

Clinical Use

- Identify patients at risk for toxicity from thiopurine drugs
- Determine need to adjust drug dosage or select alternative therapy

Clinical Background

Thiopurine drugs (azathiopurine, 6-mercaptopurine, and 6-thioguanine) are used to treat patients with leukemia, rheumatic disease, inflammatory bowel disease, or solid organ transplant. These drugs require conversion to thioguanine nucleotides to exert their therapeutic (cytotoxic) effect; however, that conversion can be blocked by methylation or oxidation.¹ The methylation pathway depends on thiopurine methyltransferase (TPMT) activity, which varies among individuals: approximately 90% have normal activity, 10% have intermediate activity, and 0.3% have low or no detectable activity.¹ Thioguanine nucleotides can accumulate in patients who have reduced TPMT activity and who are receiving standard thiopurine doses, resulting in hematopoietic toxicity (e.g., myelosuppression).^{2,3} Dosage reduction can minimize toxicity in such patients.⁴

Reduced TPMT activity can be caused by polymorphisms in the *TPMT* gene.¹ Molecular studies have identified 4 variant alleles that together account for >95% of reduced TPMT activity: *TPMT*2* (238G→C), *TPMT*3A* (460G→A and 719A→G), *TPMT*3B* (460G→A), and *TPMT*3C* (719A→G).^{3,5} Individuals with 2 variant alleles have low or no TPMT activity, while those with 1 variant allele have intermediate TPMT activity. Wild-type (*TPMT*1*) homozygotes, on the other hand, have normal enzyme activity.

Methods for measuring red blood cell (RBC) TPMT activity are available, but results may be falsely elevated by recent blood transfusions and falsely lowered by RBC aging.^{5,6} *TPMT* genotype testing can predict reduced TPMT activity^{5,7} and is not affected by these variables. The Sonora Quest Laboratories *TPMT* genotype assay uses polymerase chain reaction (PCR) amplification followed by a

single nucleotide primer extension (SNPE) to detect the 4 common *TPMT* variants.

TPMT genotyping results have predicted thiopurine drug toxicity in a variety of disorders, including rheumatic disease,⁸ acute lymphoblastic leukemia,⁷ renal transplantation,⁹ and Crohn's disease.² Genotype analysis can thus help identify patients at increased risk of hematologic toxicity, although prospective clinical studies are needed to determine appropriate starting dosage for such patients.¹

Individuals Suitable For Testing

- Patients being considered for thiopurine therapy

Specimen Requirements

5 mL room temperature whole blood in an EDTA lavender-top tube (3 mL minimum).

CPT Codes*

83890, 83900, 83892, 83896 x 4, 83912

Method

- PCR amplification followed by SNPE
 - PCR amplification of the *TPMT* gene regions
 - Multiplex SNPE reactions targeting nucleotides 238, 460, and 719
 - Hybridization through linker oligonucleotides to microspheres
 - Detection of reporter fluorescence on a specific microsphere indicates the presence of an allele
- Results reported: genotype detected
- Analytical specificity: detection of wild-type *TPMT*1* and variants *TPMT*2*, **3A*, **3B*, and **3C*; other variants are not detected
- Aliases: azathioprine toxicity; mercaptopurine toxicity; TPMT deficiency; thiopurine S-methyltransferase genotype

Interpretive Information

The wild-type *TPMT*1/TPMT*1* genotype is consistent with normal TPMT enzyme activity. Standard doses of thiopurine drugs are less likely to be toxic in individuals with this genotype. Heterozygotes with 1 wild type and 1 variant allele are predicted to have intermediate TPMT activity, are at increased risk of hematologic toxicity, and may require a lower dosage.^{1,4}

Patients who lack a wild-type allele are predicted to have low or no detectable enzyme activity and are at high risk for life-threatening hematologic toxicity if given full doses of thiopurine medication.⁴ Alternative therapy or reduced dosage should be considered for these patients.^{1,4}

This assay does not detect rare alleles. In addition, because the *TPMT*3A* allele contains the polymorphisms found in the *TPMT*3B* and *TPMT*3C* alleles, this assay cannot distinguish the *TPMT*1/TPMT*3A* (intermediate enzyme activity) from the *TPMT*3B/TPMT*3C* genotype (no or low enzyme activity).³ However, the *TPMT*3B/TPMT*3C* genotype is extremely rare in the United States.

Results should be interpreted in conjunction with other laboratory and clinical findings.

References

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*The CPT codes provided are based on AMA guidelines and are for informational purposes only. CPT coding is the sole responsibility of the billing party. Please direct any questions regarding coding to the payor being billed.

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