Regence

Medical Policy Manual Laboratory, Policy No. 64

Laboratory and Genetic Testing for Use of Fluoropyrimidine Chemotherapy (5-FU and Capecitabine) in Patients with Cancer

Effective: February 1, 2024

Next Review: November 2024
Last Review: December 2023

IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

DPYD and *TYMS* genotyping prior to treatment with fluoropyrimidines and/or dosing of 5-fluorouracil (5-FU) in cancer patients to a predetermined area under the curve (AUC) target have been proposed as methods to reduce variability in systemic exposure to fluoropyrimidines, reduce toxicity, and maximize tumor response.

MEDICAL POLICY CRITERIA

The following tests to guide fluoropyrimidine dosing and/or treatment choice in patients with cancer are considered **investigational**:

A. Assays for determining 5-fluorouracil area under the curve in order to adjust 5-FU dose for colorectal cancer patients or other cancer patients, including but not limited to My5-FU (formerly OnDose)

B. Genetic testing of dipyrimidine dehydrogenase (*DPYD)* or thymidylate synthase (*TYMS*)

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

CROSS REFERENCES

1. Expanded Molecular Testing of Cancers to Select Targeted Therapies, Genetic Testing, Policy No. 83

BACKGROUND

Variability in systemic exposure to fluoropyrimidines is thought to directly impact the tolerability and efficacy of 5-FU and capecitabine (oral 5-FU). Two approaches have been proposed for modifying use of fluoropyrimidines.

1. Dosing of 5-fluorouracil (5-FU) in cancer patients to a predetermined area under the curve (AUC) serum concentration target: Accurate AUC determination relies on sampling at a pharmacokinetically appropriate time, as well as on an accurate method of 5-FU laboratory measurement. Available measurement methods are complex, making them less amenable to routine clinical laboratory settings.

2. Genetic testing for variants affecting 5-FU metabolism: Genetic variants may affect activity of enzymes involved in 5-FU metabolism. Currently available tests assess for specific variants 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 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 inter-patient variability in systemic drug exposure, as measured by plasma concentration or, more accurately, by area under the curve techniques (AUC). AUC is a measure of the 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 have most recently been determined by high-performance liquid chromatography or liquid chromatography coupled with tandem mass spectrometry. Both methods require the expertise to develop an in-house assay and may be less amenable to routine clinical laboratory settings.

METABOLISM OF FLUOROPYRIMIDINES

5-FU and capecitabine are pyrimidine antagonists, 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. Capecitabine is a prodrug that is administered orally and converts to 5-FU in the liver.

Genetic variants in *DPYD*, located on chromosome 1, can lead to reduced 5-FU catabolism and increased toxicity. More than 200 *DPYD* variants have been identified, but evidence of pharmacogenomic prediction of 5-FU toxicity is centered on four *DPYD* variants found in European populations. *DPYD* deficiency is an autosomal co-dominantly inherited trait.[1, 2]

The anabolic pathway metabolizes 5-FU to an active form that inhibits DNA and RNA synthesis by competitive inhibition of *TYMS* or by incorporation of cytotoxic metabolites into nascent DNA. Genetic variants in *TYMS* can cause tandem repeats in the *TYMS* enhancer region (TSER). One variant leads to 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.

REGULATORY STATUS

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments (CLIA). Laboratories that offer LDTs must be licensed by CLIA for high-complexity testing.

On December 14, 2022, capecitabine tablets (Xeloda, Genentech, Inc.) became the first drug approved for a labeling update from the U.S. Food and Drug Administration (FDA) through its Project Renewal.[3] Project Renewal is an oncology-focused initiative with the goal of updating the labels of certain older medications to ensure information is scientifically current and clinically relevant. The labeling revisions include information in the Warnings and Precautions section of the label on the risk of serious adverse reactions to Xeloda due to dihydropyrimidine dehydrogenase (DPD) deficiency. The label update was in response to a Citizen's Petition.[4] In addition to the labeling update, the petition requested the FDA issue a recommendation for DPYD testing prior to fluoropyrimidine therapy, add a boxed warning to the test recommendation, and provide dose adjustment recommendations related to DPD deficiency. The FDA elected not to recommend DPYD testing, add the boxed warning, or provide dose adjustment recommendations.

EVIDENCE SUMMARY

MEASURING EXPOSURE TO 5-FU

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, impaired liver function, and the presence of inherited *DPD*-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. Although searches of large clinical

laboratories did not find tests for 5-FU AUC on their listings, it is possible that clinical laboratories in the U.S. offer tests that measure exposure to 5-FU AUC.

MODIFYING 5-FLUOROURACIL EXPOSURE TO IMPROVE OUTCOMES

Systematic Reviews

Glewis (2022) conducted a systematic review and meta-analysis of 17 studies that compared treatment outcomes of patients with *DPYD* variants that received pharmacogenomic-guided dosing (PGD) of 5-FU or capecitabine to patients that had usual dosing.[5] The study also compared outcomes of patients with *DPYD* variants to patients with wild-type alleles within the PGD cohorts. Genotyping was limited to the DPYD*2A variant in 15 of the studies. In the PGD cohorts, the incidence of grade 3-4 overall toxicity (five pooled studies) was lower ($p<0.00001$). The incidence of diarrhea (six pooled studies) was lower than the non-PGD cohorts ($p<0.0001$). The incidence of other specific treatment side effects (e.g., mucositis, neutropenia) varied among the studies. Hospitalizations were fewer in the patients in the PGD cohort but certainty of the evidence was low. The results of the meta-analysis suggest that dose reductions based on DPYD genotyping do not affect treatment response and outcomes, but evidence is limited. Differences in complete and partial response within the PGD cohort were not statistically significant in three pooled studies ($p=0.47$). Only one study reported progression-free (PFS) and overall survival (OS) outcomes. PFS and OS were similar between patients who received the full fluoropyrimidine dose (median PFS 10 months, OS 24 months) vs. those who had DPYD-dose reduction (median PFS 14 months, OS 27 months).

A systematic review and meta-analysis from 35 studies was performed by Sharma (2021) to estimate risk of treatment-related death in people with *DPYD* variants who received standard-dose fluoropyrimidine chemotherapy.[6] Genotyping was performed for at least one of four *DPYD* variants seen in European populations. The study included 13,929 patients who had solid tumors and were treated with either 5-FU or capecitabine. Genetic testing revealed 566 patients with *DPYD* variants (4.1%). The review found that patients with a pathogenic *DPYD* gene variant had a 25.6 times increased risk of treatment-related mortality (95% CI, 12.1-53.9; $I^2=8.2\%$). The absolute risk of treatment-related death related to a *DPYD* pathogenic variant was 2.3%, and 50% of treatment-related deaths occurred in patients with *DPYD* variants.

In 2016, Yang published a meta-analysis of data from two RCTs described below (Gamelin and Fety), as well as from three observational studies.[7] 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 (three 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.

Evidence supporting the use of 5-FU AUC measurement to help modify subsequent 5-FU treatment doses in order to improve response and reduce toxicity has been summarized and evaluated in a 2009 BlueCross BlueShield Association Technology Evaluation Center (TEC) Special Report.[8] Early evidence from small, cohort studies showed that 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. This association has been studied extensively in head and neck cancer and in colorectal cancer. In addition, a majority of 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 the 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 the 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 colorectal cancer patients suggested consistency of improved tumor response.[9-11] Similar, although less compelling results were seen in single-arm trials of AUC targeted 5-FU dosing in head and neck cancer.[12, 13] The best contemporary evidence in support of AUC targeted dosing consists of two randomized, controlled trials (RCTs), one enrolling patients with colorectal cancer and the other, patients with head and neck cancer. No trials of any design were identified for 5-FU dose adjustment in other malignancies.

Randomized Controlled Trials

Deng (2020) conducted an RCT of 153 patients with advanced colorectal cancer who were treated with 5-fluorouracil (FOLFOX or FOLFIRI).[14] 5-fluorouracil was dosed using BSA for all patients in the first period, then patients were randomized to receive area under the curve-guided dosing (adjusted via an algorithm) or BSA-guided dosing for subsequent periods. The percentage of patients in the therapeutic window (area under the curve between 20 to 30 mg/h/L) was 24.52% with body surface area dosing. With the area under the curve dosing, the percentage of patients in the therapeutic range was 18.42% in the first period which increased to 89.71% in the sixth (and final) period. In the area under the curve-guided dosing, grade 3 toxicities were reduced and more patients experienced a clinical benefit, defined as partial response or stable disease.

Gamelin[9] developed a chart for weekly dose adjustment based on the results of an earlier, similar single-arm study[15] in which dose was increased by prespecified increments and intervals up to a maximum dose or the first signs of toxicity. In an RCT of patients with metastatic colorectal cancer, Gamelin[16] reported significantly improved tumor response (33.6% versus 18.3%, respectively; p=0.0004) and a trend toward improved survival (40.5% versus 29.6%, respectively; p=0.08) in the experimental arm using AUC-targeted dosing. 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 to 7%).[17, 18] In the latter two trials, delivery over a longer time period for both 5-FU (22 hours vs. 8 hours) and leucovorin (two hours vs. bolus), which is characteristic of currently recommended 5-FU treatment regimens, likely minimized toxicity. The administration schedule used in the Gamelin[16] trial is “rarely used in current practice in most countries” as described in an accompanying editorial by Walko and McLeod[19] and is absent from current guidelines.[20] Additional optimization studies are needed in order to apply 5-FU exposure monitoring and AUC-targeted dose adjustment to a more standard single-agent 5-FU treatment regimen. The

new dose adjustment scheme would then require validation versus a fixed-dose regimen in a comparative trial to ensure that tumor response is at least as good as or better than a fixed-dose regimen and toxicity is reduced. If the intent is to show that dose-modulated single-agent 5-FU is comparable to combination regimens such as fixed-dose FOLFOX, then FOLFOX should be added as a third treatment arm.

Fety (1998) used a different method of dose adjustment in an RCT in patients with locally advanced head and neck cancer.[21] Overall 5-FU exposures in head and neck cancer patients were reported to be significantly reduced in the dose adjustment arm compared to 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, as the 12 protocol violations in this treatment arm (of 61 enrolled) were all related to 5-FU dose adjustment miscalculations. Because patients with protocol violations were removed from analysis, results did not reflect the “real world” results of the dose adjustment method. In addition, the induction therapy regimen used two drugs, not the current standard of three; therefore, these results are also limited in generalizability to current clinical practice.

Nonrandomized Studies

Capitain (2012) conducted a retrospective analysis of their dose adjustment protocol used in a FOLFOX regimen administered to patients with colorectal cancer (n=118) and compared with patients treated with FOLFOX administered in standard fashion according to body surface area (n=39).[22] In the dose-adjusted group, the therapeutic dose at three months was 110% of the theoretic dose. Grade 3/4 toxicity was 1.7% for diarrhea, 0.8% for mucositis, 18% for neutropenia, and 12% for thrombopenia; corresponding numbers were 12%, 15%, 25% and 10%, respectively, in the standard group. In the dose-adjusted group, the objective response rate was 70% at three months and 56% at six months; the corresponding result at 3 months for the standard group was 46%. Median overall survival and median progression-free survival in the dose-adjusted arm were 28 and 16 months, respectively; corresponding numbers for the standard group were 22 and 10 months. As the authors noted, this proof of principle study needs confirmation in a randomized trial.

Although several additional studies have been published, none were randomized comparisons.[23-28]

MY5-FU (FORMERLY ONDOSE)

Analytic Validity (Technical Performance/reproducibility)

In 2014, Freeman published a diagnostic assessment report on behalf of the National Institute for Health Care and Excellence (NICE) assessing the My5-FU™ assay for guiding dose adjustment in patients receiving 5-FU chemotherapy by continuous infusion.[29] The findings were also published in a peer-reviewed journal in 2015.[30] Additionally, a systematic review, also by Freeman, with the same conclusions, was published in 2016.[31] 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.

Büchel (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.[32] 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 (Association with Outcomes)

Kline (2014) assessed OnDose® 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.[33] 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).

For technical validation, Salamone (2008) compared OnDose directly to liquid chromatography-tandem mass spectrometry[34]; the slope of the correlation was 1.03 (ideal: 1.00) and the r-value was 0.99 (ideal: 1.00). This test is clinically validated only for patients with colorectal cancer to determine 5-FU exposure and subsequent dose modification. Myriad Genetics cited Gamelin [16] for clinical validation of AUC-targeted 5-FU dose adjustment and for information on how to modify the dose once 5-FU exposure has been determined. Gamelin used high-performance liquid chromatography, similar to liquid chromatography-tandem mass spectrometry, to measure AUC.

Thus, OnDose clinical validation was indirect; the only published clinical study using OnDose was reported in a commentary by Saam (2011) describing the results of an observational analysis of sequential patients treated with constant infusion 5-FU using current adjuvant or metastatic treatment protocols with or without bevacizumab.[23] 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 were studied longitudinally across four sequential sample submissions (i.e., four 5-FU treatment infusions), of which only about 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 (Impact on Patient Outcomes)

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

TESTING FOR GENETIC VARIANTS IN *DPYD* OR *TYMS*

Human Genome Variation Society (HGVS) nomenclature[35] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term "variant" is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as "mutation." Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

A 2009 TEC Assessment reviewed the evidence for pharmacogenetic testing to predict 5-FU toxicity.[36] *DPYD* and *TYMS* variant 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 variants in DPD or TS genes."

Analytic Validity

Myriad Genetics offered *DPYD* and *TYMS* variant testing by polymerase chain reaction (PCR) until 2011 (TheraGuide®). The entire coding sequence of *DPYD*, comprising 23 coding exons and 690 introns, were analyzed. *TYMS* was 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 were reported as high, moderate, or low risk or "genetic variant of uncertain significance."

- High risk: One of three variants (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 were not provided by Myriad Genetics. 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 three variants in *DPYD* (c.1679T>G, c.1905+1G>A, and c.2846A>T) .[37] Results are reported as positive (variant detected) or negative (no variant detected). On its website, ARUP Laboratories reports analytical sensitivity and specificity of

99 percent. Clinical sensitivity is estimated at 31 percent for *DPYD* variants analyzed and specificity is not reported. The website also notes, “Only the targeted *DPYD* variants will be detected by this panel. Rare diagnostic errors may occur due to rare sequence variations. [not detected by the test]. Genetic and non-genetic factors not detected by this test may affect 5-FU drug metabolism and efficacy and the risk for toxicity. Genotyping does not replace the need for therapeutic drug monitoring or clinical observation. Lack of detection of the targeted DPYD variants does not rule out risk for 5-FU toxicity or predict degree of responsiveness to 5-FU” Other laboratories may offer assays for *DPYD* and *TYMS* gene testing.

Clinical Validity: Toxicity

Negarandeh (2020) collected toxicity and genotype data on 88 colorectal cancer patients receiving FOLFOX and FOLFIRI regimens.[38] The *DPYD* IVS14 + 1 G > A polymorphism was identified in four patients (5.5%). There was no difference in the rate of chemotherapy-induced diarrhea, nausea, vomiting or oral mucositis between chemotherapy groups or between genotypes. The incidence of peripheral neuropathy was more common in patients undergoing FOLFOX treatment, but not different between those with and without the *DPYD* polymorphism.

Abbasian (2020) reported on 83 cancer patients treated with fluoropyrimidine-based chemotherapy who underwent *DPYD* and *TYMS* genotyping.[39] No *DPYD* polymorphisms were identified. The frequency of the TYMS +6 bp allele was 40.35% and the -6 bp allele was 59.65%. TYMS insertion and deletion polymorphisms were significantly associated with increased grade III neurotoxicity (p=0.02) and 2R/2R genotype was significantly associated with grade III anemia (p=0.009).

Varma (2019) evaluated genotype and toxicity data on 145 treatment-naïve patients with colorectal cancer.[40] The patients were genotyped and received a standard treatment schedule of CAPOX treatment. Individuals with DPYD*9A polymorphisms were found to be at higher risk for HFS, diarrhea and thrombocytopenia compared to patients with wild-type allele. The only significant association found between DPYD*6, GSTP1 ile105val polymorphisms and CAPOX related toxicities was for thrombocytopenia.

Hamzic (2019) reported a meta-analysis performed to assess the potential association between the c.742-227G > A (rs2612091) polymorphism in the Enolase Superfamily Member 1 gene (*ENOSF1*) and two variants in *TYMS* and severe fluoropyrimidine toxicity in cancer patients.[41] Four studies, including an unpublished cohort from the meta-analysis authors, met inclusion criteria. No significant publication bias was observed for the *TYMS* 6bp-indel variant and overall toxicity (Egger’s test p=0.15), but for the other two significant publication bias with overall toxicity was reported (*TYMS* 28bp-repeat and ENOSF1 c.742-227G>A, Egger’s test: $p = 0.01$ and $p < 0.01$, respectively). Of the total of 2,067 patients from the included studies, 1,012 were eligible for meta-analysis. The *TYMS* 3’UTR 6pb-indel was the only variant found to be associated with overall toxicity (OR=1.21; p=0.0215). However, all of the variants had statistically significant associations with severe hand-foot-syndrome. In addition, a multivariate analysis showed that *ENOSF1* c.742-227G > A and the *TYMS* 28bp-repeat each independently increased the rise for severe hand-foot-syndrome and patients homozygous for both variants had three-fold higher risk for severe hand-foot-syndrome compared to patients with neither variant.

Khushman (2018) performed a retrospective chart review to assess the association between DPYD*9A variants and toxicity in fluoropyrimidine-treated GI-malignancy patients.[42] Of a total of 28 patients genotyped for DPYD*9A, 13 had the variant genotype. Grade 3 to 4 toxicity

(diarrhea) was associated with the DPYD*9A variant in patients treated with full-dose fluoropyrimidines (p=0.0055).

In a 2017 combined retrospective chart review and prospective study, Castro-Rojas evaluated records of 99 patients (42 of whom were included in the analysis) with advanced CRC who received 5-FU or capecitabine and carried out a prospective study of 68 similar patients.[43] The *TYMS* variants rs45445694, rs183205964, rs2853542 and rs151264360 were analyzed, as was *TYMS* expression, response to treatment, and toxicity. The 2R allele of the rs45445694 was significantly associated with an increased risk of serious global toxicity (OR 8.29, 95% CI 1.25 to 54.71, p=0.023), whereas the variant rs2853542 was not (p=0.208). The other variants were not detected. In the prospective portion of the study, the same variants were identified. In this group, the rs45445694 2R allele was associated with a positive patient response to chemotherapy (OR 3.45, 95% CI 1.00 to 11.99, p=0.05). Based on previous studies, genotypes were categorized into low (2R/2R, 2R/3RC, 3RC/3RC) and high (2R/3RG, 3RG/3RC, 3RG/3RG) enzyme expression, and a significant association was found between the low expression variants and positive tumor response (OR 6.84, 95% CI 1.73 to 27.02, p=0.005). Severe toxicity was significantly associated with the rs45445694 2R allele (OR 4.11, 95% CI 1.19 to 14.25, p=0.00024). A multivariate logistic regression analysis for toxicity on the combined data from the retrospective and prospective studies (n=105) showed a significant association between the 2R/2R genotype adjusted by age and the risk of severe global toxicity to fluoropyrimidines (OR 5.21, 95% CI 1.86 to 14.59, p=0.0014).

In 2017, Vázquez conducted a prospective cohort study to determine the association of 5-FU toxicity with a number of variables, including methylenetetrahydrofolate reductase (MTHFR) single nucleotide polymorphism in exons 4 and 7 and 5'-untranslated region-*TYMS* VNTR genotypes.[44] Between 2013 and 2015, 197 patients were treated with 5-FU. 40.1% of patients developed severe toxicity during follow-up. According to the Cox regression model, the development of severe toxicity was significantly associated with a number of factors, including tumor type and baseline functional status, but there was no significant association with the genetic variants, MTHFR single nucleotide polymorphism in exons 4 and 7 and 5'-untranslated region-*TYMS* VNTR genotypes.

Nahid (2017) prospectively evaluated 161 patients with CRC who were treated with 5-FU based chemotherapy.[45] Of these patients, clinical follow-up was available for 139 patients. Within this population, DPYD*2A was significantly associated with grade 3 or 4 toxicity (p=0.023). The MTHFR C677T variant was associated with increased efficacy of treatment (p=0.006). The authors recommended confirmation of these findings in a larger population.

A 2017 study by Meulendijks investigated the predictive value of pretreatment serum concentrations of uracil and dihydrouracil and the association between *DYPD* and *TYMS* genetic variants and severe fluoropyrimidine toxicity.[46] Five-hundred fifty patients were treated with 5-FU following pretreatment measurement of serum uracil and dihydrouracil and detection of genetic variants. An association was found between high pretreatment concentrations of uracil and severe toxicity, but not between *DYPD* or *TYMS* variants and severe toxicity.

In a multicenter prospective nonrandomized cohort study, Boisdron-Celle (2017) assessed a multimodal approach to pretreatment screening for DPYD deficiency.[47] The screening included determining the dihydrouracil over uracil ratio, the *DPYD* variant status, and demographic parameters. Patients were divided into groups, with 718 patients receiving pretreatment screening followed by screening-based 5-FU dosing and 398 patients receiving

no screening and standard dosing. The incidence of grade 4 to 5 toxic early events was significantly higher in the standard dosing group. Grade 3 toxicity was observed in 10.8% of prescreened patients and 17.55% of standard dosing patients (p = 0.0497), and time to grade 3 or above toxicity was significantly greater in the prescreening group.

In 2016, Boige published a subanalysis of patients participating in an RCT.[48] The RCT compared treatment with FOLFOX4 and FOLFOX4 plus cetuximab. A total of 1545 patients participated in the pharmacogenetics substudy and were genotyped on 25 *DPYD* variants. The primary end point was development of grade 3 or higher FU-related adverse events (hematologic and gastrointestinal combined). Two *DPYD* variants (D949V and V73231) were significantly associated with grade 3 or higher adverse events (p<0.001 for both).

Schwab (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.[49] 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 variant *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% to 13%. Tandem repeats in *TYMS* were neither sensitive nor specific indicators of serious toxicity. Clinical factors also were examined for association with toxicity. Overall and in the group of 13 patients who were heterozygous for DPYD*2A, women were more likely than men to develop severe toxicity (overall OR, 1.9; 95% CI 1.26 to 2.87; p=0.002), most commonly mucositis. Bolus administration of 5-FU was a significant, independent predictor of severe toxicity overall.

In an accompanying editorial, Ezzedin and Diasio 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.”[50] 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[49]

	***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

	*DPYD wt/*2A*[a] (n=13)	*TYMS* VNTR 2/3 or 3/3[b] (n=521)
Specificity	NR	0.22
PPV	NR	0.06
NPV	NR	0.84
Mucositis		
Sensitivity	0.08	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 systematic review and meta-analyses,[51] and other studies,[52, 53]including two studies of homogenous patient groups enrolled in RCTs.[54, 55] Cancer types and specific variants studied varied across these reports.

In 2013, Loganayagam 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.[54, 56] Sensitivity and specificity of the three identified *DPYD* variants of the TheraGuide® 5-FU 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* variants associated with 5-FU toxicity noted a lack of consistent correspondence between deleterious variants and DPYD activity across studies.[57] 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: Efficacy

Smyth (2017) published a randomized phase 3 trial of 456 patients treated for gastroesophageal cancer either with surgery alone or with surgery augmented with 5-FU chemotherapy.[58] Of these patients, genetic tests were performed for 289 patients. The primary outcome was any association between 10 germline variants, including tandem repeats in the *TYMS* gene, and response rates, survival, or toxicity. Of the genes evaluated, none showed a variant significantly associated with chemotherapy-related toxicity. Of patients

who received chemotherapy, there was a significant association between the *TYMS* 2R/2R genotype and longer survival: for these patients, median OS was not reached during the study, while patients with *TYMS* 2R/3R or 3R/3R genotypes, respectively, had a median OS of 1.44 or 1.60 years (p=0.005). Authors noted that patients with *TYMS* 2R/2R genotype seemed to benefit from the chemotherapy treatment, with a significant interaction between treatment arm and genotype (p=0.029). No relationship between genotype and chemotherapy toxicity was noted. The trial was limited by the lack of tissue samples for all patients.

A 2013 systematic review and 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.[59] 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

Dolat (2020) reported on relationships between 5-FU clearance and markers of DPD activity, including uracilemia (U), dihydrouracilemia (UH2)/U ratio, or genotype of the gene encoding DPD (*DPYD*).[60] A total of 169 patients with gastrointestinal cancers who received 5-FU-based regimens were included. No correlation was observed between 5-FU clearance and measured U and UH2/U. The 5-FU AUC was significantly higher in patients with U < 16 ng/mL than in other patients (p=0.0016). Of the 23 patients that had U ≥ 16 ng/mL, 45% had a dose increase following 5-FU therapeutic drug monitoring (TDM) and one had a toxicity-related dose reduction.

Henricks (2019) reported on the effectiveness and safety of reduced-dose 5-FU therapy in patients with the DPYD*2A variant.[61] Overall survival, progression free survival, and toxicity were compared between 40 prospectively identified patients heterozygous for DPYD*2A treated with reduced fluoropyrimidine dosing and controls. For the survival analyses matched pair-analysis was performed, where each patient with the DPYD*2A variant was matched to a DPYD*2A wild-type patient. For the toxicity analysis, two control groups were used: a cohort of wild-type patients (n=1606) treated with full dose and a cohort of historical controls derived from the literature (n=86), i.e. patients with a DPYD*2A variant who received a full fluoropyrimidine dose. Overall survival and progression free survival were not significantly different between patients heterozygous for DPYD*2A treated with upfront reduced dosing and matched wild-type controls (p=0.47 and p=0.54, respectively). In the patients with the DPYD*2A variant who received reduced-dose 5-FU therapy, risk of toxicity was comparable to the cohort of wild-type patients (18% versus 23%, p=0.47) and was significantly lower than historical controls with the variant treated with a full 5-FU dose (77%, p<0.001). Of the patients included in Groups 1 and 2, 96% of patients were White, 1% of patients were Southeast Asian, 1.3% of patients were African, and 1.7% of patients did not have their ethnicity or race described.

A 2018 prospective multicenter safety analysis published by Henricks assessed toxicity in cancer patients treated with fluoropyrimidine-based therapy.[62] Prospective genotyping was carried out for DPYD*2A, c.2846A>T, c.1679T>G, and c.1236G>A. Initial doses were reduced by 25% in patients with c.2846A>T and c.1236G>A variants and 50% in patients with

DPYD*2A and c.1679T>G variants. The primary endpoint was the frequency of severe overall fluoropyrimidine-related toxicity. A total of 1103 patients met inclusion criteria. Of these, 85 were heterozygous *DPYD* variant allele carriers and the remainder were *DPYD* wild-type. The difference in severe toxicity between groups was statistically significant, with a greater percent of *DPYD* variant patients experiencing severe toxicity compared to wild-type patients (39% versus 23%, respectively, p=0.0013). Relative risk (RR) for severe toxicity was compared between patients with variants included in this study and a historical cohort of *DPYD* variant allele patients treated with full-dose fluoropyrimidine-based therapy. The RR for severe toxicity in the reduced-dose DPYD*2A, c.2846A>T, and c.1236G>A variant patients was 1.31 (CI 0.63 to 2.73), 2.0 (CI 1.19 to 3.34) and 1.69 (CI 1.18-2.42), respectively. For the historical controls, the RR for those variants was 2.87 (CI 2.14 to 3.86), 3.11 (CI 2.25 to 4.28), and 1.72 (CI 1.22 to 2.42), respectively. There was no toxicity in the one dose-reduced c.1679T>G variant patient and the RR in the historical control group was 4.30 (CI 2.10 to 8.80).

Lunenburg (2018) published a retrospective observational study of 828 patients who received fluoropyrimidine during chemoradiation therapy.[63] Risk of severe gastrointestinal and hematological toxicity was compared across three groups: patients with *DPYD* variants who received upfront dose reductions according to pharmacogenetic dosing guidelines (n=22), patients with *DPYD* variants treated with standard doses (n=34), and patients with wild-type *DPYD* (n=771). The patients with *DPYD* variants treated with standard doses had statistically significant increases in risk of severe gastrointestinal toxicity (adjusted OR 2.58, CI 1.02 to 6.53, p=0.045) and severe hematological toxicity (adjusted OR 4.19, CI 1.32 to 13.25, p=0.015) compared with wild-type patients. Patients with *DPYD* variants who received upfront dose reductions showed no statistically significant differences from wild-type patients in risk of either toxicity, although frequency of severe hematological toxicity was numerically greater. The mean duration of hospitalization was significantly shorter in the dose reduction group than the *DPYD* variant group with standard doses (p=0.01).

Cremolini (2017) reported chemotherapy-related adverse events experienced by patients with metastatic colon cancer who were enrolled in a the phase III TRIBE RCT and treated with first-line FOLFOXIRI plus bevacizumab or FOLFIRI plus bevacizumab.[64] 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, eight of ten patients who were *DPYD* carriers experienced grade 3 or higher adverse events and seven of the ten had a grade ≥ 3 adverse events within the first four cycles of induction therapy. Of patients bearing *DPYD* c.1905+1G/G and *DPYD* c.2846A/A genotypes, 166 out of 429 (39%) had a grade ≥ 3 adverse event in the same time period. 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.

In a 2017 publication, Dhawan reported the prevalence of *TPMT* and *DPYD* genetic variants in 500 healthy controls and the relationship between treatment response and TPMT and *DPYD* genetic variants in 500 patients with head and neck cancer.[65] The frequencies of TPMT*2, TPMT*3B, TPMT*3C, DPD IVS14+1G>A, and G1601A were 2%, 2.2%, 4.6%, 3.6%, and 3%, respectively. Treatment responses of TPMT*3B, TPMT*3C, and *DPYD* genetic variants were 62.50%, 59.26%, and 61.90%, respectively. In addition, the percent of nonresponders was higher in patients carrying a combination of these genetic variants.

In 2016 Deenen reported outcomes comparing pretreatment DPYD*2A testing with historical controls.[66] Cancer patients intending to undergo treatment with fluoropyrimidine-based therapy (5-FU or capecitabine) were enrolled as the test group. 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 (i.e., ≥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 (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. Relevant diversity was also not well represented, as 96% of patients were White, 1% of patients were Asian, and 3% of patients did not have their ethnicity or race described.

In 2014, Goff published results from an open-label, nonrandomized retrospective multicenter study that genotyped 42 adults with gastric or gastroesophageal junction cancer for TSER tandem repeats.[67] 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, 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, no complete responses). Median overall survival probability (OS) and progression-free survival probability (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 (2013) reported a study of 180 cancer patients receiving fluoropyrimidines (5-FU or capecitabine) who underwent *DPYD* analysis for the 1905+1 G>A variants by high-pressure liquid chromatography.[68] Four patients were heterozygous carriers. Of these, three patients received dose reduction of 50% to 60% but still experienced severe toxicities requiring hospitalization. One patient did not receive chemotherapy based on *DPYD* genotype and the presence of other variants found in mismatch repair genes.

PRACTICE GUIDELINE SUMMARY

NATIONAL COMPREHENSIVE CANCER NETWORK GUIDELINES

National Comprehensive Cancer Network (NCCN) guidelines do not recommend use of area under the curve guidance for 5-fluorouracil dosing or genetic testing for *DYPD* and/or *TYMS* variants in patients with colon[20], rectal[69], breast[70], gastric[71], pancreatic cancer[72], esophageal[73], or head and neck[74] cancers.

The colon cancer guideline discussion addresses the evidence for genetic testing for *DYPD* and notes the limitations of the studies, which include “great heterogeneity in specific treatment regimens and dosing decisions.” Further the guideline states, “Because fluoropyrimidines are a pillar of therapy in colorectal cancer (CRC) and it is not known with certainty that given *DYPD*

variants are associated with this risk and/or that dose adjustments do not impact efficacy, the NCCN Panel does not recommend universal pretreatment *DPYD* genotyping at this time." The discussion also includes information on the FDA-approved antidote uridine triacetate that is used to treat 5-FU and capecitabine toxicity.

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.[75] 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.

NATIONAL INSTITUTE FOR HEALTH AND CARE EXCELLENCE

In 2014, National Institute of Health and Care Excellence (NICE) published evidence-based diagnostics guidance on the My5-FU assay for guiding 5-FU chemotherapy dose adjustment.[76] 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.”

CLINICAL PHARMACOGENETICS IMPLEMENTATION CONSORTIUM

The Clinical Pharmacogenetics Implementation Consortium (CPIC) was formed in 2009 as a shared project between PharmGKB, an internet research tool developed by Stanford University, and the Pharmacogenomics Research Network of the National Institutes of Health. In 2013, CPIC published evidence-based guidelines for *DPYD* genotype and fluoropyrimidine dosing.[1] The guidelines did not address testing.

An update to the CPIC guidelines was published by Amstutz (2018).[77] As in 2013, the primary focus of the guidelines was on the *DPYD* genotype and implications for dosing of fluoropyrimidine. In the update, CPIC 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 CPIC listed the c.1905+1G>A, c.1679T>G, c.2846A>T, and c.1129-5923C>G variants, as affecting 5-FU toxicity. Updates were made to the tables available on the website in 2020.[78] 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-FU-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.

SUMMARY

There is not enough research to know if or how well 5-fluorouracil (5-FU) area under the curve (AUC) and pharmacogenetic testing for *DPYD* and/or *TYMS* genetic variants work to improve health outcomes in people with any type of cancer. This does not mean that these tests do not work, but more research is needed to know. Available evidence is limited to a few gene variants that are uncommon in the general population. No U.S.-based clinical guidelines recommend 5-FU AUC assays or *DPYD* and/or *TYMS* genetic testing for people with cancer. Therefore, 5-FU AUC and *DPYD* and/or *TYMS* genetic testing is considered investigational for all indications, including but not limited to colon and head/neck cancers.

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CODES

Codes	Number	Description
CPT	81232	DPYD (dihydropyrimidine dehydrogenase) (eg, 5-fluorouracil/5-FU and capecitabine drug metabolism), gene analysis, common variant(s) (eg, *2A, *4, *5, *6)
	81346	TYMS (thymidylate synthetase) (eg, 5-fluorouracil/5-FU drug metabolism), gene analysis, common variant(s) (eg, tandem repeat variant)
	84999	Unlisted chemistry procedure
HCPCS	S3722	Dose optimization by area-under-the-curve (AUC) analysis for infusional 5-fluorouracil (5-FU)

Date of Origin: *November 2012*