

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
 - *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

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

Table 2

TYMS Gene Variants			
Allele Frequency and Predicted Consequences			
TYMS variants	Allele	Allele frequency in indicated population	Predicted consequence in patients receiving 5-FU
3'-UTR 6 bp deletion (TTAAAG) (rs34489327; historically rs16430)	Deletion	0.295 – Caucasian	Decreased TYMS expression Increased 5-FU responsiveness Increased risk of toxicity
	Insertion (wild type)	0.705 – Caucasian	Increased TYMS expression Decreased 5-FU responsiveness Decreased risk of toxicity
5'-TSER 28bp VNTR (2R; 3R) (rs34743033) G>C SNP in second repeat of 3R allele (3RC) (rs2853542)	2R	0.41-0.48 – Caucasian, Hispanic, African American 0.19 – Chinese 0.175 – Japanese	2R/3RG <ul style="list-style-type: none"> • Increased TYMS expression • Decreased 5-FU responsiveness • Poor prognosis 2R/2R or 2R/3RC <ul style="list-style-type: none"> • Decreased TYMS expression • Increased 5-FU responsiveness • Increased risk of toxicity
	3RG	0.51 – Chinese 0.427 – Japanese 0.26-0.37 – Caucasian, Hispanic, African American	3RG/3RG, 3RG/3RC or 2R/3RG <ul style="list-style-type: none"> • Increased TYMS expression • Decreased 5-FU responsiveness • Poor prognosis
	3RC	0.399 – Japanese 0.30 – Chinese 0.15-0.33 – Caucasian, Hispanic, African American	3RG/3RC <ul style="list-style-type: none"> • Increased TYMS expression • Decreased 5-FU responsiveness • Poor prognosis 3RC/3RC or 2R/3RC <ul style="list-style-type: none"> • Decreased TYMS expression • Increased 5-FU responsiveness • Increased risk of toxicity