Laboratory and Genetic Testing for Use of 5-Fluorouracil (5-FU) in Patients With Cancer

Policy Number: 2.04.68
Origination: 5/2011
Last Review: 5/2017
Next Review: 5/2018

Policy
Blue Cross and Blue Shield of Kansas City (Blue KC) will not provide coverage for laboratory testing to allow area under the curve (AUC) targeted 5-Fluorouracil (5-FU) dosing for patients administered 5-FU for cancer. This is considered investigational.

When Policy Topic is covered
Not Applicable

When Policy Topic is not covered
My5-FU™ testing or other types of assays for determining 5-fluorouracil area under the curve in order to adjust 5-FU dose for colorectal cancer patients or other cancer patients is considered investigational.

Testing for genetic variants in dipyrimidine dehydrogenase (DPYD) or thymidylate synthase (TYMS) to guide 5-FU dosing and/or treatment choice in patients with cancer is considered investigational.

Considerations
Genetics Nomenclature Update
Human Genome Variation Society (HGVS) nomenclature is used to report information on variants found in DNA and serves as an international standard in DNA diagnostics. It is being implemented for genetic testing medical evidence review updates starting in 2017 (see Table PG1). HGVS nomenclature is recommended by HGVS, the Human Variome Project, and the HUman Genome Organization (HUGO).

The American College of Medical Genetics and Genomics (ACMG) and Association for Molecular Pathology (AMP) standards and guidelines for interpretation of sequence variants represent expert opinion from ACMG, AMP, and the College of American Pathologists. These recommendations primarily apply to genetic tests
used in clinical laboratories, including genotyping, single genes, panels, exomes, and genomes. Table PG2 shows the recommended standard terminology—“pathogenic,” “likely pathogenic,” “uncertain significance,” “likely benign,” and “benign”—to describe variants identified that cause Mendelian disorders.

<table>
<thead>
<tr>
<th>Table PG1. Nomenclature to Report on Variants Found in DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Previous</strong></td>
</tr>
<tr>
<td>Mutation</td>
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<tr>
<td>Variant</td>
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<tr>
<td>Familial variant</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Table PG2. ACMG-AMP Standards and Guidelines for Variant Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Variant Classification</strong></td>
</tr>
<tr>
<td>Pathogenic</td>
</tr>
<tr>
<td>Likely pathogenic</td>
</tr>
<tr>
<td>Variant of uncertain significance</td>
</tr>
<tr>
<td>Likely benign</td>
</tr>
<tr>
<td>Benign</td>
</tr>
</tbody>
</table>

ACMG: American College of Medical Genetics and Genomics; AMP: Association for Molecular Pathology.

**Genetic Counseling**
Genetic counseling is primarily aimed at patients who are at risk for inherited disorders, and experts recommend formal genetic counseling in most cases when genetic testing for an inherited condition is considered. The interpretation of the results of genetic tests and the understanding of risk factors can be very difficult and complex. Therefore, genetic counseling will assist individuals in understanding the possible benefits and harms of genetic testing, including the possible impact of the information on the individual’s family. Genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing. Genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.

**Description of Procedure or Service**

<table>
<thead>
<tr>
<th>Populations</th>
<th>Interventions</th>
<th>Comparators</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individuals:</td>
<td>Interventions of interest are:</td>
<td>Comparators of interest are:</td>
<td>Relevant outcomes include:</td>
</tr>
<tr>
<td>With cancer for whom treatment with 5-fluorouracil is indicated</td>
<td>Laboratory assays to determine 5-fluorouracil area under the curve</td>
<td>Standard dosing of 5-fluorouracil</td>
<td>Overall survival</td>
</tr>
<tr>
<td></td>
<td>Genetic testing for variants (eg, in DPYD and TYMS) affecting 5-fluorouracil</td>
<td>No genetic testing</td>
<td>Test accuracy</td>
</tr>
</tbody>
</table>

Individuals: | Interventions of interest are: | Comparators of interest are: | Relevant outcomes include: |
| With cancer for whom treatment with 5-fluorouracil is | Genetic testing for variants (eg, in DPYD and TYMS) affecting 5-fluorouracil | No genetic testing | Overall survival |
| | | | Test accuracy |
| | | | Test validity |
Variability in systemic exposure to 5-fluorouracil (5-FU) is thought to directly impact 5-FU tolerability and efficacy. The standard approach is dosing according to body surface area. Two alternative approaches have been proposed for modifying use of 5-FU: (1) dosing based on determined area under the curve serum concentration target and (2) genetic testing for variants affecting 5-FU metabolism. For genetic testing, currently available polymerase chain reaction tests assess specific variants in genes encoding dihydropyrimidine reductase (DPYD) and thymidylate synthase (TYMS) in the catabolic and anabolic pathways of 5-FU metabolism, respectively.

For individuals who have cancer for whom treatment with 5-FU is indicated who receive laboratory assays to determine 5-fluorouracil area under the curve, the evidence includes randomized controlled trials (RCTs), observational studies, and systematic reviews. Relevant outcomes are overall survival, disease-specific survival, test accuracy and validity and treatment-related morbidity. A systematic review of observational studies on analytic validity studies found good correlation between test results; however, reviewers concluded that selected studies had high risk of bias due to excluded samples. Several analyses of patients with colorectal cancer have evaluated clinical validity. For example, 1 study found that the rate of severe toxicity was significantly lower in patients with stage II and III cancer who chose pharmacokinetic monitoring versus body surface area (BSA) monitoring, but progression-free survival did not differ between groups in patients with stage IV or recurrent cancer. No RCTs or nonrandomized comparative studies were identified comparing health outcomes in cancer patients who did and did not have 5-FU dose adjustment using the My5-FU assay and who were treated with chemotherapy regimens used in current clinical practice. A systematic review of the available literature found a significantly higher response rate with BSA-based monitoring and no significant difference in toxicity. Most data were from observational studies and the RCTs were conducted in the 1980s when different chemotherapy protocols were used. The evidence is insufficient to determine the effects of the technology on health outcomes.

For individuals who have cancer for whom treatment with 5-FU is indicated who receive genetic testing for variants (eg, in DPYD and TYMS) affecting 5-FU metabolism, the evidence includes observational studies and systematic reviews. Relevant outcomes are overall survival, disease-specific survival, test accuracy and validity, and treatment-related morbidity. A 2010 TEC Assessment concluded that DPYD and TYMS variant testing had poor prognostic capacity to identify patients likely to experience severe 5-FU toxicity. Since publication of that Assessment, no prospective trials comparing efficacy and toxicity outcomes in patients who did and did not undergo pretreatment DPYD and/or TYMS testing have been published. One study compared outcomes in patients undergoing pretreatment DPYD testing with historical controls who did not receive testing. In that study, rates of grade 3 or higher toxicity were lower in patients who had genetic testing; however, the
study was not randomized and lacked concurrent controls. The evidence is insufficient to determine the effects of the technology on health outcomes.

**Background**

**5-fluorouracil**

The agent 5-fluorouracil (5-FU) is a widely used antineoplastic chemotherapy drug that targets thymidylate synthase (TYMS) enzyme, which is involved in DNA production. 5-FU has been used for many years to treat solid tumors (eg, colon and rectal cancer, head and neck cancer). In general, the incidence of grade 3 or 4 toxicity (mainly neutropenia, diarrhea, mucositis, and hand-foot syndrome) increases with higher systemic exposure to 5-FU. Several studies also have reported statistically significant positive associations between 5-FU exposure and tumor response. In current practice, however, 5-FU dose is reduced when symptoms of severe toxicity appear, but is seldom increased to promote efficacy.

Based on known 5-FU pharmacology, it is possible to determine a sampling scheme for area under the curve (AUC) determination and to optimize an AUC target and dose-adjustment algorithm for a particular 5-FU chemotherapy regimen and patient population. For each AUC value or range, the algorithm defines the dose adjustment during the next chemotherapy cycle most likely to achieve the target AUC without overshooting and causing severe toxicity.

In clinical research studies, 5-FU blood plasma levels most recently have been determined by high-performance liquid chromatography or liquid chromatography coupled with tandem mass spectrometry. Both methods require expertise to develop an in-house assay and may be less amenable to routine clinical laboratory settings.

**Measuring Exposure to 5-FU**

**Laboratory Testing**

Patient exposure to 5-FU is most accurately described by estimating the AUC, the total drug exposure over a defined period of time. 5-FU exposure is influenced by method of administration, circadian variation, liver function, and the presence of inherited dihydropyrimidine reductase (DPYD)–inactivating genetic variants that can greatly reduce or abolish 5-FU catabolism. As a result, both inter- and intrapatient variability in 5-FU plasma concentration during administration is high.

Determination of 5-FU AUC requires complex technology and expertise that may not be readily available in a clinical laboratory setting. In the United States, Saladax Biomedical offers a commercial immunoassay (My5-FU) that quantifies plasma 5-FU concentration from a blood sample drawn during continuous infusion at steady state (18-44 hours after the start of infusion) and provides a dose-adjustment algorithm to maintain plasma 5-FU AUC between 20 and 30 mg/h/L during the next cycle.
**Genetic Testing**

5-FU is a pyrimidine antagonist, similar in structure to the normal pyrimidine building blocks of RNA (uracil) and DNA (thymine). More than 80% of administered 5-FU is inactivated and eliminated via the catabolic pathway; the remainder is metabolized via the anabolic pathway.

Catabolism of 5-FU is controlled by the activity of DPYD. Because DPYD is a saturable enzyme, the pharmacokinetics of 5-FU are strongly influenced by the dose and schedule of administration. For example, 5-FU clearance is faster with continuous infusion than with bolus administration, resulting in very different systemic exposure to 5-FU during the course of therapy. Genetic variants in DPYD, located on chromosome 1, can lead to reduced 5-FU catabolism and increased toxicity. Many variants have been identified (eg, IVS14+1G>A [also known as DPYD*2A], 2846A>T [D949V]). DPYD deficiency is an autosomal codominantly inherited trait.

The anabolic pathway metabolizes 5-FU to an active form that inhibits DNA and RNA synthesis by competitive inhibition of TYMS or by incorporation of cytotoxic metabolites into nascent DNA. Genetic variants in TYMS can cause tandem repeats in the TYMS enhancer region (TSER). One variant leads to 3 tandem repeats (TSER*3) and has been associated with 5-FU resistance due to increased tumor TYMS expression compared with the TSER*2 variant (2 tandem repeats) and wild-type forms.

**Regulatory Status**

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments (CLIA). My5-FU™ (Saladax Biomedical) and genetic testing for variants in DPYD and TYMS for predicting risk of 5-fluorouracil toxicity and chemotherapeutic response (ARUP Laboratories) are available under the auspices of CLIA. (The LDT TheraGuide® by Myriad Genetics has been discontinued). Laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of laboratory or genetic tests for use of 5-FU.

**Rationale**

This evidence review was originally created in February 2011 and has been updated regularly with searches of the MEDLINE database. The most recent literature review was performed through January 25, 2017 (see Appendix Table 1 for genetic testing categories).

Assessment of a prognostic tool typically focuses on 3 categories of evidence: (1) its technical performance; (2) clinical validity (ie, statistically significant association between the test result and health outcomes); and (3) clinical utility (ie, demonstration that use of the prognostic information clinically can alter clinical management and/or improve health outcomes compared with patient
management without use of the prognostic tool). In some cases, it is important to evaluate whether the test provides incremental information above the standard workup in order to determine whether the test has utility in clinical practice. Following is a summary of the key literature to date.

**Laboratory Testing to determine 5-fluorouracil Area Under the Curve for Dose Adjustment**

**Clinical Context and Proposed Clinical Utility**
The proposed clinical utility is to use test results to guide 5-fluorouracil (5-FU) dosing so that the therapeutic impact is maximized and the toxicity is decreased.

The following PICOTS were used to select literature to inform this review.

**Patients**
The relevant population of interest is patients with cancer who have an indication for 5-FU treatment.

**Interventions**
Laboratory assays to determine 5-FU area under the curve (AUC).

**Comparators**
The comparator is standard dosing of 5-FU. This involves dosing according to body weight, specifically body surface area (BSA)–based dosing.

**Outcomes**
The general outcomes of interest are overall survival (OS), disease-specific survival, test accuracy and validity, and treatment-related morbidity. Reductions in treatment-related morbidity relate to 5-FU toxicity. Types of severe toxicity include neutropenia, diarrhea, mucositis, and hand-foot syndrome.

**Timing**
Specific OS and disease-specific survival outcomes may vary by type of cancer but generally 1- to 2-year survival is a short-term outcome and 5- and 10-year survival is a long-term outcome. Treatment-related morbidity can be acute toxicity (≤14 days) or late toxicity (>14 days).

**Setting**
Patients would be tested in the oncology setting.

**Analytic Validity**
In 2015, Freeman et al published a health technology assessment (HTA) on the My5-FU assay to guide dose adjustment in patients receiving 5-FU chemotherapy by continuous infusion. The report was conducted on behalf of the National Institute of Health and Care Excellence (NICE). The assessment included a review of studies on the accuracy of the My5-FU assay compared with a reference standard test, high-performance liquid chromatography (HPLC) or liquid chromatography–mass spectrometry (LC-MS). Three studies were included, as well
as information provided by the manufacturer. Risk of bias was difficult to assess due to incomplete reporting. In particular, it was unclear whether there was complete reporting of failed samples or outliers, which could result in overly optimistic estimates of accuracy.

One of the studies included in the Freeman HTA was published by Büchel et al in 2013.6 The study compared My5-FU assay performance on the Roche Cobas Integra 800 analyzer with liquid chromatography–tandem mass spectrometry (LC-MS/MS) and 3 other analyzers (Olympus AU400, Roche Cobas c6000, Thermo Fisher CDx90). Serum samples were collected from 32 patients with gastrointestinal cancers who were receiving 5-FU infusion therapy at a single center in Switzerland. My5-FU was validated for linearity (ie, correlated linearly within ≤10% of true 5-FU concentrations from 100-1750 mg/mL), precision, accuracy, recovery, sample carryover, and dilution integrity. Of several plasma compounds tested for potential interference, only lipids exceeded manufacturer’s specification. This was attributed to a freezing effect, and the authors recommended storage of plasma samples at 39°F (4°C) until analysis, or frozen for longer periods. Compared with other tests, My5-FU had a 7% proportional (ie, dose-dependent) bias toward higher values than LC-MS/MS, and a 1.6% or less proportional bias toward higher values than the other 3 analyzers.

A second study is Beumer et al (2009), which compared the My5-FU (then called OnDose) assay results to results of LC-MS/MS testing in 156 head and neck cancer and colorectal cancer (CRC) patients.7 The test results were highly correlated ($R^2=0.986$).

The third study included in the Freeman HTA was available only as an abstract. As reported in the HTA, the abstract discussed 50 patients with CRC. The correlation between results of the My5-FU test and LC-MS was high ($R^2=0.847$). Freeman et al noted that the strength of agreement was lower than in other studies.

In the 2015 HTA5 and in a 2016 article based on the HTA,8 Freeman et al concluded that there was good correlation between the results of My5-FU and reference standard tests; however, there was significant variability in test agreement and it was difficult to fully assess the quality of the 3 included studies because of missing details such as how often samples were excluded.

**Section Summary: Analytic Validity**
Several studies have compared results of the My5-FU assay and a reference standard test. A systematic review of these studies found good correlation between test results; however, reviewers concluded that there was significant variability in test agreement and a high risk of bias in selected studies due to excluded samples.

**Clinical Validity**
Kline et al (2014) assessed My5-FU in a retrospective study of patients with stage II and III (n=35) or stage IV or recurrent (n=49) CRC who received 5-FU regimens at a single center in the United States.9 Thirty-eight patients chose
pharmacokinetic monitoring with OnDose and 46 patients were dosed by BSA. Median progression-free survival (PFS) did not differ by dosing strategy in stage IV or recurrent patients (14 months with AUC monitoring vs 10 months BSA dosing; \( p=0.16 \)), but did differ in stage II and III patients (\( p=0.04 \)). Thirty-seven percent of stage IV or recurrent patients in both dosing strategy groups experienced grade 3 toxicity. Among stage II and III patients, 32% of AUC-monitored patients and 69% of BSA-dosed patients experienced grade 3 toxicity (\( p=0.04 \)). Onset of adverse events also was delayed in the AUC-monitored group (6-7 months) compared with the BSA-dose group (2 months; \( p=0.01 \)).

My5-FU was clinically validated for patients with CRC in an observational analysis reported by Saam et al (2011). Sequential patients (N=357) were treated with constant infusion 5-FU using current adjuvant or metastatic treatment protocols with or without bevacizumab. Samples were drawn at least 2 hours after the start of and before the end of each infusion and sent to Myriad Genetics for analysis. Sixty-two (17%) patients were studied longitudinally across 4 sequential sample submissions (ie, four 5-FU treatment infusions), of which 3 (5%) were within the target AUC after the first infusion. By the fourth infusion, this percentage rose to 37% and outliers were reduced. Use of bevacizumab did not affect results. Response and toxicity were not reported.

**Section Summary: Clinical Validity**

Several analyses of patients with CRC have evaluated the clinical validity of the My5-FU assay. In 1 study, the rate of severe toxicity was significantly lower in patients with stage II and III cancer who chose pharmacokinetic monitoring versus BSA monitoring but PFS did not differ between groups in patients with stage IV or recurrent cancer. In another study, among patients studied longitudinally and monitored with My5-FU, 3% were within the target AUC after the first infusion and this reached 37% by the fourth infusion.

**Clinical Utility**

The results of single-arm trials of AUC-targeted 5-FU dose adjustment in advanced CRC patients have suggested consistently improved tumor response. Similar, although less compelling, results were seen in single-arm trials of AUC-targeted 5-FU dosing in head and neck cancer. The best contemporary evidence supporting AUC-targeted dosing consists of 2 randomized controlled trials (RCTs), one enrolling patients with CRC and the other patients with head and neck cancer. No trials of any design were identified for 5-FU dose adjustment in other malignancies.

Gamelin et al (1998) developed a chart for weekly dose adjustment based on the results of an earlier, similar single-arm study in which dose was increased by prespecified increments and intervals up to a maximum dose or the first signs of toxicity. In an RCT enrolling patients with metastatic CRC, Gamelin et al (2008) reported significantly improved tumor response (33.6% vs 18.3%, respectively; \( p<0.001 \)) and a trend toward improved survival (40.5% vs 29.6%, respectively; \( p=0.08 \)) in the experimental arm using AUC-targeted dosing (by HPLC) for single-agent 5-FU compared to fixed dosing. However, trialists also reported 18% grade
3 to 4 diarrhea in the fixed-dose control arm, higher than reported in comparable arms of 2 other large chemotherapy trials (5%-7%).\textsuperscript{18,19} In the latter 2 trials, delivery over a longer time period for both 5-FU (22 hours vs 8 hours) and leucovorin (2 hours vs bolus), which is characteristic of currently recommended 5-FU treatment regimens, likely minimized toxicity.

The administration schedule used in the 2008 Gamelin trial\textsuperscript{17} is rarely currently used in clinical practice and is absent from current guidelines.\textsuperscript{10} Additional optimization studies would be needed to apply 5-FU exposure monitoring and AUC-targeted dose adjustment to a more standard single-agent 5-FU treatment regimen, with validation in a comparative trial versus a fixed-dose regimen.

Fety et al (1998), in an RCT of patients with locally advanced head and neck cancer, used a different method of dose adjustment and reported overall 5-FU exposures in head and neck cancer patients that were significantly reduced in the dose-adjustment arm compared with the fixed-dose arm.\textsuperscript{20} This reduced toxicity but did not improve clinical response. The dose-adjustment method in this trial may have been too complex, because the 12 patients with protocol violations in this treatment arm (of 61 enrolled) all were related to 5-FU dose adjustment miscalculations. Because patients with protocol violations were removed from analysis, results did not reflect “real-world” results of the dose-adjustment method. In addition, the induction therapy regimen used 2 drugs, not the current standard of 3, and, therefore, generalizability of results to current clinical practice is limited.

In 2016, Yang et al published a meta-analysis of data from the 2 RCTs described above (ie, Gamelin et al and Fety et al), as well as from 3 observational studies.\textsuperscript{21} In a pooled analysis, the overall response rate was significantly higher with pharmacokinetic AUC-monitored 5-FU therapy than with standard BSA-based monitoring (odds ratio [OR], 2.04; 95% confidence interval [CI], 1.41 to 2.95). In terms of toxicity, incidence of diarrhea (3 studies), neutropenia (3 studies), and hand-foot syndrome (2 studies) did not differ significantly between the pharmacokinetic and BSA monitoring strategies. The rate of mucositis was significantly lower in the BSA-monitored group (3 studies; OR=0.16; 95% CI, 0.04 to 0.63). Most data were from observational studies, which are subject to selection and observational biases.

**Section Summary: Clinical Utility**
No RCTs or nonrandomized comparative studies were identified comparing health outcomes in cancer patients who did and did not have 5-FU dose adjustment using the My5-FU assay and who were treated with chemotherapy regimens used in current clinical practice. A systematic review of the available literature found a significantly higher response rate with BSA-based monitoring and no significant difference in toxicity. Most data were from observational studies; RCTs were conducted in the 1980s when different chemotherapy protocols were used.
Genetic Testing for *DPYD* or *TYMS* Variants in Affecting 5-FU Dose Adjustment

**Clinical Context and Proposed Clinical Utility**
The proposed clinical utility is to use test results to guide 5-FU dosing so that the therapeutic impact is maximized and the toxicity is decreased.

The following PICOTS were used to select literature to inform this review.

**Patients**
The relevant population of interest is patients with cancer who have an indication for 5-FU treatment.

**Interventions**
Genetic testing for variants (eg, in *DPYD* and *TYMS*) affecting 5-FU metabolism.

**Comparators**
See above in the laboratory testing section.

**Outcomes**
See above in the laboratory testing section.

**Timing**
See above in the laboratory testing section.

**Setting**
Patients would be tested in the oncology setting. In addition, referral for genetic counseling is important for explanation of genetic disease, heritability, genetic risk, test performance, and possible outcomes.

**Analytic Validity**
ARUP Laboratories uses polymerase chain reaction testing to assess 5 variants—3 in *DPYD* and 2 in *TYMS*.\(^4\) Results are reported as positive (variant detected) or negative (no variant detected). On its website, ARUP Laboratories reports analytical sensitivity and specificity of 99%. The information on the ARUP website also notes: “Only targeted mutations in the *DPYD* and *TYMS* genes will be detected by this panel. Rare diagnostic errors may occur due to rare sequence variations [not detected by the test]....Genotyping does not replace the need for therapeutic drug monitoring or clinical observation.”\(^4\)

**Section Summary: Analytic Validity**
The analytic validity for the commercially available laboratory test was reported by the manufacturer to be 99% sensitive and 99% specific.

**Clinical Validity**
Toxicity

A number of studies have evaluated the association between variants in the *DPYD* and/or *TYMS* genes and 5-FU toxicity. Cancer types and specific variants studied differed across these reports. Several meta-analyses of the literature have been published. In 2014, Li et al identified 7 cohort studies with a total of 946 patients with CRC. A pooled analysis of study findings found that *DPYD* variants correlated significantly with an increased risk of 5-FU-related toxicity. Also in 2014, Rosmarin et al identified 16 studies with a total of 4,855 patients with CRC who were treated with capecitabine and other fluorouracil-based treatment regimens. Capecitabine toxicity was significantly associated with several DPYD alleles and several *TYMS* single-nucleotide variants.

A key study was published in 2008 by Schwab et al. They enrolled 683 patients who were receiving 5-FU for colon or other gastrointestinal cancers, cancers of unknown primary, or breast cancer in a genotype study. Seven different 5-FU regimens (monotherapy or in combination with folate or levamisole [not approved by the Food and Drug Administration]) administered by bolus or by infusion were included. Patients were genotyped for the *DPYD* splice site variant *DPYD*2A (IVS14+1G>A), which leads to a nonfunctional enzyme, and for *TYMS* tandem repeats. Sensitivity, specificity, and positive and negative predictive values for overall toxicity, diarrhea, mucositis, and leukopenia were calculated (see Table 1). Although heterozygosity for *DPYD*2A had 99% specificity for serious toxicity, sensitivity ranged from 6% to 13%. Tandem repeats in *TYMS* were neither sensitive nor specific indicators of serious toxicity. Clinical factors also were examined for association with toxicity. Overall and in the group of 13 patients who were heterozygous for *DPYD*2A, women were more likely than men to develop severe toxicity (overall OR=1.9; 95% CI, 1.26 to 2.87; p=0.002), most commonly mucositis. Bolus administration of 5-FU was a significant, independent predictor of severe toxicity overall.

<table>
<thead>
<tr>
<th>Toxicity</th>
<th><em>DPYD</em>2A&lt;sup&gt;a&lt;/sup&gt; (n=13)</th>
<th><em>TYMS</em> VNTR 2/3 or 3/3&lt;sup&gt;b&lt;/sup&gt; (n=521)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall toxicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>6%</td>
<td>65%</td>
</tr>
<tr>
<td>Specificity</td>
<td>99%</td>
<td>21%</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>46%</td>
<td>14%</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>85%</td>
<td>76%</td>
</tr>
<tr>
<td>Diarrhea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>NR</td>
<td>57%</td>
</tr>
<tr>
<td>Specificity</td>
<td>NR</td>
<td>22%</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>NR</td>
<td>06%</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>NR</td>
<td>84%</td>
</tr>
<tr>
<td>Mucositis</td>
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<tr>
<td>Sensitivity</td>
<td>8%</td>
<td>NR</td>
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<tr>
<td>Specificity</td>
<td>99%</td>
<td>NR</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>31%</td>
<td>NR</td>
</tr>
<tr>
<td>Negative predictive</td>
<td>93%</td>
<td>NR</td>
</tr>
<tr>
<td>Toxicity</td>
<td>DPYD*2A&lt;sup&gt;a&lt;/sup&gt; (n=13)</td>
<td>TYMS VNTR 2/3 or 3/3&lt;sup&gt;b&lt;/sup&gt; (n=521)</td>
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<tr>
<td>Leukopenia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>13%</td>
<td>NR</td>
</tr>
<tr>
<td>Specificity</td>
<td>99%</td>
<td>NR</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>31%</td>
<td>NR</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>96%</td>
<td>NR</td>
</tr>
</tbody>
</table>

NR: not reported; VNTR: variable number of tandem repeats.

<sup>a</sup> Heterozygous DPYD*2A vs wt/wt.

<sup>b</sup> Homozygous (3R/3R) or mixed heterozygous (2R/3R) triple repeats vs homozygous double repeats (2/2).

Most recently, in 2016, Boige et al published a subanalysis of patients participating in an RCT. The RCT compared treatment with FOLFOX4 and FOLFOX4 plus cetuximab. A total of 1545 patients participated in the pharmacogenetics substudy and were genotyped on 25 DPYD variants. The primary end point was development of grade 3 or higher 5-FU-related adverse events (hematologic and gastrointestinal combined). Two DPYD variants (D949V and V73231) were significantly associated with grade 3 or higher adverse events (p<0.001 for both).

**Efficacy**

A 2013 meta-analysis by Wang et al included 11 studies that assessed the association between TYMS variants (5’ tandem repeats and a single-nucleotide substitution [G>C] within triplet repeats) and survival outcomes. Patients had gastric cancer or CRC and received 5-FU with or without leucovorin with or without levamisole. Three studies (n=311 patients) were eligible for pooled analysis of OS. Statistical heterogeneity was not assessed. Patients who were homozygous for triplet repeats (3R/3R) had longer OS than patients who were homozygous for doublet repeats (2R/2R) or compound heterozygous (2R/3R).

**Section Summary: Clinical Validity**

A number of observational studies and meta-analyses of these studies have found that DPYD variants and/or TYMS single-nucleotide variants correlated significantly with an increased risk of 5-FU-related toxicity. A meta-analysis of 3 studies found a significant association between TYMS gene single-nucleotide variants and longer OS. The available studies reported statistical associations and did not prospectively evaluate health outcomes in patients with genetic variants.

**Clinical Utility**

A 2010 TEC Assessment concluded that DPYD and TYMS variant testing did not meet TEC criteria. The Assessment noted that the tests had “poor ability to identify patients likely to experience severe 5-FU toxicity. Although genotyping may identify a small fraction of patients for whom serious toxicity is a moderate to strong risk factor, most patients who develop serious toxicity do not have variants in DPD or TS genes.”

No prospective trials comparing efficacy and safety outcomes with or without pretreatment DPYD and/or TYMS testing were identified.
One prospective trial compared outcomes for pretreatment DPYD*2A testing with historical controls. This 2016 study by Deenen et al included cancer patients intending to undergo treatment with fluoropyrimidine-based therapy (5-FU or capecitabine). Genotyping for DPYD*2A was performed prior to treatment and dosing was adjusted based on the alleles identified. Patients with heterozygous variant alleles were treated with a reduced (ie, ≥50%) starting dose of fluoropyrimidine for 2 cycles, and dosage was then individualized based on tolerability. No homozygous variant allele carriers were identified. Safety outcomes were compared with historical controls. Twenty-two (1.1%) of 2038 patients were heterozygous for DPYD*2A. Eighteen (82%) of these 22 patients were treated with reduced doses of capecitabine. Five (23%; 95% CI, 10% to 53%) patients experienced grade 3 or higher toxicity. In historical controls with DPYD*2A variant alleles, the rate of grade 3 or higher toxicity was 73% (95% CI, 58% to 85%). The historical controls were more likely to be treated with 5-FU-based therapy than with capecitabine-based therapy. Limitations of the study included lack of randomization to a management strategy and use of historical, rather than concurrent, controls.

Goff et al (2014) prospectively genotyped 42 adults who had gastric or gastroesophageal junction cancer for TSER tandem repeats. Twenty-five patients who had TSER 2R/2R or 2R/3R genotypes received modified FOLFOX-6 (5-FU intravenous push and intravenous infusion with oxaliplatin and leucovorin every 2 weeks) until unacceptable toxicity or disease progression (median, 5.5 cycles); patients homozygous for triplet repeats (3R/3R) were excluded. The overall response rate in 23 evaluable patients was 39% (9 partial responses, no complete responses), which was worse than a 43% historical overall response rate in unselected patients. The overall response rate in 6 patients homozygous for doublet repeats (2R/2R) was 83% (5 partial responses, no complete responses). Median OS and PFS in the entire cohort (secondary outcomes) were 11.3 months and 6.2 months, respectively; these rates were similar to those reported in unselected populations. The study was stopped before meeting target enrollment (minimum 75 patients) due to insufficient funding.

Magnani et al (2013) reported on 180 cancer patients receiving fluoropyrimidines (5-FU or capecitabine) who underwent DPYD analysis for the 1905+1 G>A variant by HPLC. Four patients were heterozygous carriers. Of these, 3 patients received dose reduction of 50% to 60% but still experienced severe toxicities requiring hospitalization. One patient did not receive chemotherapy based on DPYD genotype and the presence of other variants found in mismatch repair genes.

**Section Summary: Clinical Utility**

A 2010 TEC Assessment concluded that DPYD and TYMS variant testing had a poor ability to identify patients likely to experience severe 5-FU toxicity. Since publication of the TEC Assessment, no prospective trials comparing efficacy and toxicity outcomes in patients who did and did not undergo pretreatment DPYD and/or TYMS testing have been published. A study comparing outcomes after pretreatment DYPD testing and historical controls found a lower rate of grade 3 or
higher toxicity in patients who underwent genetic testing. This study was limited by lack of randomization and lack of a concurrent control group.

**Summary of Evidence**
For individuals who have cancer for whom treatment with 5-fluorouracil (5-FU) is indicated who receive laboratory assays to determine 5-fluorouracil area under the curve, the evidence includes randomized controlled trials (RCTs), observational studies, and systematic reviews. Relevant outcomes are overall survival, disease-specific survival, test accuracy and validity and treatment-related morbidity. A systematic review of observational studies on analytic validity studies found good correlation between test results; however, reviewers concluded that selected studies had high risk of bias due to excluded samples. Several analyses of patients with colorectal cancer have evaluated clinical validity. For example, 1 study found that the rate of severe toxicity was significantly lower in patients with stage II and III cancer who chose pharmacokinetic monitoring versus body surface area (BSA) monitoring, but progression-free survival did not differ between groups in patients with stage IV or recurrent cancer. No RCTs or nonrandomized comparative studies were identified comparing health outcomes in cancer patients who did and did not have 5-FU dose adjustment using the My5-FU assay and who were treated with chemotherapy regimens used in current clinical practice. A systematic review of the available literature found a significantly higher response rate with BSA-based monitoring and no significant difference in toxicity. Most data were from observational studies and the RCTs were conducted in the 1980s when different chemotherapy protocols were used. The evidence is insufficient to determine the effects of the technology on health outcomes.

For individuals who have cancer for whom treatment with 5-FU is indicated who receive genetic testing for variants (eg, in *DPYD* and *TYMS*) affecting 5-FU metabolism, the evidence includes observational studies and systematic reviews. Relevant outcomes are overall survival, disease-specific survival, test accuracy and validity, and treatment-related morbidity. A 2010 TEC Assessment concluded that *DPYD* and *TYMS* variant testing had poor prognostic capacity to identify patients likely to experience severe 5-FU toxicity. Since publication of that Assessment, no prospective trials comparing efficacy and toxicity outcomes in patients who did and did not undergo pretreatment *DPYD* and/or *TYMS* testing have been published. One study compared outcomes in patients undergoing pretreatment *DPYD* testing with historical controls who did not receive testing. In that study, rates of grade 3 or higher toxicity were lower in patients who had genetic testing; however, the study was not randomized and lacked concurrent controls. The evidence is insufficient to determine the effects of the technology on health outcomes.

**Supplemental Information**

**Practice Guidelines and Position Statements**

**National Comprehensive Cancer Network Guidelines**
Although current National Comprehensive Cancer Network (NCCN) guidelines acknowledge that the
“selection, dosing, and administration of anticancer agents and the management of associated toxicities are complex,” NCCN does not recommend use of area under the curve guidance for 5-fluorouracil (5-FU) dosing or genetic testing for DPYD and/or TYMS variants in patients with colon, rectal, breast, gastric, pancreatic cancer, or head and neck cancers.

**Clinical Pharmacogenetics Implementation Consortium**
The Clinical Pharmacogenetics Implementation Consortium (CPIC) was formed in 2009 as a shared project between PharmGKB, an internet research tool developed by Stanford University, and the Pharmacogenomics Research Network of the National Institutes of Health. In 2013, CPIC published an evidence-based guideline for DPYD genotype and fluoropyrimidine dosing. The guideline did not address testing.

**National Institute for Health and Care Excellence**
In 2014, the National Institute of Health and Care Excellence published evidence-based diagnostics guidance on the 5-FU assay for 5-FU chemotherapy dose adjustment. The guidance stated: “The My5-FU assay is only recommended for use in research for guiding dose adjustment in people having fluorouracil chemotherapy by continuous infusion. The My5-FU assay shows promise and the development of robust evidence is recommended to demonstrate its utility in clinical practice.”

**U.S. Preventive Services Task Force Recommendations**
Not applicable.

**Medicare National Coverage**
There is no national coverage determination (NCD). In the absence of an NCD, coverage decisions are left to the discretion of local Medicare carriers.

**Ongoing and Unpublished Clinical Trials**
Some currently unpublished trials that might influence this review are listed in Table 2.

**Table 2. Summary of Key Trials**

<table>
<thead>
<tr>
<th>NCT No.</th>
<th>Trial Name</th>
<th>Planned Enrollment</th>
<th>Completion Date</th>
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<tbody>
<tr>
<td><strong>Ongoing</strong></td>
<td></td>
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<tr>
<td><strong>AUC-guided dosing of 5-FU</strong></td>
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<tr>
<td>NCT00943137</td>
<td>The Optimisation of 5-Fluorouracil Dose by Pharmacokinetic Monitoring in Asian Patients With Advanced Stage Cancer</td>
<td>55</td>
<td>Jun 2017</td>
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<tr>
<td>NCT02055560a</td>
<td>Retrospective Data Comparison of Toxicity and Efficacy in Colorectal Cancer (CRC) Patients Managed With and Without 5-FU Exposure Optimization Testing</td>
<td>350</td>
<td>Dec 2017</td>
</tr>
<tr>
<td><strong>DPYD and/or TYMS testing before use of fluoropyrimidines</strong></td>
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<td></td>
<td></td>
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<tr>
<td>NCT02324452</td>
<td>Safety, Feasibility and Cost-effectiveness of Genotype-directed Individualized Dosing of 5-Fluorouracil</td>
<td>1250</td>
<td>Mar 2017 (ongoing)</td>
</tr>
</tbody>
</table>
Fluopyrimidines

NCT00131599
Thymidylate Synthase Polymorphisms as a Predictor of Toxicity to 5-Fluorouracil Based Chemotherapy in Stage III Colon C 104 July 2017

AUC: area under the curve; 5-FU: 5-Fluorouracil; NCT: national clinical trial.

a Denotes industry-sponsored or cosponsored trial.

References
receiving infusional therapy containing 5-FU. Cancer. Feb 1 1996;77(3):441-451. PMID 8630950
27. Blue Cross and Blue Shield Association Technology Evaluation Center (TEC). Pharmacogenetic Testing to Predict Serious Toxicity From 5-Fluorouracil (5-FU) for Patients Administered 5-FU-Based Chemotherapy for Cancer. TEC Assessments. 2010;24:Tab 13.

Billing Coding/Physician Documentation Information

S3722 Dose optimization by area under the curve (AUC) analysis, for infusional 5-fluorouracil
81400 Molecular pathology procedure, Level 1 (eg, identification of single germline variant [eg, SNP] by techniques such as restriction enzyme digestion or melt curve analysis)
81401 Molecular pathology procedure, Level 2 (eg, 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat)
84999 Unlisted chemistry procedure

ICD10 Codes
C18.0- C18.9 Malignant neoplasm of colon code range
C19 Malignant neoplasm of rectosigmoid junction

Additional Policy Key Words
N/A

Policy Implementation/Update Information
5/1/11 New policy; considered investigational.
5/1/12 Coding Updated
5/1/12 No policy statement changes.
5/1/13 No policy statement changes.
5/1/15 Title changed to “Laboratory and Genetic Testing for Use of 5-Fluorouracil in Patients With Cancer”. Added TheraGuide® testing for genetic mutations in dipyrimidine dehydrogenase (DPYD) or thymidylate synthase (TYMS) to guide 5-FU dosing and/or treatment choice in patients with cancer is considered investigational. Changed OnDose™ to My5-FU™ in investigational statement.
5/1/16 “TheraGuide®” removed from policy statement because this test is no
longer commercially available; policy statements otherwise unchanged

Genetic testing nomenclature updated; “mutations” changed to “variants” in second policy statement. Policy statements otherwise unchanged.

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