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 methyltrasferase (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. Complementary to pretherapeutic 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 decent

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

- 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

- 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

- 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

Gene

TPMT

Inheritance

Autosomal codominant

Test Interpretation

Thiopurine Methyltransferase, RBC

Results

TMPT 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*

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 or www.pharmgkb.org

Sensitivity/Specificity

- Clinical sensitivity: 95%[1,2]
- 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 10^8 RBC (6-TGN)
- LOQ: 325 pmol/8 x 10^8 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 10^8 RBC may indicate a reduced response to therapy
- 6-TGN concentrations >400 pmol/8 x 10^8 RBC may indicate a higher risk for leukopenia
- 6-MMPN concentrations >5,700 pmol/8 x 10^8 RBC may indicate a higher risk for hepatotoxicity

References

1. Evans WE. Pharmacogenetics of thiopurine S-methyltransferase and thiopurine therapy. Ther Drug Monit. 2004;26(2):186-191. PubMed

2. Yates CR, Krynetski EY, Loennechen T, et al. Molecular diagnosis of thiopurine S-methyltransferase deficiency: genetic basis for azathioprine and mercaptopurine intolerance. Ann Intern Med. 1997;126(8):608-614. PubMed

Additional Resources

Relling MV, Schwab M, Whirl-Carrillo M, et al. Clinical Pharmacogenetics Implementation Consortium guideline for thiopurine dosing based on TPMT and NUDT15 genotypes: 2018 update. Clin Pharmacol Ther. 2019;105(5):1095-1105. PubMed

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