5-Fluorouracil Toxicity and Chemotherapeutic Response Panel

**Indications for Ordering**

Predict risk of dose-related toxicity and responsiveness to 5-FU therapy

**Test Description**

5-Fluorouracil (5-FU) Toxicity and Chemotherapeutic Response, 5 Mutations

- Polymerase chain reaction/single nucleotide extensions/fragment analysis to detect DPYD variants (c.1679 T>G, c.1905+1 G>A, and c.2846 A>T) and TYMS variants (3'-UTR deletion, and 5'-TSER)

Dihydropyrimidine Dehydrogenase (DPYD), 3 Variants

- Polymerase chain reaction/fluorescence monitoring to detect DPYD variants (c.1679T>G, c.1905+1G>A, and c.2846A>T)

**Tests to Consider**

5-Fluorouracil (5-FU) Toxicity and Chemotherapeutic Response, 5 Mutations 2007228

- Predict risk of dose-related toxicity and responsiveness to 5-FU therapy

Dihydropyrimidine Dehydrogenase (DPYD), 3 Variants 2012166

- Predict risk of dose-related toxicity to 5-FU therapy

**Disease Overview**

**Physiology**

When 5-FU is metabolized in the body

- ~80% is catabolized by the enzyme dihydropyrimidine dehydrogenase (DPD) into an inactive form, dihydro 5-FU, and excreted in urine

- Remaining drug is metabolized into an active form
  - Inhibits the synthesis of both DNA and RNA by
    - Competitive inhibition of the thymidylate synthase (TYMS) enzyme
    - Direct incorporation of cytotoxic metabolites (5-FUTP and 5-FdUTP) into nucleic acids

**Treatment issues**

- 5-FU is the most frequently used chemotherapeutic drug for the treatment of colorectal adenocarcinoma
  - Intravenous 5-FU
    - Adrucil (5-fluorouracil)
    - Oral 5-FU prodrugs
      - Xeloda (capecitabine)
      - Uftoral (tegafur/uracil)
  - Grade III-IV drug toxicity attributed to 5-FU occurs in ~16% of individuals

- Complications
  - Hematologic
  - Gastrointestinal
  - Dermatologic
  - Toxicity-related death

- Germline variants in the DPYD gene can
  - Lead to reduced 5-FU catabolism
  - Result in grade III-IV 5-FU toxicity

- TYMS enzyme is the primary target of 5-FU
  - Enzyme provides the only de novo source of thymidylate for DNA synthesis
  - Expression of TYMS is greater in rapidly proliferating cells, including cancer cells

- Germline variants in 5'-promoter enhancer region (5'-TSER) and 3'-untranslated region (3'-UTR) of the TYMS gene have been correlated with
  - Outcome
  - TYMS expression levels
  - Responsiveness to 5-FU therapy
  - Clinical testing for variants that alter 5-FU metabolism may aid in patient care

**Clinical issues (5-FU dosing)**

- Homozygous or compound heterozygous DYPD gene variants
  - Associated with DPD enzyme deficiency
  - Avoidance of fluoropyrimidine therapy is recommended
  - An alternate drug should be selected

- Heterozygous DYPD gene variants
  - Associated with 30-70% of normal DPD activity
  - Fluoropyrimidine therapy should be initiated with reduced dosing
  - ~50% of a standard dose is recommended
• Titration of dose based on patient tolerability and therapeutic drug monitoring
• Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing available at www.pharmgkb.org/gene/PA145

Genetics

Genes – DPYD, TYMS

Variants tested – see tables 1 and 2

Test Interpretation

Sensitivity/specificity
• Clinical sensitivity – ~31% for the DPYD variants analyzed (Caudle, 2013)
• Analytical sensitivity/specificity – 99%

Results
• Positive
  o DPYD gene variant detected
    ▪ Predicts decreased DPD enzymatic activity
    ▪ Associated with an increased risk for grade III-IV 5-FU toxicity
  o TYMS gene variant detected
    ▪ Predicts survival of patients with colorectal adenocarcinoma receiving 5-FU therapy
    ▪ Some TYMS genotypes may predict risk of 5-FU toxicity
• Negative
  o No variants detected in either the DPYD gene or the TYMS gene
  o No variants detected in DPYD gene – predictive of *1 functional alleles

Limitations
• Only targeted variants in the DPYD and TYMS genes will be detected by this panel
• Rare diagnostic errors may occur due to rare sequence variations
• Genetic and/or nongenetic factors not detected by this test may affect
  o 5-FU drug metabolism
  o Efficacy
  o Risk for toxicity
• Genotyping does not replace the need for therapeutic drug monitoring or clinical observation
• Lack of detection of the targeted DPYD and TYMS variants does not
  o Rule out risk for 5-FU toxicity
  o Predict degree of responsiveness to 5-FU

Reference

Table 1

<table>
<thead>
<tr>
<th>DPYD variant</th>
<th>Alternative name(s)</th>
<th>Allele frequency in indicated population</th>
<th>Predicted consequence in patients receiving 5-FU</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.1679T&gt;G</td>
<td>DPYD*13, rs55886062</td>
<td>0.001 – French Caucasian</td>
<td>Decreased DPD activity</td>
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<td>Increased toxicity risk</td>
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<tr>
<td>c.1905+1G&gt;A</td>
<td>DPYD*2A, IVS14+1 G&gt;A, rs3918290</td>
<td>0.0047-0.022 – Dutch, German, French, Turkish, Finnish Absent – Japanese, Korean, African American</td>
<td>Abolished DPD activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Greatly increased toxicity risk</td>
</tr>
<tr>
<td>c.2846A&gt;T</td>
<td>rs67376798</td>
<td>0.01 – French Caucasian</td>
<td>Decreased DPD activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Increased toxicity risk</td>
</tr>
<tr>
<td>TYMS variants</td>
<td>Allele</td>
<td>Allele frequency in indicated population</td>
<td>Predicted consequence in patients receiving 5-FU</td>
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<tr>
<td>3'-UTR</td>
<td>Deletion</td>
<td>0.295 – Caucasian</td>
<td>Decreased TYMS expression&lt;br&gt;Increased 5-FU responsiveness&lt;br&gt;Increased risk of toxicity</td>
</tr>
<tr>
<td></td>
<td>Insertion (wild type)</td>
<td>0.705 – Caucasian</td>
<td>Increased TYMS expression&lt;br&gt;Decreased 5-FU responsiveness&lt;br&gt;Decreased risk of toxicity</td>
</tr>
<tr>
<td>5'-TSER</td>
<td>2R</td>
<td>0.41-0.48 – Caucasian, Hispanic, African American&lt;br&gt;0.19 – Chinese&lt;br&gt;0.175 – Japanese</td>
<td>2R/3RG&lt;br&gt;• Increased TYMS expression&lt;br&gt;• Decreased 5-FU responsiveness&lt;br&gt;• Poor prognosis&lt;br&gt;2R/2R or 2R/3RC&lt;br&gt;• Decreased TYMS expression&lt;br&gt;• Increased 5-FU responsiveness&lt;br&gt;• Increased risk of toxicity</td>
</tr>
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<td></td>
<td>3RG</td>
<td>0.51 – Chinese&lt;br&gt;0.427 – Japanese&lt;br&gt;0.26-0.37 – Caucasian, Hispanic, African American</td>
<td>3RG/3RG, 3RG/3RC or 2R/3RG&lt;br&gt;• Increased TYMS expression&lt;br&gt;• Decreased 5-FU responsiveness&lt;br&gt;• Poor prognosis</td>
</tr>
<tr>
<td></td>
<td>3RC</td>
<td>0.399 – Japanese&lt;br&gt;0.30 – Chinese&lt;br&gt;0.15-0.33 – Caucasian, Hispanic, African American</td>
<td>3RG/3RC&lt;br&gt;• Increased TYMS expression&lt;br&gt;• Decreased 5-FU responsiveness&lt;br&gt;• Poor prognosis&lt;br&gt;3RC/3RC or 2R/3RC&lt;br&gt;• Decreased TYMS expression&lt;br&gt;• Increased 5-FU responsiveness&lt;br&gt;• Increased risk of toxicity</td>
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