***Policy replaced by MolDX***
***Effective January 1, 2016***

BlueCross BlueShield of Alabama

**Name of Blue Advantage Policy:**

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

Policy #: 253
Category: Laboratory

Latest Review Date: March 2016
Policy Grade: B

## Background:

***Blue Advantage*** *medical policy does not conflict with Local Coverage Determinations (LCDs), Local Medical Review Policies (LMRPs) or National Coverage Determinations (NCDs) or with coverage provisions in Medicare manuals, instructions or operational policy letters. In order to be covered by Blue Advantage the service shall be reasonable and necessary under Title XVIII of the Social Security Act, Section 1862(a)(1)(A). The service is considered reasonable and necessary if it is determined that the service is:*

1. *Safe and effective;*
2. *Not experimental or investigational*;*
3. *Appropriate, including duration and frequency that is considered appropriate for the service, in terms of whether it is:*
   - *Furnished in accordance with accepted standards of medical practice for the diagnosis or treatment of the patient's condition or to improve the function of a malformed body member;*
   - *Furnished in a setting appropriate to the patient's medical needs and condition;*
   - *Ordered and furnished by qualified personnel;*
   - *One that meets, but does not exceed, the patient's medical need; and*
   - *At least as beneficial as an existing and available medically appropriate alternative.*

**Routine costs of qualifying clinical trial services with dates of service on or after September 19, 2000 which meet the requirements of the Clinical Trials NCD are considered reasonable and necessary by Medicare. Providers should bill* ***Original Medicare*** *for covered services that are related to* ***clinical trials*** *that meet Medicare requirements (Refer to Medicare National Coverage Determinations Manual, Chapter 1, Section 310 and Medicare Claims Processing Manual Chapter 32, Sections 69.0-69.11).*

## Description of Procedure or Service:

Variability in systemic exposure to 5-fluorouracil (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 of 5-FU in cancer patients to a predetermined area under the curve (AUC) serum concentration target: Accurate AUC determination relies on sampling at pharmacokinetically appropriate times, as well as on accurate methods of 5-FU serum concentration measurement. Available measurement methods are complex, making them less amenable to routine clinical laboratory settings.
2. Genetic testing for mutations affecting 5-FU metabolism: Genetic mutations may affect activity of enzymes involved in 5-FU metabolism. Currently-available polymerase chain reaction (PCR) tests assess specific mutations in genes encoding dihydropyrimidine reductase (*DPYD*) and thymidylate synthase (*TYMS*), enzymes in the catabolic and anabolic pathways of 5-FU metabolism, respectively.

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 often are limited by hematologic and gastrointestinal toxicity. Moreover, patients administered the same fixed-dose, continuous-infusion regimen of 5-FU have wide intra- and inter-patient 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 have also 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 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 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 (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-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 three 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 (two tandem repeats) and wild-type forms.

## Policy:

**Effective for dates of service on or after May 19, 2011 and prior to January 1, 2016:**
**Blue Advantage** will treat **My5-FU™ testing or other types of assays for determining 5-fluorouracil area under the curve in order to adjust 5-FU dose** for cancer patients as a **non-covered benefit** and as **investigational**.

**Blue Advantage** will treat **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 as a **non-covered benefit** and as **investigational.**

*Blue Advantage does not approve or deny procedures, services, testing, or equipment for our members. Our decisions concern coverage only. The decision of whether or not to have a certain test, treatment or procedure is one made between the physician and his/her patient. Blue Advantage administers benefits based on the members' contract and medical policies. Physicians should always exercise their best medical judgment in providing the care they feel is most appropriate for their patients. Needed care should not be delayed or refused because of a coverage determination.*

## Key Points:

This policy was updated with a review of the MEDLINE database. The most recent literature review was performed through February 17, 2016.

**5-Fluorouracil and Clinical Use**
5-Fluorouracil (5-FU) is a pyrimidine analog, antineoplastic antimetabolite; 5-FU has been used for many years to treat solid tumors, eg, colorectal adenocarcinoma. The 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 two weeks (i.e., a FOLFOX [leucovorin calcium, fluorouracil, oxaliplatin] regimen) significantly increased disease-free (DFS) and overall survival (OS), the FOLFOX regimen is recommended for patients with stage III colorectal cancer. A FOLFOX regimen also improves progression-free survival (PFS) in patients with advanced (i.e., metastatic) CRC who are able to tolerate intensive versus single-agent 5-FU therapy, and FOLFOX may be considered for individual 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 three-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 two combination regimens for recurrent, unresectable, or metastatic disease.

**Measuring Exposure to 5-Fluorouracil**

Patient exposure to 5-FU is most accurately described by estimating the area under the curve (AUC), the total drug exposure over a defined period of time. 5-FU exposure is influenced by method of administration, circadian variation, liver function, and the presence of inherited dihydropyrimidine reductase (*DPYD)*-inactivating genetic variants that can greatly reduce or abolish 5-FU catabolism. As a result, both inter- and intra-patient 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 U.S., 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 20-30 mg/h/L during the next cycle. The dosing algorithm is based on that developed by Kaldate 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, two-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-Fluorouracil 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 studied extensively 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 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 two 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 in1998 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 colorectal cancer, Gamelin et al (2008) reported significantly improved tumor response (33.6% versus 18.3%, respectively; $p<0.001$) and a trend toward improved survival (40.5% versus 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 two other large chemotherapy trials (5%-7%). In the latter two trials, delivery over a longer time period for both 5-FU (22 hours vs eight hours) and LV (two 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 in order 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 resulted in reduced toxicity but no improvement in 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 two drugs, not the current standard of three and, therefore, generalizability of results to current clinical practice is limited.

In 2016, Yang et al published a meta-analysis of data from the two RCTs described above (i.e., Gamelin et al and Fety et al), as well as from three 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 (three studies), neutropenia (three studies) and hand-foot syndrome (two 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*

Analytic validity is the technical performance (i.e., reproducibility) of a test.

In 2014, Freeman et al published a diagnostic assessment report for the National Institute of Health and Care Excellence (NICE) 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, correlation between My5-FU™ and reference standards tests (high-pressure liquid chromatography or liquid chromatography–mass 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 three other analyzers (Olympus AU400®, Roche Cobas® c6000, and Thermo Fisher CDx90®). Serum samples were collected from 32 patients with gastrointestinal cancers who were receiving 5-FU infusion therapy at a single center in Switzerland. My5- FU™ was validated for linearity (i.e., correlated

linearly within 10% or less of true 5-FU concentrations from 100 mg/mL to 1750 mg/mL), precision, accuracy, recovery, sample carryover, and dilution integrity. Of several plasma compounds tested for potential interference, only lipids were found to exceed 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. In comparison with other tests, My5-FU™ had a 7% proportional (i.e., dose-dependent) bias toward higher values compared with chromatography-spectrometry, and a 1.6% or less proportional bias toward higher values compared with the other three analyzers.

*Clinical Validity*
Clinical validity is a test's association with outcomes.

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 U.S. Patients who required radiation therapy 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 (six or seven months vs two 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 two 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 four sequential sample submissions (i.e., 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.

*Clinical Utility*
Clinical utility is a test's impact on patient outcomes.

No prospective trials comparing outcomes with AUC-adjusted 5-FU dosing with standard BSA-based dosing were identified.

~~TheraGuide®~~ Testing for Genetic Mutations in DPYD or TYMS
A 2009 TEC Assessment reviewed the evidence for pharmacogenetic testing to predict 5-FU toxicity. *DPYD* and *TYMS* mutation testing did not meet TEC criteria. The author 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®. (However, once the test was removed from the market, the technical specification document was no longer available on the internet.) *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 three 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 five mutations in *DPYD* (the three identified mutations in TheraGuide® plus c. 85T>C and c.-1590T>C) and two 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 percent; 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 (Table 1). Although heterozygosity for *DPYD*2A* had 99% specificity for serious toxicity, sensitivity ranged from 6%-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 [OR]=1.9; 95% CI, 1.26 to 2.87; p=0.002), most commonly mucositis. Bolus administration of 5-FU was a significant, independent predictor of severe toxicity overall. 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)**

| | **DPYD wt/*2A[a] n=13** | **TYMS VNTR 2/3 or 3/3[b] n=521** |
|---|---|---|
| Overall toxicity | | |
| Sensitivity | 0.06 | 0.65 |
| Specificity | 0.99 | 0.21 |
| PPV | 0.46 | 0.14 |
| NPV | 0.85 | 0.76 |
| Diarrhea | | |
| Sensitivity | NR | 0.57 |
| Specificity | NR | 0.22 |
| PPV | NR | 0.06 |
| NPV | NR | 0.84 |
| Mucositis | | |
| Sensitivity | 0.8 | NR |
| Specificity | 0.99 | NR |
| PPV | 0.31 | NR |
| NPV | 0.93 | NR |
| Leukopenia | | |
| Sensitivity | 0.13 | NR |
| Specificity | 0.99 | NR |
| PPV | 0.31 | NR |
| NPV | 0.96 | NR |

NR, not reported; VNTR, variable number of tandem repeats

[a] Heterozygous *DPYD*2A* compared with wt/wt.

[b] Homozygous (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 two 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 three 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%-3% and 100%, respectively. Positive and negative predictive values were greater than 99% and 76%-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 colorectal cancer and received 5-FU with or without leucovorin 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 study, published in 2016 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 two 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 (28%; 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 that patients were not randomized to a management strategy and that historical, rather than concurrent, controls were used.

Goff et al in 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 leucovorin every two 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% (nine partial responses and no complete responses), which was worse than a 43% historical overall response rate in unselected patients. Overall response rate in six patients homozygous for doublet repeats (2R/2R) was 83% (five partial responses and 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, three patients received dose reduction of 50%-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.

**Summary of Evidence**

The evidence for laboratory assays to determine 5-fluorouracil area under the curve in individuals who have cancer for whom treatment with 5-fluorouracil is indicated, includes several studies on analytic validity and clinical validity. Relevant outcomes are overall survival, test accuracy, test validity, quality of life and treatment-related morbidity. There was one clinical validity study reporting clinical response or toxicity and findings of this study are not sufficient to draw conclusions on whether use of the test is associated with clinical outcomes. No prospective trials comparing efficacy and safety outcomes with area under the curve (AUC)-adjusted 5-FU dosing with 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, e.g., in DPYD and TYMS, affecting 5-fluorouracil metabolism in individuals who have cancer for whom treatment with 5-fluorouracil is indicated includes several studies on analytic validity, clinical validity and clinical utility. Relevant outcomes are overall survival, test accuracy, test 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 mutations for DPYD 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 rate of Grade 3 or higher toxicity was lower in the patients who underwent genetic testing; however, the study was limited by lack of randomization or concurrent controls. The evidence is insufficient to determine the effects of the technology on health outcomes.

**Practice Guidelines and Position Statements**

National Comprehensive Cancer Network Guidelines

Although current NCCN guidelines acknowledge that the "selection, dosing, and administration of anticancer agents and the management of associated toxicities are complex," they do not recommend AUC-guided 5-FU dosing or genetic testing for *DPYD* and/or *TYMS* mutations in patients with colon, rectal, breast, gastric, pancreatic cancer, or head and neck cancers.

Clinical Pharmacogenetics Implementation Consortium

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

**National Institute for Health and Care Excellence (NICE)**

In 2014, NICE published evidence-based diagnostics guidance on the My5-FU assay for guiding 5-FU chemotherapy dose adjustment. The guidance states, "The My5-FU assay is only recommended for use in research for guiding dose adjustment in people having fluorouracil chemotherapy by continuous infusion. The My5-FU assay shows promise and the development of robust evidence is recommended to demonstrate its utility in clinical practice."

**U.S. Preventive Services Task Force Recommendations**

Not applicable.

## Key Words:

Area Under the Curve (AUC) Testing, 5-Fluorouracil (5-FU) Dosing, OnDose™, My5-FU, TheraGuide

## Approved by Governing Bodies:

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratories offering such tests as a clinical service must meet general regulatory standards of the Clinical Laboratory Improvement Act (CLIA) and must be licensed by CLIA for high-complexity testing. Both Saladax Biomedical and Myriad Genetics are CLIA-licensed laboratories. Currently, U.S. Food and Drug Administration (FDA)-approved tests for 5-FU AUC measurement and for *DPYD*/*TYMS* mutation testing are unavailable. My5-FU™ is offered by Saladax Biomedical as a laboratory-developed test; other clinical laboratories may offer in-house assays to measure 5-FU AUC. Similarly, TheraGuide® was offered by Myriad Genetics as 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, seven mutations test).

## Benefit Application:

Coverage is subject to member's specific benefits. Group specific policy will supersede this policy when applicable.

## Current Coding:

CPT Codes

| | |
|---|---|
| **81400** | Molecular pathology procedure, Level 1(eg, identification of single germline variant [eg, SNP] by techniques such as restriction enzyme digestion or melt curve analysis) – includes *DPYD (dihydropyrimidine dehydrogenase)* (eg, 5-fluorouracil/5-FU and capecitabine drug metabolism), IVS14+1G>A variant |
| **81401** | Molecular pathology procedure, Level 2 (eg, 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat) – includes *TYMS (thymidylate synthetase)* (eg, 5-fluorouracil/5-FU drug metabolism), tandem repeat variant |
| **84999** | Unlisted chemistry procedure |

HCPCS Codes:

| | |
|---|---|
| **S3722** | Dose optimization by area under the curve (AUC) analysis, for infusional 5-fluorouracil **(Effective 01/01/2012)** |

## References:


1. Amstutz U, Froehlich TK, Largiader CR. Dihydropyrimidine dehydrogenase gene as a major predictor of severe 5-fluorouracil toxicity. Pharmacogenomics 2011; 12(9):1321-36.
2. Andre T, Boni C, Navarro M et al. Improved overall survival with oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment in stage II or III colon cancer in the MOSAIC trial. J Clin Oncol 2009; 27(19):3109-16.
3. ARUP Laboratories. 5-Fluorouracil (5-FU) Toxicity and Chemotherapeutic Response, 7 Mutations. Available online at: //ltd.aruplab.com/Tests/Pub/2007228.
4. Beneton M, Chapet S, Blasco H et al. Relationship between 5-fluorouracil exposure and outcome in patients receiving continuous venous infusion with or without concomitant radiotherapy. Br J Clin Pharmacol 2007; 64(5):613-21.
5. Beumer JH, Boisdron-Celle M, Clarke W et al. Multicenter evaluation of a novel nanoparticle immunoassay for 5-fluorouracil on the Olympus AU400 analyzer. Therap Drug Monit 2009; 31(6):688-94.
6. Blue Cross and Blue Shield Association Technology Evaluation Center (TEC). TEC Special Report: Laboratory Testing to Allow Area Under The Curve (AUC) –Targeted 5-Fluorouracil Dosing for Patients Administered Chemotherapy for Cancer. TEC Assessments 2009; Volume 24, Tab 10.
7. 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.
8. Boisdron-Celle M, Craipeau C, Brienza S et al. Influence of oxaliplatin on 5-fluorouracil plasma clearance and clinical consequences. Cancer Chemother Pharmacol 2002; 49(3):235-43.
9. Buchel B, Sistonen J, Joerger M et al. Comparative evaluation of the My5-FU immunoassay and LC-MS/MS in monitoring the 5-fluorouracil plasma levels in cancer patients. Clin Chem Lab Med 2013; 51(8):1681-8.
10. Capitain O, Asevoaia A, Boisdron-Celle M et al. Individual fluorouracil dose adjustment in FOLFOX based on pharmacokinetic follow-up compared with conventional body-area-surface dosing: a phase II, proof-of-concept study. Clin Colorectal Cancer 2012; 11(4):263-7.
11. Caudle KE, Thorn CF, Klein TE et al. Clinical Pharmacogenetics Implementation Consortium guidelines for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing. Clin Pharmacol Therap 2013; 94(6):640-5.
12. de Gramont A, Figer A, Seymour M et al. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. J Clin Oncol 2000; 18(16):2938-47.
13. Deenen MJ, Meulendijks D, Cats A, et al. Upfront Genotyping of DPYD*2A to Individualize Fluoropyrimidine Therapy: A Safety and Cost Analysis. J Clin Oncol. Jan 20 2016; 34(3):227-234.
14. Ezzeldin HH, Diasio RB. Predicting Fluorouracil Toxicity: Can We Finally Do It? J Clin Oncol 2008; 26(13):2080-82.
15. Fety R, Rolland F, Barberi-Heyob M et al. Clinical impact of pharmacokinetically-guided dose adaptation of 5-fluorouracil: results from a multicentric randomized trial in patients with locally advanced head and neck carcinomas. Clin Cancer Res 1998; 4(9):2039-45.
16. Freeman K, Connock M, Cummins E, et al. Fluorouracil plasma monitoring: systematic review and economic evaluation of the My5-FU assay for guiding dose adjustment in patients receiving fluorouracil chemotherapy by continuous infusion. Health Technol Assess. Nov 2015; 19(91):1-322.
17. Freeman K, Connock M, Cummins E et al. Fluorouracil plasma monitoring: the My5-FU assay for guiding dose adjustment in patients receiving fluorouracil chemotherapy by continuous infusion. Diagnostic Assessment Report commissioned by the NIHR HTA Programme on behalf of the National Institute for Health and Care Excellence. June 2014. www.nice.org.uk/guidance/dg16/evidence.
18. Froehlich TK, Amstutz U, Aebi S, et al. Clinical importance of risk variants in the dihydropyrimidine dehydrogenase gene for the prediction of early-onset fluoropyrimidine toxicity. Int J Cancer. Feb 1 2015; 136 (3):730-739.
19. Gamelin E, Boisdron-Celle M, Delva R et al. Long-term weekly treatment of colorectal metastatic cancer with fluorouracil and leucovorin: results of a multicentric prospective trial of fluorouracil dosage optimization by pharmacokinetic monitoring in 152 patients. J Clin Oncol 1998; 16(4):1470-8.
20. Gamelin E, Delva R, Jacob J et al. Individual fluorouracil dose adjustment based on pharmacokinetic follow-up compared with conventional dosage: results of a multicenter randomized trial of patients with metastatic colorectal cancer. J Clin Oncol 2008; 26(13):2099-105.

21. Gamelin EC, Danquechin-Dorval EM, Dumesnil YF et al. Relationship between 5-fluorouracil (5-FU) dose intensity and therapeutic response in patients with advanced colorectal cancer receiving infusional therapy containing 5-FU. Cancer 1996; 77(3):441-51.
22. Giacchetti S, Perpoint B, Zidani R et al. Phase III multicenter randomized trial of oxaliplatin added to chronomodulated fluorouracil-leucovorin as first-line treatment of metastatic colorectal cancer. J Clin Oncol 2000; 18(1):136-47.
23. Goff LW, Thakkar N, Du L, et al. Thymidylate synthase genotype-directed chemotherapy for patients with gastric and gastroesophageal junction cancers. PLoS One. 2014; 9(9):e107424.
24. Grem JL. 5-Fluorouracil and its biomodulation in the management of colorectal cancer. In: Saltz LB, ed. Colorectal Cancer: Multimodality Management. Totowa, NJ: Humana Press; 2002.
25. Kaldate RR, Haregewoin A, Grier CE et al. Modeling the 5-fluorouracil area under the curve versus dose relationship to develop a pharmacokinetic dosing algorithm for colorectal cancer patients receiving FOLFOX6. Oncologist 2012; 17(3):296-302.
26. Keiser WL. The role of pharmacogenetics in the management of fluorouracil-based toxicity. Commun Oncol 2008; 5(suppl 12):1–8. Available online at: www.oncologypractice.com/co/journal/abstracts/0510s1201.html.
27. Kline CL, Schiccitano A, Zhu J et al. Personalized Dosing via Pharmacokinetic Monitoring of 5- Fluorouracil Might Reduce Toxicity in Early- or Late-Stage Colorectal Cancer Patients Treated With Infusional 5-Fluorouracil-Based Chemotherapy Regimens. Clin Colorectal Cancer 2013.
28. Konings IR, Sleijfer S, Mathijssen RH et al. Increasing tumoral 5-fluorouracil concentrations during a 5-day continuous infusion: a microdialysis study. Cancer Chemother Pharmacol 2011; 67(5):1055-62.
29. Lee AM, Shi Q, Pavey E, et al. DPYD variants as predictors of 5-fluorouracil toxicity in adjuvant colon cancer treatment (NCCTG N0147). J Natl Cancer Inst. Dec 2014; 106 (12).
30. Li Q, Liu Y, Zhang HM, et al. Influence of DPYD Genetic Polymorphisms on 5-Fluorouracil Toxicities in Patients with Colorectal Cancer: A Meta-Analysis. Gastroenterol Res Pract. 2014; 2014:827989.
31. Loganayagam A, Arenas Hernandez M, Corrigan A et al. Pharmacogenetic variants in the DPYD, TYMS, CDA and MTHFR genes are clinically significant predictors of fluoropyrimidine toxicity. Br J Cancer 2013; 108(12):2505-15.
32. Magnani E, Farnetti E, Nicoli D et al. Fluoropyrimidine toxicity in patients with dihydropyrimidine dehydrogenase splice site variant: the need for further revision of dose and schedule. Intern Emerg Med 2013; 8(5):417-23.
33. Milano G, Etienne MC, Renee N et al. Relationship between fluorouracil systemic exposure and tumor response and patient survival. J Clin Oncol 1994; 12(6):1291-5.
34. Myriad Pro™. TheraGuide® 5-FU FAQs. Available online at: www.myriadpro.com/additional-products/chemotoxicity/theraguide-5-fu-faqs/.
35. Myriad Pro™. TheraGuide® 5-FU Technical Specifications, February 2009. Available online at: www.myriadpro.com/additional-products/chemotoxicity/managing-chemotoxicity/.
36. National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology 2014. Available online at: //www.nccn.org/index.asp.
37. National Institute for Health and Care Excellence. NICE diagnostics guidance [DG16]: Fluorouracil chemotherapy: The My5-FU assay for guiding dose adjustment, December 2014. www.nice.org.uk/guidance/dg16.

38. OnDose® Technical Specifications, Myriad Genetic Laboratories, Inc. June 2010. Available at: www.myriad.com/lib/technical-specifications/OnDose%20Tech%20Specs_6_10.pdf.
39. Posner M, Vermorken JB. Induction therapy in the modern era of combined-modality therapy for locally advanced head and neck cancer. Semin Oncol 2008; 35(3):221-8.
40. PRNewswire. Saladax Biomedical Laboratories to Offer the Full Portfolio of MyCare™ Therapeutic Dose Management Assays in the United States. February 11, 2013. Available online at: www.prnewswire.com/news-releases/saladax-biomedical-laboratories-to-offer-the-full-portfolio-of-mycare-therapeutic-dose-management-assays-in-the-united-states-190681031.html.
41. Rosmarin D, Palles C, Church D, et al. Genetic markers of toxicity from capecitabine and other fluorouracil-based regimens: investigation in the QUASAR2 study, systematic review, and meta-analysis. J Clin Oncol. Apr 1 2014; 32(10):1031-1039.
42. Saam J, Critchfield GC, Hamilton SA et al. Body surface area-based dosing of 5-fluoruracil results in extensive interindividual variability in 5-fluorouracil exposure in colorectal cancer patients on FOLFOX regimens. Clin Colorectal Cancer 2011; 10(3):203-6.
43. Saladax Biomedical, Inc. MyPatient MyDecision My5-FU™ Brochure. Available online at: www.mycaretests.com/hcp-resources/mycare-tests-menu/my5-fu/brochure/.
44. Salamone SJ, Beumer JH, Egorin MJ et al. A multi-center evaluation of a rapid immunoassay to quantitate 5-fluorouracil (5-FU) in plasma. HOPA/ISOPP 2008 Conference June 2008.
45. Santini J, Milano G, Thyss A et al. 5-FU therapeutic monitoring with dose adjustment leads to an improved therapeutic index in head and neck cancer. Br J Cancer 1989; 59(2):287-90.
46. Schwab M, Zanger UM, Marx C et al. Role of Genetic and Nongenetic Factors for Fluorouracil Treatment-Related Severe Toxicity: A Prospective Clinical Trial by the German 5-FU Toxicity Study Group. J Clin Oncol 2008; 26(13):2131-38.
47. Sun W, Yan C, Jia S, et al. Correlation analysis of peripheral DPYD gene polymorphism with 5-fluorouracil susceptibility and side effects in colon cancer patients. Int J Clin Exp Med. 2014; 7(12):5857-5861.
48. Teva Parenteral Medicines, Inc. Adrucil® (fluorouracil) injection prescribing information, August 2012. Available online at: dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=e0794add-67a7-4308- 93e9-f889472716cc.
49. Walko CM, McLeod HL. Will we ever be ready for blood level-guided therapy? J Clin Oncol 2008; 26(13):2078-9.
50. Wang YC, Xue HP, Wang ZH et al. An integrated analysis of the association between Ts gene polymorphisms and clinical outcome in gastric and colorectal cancer patients treated with 5-FU-based regimens. Mol Biol Rep 2013; 40(7):4637-44.
51. Yang R, Zhang Y, Zhou H, et al. Individual 5-Fluorouracil Dose Adjustment via Pharmacokinetic Monitoring Versus Conventional Body-Area-Surface Method: A Meta-Analysis. Ther Drug Monit. Feb 2016; 38(1):79-86.
52. Ychou M, Duffour J, Kramar A et al. Individual 5-FU dose adaptation in metastatic colorectal cancer: results of a phase II study using a bimonthly pharmacokinetically intensified LV5FU2 regimen. Cancer Chemother Pharmacol 2003; 52(4):282-90.

## Policy History:

Adopted for Blue Advantage, March 2011
Available for comment April 4 – May 18, 2011
Medical Policy Group, December 2011
Medical Policy Group, March 2012
Medical Policy Group, April 2013
Medical Policy Group, March 2015
Medical Policy Group, March 2016

---

*This medical policy is not an authorization, certification, explanation of benefits, or a contract. Eligibility and benefits are determined on a case-by-case basis according to the terms of the member's plan in effect as of the date services are rendered. All medical policies are based on (i) research of current medical literature and (ii) review of common medical practices in the treatment and diagnosis of disease as of the date hereof. Physicians and other providers are solely responsible for all aspects of medical care and treatment, including the type, quality, and levels of care and treatment.*

*This policy is intended to be used for adjudication of claims (including pre-admission certification, pre-determinations, and pre-procedure review) in Blue Cross and Blue Shield's administration of plan contracts.*