

## Thiopurine Drug Therapy

Thiopurine drug therapy is used for autoimmune diseases, inflammatory bowel disease, acute lymphoblastic leukemia, and to prevent rejection after solid organ transplant. The inactivation of thiopurine drugs is catalyzed in part by thiopurine methyltransferase (TPMT) and nudix hydrolase 15 (NUDT15).

Variants in the *TPMT* and/or *NUDT15* genes are associated with an accumulation of cytotoxic metabolites leading to increased risk of drug-related toxicity with standard doses of thiopurine drugs, and the effects on thiopurine catabolism can be additive.

The enzyme activity phenotype of TPMT can also be measured directly when performed prior to drug administration. Complimentary to pre-therapeutic tests, concentrations of thiopurines and metabolites can be measured after initiation of therapy to optimize dose.

### 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
- Frequency of *NUDT15* alleles with decreased function is less than 1% in most populations, and is relevant primarily to people of Asian descent

#### 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

### TESTS TO CONSIDER

#### [Thiopurine Methyltransferase, RBC 0092066](#)

Method: Enzymatic/Quantitative Liquid Chromatography-Tandem Mass Spectrometry

Indications for ordering:

- 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

#### [TPMT and NUDT15 3001535](#)

Method: Polymerase Chain Reaction/Fluorescence Monitoring

Indications for ordering:

- Genotyping test to assess genetic risk 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 2014484](#)

Method: Quantitative Liquid Chromatography/Tandem Mass Spectrometry

Indications for Ordering:

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

- Ulcerative colitis drugs
  - Mesalamine
  - Olsalazine
  - Sulfasalazine
- NUDT15 catalyzes the conversion of cytotoxic 6-TG triphosphate metabolites to the less toxic 6-TG monophosphate.
  - Variants reduce enzyme activity and contribute to excessive myelosuppression.

### Treatment Issues

- AZA, 6-MP, and 6-TG are inactive prodrugs
  - 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 <https://cpicpgx.org/guidelines/>
  - [CPIC Guideline for TPMT and NUDT15 genotypes and thiopurine dosing](#)

## GENETICS

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### Gene

*TPMT*

### Inheritance

Autosomal codominant

## TEST INTERPRETATION

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### 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

### TPMT and NUDT15

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Variants Tested	
Gene (Transcript)	Allele
TPMT (NM_000367)	TPMT*2: rs1800462, c.238G>C
	TPMT*3A: rs1800460, c.460G>A; rs1142345, c.719A>G
	TPMT*3B: rs1800460, c.460G>A
	TPMT*3C: rs1142345, c.719A>G
	TPMT*4: rs1800584, c.626-1G>A
NUDT15 (NM_018283)	NUDT15*2 or *3: rs116855232, c.415C>T
	NUDT15*4: rs147390019, c.416G>A

Allele frequencies and phenotype predictions available at [www.pharmvar.org](http://www.pharmvar.org) or [www.pharmgkb.org](http://www.pharmgkb.org)

### Sensitivity/Specificity

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

### Results

- TPMT and/or NUDT15 variants detected
  - One variant allele predicts the intermediate metabolizer phenotype
  - Two variant alleles predict the poor metabolizer phenotype
- Negative – no variants detected is predictive of \*1 functional alleles

### Limitations

- Only targeted TPMT and NUDT15 variants will be detected by this test
- Diagnostic errors can occur due to rare sequence variations
- Genotyping in individuals who have received allogenic stem cell/BM transplant will reflect donor status
- Because the complex TPMT\*3A allele contains the variants found in the \*3B and \*3C alleles, genotyping cannot distinguish the \*3A /negative genotype (intermediate TPMT activity) from the rare \*3B/\*3C genotype (no or low TPMT activity); the \*3A /negative genotype is assumed when both \*3B and \*3C are detected
- Thiopurine drug metabolism and risk for adverse reactions to thiopurines 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 and clinical observation

### Thiopurine Metabolites by LC-MS/MS

#### Limit of Quantification (LOQ)

- LOQ – 12.5 pmol/8 x 10<sup>8</sup> RBC (6-TGN)
- LOQ – 325 pmol/8 x 10<sup>8</sup> RBC (6-methylmercaptopurine nucleotide [6-MMPN])

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

- 6-TGN concentrations <235 pmol/8 x 10<sup>8</sup> RBC may indicate a reduced response to therapy
- 6-TGN concentrations >400 pmol/8 x 10<sup>8</sup> RBC may indicate a higher risk for leukopenia
- 6-MMPN concentrations >5,700 pmol/8 x 10<sup>8</sup> RBC may indicate a higher risk for hepatotoxicity

### REFERENCES

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