# 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

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- 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
  - Excreted in the 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
  - *DPYD* gene variant detected
    - Predicts decreased DPD enzymatic activity
    - Associated with an increased risk for grade III-IV 5-FU toxicity
  - *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
  - No variants detected in either the *DPYD* gene or the *TYMS* gene
  - 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
  - 5-FU drug metabolism
  - Efficacy
  - 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
  - Rule out risk for 5-FU toxicity
  - Predict degree of responsiveness to 5-FU

## Reference

Caudle KE, Thorn CF, et al. Clinical Pharmacogenetics Implementation Consortium guidelines for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing. Clin Pharmacol Ther. 2013;94(6):640-645

**Table 1**

| ***DPYD* Gene Variants**<br>**Nomenclature, Allele Frequency, and Predicted Consequences** | | | |
|---|---|---|---|
| ***DPYD* variants** | **Alternative name(s)** | **Allele frequency in indicated population** | **Predicted consequence in patients receiving 5-FU** |
| c.1679T>G | *DPYD**13, rs55886062 | 0.001 – French Caucasians | Decreased DPD activity<br>Increased toxicity risk |
| c.1905+1G>A | *DPYD**2A, IVS14+1 G>A, rs3918290 | 0.0047-0.022 – Dutch, German, French, Turkish, Finnish<br>Absent – Japanese, Korean, African American | Abolished DPD activity<br>Greatly increased toxicity risk |
| c.2846A>T | rs67376798 | 0.01 – French Caucasians | Decreased DPD activity<br>Increased toxicity risk |

**Table 2**

| ***TYMS* Gene Variants**<br>**Allele Frequency and Predicted Consequences** | | | |
|---|---|---|---|
| ***TYMS* variants** | **Allele** | **Allele frequency in indicated population** | **Predicted consequence in patients receiving 5-FU** |
| **3'-UTR**<br>6 bp deletion (TTAAAG) (rs34489327; historically rs16430) | DELETION | • 0.295 – Caucasian | • Decreased TYMS expression<br>• Increased 5-FU responsiveness<br>• Increased risk of toxicity |
| | INSERTION (wild type) | • 0.705 – Caucasian | • Increased TYMS expression<br>• Decreased 5-FU responsiveness<br>• Decreased risk of toxicity |
| **5'-TSER**<br>28bp VNTR (2R; 3R) (rs34743033)<br><br>G>C SNP in 2nd repeat of 3R allele (3RC) (rs2853542) | 2R | • 0.41-0.48 – Caucasian, Hispanic, African American<br>• 0.19 – Chinese<br>• 0.175 – Japanese | 2R/3RG<br>• Increased TYMS expression<br>• Decreased 5-FU responsiveness<br>• Poor prognosis<br>2R/2R or 2R/3RC<br>• Decreased TYMS expression<br>• Increased 5-FU responsiveness<br>• Increased risk of toxicity |
| | 3RG | • 0.51 – Chinese<br>• 0.427 – Japanese<br>• 0.26-0.37 – Caucasian, Hispanic, African American | 3RG/3RG, 3RG/3RC or 2R/3RG<br>• Increased TYMS expression<br>• Decreased 5-FU responsiveness<br>• Poor prognosis |
| | 3RC | • 0.399 – Japanese<br>• 0.30 – Chinese<br>• 0.15-0.33 – Caucasian, Hispanic, African American | 3RG/3RC<br>• Increased TYMS expression<br>• Decreased 5-FU responsiveness<br>• Poor prognosis<br>3RC/3RC or 2R/3RC<br>• Decreased TYMS expression<br>• Increased 5-FU responsiveness<br>• Increased risk of toxicity |