Policy Statement

My5-fluorouracil™ assay testing or other types of assays for determining 5-fluorouracil (5-FU) area under the curve in order to adjust 5-fluorouracil dose for colorectal cancer patients or other cancer patients is considered investigational.

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

Policy Guidelines

Genetics Nomenclature Update
The 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). The Society’s nomenclature is recommended by the Human Variome Project, the Human Genome Organization (HUGO), and by the Human Genome Variation Society itself.

The American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) standards and guidelines for interpretation of sequence variants represent expert opinion from both organizations, in addition to 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>Previous Definition</th>
<th>Updated Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation Disease-associated variant</td>
<td>Disease-associated change in the DNA sequence</td>
</tr>
<tr>
<td>Variant</td>
<td>Change in the DNA sequence</td>
</tr>
<tr>
<td>Familial variant Disease-associated variant identified in a proband for use in subsequent targeted genetic testing in first-degree relatives</td>
<td></td>
</tr>
</tbody>
</table>

Table PG2. ACMG - AMP Standards and Guidelines for Variant Classification

<table>
<thead>
<tr>
<th>Variant Classification</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogenic</td>
<td>Disease-causing change in the DNA sequence</td>
</tr>
<tr>
<td>Likely pathogenic</td>
<td>Likely disease-causing change in the DNA sequence</td>
</tr>
<tr>
<td>Variant of uncertain significance</td>
<td>Change in DNA sequence with uncertain effects on disease</td>
</tr>
<tr>
<td>Likely benign</td>
<td>Likely benign change in the DNA sequence</td>
</tr>
<tr>
<td>Benign</td>
<td>Benign change in the DNA sequence</td>
</tr>
</tbody>
</table>

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

Genetic Counseling
Experts recommend formal genetic counseling for patients who are at risk for inherited disorders and who wish to undergo genetic testing. Interpreting the results of genetic tests and understanding risk factors can be difficult for some patients; genetic counseling helps individuals understand the impact of genetic testing, including the possible effects the test results could have on the individual or their family members. It should be noted that genetic counseling may
alter the utilization of genetic testing substantially and may reduce inappropriate testing; further, genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.

Coding

Effective January 1, 2018, the following specific CPT codes may be used:

- **81230**: CYP3A4 (cytochrome P450 family 3 subfamily A member 4) (e.g., drug metabolism), gene analysis, common variant(s) (e.g., *2, *22)
- **81231**: CYP3A5 (cytochrome P450 family 3 subfamily A member 5) (e.g., drug metabolism), gene analysis, common variants (e.g., *2, *3, *4, *5, *6, *7)
- **81232**: DPYD (dihydropyrimidine dehydrogenase) (e.g., 5-fluorouracil/5-FU and capecitabine drug metabolism), gene analysis, common variant(s) (e.g., *2A, *4, *5, *6)
- **81346**: TYMS (thymidylate synthetase) (e.g., 5-fluorouracil/5-FU drug metabolism), gene analysis, common variant(s) (e.g., tandem repeat variant)

The following is a specific HCPCS “S” code for the My5-FU test:

- **S3722**: Dose optimization by area under the curve (AUC) analysis, for infusional 5-fluorouracil

### Description

Variability in systemic exposure to 5-fluorouracil chemotherapy is thought to directly impact 5-fluorouracil tolerability and efficacy. The standard approach is dosing according to body surface area. Two alternative approaches have been proposed for modifying use of 5-fluorouracil: (1) dosing based on the determined area under the curve serum concentration target and (2) genetic testing for variants affecting 5-fluorouracil metabolism. For genetic testing, currently available polymerase chain reaction tests assess specific variants in genes encoding dihydropyrimidine reductase (DPYD) and thymidylate synthetase (TYMS) in the catabolic and anabolic pathways of 5-fluorouracil metabolism, respectively.

### Related Policies

- N/A

### Benefit Application

Benefit determinations should be based in all cases on the applicable contract language. To the extent there are any conflicts between these guidelines and the contract language, the contract language will control. Please refer to the member’s contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

Some state or federal mandates (e.g., Federal Employee Program [FEP]) prohibits plans from denying Food and Drug Administration (FDA)-approved technologies as investigational. In these instances, plans may have to consider the coverage eligibility of FDA-approved technologies on the basis of medical necessity alone.

### Regulatory Status

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

**Rationale**

**Background**

5-fluorouracil

The agent 5-fluorouracil is a widely used antineoplastic chemotherapy drug that targets thymidylate synthase (TYMS) enzyme, which is involved in DNA production. 5-fluorouracil has been used for many years to treat solid tumors (e.g., 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-fluorouracil. Several studies also have reported statistically significant positive associations between 5-fluorouracil exposure and tumor response. In current practice, however, 5-fluorouracil dose is reduced when symptoms of severe toxicity appear but is seldom increased to promote efficacy.

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

In clinical research studies, 5-fluorouracil 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.

**Literature Review**

The primary goal of therapeutic drug monitoring, pharmacogenomics testing, and personalized medicine is to achieve better clinical outcomes compared with the standard of care. Drug response varies greatly between individuals, and genetic factors are known to play a role. However, in most cases, the genetic variation only explains a modest portion of the variance in the individual response because clinical outcomes are also affected by a wide variety of factors including alternate pathways of metabolism and patient- and disease-related factors that may affect absorption, distribution, and elimination of the drug. Therefore, assessment of clinical utility cannot be made by a chain of evidence from clinical validity data alone. In such cases, evidence evaluation requires studies that directly demonstrate that the therapeutic drug monitoring strategy or pharmacogenomic test alters clinical outcomes; it is not sufficient to demonstrate that the test predicts a disorder or a phenotype. The review of evidence in the following sections will focus on direct evidence of clinical utility.

Evidence reviews assess whether a medical test is clinically useful. A useful test provides information to make a clinical management decision that improves the net health outcome. That is, the balance of benefits and harms is better when the test is used to manage the condition than when another test or no test is used to manage the condition.

The first step in assessing a medical test is to formulate the clinical context and purpose of the test. The test must be technically reliable, clinically valid, and clinically useful for that purpose. Evidence reviews assess the evidence on whether a test is clinically valid and clinically useful. Technical reliability is outside the scope of these reviews, and credible information on technical reliability is available from other sources.
Laboratory Testing to determine 5-fluorouracil Area Under the Curve for Dose Adjustment

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

The question addressed in this evidence review is: Can lab tests be used to guide 5-fluorouracil dosing to maximize therapeutic impact and minimize toxicity?

The following PICO was used to select literature to inform this review.

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

**Interventions**
The test being considered is laboratory assays to determine 5-fluorouracil area under the curve.

Patient exposure to 5-fluorouracil is most accurately described by estimating the area under the curve, the total drug exposure over a defined period of time. 5-fluorouracil exposure is influenced by the 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-fluorouracil catabolism. As a result, both inter- and intrapatient variability in 5-fluorouracil plasma concentration during administration is high.

Determination of 5-fluorouracil area under the curve requires complex technology and expertise that may not be readily available in a clinical laboratory setting. In the U.S., a commercial immunoassay (My5-fluorouracil) can quantify plasma 5-fluorouracil concentration from a blood sample drawn during continuous infusion at steady state (18-44 hours after the start of infusion) and provide a dose-adjustment algorithm to maintain plasma 5-fluorouracil area under the curve between 20 and 30 mg/h/L during the next cycle.

The association between area under the curve-monitored (My5-fluorouracil) versus body surface area (body surface area) dosing strategies has been examined in colorectal cancer patients who received 5-fluorouracil regimens.

**Comparators**
The following practice is currently being used to make decisions about dosing of 5-fluorouracil. This involves standard dosing by body weight, specifically body surface area-based dosing.

Body surface area-based dosing is associated with wide variability in pharmacokinetic parameters leading to significant differences in individual exposure. Nevertheless, body surface area-based dosing is the standard for most chemotherapeutic agents.

**Outcomes**
There is a relatively narrow therapeutic window for 5-fluorouracil and levels of exposure leading to toxicity and efficacy overlap. Therefore, both safety and efficacy outcomes are of interest in evaluating evidence.

The outcomes of interest related to 5-fluorouracil toxicity are types of severe toxicity such as cardiotoxicity, neutropenia, diarrhea, mucositis, and hand-foot syndrome.

**Clinically Useful**
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.
Study Selection Criteria
As previously described, the review of evidence focuses on direct evidence of clinical utility including studies that report efficacy and safety outcomes.

Methodologically credible studies were selected using the following principles:

a. To assess efficacy outcomes, comparative controlled prospective trials were sought, with a preference for randomized controlled trials (RCTs).

b. In the absence of such trials, comparative observational studies were sought, with a preference for prospective studies.

c. To assess longer-term outcomes and adverse effects, single-arm studies were sought that capture longer periods of follow-up and/or larger populations.

d. Duplicative or studies overlapping populations were excluded.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

The results of single-arm trials of area under the curve-targeted 5-fluorouracil dose adjustment in advanced colorectal cancer patients have suggested consistently improved tumor response.4,5,6 Similar, although less compelling, results were seen in single-arm trials of area under the curve-targeted 5-fluorouracil dosing in head and neck cancer.7,8 The best contemporary evidence supporting area under the curve-targeted dosing consists of 2 RCTs, 1 enrolling patients with colorectal cancer and the other enrolling patients with head and neck cancer. No trials of any design were identified for 5-fluorouracil 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 (1996)9 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 colorectal cancer, 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 area under the curve-targeted dosing (by high-performance liquid chromatography) for single-agent 5-fluorouracil compared with fixed dosing.10 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%).11,12 In the latter 2 trials, the delivery over a longer time period for both 5-fluorouracil (22 hours vs. 8 hours) and leucovorin (2 hours vs. bolus), which is characteristic of currently recommended 5-fluorouracil treatment regimens, likely minimized toxicity.

The administration schedule used in the Gamelin et al (2008) trial is rarely currently used in clinical practice and is absent from current guidelines.3 Additional optimization studies would be needed to apply 5-fluorouracil exposure monitoring and area under the curve-targeted dose adjustment to a more standard single-agent 5-fluorouracil 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-fluorouracil exposures in head and neck cancer patients that were significantly reduced in the dose-adjustment arm compared with the fixed-dose arm.13 This reduced toxicity but did not improve clinical response. The dose-adjustment method in this trial might have been too complex because the 12 patients with protocol violations in this treatment arm (of 61 enrolled) all were related to 5-fluorouracil dose adjustment miscalculations. Because patients with protocol violations were removed from the analysis, results did not reflect “real-world” results of the dose-adjustment method. Also, the induction therapy regimen used 2 drugs, not the current standard of 3, therefore, the generalizability of results to current clinical practice is limited.
Yang et al (2016) published a meta-analysis of data from the 2 RCTs described above (i.e., Gamelin et al [2008] and Fety et al [1998]), as well as from 3 observational studies. In a pooled analysis, the overall response rate was significantly higher with pharmacokinetic area under the curve-monitored 5-fluorouracil therapy than with standard body surface area-based monitoring (odds ratio, 2.04; 95% confidence interval, 1.41 to 2.95). In terms of toxicity, the incidence of diarrhea (3 studies), neutropenia (3 studies), and hand-foot syndrome (2 studies) did not differ significantly between the pharmacokinetic and body surface area monitoring strategies. The rate of mucositis was significantly lower in the body surface area-monitored group (3 studies; odds ratio, 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: Clinically Useful
No RCTs or nonrandomized comparative studies were identified comparing health outcomes in cancer patients who did and did not have 5-fluorouracil dose adjustment using the My5-fluorouracil 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 body surface area-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.

Testing for DPYD or TYMS Variants Affecting 5-fluorouracil Dose Adjustment
Clinical Context and Proposed Clinical Utility
The proposed clinical utility of genetic testing is to use test results to guide 5-fluorouracil dosing so that the therapeutic impact is maximized and the toxicity is decreased.

The question addressed in this evidence review is: Can genetic tests guide 5-fluorouracil dosing to maximize therapeutic impact and minimize toxicity?

The following PICO was used to select literature to inform this review.

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

Interventions
The test being considered is genetic testing for variants (e.g., in DPYD and TYMS) affecting 5-fluorouracil metabolism.

5-fluorouracil 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-fluorouracil is inactivated and eliminated via the catabolic pathway; the remainder is metabolized via the anabolic pathway.

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

The anabolic pathway metabolizes 5-fluorouracil 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-
fluorouracil resistance due to increased tumor TYMS expression compared with the TSER*2 variant (2 tandem repeats) and wild-type forms.

A number of studies have evaluated the association between variants in the DPYD and/or TYMS genes and 5-fluorouracil toxicity. Cancer types and specific variants studied differed across these reports.18-23.

Comparators
The following practice is currently being used to make decisions about dosing of 5-fluorouracil. This involves standard dosing by body weight, specifically body surface area-based dosing.

Outcomes
There is a relatively narrow therapeutic window for 5-fluorouracil and levels of exposure leading to toxicity and efficacy overlap. The beneficial outcome of a true-positive (identifying a variant that would have caused severe toxicity) is prevention of toxicity. However, the harmful outcome of a false-positive is withholding or premature cessation of effective chemotherapy which may compromise chemotherapy effectiveness.

Therefore, both safety and efficacy outcomes are of interest in evaluating evidence. The outcomes of interest related to 5-fluorouracil toxicity are types of severe toxicity such as cardiotoxicity, neutropenia, diarrhea, mucositis, and hand-foot syndrome.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing. Study selection criteria were previously described.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

A Blue Cross Blue Shield Association Technology Evaluation Center (TEC) Assessment (2010) concluded that DPYD and TYMS variant testing did not meet TEC criteria.24, The Assessment noted that the tests had “poor ability to identify patients likely to experience severe 5-fluorouracil toxicity. Although genotyping may identify a small fraction of patients for whom serious toxicity is a moderate to a strong risk factor, most patients who develop serious toxicity do not have variants in DPD or TS genes.”24

Several recent prospective observational studies have reported safety and effectiveness outcomes in patients who received genetic testing prior to receiving a 5-fluorouracil-based chemotherapy regimen. Characteristics and results of these studies are shown in Tables 1 and 2. Three of these, conducted by the same research group in the Netherlands, used historical controls,25,26,27, and 1 also included a matched-pairs analysis using previously-collected data.26 The others were single-arm, uncontrolled studies.28,29,30 No prospective trials comparing efficacy and safety outcomes using concurrent control groups with or without pretreatment DPYD and/or TYMS testing were identified.

Henricks et al (2019) included 3 comparison groups in a prospective cohort study in which patients received genotyping prior to treatment as part of routine care.26, Group 1 (n=40) were DPYD*2A carriers treated with an approximately 50% reduced fluoropyrimidine dose. Group 2 (n=1606) were wild-type patients who had been identified as part of an earlier study (Deenan et al [2016];25 discussed below) and treated with a standard dose. Group 3 (n=86) were DPYD*2A carriers, identified from the literature, treated with a standard dose. Safety outcomes of the first 18 of the 40 patients in Group 1 were previously reported in Deenan et al (2016).25 Patients in
Group 1 were matched to those in Group 2 for the primary analysis for covariates known to influence treatment outcome. The primary effectiveness endpoint was overall survival. Secondary endpoints were progression-free survival and tumor response.

In matched-pair comparisons, Groups 1 and 2 did not differ on overall survival (hazard ratio 0.82; 95% confidence interval 0.47 to 1.43; P=0.47), PFS (hazard ratio 0.83; 95% confidence interval 0.47 to 1.50; p=0.54), or tumor response (0% vs. 5% complete response; 20% vs. 34% partial response; p>0.99), suggesting that the lower dose did not have a detrimental effect on treatment response in DPYD*2A carriers. The incidence of treatment-related toxicity, including overall toxicity, gastrointestinal toxicity, hematological toxicity, and hand-foot syndrome, was higher in the genotype-guided dosing group compared to wild-type patients, but differences were not statistically significant. Compared to the historical literature cohort who had received standard dosing, Group 1 patients had a lower risk of severe toxicity (77% vs. 18%; P<0.001). There were no treatment-related deaths in the genotype-guided group, compared to 7 of 86 (8%) in the historical cohort. This study had several methodological limitations. Although patients were prospectively genotyped, data collection of outcomes was retrospective. A historical control group was used for the assessment of adverse events. There was a relatively large amount of missing data, small sample size, and the study was underpowered. Because it was conducted at a single-institution, its results may not be generalizable to other settings.

Deenan et al (2016) compared outcomes for pretreatment DPYD*2A testing with historical controls.25, The study included cancer patients intending to undergo treatment with fluoropyrimidine-based therapy (5-fluorouracil or capecitabine).25, Genotyping for DPYD*2A was performed before treatment, and dosing was adjusted based on the alleles identified. Patients with heterozygous variant alleles were treated with a reduced (i.e., ≥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% confidence interval, 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% confidence interval, 58% to 85%). The historical controls were more likely to be treated with 5-fluorouracil based therapy than with capecitabine-based therapy. Trial limitations included lack of randomization to a management strategy and use of historical, rather than concurrent, controls.

Henricks et al (2018) conducted a prospective study of adult patients with cancer who were intended to start fluoropyrimidine-based therapy.27, Patients were enrolled from 17 hospitals in the Netherlands. Dose reductions were based on genotyping: Heterozygous DPYD variant allele carriers received an initial dose reduction of either 25% (for c.2846A>T and c.1236G>A) or 50% (for DPYD*2A and c.1679T>G). The researchers compared adverse events in the prospectively genotyped group who received genotype-based dosing, wild-type patients identified through prospective genotyping, and a historical control group of patients from a previously published meta-analysis who were DPYD variant carriers but did not receive genotype-guided dosing. The primary outcome was the frequency of severe treatment-related toxicity. Survival and response were not assessed. There was a higher incidence of grade 3 or higher toxicity in the genotype-dosing group compared to wild-type patients (39% vs. 23% p=0.0013). The relative risk for severe toxicity in DPYD*2A carriers who did not have genotype-guided dosing was 2.87 (95% confidence interval 2.14 to 3.86), compared to 1.31 (0.63 to 2.73) in the cohort that received genotype-based dosing. The main limitation of this study is its use of a historical control group, with no control for confounders in the analysis.

Cremolini et al (2018) reported chemotherapy-related adverse events experienced by patients with metastatic colon cancer who were enrolled in the phase III RCT and treated with first-line FOLFOXIRI plus bevacizumab or FOLFIRI plus bevacizumab. Of 508 randomized patients, 443 (87%) were genotyped for DPYD and UGT1A1 variants. All received study treatments as
planned; dosage was not adjusted based on genotyping. All patients received study treatments at planned doses. Overall 8 of 10 patients who were DPYD carriers experienced grade 3 or higher adverse events. An advantage of this study was that it used prospectively and systematically collected data on adverse events. It is limited by the lack of a comparison group and because genotype-based dosing was not used.

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 a modified 5-fluorouracil chemotherapy regimen 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 overall survival and progression-free survival in the entire cohort (secondary outcomes) was 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-fluorouracil or capecitabine) who underwent DPYD analysis for the 1905+1 G > A variant by high-performance liquid chromatography. Four patients were heterozygous carriers. Of these, 3 patients received a 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.

### Table 1. Summary of Key Nonrandomized Trials Characteristics

<table>
<thead>
<tr>
<th>Study</th>
<th>Study Type</th>
<th>Country</th>
<th>Dates</th>
<th>Participants</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Henricks et al (2019)</td>
<td>Prospective, retrospective data collection, historical control groups</td>
<td>Netherlands</td>
<td>2007-2015</td>
<td>Patients intended to be treated with FU-based chemotherapy (n=1732)</td>
<td>Genotyping for DPYD*2A</td>
</tr>
<tr>
<td>Henricks et al (2018)</td>
<td>Prospective, with historical control</td>
<td>Netherlands</td>
<td>2015-2017</td>
<td>Patients intended to be treated with FU-based chemotherapy (n=1181)</td>
<td>Genotyping for DPYD*2A,</td>
</tr>
<tr>
<td>Cremolini et al (2018)</td>
<td>Prospective, uncontrolled</td>
<td>Italy</td>
<td>2008-2011</td>
<td>Patients with metastatic colorectal cancer who were treated with 5-fluorouracil and irinotecan-based chemotherapy in an RCT (n=443)</td>
<td>Genotyping for DPYD*2A</td>
</tr>
<tr>
<td>Deenen et al (2016)</td>
<td>Prospective, with historical control</td>
<td>Netherlands</td>
<td>2007-2011</td>
<td>Patients intended to be treated with FU-based chemotherapy (n=2038)</td>
<td>Genotyping for DPYD*2A</td>
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<td>Goff et al (2014)</td>
<td>Prospective, uncontrolled</td>
<td>U.S.</td>
<td>2008-2010</td>
<td>Adults with gastric or gastroesophageal junction cancer (n=25)</td>
<td>Genotyping for TSER tandem repeats</td>
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<tr>
<td>Magnani et al (2013)</td>
<td>Prospective, uncontrolled</td>
<td>Italy</td>
<td>2011-2012</td>
<td>Patients diagnosed with gastrointestinal, breast, head and neck, and other tumors (n=180)</td>
<td>DPYD analysis</td>
</tr>
</tbody>
</table>

FU: fluoropyrimidine; 5-fluorouracil: 5-fluorouracil; RCT: randomized controlled trial.
### Table 2. Summary of Key Nonrandomized Trials Results

<table>
<thead>
<tr>
<th>Study</th>
<th>Heterozygous Carrier Patients</th>
<th>Grade 3 Toxicity</th>
<th>Overall Response Rate</th>
<th>Median Overall Survival</th>
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<tbody>
<tr>
<td>Henricks et al (2019)</td>
<td></td>
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<tr>
<td><strong>Group 1: DPYD*2A carriers, reduced dose (n=40)</strong></td>
<td>40</td>
<td>7/40 (18%)</td>
<td>0% complete response, 20% partial response, 40% stable</td>
<td>27 mo (range 1-83 mo)</td>
</tr>
<tr>
<td><strong>Group 2: Wild-type, standard dose (n=1606)</strong></td>
<td>NA</td>
<td>372/1606 (23%)</td>
<td>5% complete response, 29% partial response, 14% stable</td>
<td>24 mo (range 0.7 to 97 mo)</td>
</tr>
<tr>
<td><strong>Group 3: DPYD*2A carriers, standard dose (n=86)</strong></td>
<td>86</td>
<td>66/86 (77%)</td>
<td>NR</td>
<td></td>
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<tr>
<td>Hazard ratio (95% CI)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>Group 1 vs. Group 2:</strong> 0.82 (0.47 to 1.43)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Group 1 vs. Group 3:</strong> &lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Group 1 vs. Group 2:</strong> &gt;0.99</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Henricks et al (2018)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPYD*2A carriers, genotype-guided dosing</td>
<td>85/1181 (7.7%)</td>
<td>33/85 (39%)</td>
<td>RR 1.31 (95% CI 0.63 to 2.73)</td>
<td></td>
</tr>
<tr>
<td>Historical control (DPYD*2A carriers, standard dose)</td>
<td>RR 2.87 (95% CI 2.14 to 3.86)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative risk (95% CI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Historical control (wild-type, standard dosing)</td>
<td>231/1018 (23%); p&lt;0.0013 vs. genotype guided dosing cohort</td>
<td>NR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cremolini et al (2018)</td>
<td>10/439 (2.2%)</td>
<td>8/10 (80%)</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Deenen et al (2016)</td>
<td>22/2038 (1.1%)</td>
<td>28%</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goff et al (2014)</td>
<td>NR</td>
<td>NR</td>
<td>39.1% (9 partial responses, no complete responses)</td>
<td>11.3 mo; 6.2 mo</td>
</tr>
<tr>
<td>95% CI</td>
<td></td>
<td></td>
<td>22.2-59.2</td>
<td></td>
</tr>
</tbody>
</table>
### Table 3. Relevance Limitations

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Intervention</th>
<th>Comparator</th>
<th>Outcomes</th>
<th>Duration of Follow-Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Henricks et al (2019)</td>
<td>26,</td>
<td>historical control group</td>
<td>1. no effectiveness outcomes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Henricks et al (2018)</td>
<td>27,</td>
<td>historical control group</td>
<td>1. no effectiveness outcomes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cremolini et al (2018)</td>
<td>30,</td>
<td>3. genotype-based dosing not used</td>
<td>no control group</td>
<td>1. no effectiveness outcomes</td>
<td></td>
</tr>
<tr>
<td>Deenen et al (2016)</td>
<td>25,</td>
<td>historical control group</td>
<td>1. no effectiveness outcomes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goff et al (2014)</td>
<td>28,</td>
<td>no control group</td>
<td>1. no effectiveness outcomes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnani et al (2013)</td>
<td>29,</td>
<td>no control group</td>
<td>1. no effectiveness outcomes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The study limitations stated in this table are those notable in the current review; this is not a comprehensive limitations assessment.

- **Population key:**
  1. Intended use population unclear
  2. Clinical context is unclear
  3. Study population is unclear
  4. Study population not representative of intended use

- **Intervention key:**
  1. Classification thresholds not defined
  2. Version used unclear
  3. Not intervention of interest

- **Comparator key:**
  1. Classification thresholds not defined
  2. Not compared to credible reference standard
  3. Not compared to other tests in use for same purpose

- **Outcomes key:**
  1. Study does not directly assess key health outcome
  2. Evidence chain or decision model not explicated
  3. Key clinical validity outcomes not reported (sensitivity, specificity, and predictive values)
  4. Reclassification of diagnostic or risk categories not reported
  5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests)

- **Duration of Follow-Up key:**
  1. Follow-up duration not sufficient with respect to natural history of disease (true-positives, true-negatives, false-positives, false-negatives cannot be determined)

### Table 4. Study Design and Conduct Limitations

<table>
<thead>
<tr>
<th>Study</th>
<th>Selection</th>
<th>Blinding</th>
<th>Delivery of Test</th>
<th>Selective Reporting</th>
<th>Data Completeness</th>
<th>Statistical Analysis</th>
</tr>
</thead>
</table>

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The study limitations stated in this table are those notable in the current review; this is not a comprehensive limitations assessment.

- **Selection key:** 1. Selection not described; 2. Selection not random or consecutive (i.e., convenience).
- **Blinding key:** 1. Not blinded to results of reference or other comparator tests.
- **Delivery key:** 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
- **Selective Reporting key:** 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
- **Data Completeness key:** 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
- **Statistical key:** 1. Confidence intervals and/or p values not reported; 2. Comparison with other tests not reported.

### Section Summary: Clinically Useful

A 2010, Blue Cross Blue Shield Association Technology Evaluation Center (TEC) Assessment concluded that DPYD and TYMS variant testing had a poor ability to identify patients likely to experience severe 5-fluorouracil toxicity. Since the publication of the Blue Cross Blue Shield Association Technology Evaluation Center (TEC) Assessment, no prospective trials comparing the efficacy and toxicity outcomes in patients who did and did not undergo pretreatment DPYD and/or TYMS testing have been published. Three prospective observational studies used a historical control group and 1 also used a matched-pairs analysis to compare outcomes in patients who received genotype-based dosing to those who received standard dosing. No differences in overall survival, progression-free survival or tumor progression were observed. Risk of serious toxicity was higher in DPYD allele carriers who received genotype-based dosing compared to wild-type patients but lower when compared to historical controls who were carriers but received standard dosing. The evidence is limited by retrospective data collection, use of historical control groups, small sample sizes, and missing data.

### Summary of Evidence

For individuals who have cancer for whom treatment with 5-fluorouracil 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. Several analyses of patients with colorectal cancer have evaluated clinical validity. One study, for example, 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 monitoring but progression-free survival and tumor progression were observed. Risk of serious toxicity was higher in DPYD allele carriers who received genotype-based dosing compared to wild-type patients but lower when compared to historical controls who were carriers but received standard dosing. The evidence is limited by retrospective data collection, use of historical control groups, small sample sizes, and missing data.
survival, disease-specific survival, test accuracy and validity, and treatment-related morbidity. A Blue Cross Blue Shield Association Technology Evaluation Center (TEC) Assessment (2010) concluded that DPYD and TYMS variant testing had poor prognostic capacity to identify patients likely to experience severe 5-fluorouracil toxicity. Since the publication of that Assessment, no prospective trials comparing the efficacy and toxicity outcomes in patients who did and did not undergo pretreatment DPYD and/or TYMS testing have been published. Three prospective observational studies used a historical control group and 1 also used a matched-pairs analysis to compare outcomes in patients who received genotype-based dosing to those who received standard dosing. No differences in overall survival, progression-free survival, or tumor progression were observed. Risk of serious toxicity was higher in DPYD allele carriers who received genotype-based dosing compared to wild-type patients but lower when compared to historical controls who were carriers but received standard dosing. The evidence is limited by retrospective data collection, use of historical control groups, small sample sizes, and missing data. 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**

National Comprehensive Cancer Network guidelines do not recommend use of area under the curve guidance for 5-fluorouracil dosing or genetic testing for DPYD and/or TYMS variants in patients with colon, rectal, breast, gastric, pancreatic cancer, or head and neck cancers.

**International Association of Therapeutic Drug Monitoring and Clinical Toxicology**

In 2019, the International Association of Therapeutic Drug Monitoring and Clinical Toxicology published recommendations for therapeutic drug monitoring of 5-Fluorouracil therapy. The work was supported in part by grants from the National Cancer Institute National Institutes of Health. Several authors reported relationships with Saladax, the manufacturer of the My5-fluorouracil test. The committee concluded that there was sufficient evidence to strongly recommend therapeutic drug monitoring for the management of 5-fluorouracil therapy in patients with early or advanced colorectal cancer and patients with squamous cell carcinoma of head-and-neck cancer receiving common 5-fluorouracil dosing regimens.

**Clinical Pharmacogenetics Implementation Consortium**

In 2009, the Clinical Pharmacogenetics Implementation Consortium was formed 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, the Clinical Pharmacogenetics Implementation Consortium published evidence-based guidelines for DPYD genotype and fluoropyrimidine dosing. The guidelines did not address testing.

An update to the Clinical Pharmacogenetics Implementation Consortium (2017) guidelines was published by Amstutz et al (2018). As in 2013, the primary focus of the guidelines was on the DPYD genotype and implications for dosing of fluoropyrimidine. In the 2017 update, the Clinical Pharmacogenetics Implementation Consortium noted that genetic testing for DPYD may include “resequencing of the complete coding regions” or may be confined to analysis of particular risk variants, among which Clinical Pharmacogenetics Implementation Consortium listed the c.1905+1G>A, c.1679T>G, c.2846A>T, and c.1129-5923C>G variants, as affecting 5-fluorouracil toxicity. The guideline further noted that, while other genes (TYMS, MTHFR) may be tested for variants, the clinical utility of such tests is yet unproven. In patients who have undergone genetic testing and who are known carriers of a DPYD risk variant, the guidelines recommended that caregivers strongly reduce the dosage of 5-fluorouracil-based treatments, or exclude them, depending on the patient’s level of DPYD activity. CPIC advised follow-up therapeutic drug monitoring to guard against underdosing and cautioned that genetic tests could be limited to known risk variants and, therefore, not identify other DPYD variants.
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-fluorouracil assay for 5-fluorouracil chemotherapy dose adjustment. The guidance stated: “The My5-fluorouracil assay is only recommended for use in research for guiding dose adjustment in people having fluorouracil chemotherapy by continuous infusion. The My5-fluorouracil 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. In the absence of a national coverage determination, coverage decisions are left to the discretion of local Medicare carriers.

Ongoing and Unpublished Clinical Trials
There are currently no relevant ongoing trials. Some unpublished trials that might influence this review are listed in Table 5.

Table 5. Summary of Key Trials

<table>
<thead>
<tr>
<th>NCT No.</th>
<th>Trial Name</th>
<th>Planned Enrollment</th>
<th>Completion Date</th>
</tr>
</thead>
<tbody>
<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>June 2017</td>
</tr>
<tr>
<td>NCT02055560a</td>
<td>Retrospective Data Comparison of Toxicity and Efficacy in Colorectal Cancer (CRC) Patients Managed With and Without 5-fluorouracil Exposure Optimization Testing</td>
<td>350</td>
<td>Dec 2017(unknown)</td>
</tr>
</tbody>
</table>

NCT: national clinical trial.

References
24. 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.


PMID 29152729

**Documentation for Clinical Review**

- No records required

**Coding**

This Policy relates only to the services or supplies described herein. Benefits may vary according to product design; therefore, contract language should be reviewed before applying the terms of the Policy. Inclusion or exclusion of codes does not constitute or imply member coverage or provider reimbursement.

IE

The following services may be considered investigational.

<table>
<thead>
<tr>
<th>Type</th>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPT</td>
<td>81230</td>
<td>CYP3A4 (cytochrome P450 family 3 subfamily A member 4) (e.g., drug metabolism), gene analysis, common variant(s) (e.g., *2, *22)</td>
</tr>
<tr>
<td></td>
<td>81231</td>
<td>CYP3A5 (cytochrome P450 family 3 subfamily A member 5) (e.g., drug metabolism), gene analysis, common variants (e.g., *2, *3, *4, *5, *6, *7)</td>
</tr>
<tr>
<td></td>
<td>81232</td>
<td>DPYD (dihydropyrimidine dehydrogenase) (e.g., 5-fluorouracil/5-FU and capecitabine drug metabolism), gene analysis, common variant(s) (e.g., *2A, *4, *5, *6)</td>
</tr>
<tr>
<td>HCPCS</td>
<td>S3722</td>
<td>Dose optimization by area under the curve (AUC) analysis, for infusional 5-fluorouracil</td>
</tr>
</tbody>
</table>

**Policy History**

This section provides a chronological history of the activities, updates and changes that have occurred with this Medical Policy.

<table>
<thead>
<tr>
<th>Effective Date</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>07/30/2015</td>
<td>BCBSA Medical Policy adoption</td>
</tr>
<tr>
<td>06/01/2016</td>
<td>Policy revision without position change</td>
</tr>
<tr>
<td>05/01/2017</td>
<td>Policy revision without position change</td>
</tr>
<tr>
<td>02/01/2018</td>
<td>Coding update</td>
</tr>
<tr>
<td>05/01/2018</td>
<td>Policy revision without position change</td>
</tr>
<tr>
<td>05/01/2019</td>
<td>Policy revision without position change</td>
</tr>
<tr>
<td>10/01/2019</td>
<td>Policy revision without position change</td>
</tr>
<tr>
<td>06/01/2020</td>
<td>Annual review. No change to policy statement. Literature review updated.</td>
</tr>
</tbody>
</table>
Definitions of Decision Determinations

**Medically Necessary**: Services that are Medically Necessary include only those which have been established as safe and effective, are furnished under generally accepted professional standards to treat illness, injury or medical condition, and which, as determined by Blue Shield, are: (a) consistent with Blue Shield medical policy; (b) consistent with the symptoms or diagnosis; (c) not furnished primarily for the convenience of the patient, the attending Physician or other provider; (d) furnished at the most appropriate level which can be provided safely and effectively to the patient; and (e) not more costly than an alternative service or sequence of services at least as likely to produce equivalent therapeutic or diagnostic results as to the diagnosis or treatment of the Member’s illness, injury, or disease.

**Investigational/Experimental**: A treatment, procedure, or drug is investigational when it has not been recognized as safe and effective for use in treating the particular condition in accordance with generally accepted professional medical standards. This includes services where approval by the federal or state governmental is required prior to use, but has not yet been granted.

**Split Evaluation**: Blue Shield of California/Blue Shield of California Life & Health Insurance Company (Blue Shield) policy review can result in a split evaluation, where a treatment, procedure, or drug will be considered to be investigational for certain indications or conditions, but will be deemed safe and effective for other indications or conditions, and therefore potentially medically necessary in those instances.

**Prior Authorization Requirements (as applicable to your plan)**

Within five days before the actual date of service, the provider must confirm with Blue Shield that the member's health plan coverage is still in effect. Blue Shield reserves the right to revoke an authorization prior to services being rendered based on cancellation of the member's eligibility. Final determination of benefits will be made after review of the claim for limitations or exclusions.

Questions regarding the applicability of this policy should be directed to the Prior Authorization Department at (800) 541-6652, or the Transplant Case Management Department at (800) 637-2066 ext. 3507708 or visit the provider portal at www.blueshieldca.com/provider.

Disclaimer: This medical policy is a guide in evaluating the medical necessity of a particular service or treatment. Blue Shield of California may consider published peer-reviewed scientific literature, national guidelines, and local standards of practice in developing its medical policy. Federal and state law, as well as contract language, including definitions and specific contract provisions/exclusions, take precedence over medical policy and must be considered first in determining covered services. Member contracts may differ in their benefits. Blue Shield reserves the right to review and update policies as appropriate.