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

Policy # 00291
Original Effective Date: 03/16/2011
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Applies to all products administered or underwritten by Blue Cross and Blue Shield of Louisiana and its subsidiary, HMO Louisiana, Inc. (collectively referred to as the "Company"), unless otherwise provided in the applicable contract. Medical technology is constantly evolving, and we reserve the right to review and update Medical Policy periodically.

When Services Are Considered Investigational
Coverage is not available for investigational medical treatments or procedures, drugs, devices or biological products.

Based on review of available data, the Company considers My5-FU™† testing or other types of assays for determining 5-fluorouracil (5-FU) area under the curve (AUC) in order to adjust 5-FU dose for colorectal cancer patients or other cancer patients to be investigational.*

Based on review of available data, the Company considers 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 to be investigational.*

Background/Overview
Variability in systemic exposure to 5-FU is thought to directly impact 5-FU tolerability and efficacy. Two approaches have been proposed for modifying use of 5-FU: (1) dosing based on determined AUC serum concentration target and (2) genetic testing for mutations affecting 5-FU metabolism. Accurate AUC determination relies on sampling at pharmacokinetically appropriate times and on accurate methods of 5-FU serum concentration measurement. Available measurement methods are complex, making them less amenable to routine clinical laboratory settings. Genetic mutations may affect activity of enzymes involved in 5-FU metabolism. Currently available polymerase chain reaction tests assess specific mutations in genes encoding DPYD and TYMS in the catabolic and anabolic pathways of 5-FU metabolism, respectively.

The agent 5-FU is a widely used antineoplastic chemotherapy drug that targets TYMS, an enzyme involved in DNA production. 5-FU has a narrow therapeutic index; doses recommended for effectiveness are often limited by hematologic and gastrointestinal toxicity. Moreover, patients administered the same fixed-dose, continuous-infusion regimen of 5-FU have wide intra- and interpatient variability in systemic drug exposure, as measured by plasma concentration or, more accurately, by AUC techniques. AUC is a measure of systemic drug exposure in an individual over a defined period of time.

In general, the incidence of grade 3 to 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 AUC determination and to optimize an AUC target and dose adjustment algorithm for a particular 5-FU chemotherapy regimen.
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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.

Metabolism of 5-Fluorouracil
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 compared with bolus administration, resulting in very different systemic exposure to 5-FU during the course of therapy. Genetic mutations 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 mutations 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 in comparison with the *TSER*2 variant (2 tandem repeats) and wild-type forms.

FDA or Other Governmental Regulatory Approval

U.S. Food and Drug Administration (FDA)
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). Laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. To date, the FDA has chosen not to require any regulatory review of this test. My5-FU (Saladax Biomedical) is available under the auspices of CLIA. Other clinical laboratories may offer in-house assays to measure 5-FU AUC. Similarly, TheraGuide® (Myriad Genetics) was a laboratory-developed test but has been discontinued. Other laboratories may offer in-house assays for *DPYD* and *TYMS* mutation testing and ARUP Laboratories offers a test that is equivalent to TheraGuide (5-FU toxicity and chemotherapeutic response, 7 mutations test).

Centers for Medicare and Medicaid Services (CMS)
There is no national coverage determination (NCD). In the absence of an NCD, coverage decisions are left to the discretion of local Medicare carriers.
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Rationale/Source
This policy was originally created in 2011 and was updated regularly with searches of the MEDLINE database. The most recent literature review was performed through February 2, 2016. Following is a summary of the key literature to date.

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.

5-Fluorouracil and Clinical Use
5-Fluorouracil is a pyrimidine analog, antineoplastic antimetabolite; 5-FU has been used for many years to treat solid tumors, eg, colorectal adenocarcinoma. The U.S. FDA-approved indication of 5-FU is for “palliative management of carcinoma of the colon, rectum, breast, stomach, and pancreas.”

Colon Cancer
Potentiated by leucovorin (LV), 5-FU is the basis for several standard treatment regimens currently recommended by the National Comprehensive Cancer Network (NCCN) for the treatment of colorectal cancer (CRC). For stage II CRC, NCCN recommends adjuvant therapy primarily for disease with high-risk features, individualized for each patient; for stage III disease, oxaliplatin in combination with 5-FU/LV is the preferred standard of care. Based on results from the 2009 European Multicenter International Study of Oxaliplatin/5-Fluorouracil/Leucovorin in the Adjuvant Treatment of Colon Cancer (MOSAIC) trial, in which the addition of oxaliplatin to a regimen of LV and infusional 5-FU every 2 weeks (ie, a FOLFOX [leucovorin calcium, fluorouracil, oxaliplatin] regimen) significantly increased disease-free and overall survival (OS), the FOLFOX regimen is recommended for patients with stage III CRC. A FOLFOX regimen also improves progression-free survival (PFS) in patients with advanced (ie, metastatic) CRC who are able to tolerate intensive versus single-agent 5-FU therapy, and FOLFOX may be considered for patients with high-risk stage II disease. Other 5-FU-based combination chemotherapy regimens are options in advanced disease. In patients with advanced or metastatic colon cancer, bolus 5-FU regimens seem to be more toxic than infusional regimens and are considered inappropriate when coadministered with either irinotecan (a topoisomerase inhibitor) or oxaliplatin.

Head and Neck Cancers
5-FU has for many years been a component, with cisplatin, of induction therapy for squamous cell carcinoma of the head and neck in patients with advanced locoregional disease, yielding high rates of overall and complete clinical response. The addition of docetaxel was shown to improve survival, and this 3-drug combination is now considered the standard of care for induction chemotherapy. Typical 5-FU administration is by continuous infusion. 5-FU also is a component of several combination chemotherapy regimens used for primary systemic therapy in conjunction with radiotherapy, and of 2 combination regimens for recurrent, unresectable, or metastatic disease.
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Measuring Exposure to 5-FU
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 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 the course of administration is high.

As noted, 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 to 30 mg/h/L during the next cycle. The dosing algorithm is based on that developed by Kaldat et al (2012) using OnDose (now called My5-FU) in patients with CRC treated with FOLFOX. Technical specifications for OnDose can still be found on the Myriad Genetics website, which describes the test as a “competitive, homogeneous, 2-reagent nanoparticle agglutination immunoassay.” Although a search of large clinical laboratories did not find tests for 5-FU AUC listed, it is possible that other clinical laboratories measure 5-FU levels by methods other than the specific method used by Saladax Biomedical.

Modifying 5-FU Exposure to Improve Outcomes
A 2009 TEC Special Report reviewed the evidence for 5-FU AUC measurement to help modify subsequent 5-FU treatment doses to improve response and reduce toxicity. Early evidence from small, cohort studies showed that in general, the incidence of grade 3 to 4 toxicity (mainly neutropenia, diarrhea, mucositis, hand-foot syndrome) increased with higher systemic exposure to 5-FU. This association has been extensively studied in head and neck cancer and in CRC. In addition, most studies reported statistically significant positive associations between 5-FU exposure and tumor response.

Based on these early results, various strategies have been tried to reduce variability in 5-FU pharmacokinetics, improve treatment efficacy, and decrease toxicity. In particular, individual pharmacokinetic dose adaptation can be accomplished by monitoring plasma 5-FU area under the curve (AUC) at steady state during each treatment cycle and adjusting administered 5-FU dose for the next treatment cycle to achieve a target AUC value established as maximally efficacious and minimally toxic. The hypothesis is that individual 5-FU dose modulation to a target AUC value that is just below the threshold for severe toxicity could minimize toxicity while improving response.

The results of single-arm trials of AUC-targeted 5-FU dose adjustment in advanced CRC patients 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 in support of 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 high-performance liquid chromatography) for single-agent 5-FU. However, the authors 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%). In the latter 2 trials, delivery over a longer time period for both 5-FU (22 hours vs 8 hours) and LV (2 hours vs bolus), which is characteristic of currently recommended 5-FU treatment regimens, likely minimized toxicity. The administration schedule used in the Gamelin et al (2008) trial is “rarely used in current practice in most countries” as described in an accompanying editorial by Walko and McLeod and is absent from current guidelines. 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 in 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. 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. In a pooled analysis, the overall response rate was significantly higher with pharmacokinetic AUC-monitored 5-FU therapy than with standard body surface area (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.

Test Performance
My5-FU
Analytic Validity
In 2014, Freeman et al published a diagnostic assessment report for the National Institute of Health and Care Excellence on the My5-FU assay for guiding dose adjustment in patients receiving 5-FU chemotherapy by continuous infusion. Evidence for analytic validity included validation data provided by the manufacturer, which were judged to have a high risk of bias. Overall, the correlation between My5-FU and reference standard tests (high-pressure liquid chromatography or liquid chromatography–mass...
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Spectrometry) was considered good. It was unclear whether observed variability between My5-FU and reference standard tests is clinically significant. Findings from the NICE report were published in a peer-reviewed journal in 2015.

Beumer et al (2009) compared OnDose (now called My5-FU) assay results with liquid chromatography-tandem mass spectrometry results; the slope of the correlation was 1.04 (ideal=1.00) and the r value was 0.99 (ideal=1.00).

Büchel et al (2013) compared My5-FU assay performance on the Roche Cobas® Integra 800 analyzer with liquid chromatography-tandem mass spectrometry 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—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 chromatography-spectrometry, and a 1.6% or less proportional bias toward higher values than the other 3 analyzers.

Clinical Validity
Kline et al (2013) assessed OnDose (now called My5-FU) in a retrospective study of patients with stage II/III (n=35) or stage IV or recurrent (n=49) CRC who received 5-FU regimens at a single center in the United States. Patients who required radiotherapy were excluded. Thirty-eight patients chose pharmacokinetic monitoring with OnDose, and 46 patients were dosed by body surface area (BSA). Median PFS did not differ by dosing strategy in stage IV or recurrent patients (14 months with AUC monitoring vs 10 months BSA dosing; log-rank test, p=0.16), but did differ in stage II/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/III patients, 32% of AUC-monitored patients and 69% of BSA-dosed patients experienced grade 3 toxicity (Fisher exact test, p=0.04). Onset of adverse events also was delayed in the AUC-monitored group (6 or 7 months vs 2 months in the BSA-dose group; log-rank test, p=0.01).

OnDose (now called My5-FU) was clinically validated for patients with CRC in an observational analysis reported as a commentary 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 Laboratories for analysis. Sixty-two patients (17%) were studied longitudinally across 4 sequential sample submissions (ie, four 5-FU treatment infusions), of which 5% were within the target AUC after the first infusion. By the fourth infusion, this number rose to 37% and outliers were reduced. The use of bevacizumab did not affect results. No information on response or toxicity was reported.
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Clinical Utility
No prospective trials comparing outcomes with AUC-adjusted 5-FU dosing with standard BSA-based dosing were identified.

Testing for Genetic Mutations in DPYD or TYMS
A 2010 TEC Assessment reviewed the evidence for pharmacogenetic testing to predict 5-FU toxicity. DPYD and TYMS mutation 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 mutations in DPD or TS genes.”

Analytic Validity
The Myriad Genetics website reports technical specifications for TheraGuide. DPYD and TYMS mutation testing both are PCR tests. The entire coding sequence of DPYD, comprising 23 coding exons and 690 introns, is analyzed. TYMS is analyzed for the number of base pair tandem repeats in the 5’ untranslated region. Analytic specificity and sensitivity were assessed in 60 samples from unselected individuals. No false positives or false negatives were reported. The estimated incidence of errors that may be due to specimen handling, amplification reactions, or analysis is less than 1%. Testing results are reported as high, moderate, or low risk or “genetic variant of uncertain significance.”

- High risk: One of 3 mutations (IVS14 +1 G>A [also known as c.1905+1 G>A and DPYD*2A], c.2846A>T [D949V], or c.1679T>G [I560S and DPYD*13]) or other “variants with significant evidence indicating that they adversely affect protein production or function” is present in DPYD, regardless of TYMS genotype.
- Moderate risk: Two tandem repeats (2R/2R) are present in TYMS, and the DPYD result is low risk.
- Low risk: Both DPYD and TYMS must have low-risk genotypes. For DPYD, this includes variants not predicted to affect protein production or function. For TYMS, this includes 2R/3R and 3R/3R genotypes.
- Genetic variants of uncertain significance: Missense and/or intronic variants with uncertain clinical relevance are detected.

Specific recommendations for treatment selection and/or 5-FU dose modification or discontinuation based on genetic testing results are not provided. Some authors have developed dosing paradigms based on DPYD results, but these have not been prospectively correlated with outcomes such as reduced toxicity.

ARUP Laboratories uses PCR to assess 5 mutations in DPYD (the 3 identified mutations in TheraGuide plus c. 85T>C and c.-1590T>C) and 2 mutations in TYMS (5’ promoter-enhancer region and 3’ untranslated region. Results are reported as positive (mutation detected) or negative (no mutation detected). On its website, ARUP Laboratories reports analytical sensitivity and specificity of 99%; clinical sensitivity and specificity are unknown. The website also notes, “Only targeted mutations in the DPYD and TYMS genes will be detected by this panel. Diagnostic errors can occur due to rare sequence variations [not detected by the test]. Genotyping does not replace the need for therapeutic drug monitoring or clinical observation.”
Clinical Validity: Toxicity

Schwab et al (2008) 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 FDA-approved]) administered by bolus or by infusion were included. Patients were genotyped for the DPYD splice site mutation DPYD*2A (IVS14+1G>A), which leads to a nonfunctional enzyme, and for TYMS tandem repeats. Sensitivity, specificity, and positive and negative predictive value 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 odds ratio, 1.9; 95% confidence interval, 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. In an accompanying editorial, Ezzedin and Diasio (2008) observed that “genetic tests proposed for the prediction of patients at risk of developing toxicity to FU remain underdeveloped, with a high percentage of false-negative predictions because of the absence of a comprehensive molecular approach that could account for all elements associated with FU toxicity (genetic, epigenetic, and nongenetic), including impairment of cell signaling pathways and/or DNA damage response, which may significantly influence the cellular response to FU.” The editorialists also commented that “the recent use of multiple treatment modalities in cancer patients has further complicated the development of a straightforward predictive test.”

| Table 1. Grade 3/4 Adverse Events and DPYD/TYMS Genotype in Schwab et al (2008) |
|---------------------------------|-----------------------------|-----------------------------|
| Overall toxicity               | DPYD wt/“2A” (n=13)         | TYMS VNTR 2/3 or 3/3” (n=521) |
| Sensitivity                    | 0.06                        | 0.65                        |
| Specificity                    | 0.99                        | 0.21                        |
| Positive predictive value      | 0.46                        | 0.14                        |
| Negative predictive value      | 0.85                        | 0.76                        |
| Diarrhea                       |                             |                             |
| Sensitivity                    | NR                          | 0.57                        |
| Specificity                    | NR                          | 0.22                        |
| Positive predictive value      | NR                          | 0.06                        |
| Negative predictive value      | NR                          | 0.84                        |
| Mucositis                      |                             |                             |
| Sensitivity                    | 0.08                        | NR                          |
| Specificity                    | 0.99                        | NR                          |
| Positive predictive value      | 0.31                        | NR                          |
| Negative predictive value      | 0.93                        | NR                          |
| Leukopenia                     |                             |                             |
| Sensitivity                    | 0.13                        | NR                          |
| Specificity                    | 0.99                        | NR                          |
| Positive predictive value      | 0.31                        | NR                          |
| Negative predictive value      | 0.96                        | NR                          |

NR: not reported; VNTR: variable number of tandem repeats.
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äheterozygous DPYD*2A compared with wt/wt.
bHomozygous (3R/3R) or mixed heterozygous (2R/3R) triple repeats compared with homozygous double repeats (2/2).

Similar associations between 5-FU toxicity and polymorphisms in DPYD and TYMS have been confirmed in subsequent meta-analyses, and other studies, including 2 studies of homogenous patient groups enrolled in RCTs. Cancer types and specific mutations studied varied across these reports.

In 2013, Loganayagam et al reported similar results from a study of 430 patients treated with 5-FU-based (43%) or capecitabine-based chemotherapy (57%) for colorectal or other gastrointestinal cancers or cancers of unknown primary. Sensitivity and specificity of the 3 identified DPYD mutations of the TheraGuide test (c.1905+1 G>A, c.2846A>T, and c.1679T>G) for grade 3/4 diarrhea, mucositis, or neutropenia were 1% to 3% and 100%, respectively. Positive and negative predictive values were greater than 99% and 76% to 77%, respectively.

A 2011 review of DPYD mutations associated with 5-FU toxicity noted a lack of consistent correspondence between deleterious variants and DPYD activity across studies. The authors attributed this to variation in allele frequencies across geographic populations studied, nonstandard toxicity assessments, and differences in 5-FU chemotherapy regimens.

Clinical Validity: Survival

A 2013 meta-analysis from China included 11 studies that assessed TYMS mutations (5’ tandem repeats and a single nucleotide substitution [G>C] within triplet repeats) and survival outcomes. Patients had gastric or CRC and received 5-FU with or without LV with or without levamisole. Three studies (total N=311) were eligible for pooled analysis of OS. Statistical heterogeneity was not assessed. Patients who were homozygous for triplet repeats (3R/3R) had improved OS compared with patients who were homozygous for doublet repeats (2R/2R) or compound heterozygous (2R/3R), contrary to expectation.

Clinical Utility

No prospective trials comparing efficacy and safety outcomes with or without pretreatment TheraGuide testing or DPYD and/or TYMS testing were identified.

One prospective trial compared outcomes with 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 include lack of randomization to a management strategy and use of historical, rather than concurrent, controls.
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Goff et al (2014) prospectively genotyped 42 adults with 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 LV every 2 weeks) until unacceptable toxicity or disease progression (median, 5.5 cycles); patients homozygous for triplet repeats (3R/3R) were excluded. 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. 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, 11.3 and 6.2 months, respectively) also were similar to those reported in unselected populations. The study was stopped early before meeting target enrollment (minimum 75 patients) due to insufficient funding.

Magnani et al (2013) reported a study of 180 cancer patients receiving fluoropyrimidines (5-FU or capecitabine) who underwent DPYD analysis for the 1905+1 G>A mutation by high-pressure liquid chromatography. 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 mutations found in mismatch repair genes.

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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<td>AUC-guided dosing of 5-FU</td>
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<td>Retrospective Data Comparison of Toxicity and Efficacy in Colorectal Cancer (CRC) Patients Managed With and Without 5-FU Exposure Optimization Testing</td>
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<td>NCT00943137</td>
<td>The Optimisation of 5-Fluorouracil Dose by Pharmacokinetic Monitoring in Asian Patients With Advanced Stage Cancer</td>
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<td>Jun 2017</td>
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<td></td>
<td><strong>DPYD and/or TYMS testing before use of fluoropyrimidines</strong></td>
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<td>NCT00131599</td>
<td>Thymidylate Synthase Polymorphisms as a Predictor of Toxicity to 5-Fluorouracil Based Chemotherapy in Stage III Colon C</td>
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<td>Aug 2015 (active)</td>
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<td>NCT02324452</td>
<td>Safety, Feasibility and Cost-effectiveness of Genotype-directed Individualized Dosing of Fluoropyrimidines</td>
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</table>

AUC: area under the curve; 5-FU: 5-Fluorouracil; NCT: national clinical trial.
* Denotes industry-sponsored or cosponsored trial.

Summary of Evidence
The evidence for laboratory assays to determine 5-FU AUC in individuals who have cancer for which treatment with 5-fluorouracil is indicated includes several studies on analytic validity and clinical validity. Relevant outcomes are overall survival, test accuracy and validity, quality of life, and treatment-related morbidity. One clinical validity study has reported clinical response or toxicity, and findings are not sufficient
to draw conclusions on whether use of the test is associated with improved health outcomes. No prospective trials comparing efficacy and safety outcomes between AUC-adjusted 5-FU dosing and standard dosing were identified. The evidence is insufficient to determine the effects of the technology on health outcomes.

The evidence for genetic testing for mutations (eg, in DPYD and TYMS) affecting 5-FU metabolism in individuals who have cancer for which treatment with 5-FU is indicated includes several studies on analytic validity, clinical validity, and clinical utility. Relevant outcomes are overall survival, test accuracy and validity, quality of life, and treatment-related morbidity. A large clinical validity study found that TYMS mutations were not sensitive or specific indicators of serious toxicity, and DPYD mutations were specific but not sensitive. No prospective trials comparing efficacy and safety outcomes with or without pretreatment DPYD and/or TYMS testing were identified. One study compared outcomes in patients undergoing pretreatment DPYD testing with historical controls who did not receive testing. In this study, the rates of grade 3 or higher toxicity were lower in the patients who had genetic testing; however, the study lacked randomization and concurrent controls. The evidence is insufficient to determine the effects of the technology on health outcomes.

References
Laboratory and Genetic Testing for Use of 5-Fluorouracil in Patients With Cancer

Policy # 00291
Original Effective Date: 03/16/2011
Current Effective Date: 08/17/2016


34. 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; volume 24, Tab 13

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Original Effective Date: 03/16/2011
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44. Amstutz U, Froehlich TK, Largiader CR. Dihydropyrimidine dehydrogenase gene as a major predictor of severe 5-fluorouracil toxicity. Pharmacogenomics. Sep 2011;12(9):1321-1336. PMID 21919607

Policy History
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Current Effective Date: 08/17/2016
03/03/2011 Medical Policy Committee review
03/16/2011 Medical Policy Implementation Committee approval. New Policy.
03/01/2012 Medical Policy Committee review
03/21/2012 Medical Policy Implementation Committee approval. Coverage eligibility unchanged.
05/02/2013 Medical Policy Committee review
05/22/2013 Medical Policy Implementation Committee approval. Coverage eligibility unchanged.
06/05/2014 Medical Policy Committee review
06/18/2014 Medical Policy Implementation Committee approval. Investigational OnDose statement modified to reflect new test name, My5-FU. Investigational statement for TheraGuide testing for genetic mutations in DPYD or TYMS added. Title changed from “Laboratory Testing to Allow Area Under the Curve (AUC) Targeted 5-Fluorouracil (5-FU) Dosing for Patients Administered 5-FU for Cancer” to “Laboratory and Genetic Testing for Use of 5-Fluorouracil in Patients With Cancer” to reflect incorporation of new test.
08/06/2015 Medical Policy Committee review

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08/19/2015  Medical Policy Implementation Committee approval. Coverage eligibility unchanged.
08/04/2016  Medical Policy Committee review
08/17/2016  Medical Policy Implementation Committee approval. Removed TheraGuide test from policy statement as it is no longer available.
01/01/2017  Coding update: Removing ICD-9 Diagnosis Codes
Next Scheduled Review Date:  08/2017

Coding

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Codes used to identify services associated with this policy may include (but may not be limited to) the following:

<table>
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<th>Code Type</th>
<th>Code</th>
</tr>
</thead>
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<tr>
<td>CPT</td>
<td>81400, 81401</td>
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<tr>
<td>HCPCS</td>
<td>S3722</td>
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<tr>
<td>ICD-10 Diagnosis</td>
<td>All related diagnoses</td>
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</tbody>
</table>

*Investigational – A medical treatment, procedure, drug, device, or biological product is Investigational if the effectiveness has not been clearly tested and it has not been incorporated into standard medical practice. Any determination we make that a medical treatment, procedure, drug, device, or biological product is Investigational will be based on a consideration of the following:

A. Whether the medical treatment, procedure, drug, device, or biological product can be lawfully marketed without approval of the U.S. FDA and whether such approval has been granted at the time the medical treatment, procedure, drug, device, or biological product is sought to be furnished; or

B. Whether the medical treatment, procedure, drug, device, or biological product requires further studies or clinical trials to determine its maximum tolerated dose, toxicity, safety, effectiveness, or effectiveness as compared with the standard means of treatment or diagnosis, must improve health outcomes, according to the consensus of opinion among experts as shown by reliable evidence, including:

1. Consultation with the Blue Cross and Blue Shield Association TEC or other nonaffiliated technology evaluation center(s);  
2. Credible scientific evidence published in peer-reviewed medical literature generally recognized by the relevant medical community; or
3. Reference to federal regulations.
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