. 1996 |
| Irinotecan | Inactivation by uridine diphosphate glucuronosyltransferase | 50-fold | Inherited | Iyer et al. 1998 |
| Cyclophosphamide | Activation by cytochrome P450 | 4–9-fold | Inherited | Chang et al. 1997 |

The potential severity of thiopurine toxicity is illustrated by the case report of fatal hematopoietic toxicity when full doses of thiopurines were given to a TPMT-deficient patient (Schutz et al. 1993). In contrast, TPMT-deficient patients can be treated successfully with thiopurine medications if their thiopurine dosage is decreased to 6%–10% of the conventional dosage (Evans et al. 1991). Although the greatest risk of toxicity is in patients with TPMT deficiency, there are data indicating that patients with heterozygous phenotypes are at intermediate risk of thiopurine toxicity (M. V. Relling and W. E. Evans, unpublished data).

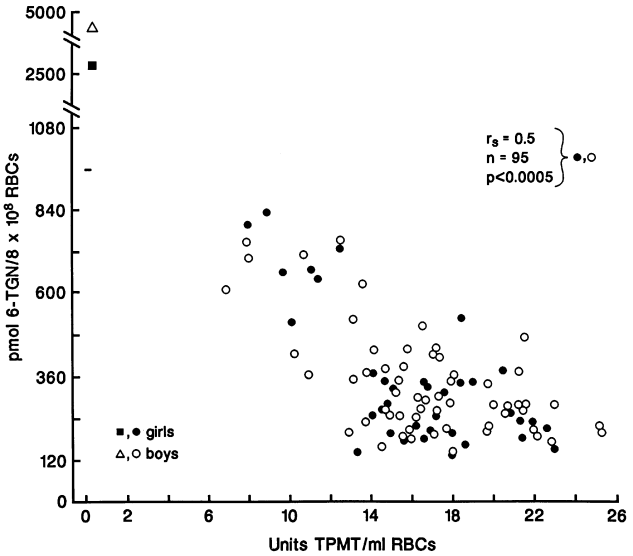


**Figure 1** Relationship between TPMT activity in erythrocytes and TGN accumulation in erythrocytes of children with ALL receiving uniform MP therapy. Values depicted by circles are from the study by Lennard et al. (1990; reproduced with publisher's permission), and the values depicted by the square and the triangle are for two TPMT-deficient patients from St. Jude Children's Research Hospital (described in Evans et al. 1991; McLeod et al. 1993).

Whereas high TGN levels have been clearly associated with thiopurine toxicity (Lennard et al. 1986; Evans et al. 1991), low erythrocyte TGN concentrations can affect the long-term outcome of pediatric acute lymphoblastic leukemia (ALL) (Lennard and Lilleyman 1989). Among 95 children with ALL, those with red blood cell (RBC) TGN levels less than the population median had significantly higher TPMT activity, compared with those having TGN levels greater than the population median value (Lennard et al. 1990), and, more importantly, children with lower TGN concentrations had a higher risk of disease relapse. TPMT activity also may be an important factor in determining long-term graft survival in azathioprine-treated patients; it has been suggested that those with high TPMT activity might benefit from doses near the upper limit of the dosage generally recommended (Chocair et al. 1992). To optimize thiopurine dosages, RBC TGN levels should be monitored or TPMT phenotype or genotype should be determined in ALL patients treated with extensive MP therapy and in transplant patients treated with azathioprine.

## Genetic Variations in TPMT Activity

TPMT activity in humans, which is inherited as an autosomal codominant trait, exhibits genetic polymorphism; ~10% of Caucasian and African-American populations inherit intermediate activity (i.e., heterozygotes) and ~1 in 300 inherit TPMT deficiency (fig. 2) (Weinshilboum and Sladek 1980; McLeod et al. 1994). The genetic basis for the TPMT polymorphism has now been

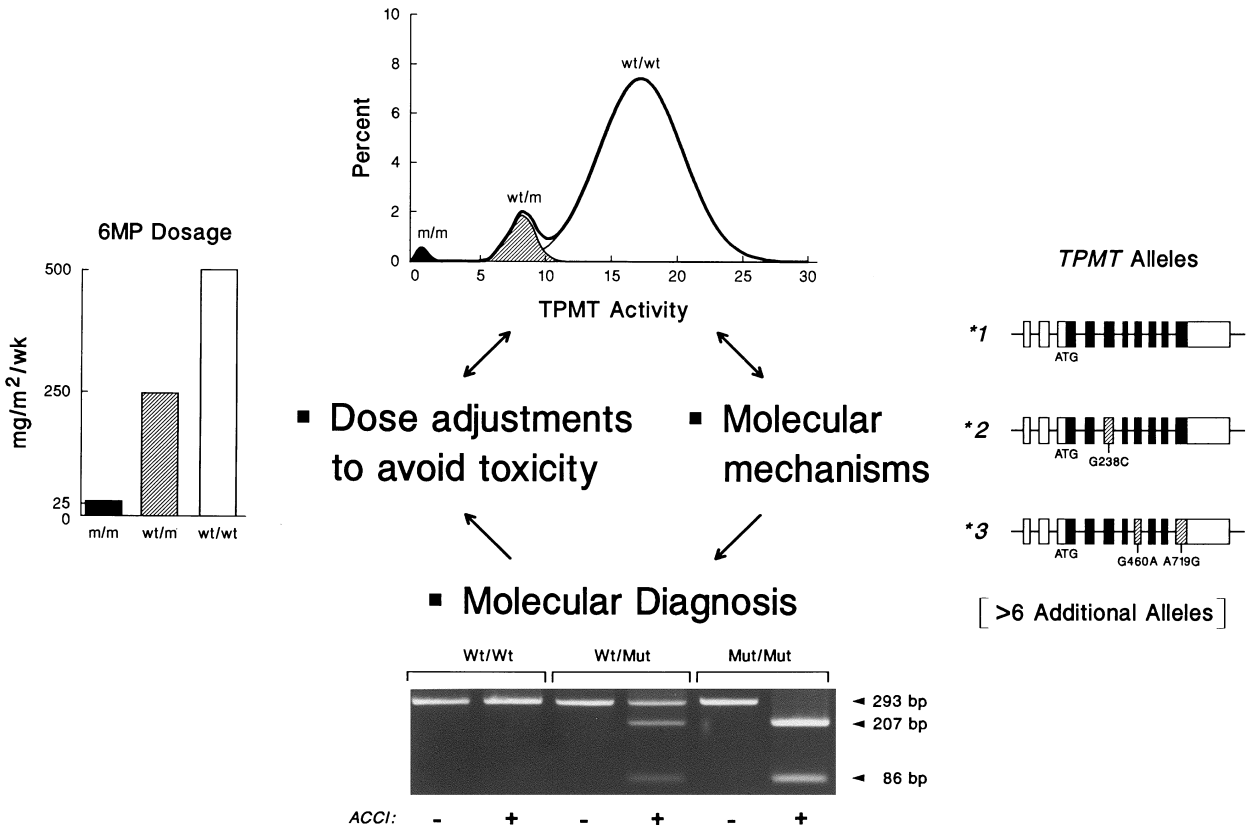


**Figure 2** Genetic polymorphism of TPMT activity and thiopurine therapy, from bench to bedside and back. *Top panel,* Distribution of TPMT activity in most populations (adapted from Krynetski et al. 1996). *Right panel,* Structure (10 exons) and positions of mutations (in exon 5 for *TPMT*2* and in exons 7 and 10 for *TPMT*3A*) in the most prevalent mutant alleles of *TPMT,* in Caucasians (more than six others have now been reported in various ethnic groups). *Bottom panel,* Results of PCR-based techniques for detection of inactivating mutations at the human *TPMT* gene locus. *Left panel,* Potential extent of MP-dosage reduction to avoid toxicity in patients with inherited deficiency of TPMT activity.

clearly established by the recent identification of inactivating mutations in the human *TPMT* gene (Krynetski et al. 1995; Tai et al. 1996; Szumlanski et al. 1996; Otterness et al. 1997). Currently, at least eight variant alleles associated with inheritance of low enzymatic activity have been reported. These alleles contain point mutations leading to amino acid substitutions (*TPMT*2, -*3A, -*3B, -*3C, -*3D, -*5,* and *-*6*), formation of a premature stop codon (*TPMT*3D*), or destruction of a splice site (*TPMT*4*). The consequences of amino acid substitutions in alleles *TPMT*2, -*3A, -*3B,* and *-*3C* have been characterized extensively, both in vitro and in vivo (Tai et al. 1996, 1997).

Although inheritance is the principal determinant of TPMT activity, other factors also may influence its in vivo activity. For example, there are data indicating that patient age, renal function, and thiopurine administration (Lennard et al. 1990; McLeod et al. 1994) alter TPMT activity in erythrocytes. Moreover, tissue-specific expression of TPMT mRNA also has been reported (Krynetski et al. 1995), with the highest expression of TPMT mRNA in the liver and kidneys and relatively low levels in the brain and lungs. However, the molecular mechanisms and clinical importance of these variations in TPMT activity remain undefined.

## Mechanism of TPMT Deficiency

Heterologous expression of wild-type and several mutant forms of TPMT (*TPMT*2, -3A, -3B,* and *-3C*) in yeast and mammalian cells and northern analysis of patient samples have indicated that the level of mRNA is not affected by these mutations (Krynetski et al. 1995; Tai et al. 1997). Similarly, the synthesis rate of the TPMT protein in yeast, as well as in vitro translation in rabbit reticulocyte lysate, was not different for mutant *TPMT* cDNAs, compared with wild-type *TPMT* cDNA. In contrast, mutant TPMT proteins (i.e., *TPMT*2* and *TPMT*3A*) were shown to undergo rapid proteolysis via an ATP-dependent proteasomal mechanism (Tai et

al. 1997), with degradation half-lives of ~0.25 h for the mutant proteins, compared with 18 h for the wild-type protein. Furthermore, western analysis of TPMT protein in erythrocytes of patients revealed a strong correlation between TPMT protein levels and catalytic activity. It should be recognized that the natural substrate for TPMT has not been identified. However, because enhanced protein degradation is a primary mechanism for loss of TPMT activity, with *TPMT*2* and *TPMT*3A,* which are the predominant mutant alleles in Caucasians (Yates et al. 1997), individuals inheriting these mutant alleles will have deficient metabolism of thiopurines and as-yet-unknown endogenous or environmental substrates.

### Diagnosis of TPMT Deficiency in Patients

One method for assessing TPMT activity in patients is by means of a biochemical assay of erythrocyte lysates, by measuring the methylation of 6-MP with ($^{14}$C-methyl)-S-adenosylmethionine as the methyl donor (Weinshilboum et al. 1978). Erythrocytes typically are used as surrogates for other drug-metabolizing tissues, since a strong correlation has been established between TPMT activity in erythrocytes and other tissues, including ALL lymphoblasts (Van Loon and Weinshilboum 1982; Woodson et al. 1982; Szumlanski et al. 1992; McLeod et al. 1995). It should be noted that, in patients who have received an RBC transfusion within ~60 d, the measured TPMT activity may be spurious if a TPMT-deficient or heterozygous patient is transfused with blood from a homozygous wild-type individual. This is illustrated by a TPMT-deficient patient at our hospital (genotype *TPMT*3A/*3A*), who had a measured TPMT activity of 9.8 U/ml pRBC 12 d after receiving two units of packed erythrocytes, compared with undetectable activity 4 mo after the erythrocyte transfusions. Thus, this patient appeared to be a TPMT heterozygote, on the basis of erythrocyte TPMT activity, because she had been transfused with erythrocytes from an individual with homozygous wild-type TPMT activity.

Because it is not uncommon for newly diagnosed cancer patients to receive RBC transfusions, molecular genetic methods have been developed to diagnose TPMT-deficient patients, by use of genomic DNA (Yates et al. 1997). In a study of Caucasians, the *TPMT*2* and *TPMT*3* alleles accounted for >80% of mutant alleles and yielded >95% concordance between genotype and phenotype (Yates et al. 1997). Additional rare mutant alleles have now been identified (Otterness et al. 1997), some of which may be more prevalent mutant alleles in other ethnic groups. These discoveries will further enhance the reliability of genotyping methods and will permit the development of more robust PCR-based genotyping systems to identify TPMT-deficient patients prior to therapy with MP, azathioprine, or TG, including patients who have received donor RBC transfusions. Furthermore, "DNA chip" technology has the potential to completely automate TPMT-genotype determination and to provide rapid screening for a larger number of mutant alleles. Given the importance of MP for curative therapy of ALL and the role of azathioprine immunosuppression in organ-transplant recipients (Hollander et al. 1995), a DNA-based method to prospectively diagnose TPMT deficiency offers a clinically important strategy to minimize the risk of potentially life-threatening hematopoietic toxicity.

TPMT illustrates the potential clinical importance of genetic polymorphisms in drug metabolism as determinants of toxicity and efficacy of anticancer therapy (fig. 2). Characterization of the molecular mechanisms of this inherited trait has made it possible to accurately identify patients who are at high risk of toxicity, thereby providing a rational way of choosing the dosage of these important antileukemic and immunosuppressive medications. Furthermore, adjusting the dose of MP (i.e., a 10–15 × decrease as compared with conventional doses) makes thiopurine therapy as tolerable and effective in TPMT-deficient patients as it is in those with normal TPMT activity. Similar studies of other anticancer agents that are known substrates of polymorphic enzymes (e.g., 5-fluorouracil, amonafide, and busulfan) may offer additional mechanism-based guidelines for increasing the efficacy or decreasing the toxicity of cancer chemotherapy.

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