

Thiopurine Drug Therapy

Indications for Ordering

Thiopurine Methyltransferase, RBC

- Phenotype test to assess risk for severe myelosuppression with standard dosing of thiopurine drugs
- Use for individuals being considered for thiopurine therapy
- Must be performed before thiopurine therapy is initiated
- Can also detect rapid metabolizer phenotype

Thiopurine Methyltransferase (TPMT) Genotyping, 4 Variants

- Genotype test to assess risk, due to genetics, for severe myelosuppression with standard dosing of thiopurine drugs
- Use for individuals being considered for thiopurine therapy or who have had an adverse reaction to thiopurine therapy
- Preferred test for patients with recent heterologous blood transfusion
- Can be performed irrespective of thiopurine therapy

Thiopurine Metabolites by LC-MS/MS

- Use to optimize therapy for thiopurine drugs
- Can identify thiopurine metabolite concentrations that may lead to toxicity

Test Description

Thiopurine Methyltransferase, RBC

- Enzymatic/quantitative liquid chromatography/tandem mass spectrometry

Thiopurine Methyltransferase (TPMT) Genotyping, 4 Variants

- Polymerase chain reaction/fluorescence monitoring

Thiopurine Metabolites by LC-MS/MS

- Quantitative liquid chromatography/tandem mass spectrometry

Tests to Consider

[Thiopurine Methyltransferase, RBC 0092066](#)

- Phenotype test to assess risk for severe myelosuppression with standard dosing of thiopurine drugs

[Thiopurine Methyltransferase \(TPMT\) Genotyping, 4 Variants 2012233](#)

- Genotype test to assess risk, due to genetics, for severe myelosuppression with standard dosing of thiopurine drugs

[Thiopurine Metabolites by LC-MS/MS 2014484](#)

- Optimize therapy for thiopurine drugs
- Performed at Quest Diagnostics

Disease Overview

Prevalence

- Very low/absent TPMT activity – ~3/1,000 individuals
- Intermediate TPMT activity – ~10% of Caucasian individuals
- Normal TPMT activity – ~90% of individuals
- High TPMT activity – unknown

Physiology

- Thiopurine drugs are purine antimetabolites and include
 - Azathioprine (AZA) (Imuran)
 - 6-mercaptopurine (6-MP) (Purinethol)
 - 6-thioguanine (6-TG) (Tabloid)
- Thiopurines must be metabolized to 6-thioguanine nucleotides (6-TGN) for activity
 - Proportion of active 6-TGN is regulated by the balance between activation and inactivation mechanisms
- Primary metabolic route for inactivation of thiopurine drugs is catalyzed by TPMT
 - Low TPMT activity – more 6-MP may be converted into active (cytotoxic) 6-TGN, which accumulates
 - Excess 6-TG in bone marrow (BM) inhibits purine synthesis
 - Inhibits cell proliferation
 - Contributes to excessive myelosuppression
- TPMT enzyme can be inhibited by common drugs
 - NSAIDs
 - Ibuprofen
 - Ketoprofen
 - Naproxen
 - Mefenamic acid
 - Diuretics
 - Furosemide
 - Thiazides
 - Ulcerative colitis drugs
 - Mesalamine
 - Olsalazine
 - Sulfasalazine

Treatment issues

- AZA, 6-MP, and 6-TG are inactive prodrugs
 - Use to treat
 - Acute lymphoblastic leukemia
 - Autoimmune diseases
 - Inflammatory bowel disease
 - Use to prevent rejection after solid organ transplant
 - Individuals with very low/no TPMT enzyme activity universally experience severe myelosuppression when receiving conventional thiopurine doses
 - Reduced drug dosing in individuals with very low to intermediate TPMT activity may reduce risk for myelosuppression
- ~30-60% of individuals with intermediate TPMT activity who receive conventional thiopurine doses experience moderate to severe myelosuppression
- Thiopurine dosing should rely on disease-specific guidelines and degree of myelosuppression
- Guidelines for thiopurine dosing by the Clinical Pharmacogenetics Implementation Consortium (CPIC) can be found at www.pharmgkb.org/gene/PA356/guideline

Test Interpretation

Thiopurine Methyltransferase, RBC

Results – TPMT activity with standard dosing of thiopurine drugs

- Normal – 24.0-44.0 U/mL
 - Low risk of BM toxicity (myelosuppression) predicted
 - No dose adjustment recommended
- Low – <17.0 U/mL
 - High risk of BM toxicity (myelosuppression) predicted
 - Recommended to avoid use of thiopurine drugs
- Intermediate – 17.0-23.9 U/mL
 - Intermediate risk of BM toxicity (myelosuppression) predicted
 - Dose reduction (30-70%) may be required
 - Therapeutic drug management recommended
- High – >44.0 U/mL
 - No risk for BM toxicity (myelosuppression) predicted
 - Risk of therapeutic failure due to excessive inactivation of thiopurine drugs
 - Higher than normal standard dose may be required
 - Therapeutic drug management recommended

Limitations

- Does not replace clinical monitoring
- TPMT inhibitors may contribute to falsely low phenotype test results
- TPMT phenotype should be assessed prior to treatment with thiopurine drugs
- Blood transfusion within 30 days may reflect donor status

Thiopurine Methyltransferase (TPMT) Genotyping, 4 Variants

Sensitivity/specificity

- Clinical sensitivity – 95% (Evans, 2004; Yates, 1997)
- Analytical sensitivity/specificity – 99%

Results

- Positive
 - Homozygosity or compound heterozygosity detected for *TPMT* deficiency alleles
 - Associated with very low/no TPMT enzyme activity
 - Very high risk for thiopurine drug-related toxicity with conventional doses
 - Heterozygosity detected for *TPMT* deficiency allele
 - Associated with intermediate TPMT enzyme activity
 - Increased risk for thiopurine drug-related toxicity with conventional doses
- Negative
 - No variants detected
 - Negative; predictive of *1 functional alleles
 - Predicts normal TPMT enzyme activity and normal risk for thiopurine drug-related toxicity with conventional doses
- Inconclusive
 - Phase of identified variants cannot be determined
 - Genotyping cannot distinguish the *1/*3A genotype (intermediate TPMT activity) from the *3B/*3C genotype (low or no TPMT activity)
 - *3B/*3C genotype is very rare (~ 1/500,000 Caucasian individuals)
 - TPMT phenotyping and/or monitoring thiopurine metabolite concentrations is recommended to optimize therapy

Limitations

- Only targeted *TPMT* allele variants will be detected by this panel
- Diagnostic errors can occur due to rare sequence variations
- Genotyping in individuals who have received allogeneic stem cell/BM transplant will reflect donor status
- Genotyping cannot distinguish the *1/*3A genotype from the *3B/*3C genotype
- Thiopurine drug metabolism and risk for toxicity may be affected by genetic and nongenetic factors that are not evaluated by this test
- Test does not assess for *TPMT* allele variants associated with ultrahigh enzyme activity
- Genotyping does not replace the need for therapeutic drug monitoring or clinical observation

Thiopurine Metabolites by LC-MS/MS

Limit of quantification (LOQ)

- LOQ – 12.5 pmol/8 x 10⁸ RBC (6-TGN)
- LOQ – 325 pmol/8 x 10⁸ RBC (6-methylmercaptopurine nucleotide [6-MMPN])

Components	Therapeutic Range
6-TGN RBC	235-400 pmol/8 x 10 ⁸ RBC
6-MMPN RBC	<5,700 pmol/8 x 10 ⁸ RBC

- 6-TGN concentrations <235 pmol/8 x 10⁸ RBC may indicate a reduced response to therapy
- 6-TGN concentrations >400 pmol/8 x 10⁸ RBC may indicate a higher risk for leukopenia
- 6-MMPN concentrations >5,700 pmol/8 x 10⁸ RBC may indicate a higher risk for hepatotoxicity

Genetics

Gene – *TPMT*

Inheritance – autosomal codominant

Penetrance – drug dependent

Allele variants

- >20 *TPMT* deficiency alleles identified to date, though most are very rare
- *TPMT* deficiency alleles tested
 - *2 (c.238G>C; p.Ala80Pro)
 - *3A (c.[460G>A;719A>G]; p.[Ala154Thr;Tyr240Cys])
 - *3B (c.460G>A ; p.Ala154Thr)
 - *3C (c.719A>G; p.Tyr240Cys)

Allele frequencies

- *TPMT**2
 - African – 0.000792
 - Asian – 0.0
 - Caucasian – 0.00190
 - Mediterranean – 0.00408
 - Mexican – 0.00592
 - Middle Eastern – 0.00749
- *TPMT**3A
 - African – 0.00198
 - Asian – 0.0001118
 - Caucasian – 0.0356
 - Mediterranean – 0.0254
 - Mexican – 0.0533
 - Middle Eastern – 0.0114
- *TPMT**3B
 - African – 0.0
 - Asian – 0.0
 - Caucasian – 0.000461
 - Mediterranean – 0.00426
 - Mexican – 0.00690
 - Middle Eastern – 0.00562
- *TPMT**3C
 - African – 0.0495
 - Asian – 0.0157
 - Caucasian – 0.004205
 - Mediterranean – 0.00545
 - Mexican – 0.00888
 - Middle Eastern – 0.00562

References

- Evans WE. Pharmacogenetics of thiopurine S-methyltransferase and thiopurine therapy. *Ther Drug Monit.* 2004;26(2):186-191
- Relling MV, Gardner EE, et al. Clinical Pharmacogenetics Implementation Consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing: 2013 update. *Clin Pharmacol Ther.* 2013;93(4):324-325
- Yates CR, Krynetski EY, et al. Molecular diagnosis of thiopurine S-methyltransferase deficiency: genetic basis for azathioprine and mercaptopurine intolerance. *Ann Intern Med.* 1997;126(8):608-614