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 San Juan Capistrano Inc.

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

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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

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 false-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 allogenic 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

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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	230-400 pmol/8 x 10^8 RBC
6-MMPN RBC	<5,700 pmol/8 x 10^8 RBC

- 6-TGN concentrations <230 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

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

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