



Assessment
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Pharmacogenetic Testing to Predict Serious Toxicity From 5-Fluorouracil (5-FU) for Patients Administered 5-FU-Based Chemotherapy for Cancer

Executive Summary

Background

Severe toxicity occurs in about 30% of patients treated with 5-fluorouracil (FU)-based chemotherapy regimens. 5-FU has a narrow therapeutic window and the drug concentration required for tumor response is within the general range where toxicity may occur. Inherited genetic variability in key enzymes involved in the 5-FU metabolic pathway may be related to the variable patient experience of toxicity. Dihydropyrimidine dehydrogenase (DPD) is a saturable and rate-limiting enzyme in the 5-FU catabolic pathway. In the anabolic pathway, 5-FU is converted into several active metabolites, one of which inhibits the action of thymidylate synthase (TS), a key enzyme in normal and tumor DNA synthesis. Genetic polymorphisms in the genes coding for DPD and TS may result in enzyme products with different activity levels, resulting in 5-FU excess, the accumulation of 5-FU anabolic products, and severe toxicity.

Objective

This Assessment will evaluate the evidence for pharmacogenetic testing to predict 5-FU toxicity. In this application, pharmacogenetic testing is defined as the use of genetic polymorphisms in the genes coding for DPD and TS as predictors of toxicity at standard doses, and as indicators for reducing 5-FU dose to avoid toxicity without reducing efficacy.

Search Strategy

MEDLINE® was searched (via PubMed) using the following two strategies:

- “Fluorouracil”[MeSH] AND (“Thymidylate Synthase”[MeSH] OR (“Dihydrouracil Dehydrogenase (NAD+)”[MeSH] OR “Dihydrouracil Dehydrogenase (NADP)”[MeSH])), limited to human subjects, clinical trials, and the English language.
- “Fluorouracil”[MeSH] AND (“Thymidylate Synthase”[MeSH] OR (“Dihydrouracil Dehydrogenase (NAD+)”[MeSH] OR “Dihydrouracil Dehydrogenase (NADP)”[MeSH])) AND gene* AND tox*, limited to human subjects and the English language.

In addition, bibliographies from recent review articles and clinical studies were hand-searched for relevant studies. For information on assay technical performance, the grey literature was also searched, in particular, websites of commercial laboratories offering DPD and/or TS genotyping.

Selection Criteria

For clinical utility (effect of using DPD and TS pharmacogenetic tests to determine 5-FU dosing on patient toxicity outcomes), controlled trials that compared standard fixed-dose 5-FU regimens versus 5-FU dose adjusted, if needed, based on the results of pharmacogenetic testing and predicted 5-FU toxicity results.

For clinical validity (ability of DPD and TS pharmacogenetic tests to predict 5-FU patient toxicity), prospective cohort studies that reported or contained the data to calculate sensitivity, specificity, and predictive values of the genetic variants were preferred. Retrospective cohort studies that provided

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relevant information were also considered. Studies that evaluated highly selected populations (e.g., healthy controls vs. selected patients with 5-FU grade 3 to 4 toxicity reactions or case series of patients with 5-FU severe toxicity) were not included.

For analytic validity (technical performance of DPD and TS pharmacogenetic tests), any information from the published or grey literature that provided information on assay methods and technical performance was included.

Main Results

Ten cohort studies, mostly prospective, that evaluated the clinical validity of DPD pharmacogenetic testing were included in this Assessment. Sample sizes ranged from 21 to 683; total for all studies was 2,187. Patients had primarily colorectal cancer, other gastrointestinal cancer, head and neck, or breast cancer, and were treated with 5-FU monotherapy or combination chemotherapy. Most studies tested for only the most common variant of *DPYD*, the gene that codes for DPD (*DPYD* *2A) or a small number of known genetic variants, or scanned *DPYD* exon 14 for variants (includes the *2A variant). Two studies scanned the entire *DPYD* coding region and flanking regions. Because of the variety of approaches, studies report finding different variants; in addition, variants found may also be influenced by geography and ethnic mix. These differences affect the results of the sensitivity, specificity, and predictive value calculations, where data reported allowed these to be made. Nevertheless, some general observations are possible. The vast majority of patients who have mutations that completely or partially inactivate DPD are heterozygotes (mutation on only one gene allele); homozygotes (same mutation on both gene alleles) or compound heterozygotes (more than one active mutation, especially if on different alleles) are very rare. Thus, the results for this Assessment apply primarily to heterozygotes. Sensitivity is very low in all cases, because the majority of patients with severe toxicity have no detectable *DPYD* genetic variants. Specificity is quite high in most studies, because genetic variants are uncommon and most results are true negative.

Seven cohort studies examined the clinical validity of one or more of the 3 types of genetic variants found in the gene that codes for TS (*TYMS*); 3 of these studies examined 2 or 3 of these variants in combination. None of these 3 studies reported significant results for *TYMS* genetic variants, alone or in combination, as predictors of severe (grade 3–4) 5-FU toxicity. Results for each of the types of *TYMS* genetic variants considered individually are generally poor, as well as highly variable across studies in terms of sensitivity, specificity, and predictive values.

One study, considered one of the best studies to address pharmacogenetic testing for 5-FU toxicity due primarily to its large patient enrollment and to its focus on 5-FU monotherapy, thus avoiding confounding toxicity from other chemotherapy drugs, addressed not only *DPYD* and *TYMS* genetic variants, but several other possible factors influencing toxicity in a multivariable regression analysis. Results indicated the strongest influences were from an interaction between *DPYD* variants and sex (such that *DPYD**2A variants were significant only in male patients), and from leucovorin administration (yes/no); there were also significant influences from method of 5-FU administration, and from variants of the *TYMS* and *MTHFR* genes (*MTHFR* codes for 5,10-methylene-tetrahydrofolate reductase; another enzyme in the 5-FU metabolic pathway). From these results, the authors constructed a nomogram for estimating 5-FU toxicity risk; however, use of this nomogram was not tested prospectively in the reported study.

The interaction between *DPYD* variants and sex was surprising and was not explained by DPD enzyme activity or protein content in the liver, or by sex-specific promoter methylation. The possibility that the result was random and due to small numbers of gene variant carriers cannot be ignored.

No published studies addressed clinical utility, that is, whether reducing the starting dose of 5-FU when serious toxicity is predicted by pretreatment pharmacogenetic testing reduces episodes of serious toxicity without reducing treatment response compared to standard dosing and dose adjustment according to symptoms.

No published studies addressed analytic validity, or the technical performance of commercially available DPD and TS pharmacogenetic assays. One commercial laboratory provides a limited summary of technical specifications for their assays on their website. False-negative and false-positive results are estimated to be less than 1%.

Author's Conclusions and Comments

It has been tempting to postulate alterations in activity of key enzymes such as DPD and TS in the 5-FU metabolic pathway as the causal basis for 5-FU toxicity, and specific genetic variants of the genes coding for those enzymes as the starting points in the causal chain. Indeed, patients who are homozygous (i.e., have the same variant sequence in both gene copies) for DPD-inactivating mutations in the DPD gene uniformly experience early, severe, and potentially fatal toxicity reactions when administered standard 5-FU doses. However, homozygosity or compound heterozygosity (more than 1 variant sequence, distributed across both gene copies) for DPD-inactivating mutations was rarely reported in the studies included in this Assessment and heterozygous *DPYD* variants were observed in relatively small proportions of patients with grade 3 to 4 toxicity. Moreover, not all patients with *DPYD* variants experience toxicity (even when variants assessed are limited to those with prior associations with toxicity). The clinical validity evidence for each of the 3 types of TS gene variants is similarly poor in terms of the ability of *TYMS* variants to predict which patients are likely to experience severe 5-FU toxicity.

In summary, testing for genetic variants of the genes coding for DPD and TS enzymes has poor predictive value for 5-FU toxicity and no studies have shown that it is useful in directing 5-FU dose reductions to lower toxicity without adversely affecting tumor response.

Based on the available evidence, the Blue Cross and Blue Shield Association Medical Advisory Panel made the following judgments about whether the use of pharmacogenetic testing to predict serious toxicity from 5-FU for patients administered 5-FU-based chemotherapy for cancer meets the Blue Cross and Blue Shield Association's Technology Evaluation Center (TEC) criteria.

1. The technology must have final approval from the appropriate governmental regulatory bodies.

There are no assay kits approved by the U.S. Food and Drug Administration (FDA) for genetic testing for *DPYD* or *TYMS* genotypes, nor are any kits being actively manufactured and marketed for distribution. Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; such tests must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). The laboratory offering the service must be licensed by CLIA for high-complexity testing. The FDA is currently considering active regulation of at least some types of laboratory-developed tests.

2. The scientific evidence must permit conclusions concerning the effect of the technology on health outcomes.

The evidence for this Assessment consists of cohort studies that address the clinical validity of DPD and TS pharmacogenetic testing. In general, both assays have 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. No studies address the clinical utility of reducing the initial 5-FU dose in patients with inactivating mutations and maximizing subsequent doses while avoiding toxicity. The evidence is insufficient to permit conclusions regarding the effect of DPD and TS pharmacogenetic testing on benefits (reduced toxicity) and potential harms (poorer response to treatment).

3. The technology must improve the net health outcome; and

4. The technology must be as beneficial as any established alternatives.

There is insufficient evidence to permit conclusions regarding the use of pharmacogenetic testing to predict serious toxicity from 5-FU for patients administered 5-FU-based chemotherapy for cancer.

5. The improvement must be attainable outside the investigational settings.

Whether or not the use of use of pharmacogenetic testing to predict serious toxicity from 5-FU for patients administered 5-FU-based chemotherapy for cancer improves health outcomes has not been demonstrated in the investigational setting.

For the above reasons, the use of pharmacogenetic testing to predict serious toxicity from 5-FU for patients administered 5-FU-based chemotherapy for cancer does not meet the TEC criteria.

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Assessment Objective

Severe toxicity occurs in about 30% of patients treated with 5-fluorouracil (5-FU)-based chemotherapy regimens. 5-FU has a narrow therapeutic window and the drug concentration required for tumor response is within the general range where toxicity may occur. Inherited genetic variability in key enzymes involved in the 5-FU metabolic pathway may be related to the variable patient experience of toxicity. Dihydropyrimidine dehydrogenase (DPD) is a saturable and rate-limiting enzyme in the 5-FU catabolic pathway. In the anabolic pathway, 5-FU is converted into several active metabolites, one of which inhibits the action of thymidylate synthase (TS), a key enzyme in normal and tumor DNA synthesis. Genetic polymorphisms in the genes coding for DPD and TS may result in enzyme products with different activity levels, resulting in 5-FU excess, the accumulation of 5-FU anabolic products, and severe toxicity.

This Assessment will evaluate the evidence for pharmacogenetic testing to predict 5-FU toxicity. In this application, pharmacogenetic testing is defined as the use of genetic polymorphisms in the genes coding for DPD and TS as predictors of toxicity at standard doses, and as indicators for reducing 5-FU dose to avoid toxicity without reducing efficacy.

Background

Inherited interindividual variability in rates of drug metabolism may result in large interpatient differences in systemic exposure, resulting in toxicity for some, lack of efficacy for others, and a satisfactory response mainly for those close to average population metabolism. Chemotherapy drugs commonly have a narrow therapeutic index that may overlap with the range of systemic exposure that results in severe toxicity, making effective dosing extremely difficult.

Inherited polymorphisms in the genes coding for key molecules responsible for metabolism of chemotherapy drugs may result in increased drug metabolism and elimination, reducing exposure and possibly reducing efficacy; or conversely may result in reduced drug metabolism and elimination, increasing systemic exposure and predisposing to severe toxicity. These genetic variants (also termed polymorphisms) affect the pharmacokinetic characteristics of the drug. In a few cases, inherited genetic

variants may result in a modified drug target and thus affect the pharmacodynamic characteristics of the drug.

5-FU Metabolism and Toxicity

5-FU is one of the most commonly prescribed components in chemotherapy regimens for a variety of cancer types such as colorectal, breast, and head and neck cancers. 5-FU must first be anabolized to the nucleotide level, where it interferes with DNA synthesis, primarily by inhibiting TS. 5-FU also interferes with RNA synthesis; both DNA and RNA synthesis are essential to the growth of the tumor. 5-FU can also be administered in the form of the prodrug capecitabine, which is given orally, is safer, and is more effective (Seck et al. 2005). Capecitabine is preferentially converted within the tumor site by thymidine phosphorylase (TP) into 5-FU (van Kuilenburg et al. 2004).

5-FU metabolism is divided into opposing anabolic and catabolic pathways. In the catabolic pathway, DPD rapidly degrades 85% or more of administered 5-FU to inactive compounds, primarily in the liver where DPD is abundantly expressed (van Kuilenburg 2004) and normally catabolizes the pyrimidine bases uracil and thymine (DNA building blocks). Both anabolic and catabolic pathways for 5-FU must be taken into account when considering net antitumor activity. 5-FU has a narrow therapeutic window. However, DPD is a saturable, rate-limiting enzyme and 5-FU can accumulate rapidly; in 5-FU excess, anabolic products accumulate and may cause World Health Organization (WHO) grade 3 to 4 toxicity (e.g., neutropenia, diarrhea, hand-foot syndrome). Examination of 5-FU pharmacokinetic parameters has shown that the drug concentration required for tumor response is within the general range where toxicity occurs (Ploylearmsaeng et al. 2006). The estimated threshold for severe toxicity is similar across different 5-FU-based treatment regimens (van Kuilenburg 2004), although severe grade 3 to 4 hematologic toxicity is more likely with bolus delivery rather than when given by continuous infusion (Meta-Analysis Group in Cancer 1998).

The efficacy of 5-FU chemotherapy increases with dose escalation, as does the likelihood of adverse events. Severe grade 3 to 4 toxicity occurs in about 30% of patients treated with 5-FU; the mortality rate is about 0.5% (Meta-Analysis Group in Cancer 1998). Patients with systemic low DPD activity, resulting in reduced

5-FU clearance, also have increased 5-FU systemic exposure and an increased risk of severe 5-FU toxicity. Based on several reports, a threshold of 70% of the mean DPD activity of a control population has been proposed as a threshold limit for DPD activity; using this threshold, approximately 14% of the population would be at risk of severe 5-FU-related toxicity using standard dosing (van Kuilenburg 2004).

Genetics of 5-FU Metabolism and Toxicity

DPD Genetic Variants. DPD activity may be low due to severely impaired liver function, patient age, elapsed time during 5-FU infusion (Etienne et al. 1998) or due to the presence of inherited DPD genetic variants. The DPD gene (*DPYD*) is large and complex, containing 23 exons*; at least 39 sequence variations have been identified (Shimoyama 2009), some of which (but not all) have been associated with loss of DPD activity and severe 5-FU toxicity. The most common, a G to A splice site substitution (IVS14+1G>A, also designated *DPYD*2A*), results in a nonfunctional protein product. General population allele frequencies of the *DPYD*2A* mutation range from 0 to nearly 3% in limited studies of different ethnic populations (van Kuilenburg 2004). The frequency of all known inactivating mutations in the general population is in the range of 3–5%; however, most patients are heterozygous for these mutations and produce some functional enzyme. The prevalence of homozygosity for inactivating mutations, resulting in no DPD activity and placing patients at highest risk for severe and possibly lethal toxicity (van Kuilenburg et al. 2001), is estimated to be about 0.1% (Miller and McLeod 2007).

The FDA-approved labels for both 5-FU and the 5-FU prodrug capecitabine (Xeloda®, Genentech) contain the following warning: “Rarely, unexpected, severe toxicity (e.g., stomatitis, diarrhea, neutropenia, and neurotoxicity) associated with 5-fluorouracil has been attributed to deficiency of dipyrimidine dehydrogenase activity.” In addition, the capecitabine label contains the following statements; “Xeloda® is contraindicated in patients with known dihydropyrimidine dehydrogenase (DPD) deficiency.” However, the labels are silent regarding testing for DPD deficiency in advance of treatment, nor do they provide guidance on how to reduce 5-FU dosage when *DPYD* mutations are detected.

TS Genetic Variants. An active metabolite of 5-FU interferes with DNA synthesis by forming stable complexes with TS and a cofactor (folate), thereby blocking the conversion of uracil to thymine, a necessary DNA building block. A variable tandem repeat polymorphism of a 28-base pair sequence is present most commonly as a double-tandem repeat (2R) or as a triple-tandem repeat (3R) in the *TYMS* 5' promoter region. However the number of tandem repeats can vary from 2 to 9 and differs extensively with ethnicity (Shimoyama 2009). Moreover, a G>C single nucleotide polymorphism (SNP) in the second tandem repeat results in 3 possible alleles, with different effects on transcriptional regulation: 2R, 3RG, and 3RC where 2R and 3RC confer similarly low TS gene expression (Mandola et al. 2003; Kawakami and Watanabe 2003). Recently, another G>C SNP was reported in the first tandem repeat, resulting in decreased *TYMS* transcription (Lincz et al. 2007). Finally, a 6-base pair insertion/deletion polymorphism in the 3' untranslated region (UTR) has been identified (Mandola 2004). These polymorphisms have been associated with altered TS expression and in some studies, 5-FU response or toxicity.

Other Genetic Influences. Other genetic or epigenetic variability may also help explain some cases of severe 5-FU toxicity. Several SNPs in the methylenetetrahydrofolate reductase gene (*MTHFR*), which codes for the reduced folate cofactor essential for TS inhibition, may play a role. An inherited inactivating mutation in dihydropyrimidinase, the second enzyme after DPD in the 5-FU catabolic pathway, has been reported in a breast cancer patient with severe 5-FU toxicity (van Kuilenburg et al. 2003). Methylation of the *DPYD* promoter region may also cause down regulation of DPD activity and 5-FU toxicity (Ezzeldin et al. 2005). Clinical parameters such as performance status and age have been reported to predict toxicity (Meta-Analysis Group in Cancer 1998) but have not been analyzed in combination with genetic mutations. Because both genetic and clinical factors may influence 5-FU metabolism and subsequent toxicity, other markers such as uracil metabolites and/or their ratios have been researched as markers of 5-FU related toxicity (e.g., Gamelin et al. 1999; Zhou et al. 2007). More recently,

*Exons are protein-coding regions of genes, which are separated by introns, nonprotein-coding regions. Introns are transcribed into precursor messenger RNA (mRNA), but are removed prior to translation of mature mRNA into protein.

Table 1. Examples of Clinical Laboratories that Offer Pharmacogenetic Testing for 5-FU Toxicity

| Laboratory | Test Name | DPD (<i>DPYD</i> *2A analysis only unless otherwise specified) | TS |
|---|--|---|---|
| Myriad Genetics | TheraGuide™ | ✓ (full sequencing of the <i>DPYD</i> gene; detects all mutations) | ✓ (analysis of tandem repeat mutations in the <i>TYMS</i> gene promoter region) |
| Specialty Laboratories | DPD 5-FU GenotypR™ | ✓ | |
| Laboratory Corporation of America (LabCorp) | DPD 5-Fluorouracil Toxicity | ✓ | |
| Quest Diagnostics | Dihydropyrimidine Dehydrogenase (DPD) Gene Mutation Analysis | ✓ | |
| Genelex | DPD Enzyme Deficiency Test for Fluorouracil | ✓ | |
| Molecular Diagnostics Laboratories | DPD Enzyme Deficiency | ✓ | |

measurement of $^{13}\text{CO}_2$ in expired air after ingesting a solution of 2- ^{13}C -uracil has been investigated as a way to evaluate the integrity of the uracil catabolic pathway and detect individuals who are likely to experience toxicity at a given 5-FU dosage (Ito et al. 2005; Mattison et al. 2006). Neither method has been validated for widespread use in clinical practice.

Clinical Utility of 5-FU Pharmacogenomic Testing. While several studies have reported evidence of associations between genetic polymorphisms and toxicity, few if any have prospectively evaluated the utility of testing for genetic variants related to 5-FU metabolism (i.e., pharmacogenetic testing), and changing 5-FU dose based on test results to avoid serious toxicity reactions and maximize effectiveness. The American Society of Clinical Oncology update of recommendations for the use of tumor markers in gastrointestinal cancer state that the “data are insufficient to recommend the routine use of ... thymidine synthase, dihydropyrimidine dehydrogenase...in the management of patients with colorectal cancer” (Locker et al. 2006).

Pharmacogenetic testing is routinely available in several commercial laboratories for the DPD gene, although testing is often limited to the *DPYD**2A mutation, and in a few laboratories for the TS gene. Examples are given in Table 1. Where a broader screen than just the *DPYD**2A variant is used, it is especially important to be

able to discriminate between *DPYD* variants that have no functional consequences, and those that significantly affect DPD activity. For example, the assay offered by Myriad Genetics identifies 3 known mutations as “high risk,” certain others predicted not to affect protein function as “low risk,” and a final category for variants whose clinical significance has not yet been determined.

FDA Status

There are no assay kits approved by the U.S. Food and Drug Administration (FDA) for genetic testing for *DPYD* or *TYMS* genotypes, nor are any kits being actively manufactured and marketed for distribution. Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; such tests must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). The laboratory offering the service must be licensed by CLIA for high-complexity testing. The FDA is currently considering active regulation of at least some types of laboratory-developed tests.

Methods

Search Methods

MEDLINE® was searched (via PubMed) using the following two strategies:

- “Fluorouracil”[MeSH] AND (“Thymidylate Synthase”[MeSH] OR (“Dihydrouracil

Dehydrogenase (NAD⁺)”[MeSH] OR “Dihydrouracil Dehydrogenase (NADP)”[MeSH]), limited to human subjects, clinical trials, and the English language.

- “Fluorouracil”[MeSH] AND (“Thymidylate Synthase”[MeSH] OR (“Dihydrouracil Dehydrogenase (NAD⁺)”[Mesh] OR “Dihydrouracil Dehydrogenase (NADP)”[MeSH])) AND gene* AND tox*, limited to human subjects and the English language.

Searches were done through October 2009; focused updates were done in July 2010. In addition, bibliographies from recent review articles and clinical studies were hand-searched for relevant studies. For information on assay technical performance, the grey literature was also searched, in particular, websites of commercial laboratories offering DPD and/or TS genotyping.

Study Selection

For clinical utility, controlled trials that compared standard fixed dose 5-FU regimens versus 5-FU dose adjusted, if needed, based on the results of pharmacogenetic testing and predicted 5-FU toxicity results.

For clinical validity, prospective cohort studies that reported or contained the data to calculate sensitivity, specificity, and predictive values of the genetic variants were preferred.

Retrospective cohort studies that provided relevant information were also considered. Studies that evaluated highly selected populations (e.g., healthy controls vs. selected patients with 5-FU grade 3 to 4 toxicity reactions or case series of patients with 5-FU severe toxicity) were not included.

For analytic validity, any information from the published or grey literature that provided information on assay methods and technical performance was included.

Medical Advisory Panel Review

This Assessment was reviewed by the Blue Cross and Blue Shield Association Medical Advisory Panel (MAP) on October 1, 2009. To maintain the timeliness of the scientific information in this Assessment, literature search updates were performed subsequent to the Panel’s review (see “Search Methods”). If the search updates identified any additional studies that met the criteria for detailed review, the results of these studies were included in the

text where appropriate. There were no studies that would change the conclusions of this Assessment.

Formulation of the Assessment

Patient Indications

Cancer patients undergoing chemotherapy with 5-FU-based regimens

Technologies to be Compared

Standard fixed-dose 5-FU regimens versus 5-FU dose adjusted (i.e., decreased), if needed, based on the results of pharmacogenetic testing and predicted 5-FU toxicity results.

Health Outcomes

Benefits. Reduced toxicity.

Harms. Reduced response rate.

Specific Assessment Question

Do genetic variants of *DPYD* and/or *TYMS* accurately predict toxicity from 5-FU-based chemotherapy? (clinical validity)

Does reducing the starting dose of 5-FU when serious toxicity is predicted by pretreatment pharmacogenetic testing reduce episodes of serious toxicity without reducing treatment response compared to standard dosing and dose adjustment according to symptoms? (clinical utility)

What is the technical accuracy and reliability of the tests for *DPYD* and *TYMS* gene variants? (analytic validity)

Review of Evidence

Do genetic variants of *DPYD* and/or *TYMS* accurately predict toxicity from 5-FU-based chemotherapy? (clinical validity)

DPYD Pharmacogenetic Testing

Ten cohort studies, mostly prospective, that evaluated *DPYD* pharmacogenetic testing were included in this Assessment (Table 2). Sample sizes ranged from 21 to 683. Patients had primarily colorectal cancer, other gastrointestinal cancer, head and neck, or breast cancer, and were treated with 5-FU monotherapy or combination chemotherapy. Most studies tested for only the most common *DPYD* genetic variant (*2A, see Background) or a small number of known genetic variants, or scanned *DPYD* exon 14 for variants (includes the *2A variant). Two

studies scanned the entire *DPYD* coding region and flanking intronic regions (Amstutz et al. 2009; Gross et al. 2008). Because of the variety of approaches, studies report finding different variants; in addition, variants found may also be influenced by geography and ethnic mix. These differences affect the results of the sensitivity, specificity, and predictive value calculations, where data reported allowed these to be made (Table 2). Nevertheless, some general observations are possible. The vast majority of patients who have mutations that completely or partially inactivate DPD are heterozygotes (mutation on only one gene allele); homozygotes (same mutation on both gene alleles) or compound heterozygotes (more than one active mutation on different alleles) are very rare. Thus, the results in Table 2 apply primarily to heterozygotes. Sensitivity is very low in all cases, because the majority of patients with severe toxicity have no detectable *DPYD* genetic variants. Specificity is quite high in most studies, because genetic variants are uncommon and most results are true negative.

Schwab et al. (2008) conducted the largest study (n=685), which was a multicenter, prospective cohort study in a population of cancer patients treated with 5-FU monotherapy*. Patients had different types of cancer including colorectal (upper colon, n=235; rectum, n=385), other gastrointestinal (n=35), breast (n=16), and unknown primary (n=14). All patients were tested for the *DPYD**2A mutation. *DPYD**2A testing had very poor sensitivity and positive predictive value for grade 3 to 4 5-FU toxicity (Table 2). In the overall population of 685 patients, the allele frequency was only about 1% and none of the patients with the allele were homozygous (heterozygous genotype frequency, 2%). The authors also sequenced all *DPYD* coding exons and flanking intronic regions in a subset of patients and found 12 additional exonic mutations, 2 of which were related to toxicity in 4 of 6 patients, but which were not incorporated into their analysis. In a multivariable logistic regression model, which included other predictors of 5-FU toxicity, *DPYD* genotype remained an independent and significant predictor.

Morel et al. (2006) also conducted a relatively large, prospective cohort study (n=487) in

patients with advanced colorectal, head and neck, or breast cancer. Patients were treated with 5-FU-based chemotherapy, mainly combination regimens that included oxaliplatin, irinotecan, or other drugs according to the type of cancer. The authors tested patients for 22 variants previously reported in the literature, finding 9 in cohort patients. The *2A variant was found in 10 patients (homozygous in 1 patient), representing a higher frequency than in the Schwab et al. (2008) study. Two other variants were also associated with toxicity and their ability to predict toxicity was calculated together (Table 2). Although the exact numbers differ from those reported by Schwab et al. (2008), positive predictive value remains low because genetic variants are rare and not all patients with variant genes experienced toxicity. However, the presence of one of the 3 relevant variants was highly correlated with early and severe toxicity at a p value of 2.8×10^{-10} .

Amstutz et al. (2009) and Gross et al. (2008) are the only studies that scanned the entire coding and flanking regions of the *DPYD* gene for genetic variants associated with toxicity (as do some commercial laboratories). Amstutz prospectively enrolled only 111 patients with a variety of solid malignancies scheduled for 5-FU-based chemotherapy. After an initial evaluation, the authors combined all *DPYD* variants associated with severe toxicity and conducted a combined haplotype analysis (Table 2). Despite the comprehensive scan, clinical validity descriptors were essentially no better than Schwab et al. (2008). In addition, the study could not confirm the previously reported association of 5-FU toxicity with known deleterious genetic variants (e.g., Morel et al. 2006, see Table 2).

The Gross et al. (2008) study was retrospective and also somewhat small (n=128). Because the wording is not clear, it is possible that included patients were preselected. Most patients were treated with 5-FU-based combination rather than monotherapy. Unfortunately, reported data did not allow calculation of sensitivity, specificity, or predictive values. The *2A mutation occurred in 5 patients, all of whom experienced toxicity; this and the 2846A>T variant were found in common with the Morel et al. (2006) study but no variants were found in common with Amstutz et al. (2009).

*Patients were treated from March 2000 to July 2005; at this time, 5-FU was “was commonly used in clinical practice as standard treatment regimen” according to the authors. However, it was reported that 80 patients received additional chemotherapy (e.g., irinotecan, oxaliplatin) during the course of the trial (Schwab et al. 2008, online Appendix).

Table 2. Summary of Published Studies Reporting Clinical Validity of DPYD Gene Variants Relevant for Severe 5-FU Toxicity¹

| Study | Patients | N | Genotype or Variant and Comparison | Genotype or Allele Frequency (%) | Clinical Validity for All Grade 3–4 Toxicity (%) | | | |
|---|---|-----|---|----------------------------------|---|-------------|-----|-----|
| | | | | | Sensitivity | Specificity | PPV | NPV |
| Amstutz et al. 2009 Prospective cohort study Switzerland | Patients with solid malignancies (colorectal cancer, other gastrointestinal tumors, nongastrointestinal tumors) scheduled for adjuvant or palliative 5-FU-based chemotherapy (complete <i>DPYD</i> coding region sequencing) | 111 | IVS5 + 18G>A IVS6 + 139G>A IVS9—51T>G c.1236G>A haplotype (all listed mutations) vs. wt | haplotype frequency: 3.6 | 17 | 97 | 57 | 81 |
| Schwab et al. 2008 Prospective cohort study Germany | Patients receiving 5-FU <u>monotherapy</u> with LV or levamisole; cancer types included colorectal, other GI, breast, or unknown primary | 683 | wt/*2A vs. wt/wt (no homozygotes) | 1.9 | 5.5 | 99 | 46 | 84 |
| Gross et al. 2008 Retrospective cohort study Germany | Patients with breast, gastroesophageal and colorectal cancer treated with 5-FU-based chemotherapy, mostly combination regimens; at least 53 specifically selected for toxicity reactions (complete <i>DPYD</i> coding region sequencing) | 128 | 496A>G *2A 1109delTA 2846A>T heterozygote vs. homozygote not indicated | 6.6 ? 0.4 0.4 | OR _{grade 3–4} =4.56, 95% CI 1.95–10.45 Found only in patients with severe toxicity Found in 1 patient with fatal toxicity in 1st cycle Found in 1 patient with grade IV enterotoxicity | | | |
| Sulzyc-Bielicka et al. 2008 Retrospective cohort study Poland | Consecutive colorectal cancer patients treated from 1998–2005 with 5-FU chemotherapy (4/252 patients had grade 3–4 neutropenia) | 252 | wt/*2A vs. wt/wt | 0.4 | 25 | 100 | 100 | 99 |

Table 2. Summary of Published Studies Reporting Clinical Validity of *DPYD* Gene Variants Relevant for Severe 5-FU Toxicity¹ (cont'd)

| Study | Patients | N | Genotype or Variant and Comparison | Genotype or Allele Frequency (%) | Clinical Validity for All Grade 3–4 Toxicity (%) | | | |
|--|--|-----|--|----------------------------------|--|-------------------------|-------------------------|-------------------------|
| | | | | | Sensitivity | Specificity | PPV | NPV |
| Zhang et al. 2007 Prospective cohort study China | Patients with gastric or colon cancer being treated with 5-FU plus oxaliplatin | 75 | 1627A>G (*5; 11/34 were homozygotes with partial DPD deficiency) | 30 | GI: 29 Leukopenia:30 | GI: 92 Leukopenia:91 | GI: 64 Leukopenia:55 | GI: 73 Leukopenia:78 |
| Morel et al. 2006 Prospective cohort study France | Patients treated for advanced carcinomas with 5-FU-based chemotherapy (including combo regimens such as FOLFOX and FOLFIRI); cancer types included colorectal, head and neck, and breast | 487 | *2A 2846A>T 1679T>G heterozygotes + homozygotes (1; *2A) vs. wt | 1.1 1.0 0.1 | 31 | 98 | 62 | 94 |
| Boisdron-Celle et al. 2007 Prospective cohort study France | Patients treated for advanced colorectal carcinomas or in the adjuvant setting by 5-FU/leucovorin chemotherapy; no prior 5-FU therapy | 252 | *2A 2846A>T *2A + 2846A>T heterozygotes (1 with multiple heterozygous mutations) vs. wt | 0.4 1.4 0.2 | 33 | 99 | 70 | 94 |
| Largillier et al. 2006 Prospective cohort study France | Consecutive advanced breast cancer patients receiving oral capecitabine alone | 105 | wt/*2A vs. wt/wt (cycle 1) | 0.95 | 5.3 | 100 | 100 | 83 |

Table 2. Summary of Published Studies Reporting Clinical Validity of *DPYD* Gene Variants Relevant for Severe 5-FU Toxicity¹ (cont'd)

| Study | Patients | N | Genotype or Variant and Comparison | Genotype or Allele Frequency (%) | Clinical Validity for All Grade 3–4 Toxicity (%) | | | |
|--------------------------|--|----|--|----------------------------------|--|-------------|-----|-----|
| | | | | | Sensitivity | Specificity | PPV | NPV |
| Salgueiro et al. 2004 | Consecutive colorectal cancer patients treated with 5-FU after surgery | 73 | *2A + 1845G>T | 1.4 | 25 | 100 | 100 | 92 |
| Prospective cohort study | | | heterozygotes vs. wt | | | | | |
| Portugal | | | | | | | | |
| Zhu et al. 2004 | Patients with stage III/IV colorectal adenocarcinoma and no prior 5-FU treatment | 21 | 496A>G | 3.1 | P=0.80 for association with grade 3–4 toxicity p=0.41 p=0.51 p=0.18 p=0.87 p=0.33 | | | |
| Prospective cohort study | | | 1627A>G | 17 | | | | |
| | | | 3351T>C (H, 1) | 9.4 | | | | |
| | | | 3649G>A | 7.8 | | | | |
| U.S. | | | 3844A>G (H, 5) | 38 | | | | |
| | | | 3856T>C (H, 4) | 38 | | | | |
| | | | 'H' indicates homozygote(s) found and number | | | | | |

¹Not included are results for common mutations such as 85T>C, for which reporting studies indicate no association with toxicity (Amstutz et al. 2009; Morel et al. 2006; Zhu et al. 2004) or with DPD half-life (Zhang et al. 2007).

Abbreviations: NPV: negative predictive value; PPV: positive-predictive value; wt: wild type

Boisdron-Celle et al. (2007) studied not only *DPYD* genotyping (3 *2A heterozygotes and 11 with other significant *DPYD* variants, all heterozygotes), but other predictors of toxicity as well, recommending a 2-step process beginning with genotyping and following up with measurements of uracil and its metabolites in order to achieve a clinically useful predictive algorithm.

***TYMS* Pharmacogenetic Testing**

Seven cohort studies examined one or more of the 3 types of *TYMS* genetic variants (Table 3); 3 of these studies examined 2 or 3 of these variants in combination (Gusella et al. 2009; Sharma et al. 2008; Largillier et al. 2006; see Table 3, “Analysis of *TYMS* variant haplotypes”). None of these studies reported significant results for *TYMS* genetic variants, alone or in combination, as predictors of 5-FU toxicity. Lecomte et al. (2004) conducted a haplotype analysis of the tandem repeat and the 3' UTR insertion/deletion variant and reported that only the 2R/3' UTR insertion haplotype was significantly associated with toxicity (OR 8.1; 95% CI: 1.6–41.8).

Results for each of the types of *TYMS* genetic variants considered individually are generally poor in terms of sensitivity, specificity, and predictive values (Table 3). Schwab et al. (2008) reported a significant, although not very strong association between the *TYMS* homozygous double tandem repeat variant and severe toxicity (OR 1.6; 95% CI: 1.08–2.22). Lecomte et al. (2004) reported a similarly significant result ($p=0.02$) for the 2R/2R variant. Ichikawa et al. (2006) also report strong and significant results for the 2R/2R association with grade 3–4 neutropenia (multivariable OR 19; 95% CI: 2.2–334) and with grade 3–4 diarrhea (multivariable OR 11; 95% CI: 1.6–117) but with a high degree of uncertainty in the estimate.

Gusella et al. (2009) studied several potential predictors of 5-FU toxicity and reported that, although none of the *TYMS* genetic variants explained toxicity outcomes, low 5-FU clearance was the strongest single predictor of severe toxicity ($p<0.0001$).

Does reducing the starting dose of 5-FU when serious toxicity is predicted by pre-treatment pharmacogenetic testing reduce episodes of serious toxicity without reducing treatment response compared to standard dosing and dose adjustment according to symptoms? (clinical utility)

There are no published studies that address this question.

The vast majority of patients identified with *DPYD* variants are heterozygotes (one variant, one normal gene copy) and have reduced but not completely absent DPD activity. Not all *DPYD* variant heterozygotes have serious toxicity reactions; however, positive predictive value was at least 60% in larger studies testing multiple *DPYD* variants (Table 2), and 46% in the largest multicenter prospective study of the *DPYD**2A variant (Schwab et al. 2008). In addition, it has been reported that the onset of toxicity occurs approximately twice as fast in patients with a partial DPD deficiency, compared with patients with a normal DPD activity (van Kuilenburg et al. 2000). Therefore, genetic testing in advance of initial dosing might identify a high-risk group for pretreatment pharmacokinetic studies or initial treatment with a lower 5-FU dose and a slow increase in dose with close monitoring. Such an approach, however, has not been systematically studied for effect on toxicity or on efficacy.

Those rare patients homozygous for one inactivating *DPYD* variant or heterozygous for more than one variant (if biallelic, i.e., on different alleles) resulting in near to complete loss of DPD activity are at highest risk for early serious and potentially fatal reactions at normal 5-FU doses. Many of these cases have been reported based solely on pharmacokinetic information, prior to the availability of *DPYD* genotyping. Some examples with genotype confirmation from cohort studies and case reports are presented in Table 4. In each case, toxicity was early, severe, and likely lethal without extreme supportive measures. Identification of such patients in advance of treatment would be beneficial as 5-FU exposure should be avoided completely. However, genotyping may not be the best way to identify DPD-deficient patients. First, in the two largest prospective cohort studies (Schwab et al. 2008; Morel et al. 2006), only one homozygote variant was found in a total of 1,170 patients. Second, genetic modifications not detected by standard *DPYD* genotyping may account for some DPD deficiencies. Noguchi et al. (2004) reported that aberrant methylation of the promoter region of the DPD gene was responsible for *DPYD* repression in cell lines with low DPD activity. Zhang et al. (2007) found that *DPYD* promoter methylation was also associated with inhibition of promoter binding of SP1, a transactivator of *DPYD*. The

Table 3. Summary of Published Studies Reporting Clinical Validity of *TYMS* Gene Variants for Severe 5-FU Toxicity

| Study | Patients | N | Genotype Comparison | Genotype Frequency (%) | Clinical Validity for All Grade 3–4 Toxicity | | | |
|---|---|-----|-------------------------|------------------------|--|-------------|-----|-----|
| | | | | | Sensitivity | Specificity | PPV | NPV |
| Analysis of <i>TYMS</i> Tandem Repeats | | | | | | | | |
| Gusella et al. 2009 Prospective cohort study | Consecutive Duke's stage B2 and C colorectal cancer patients on 5-FU/leucovorin adjuvant chemotherapy between 1999 and 2008 | 130 | 2R/2R vs. 2R/3R + 3R/3R | 19 | 22 | 82 | 40 | 67 |
| Schwab et al. 2008 Multicenter prospective cohort study | Patients receiving 5-FU monotherapy with LV or levamisole; cancer types included colorectal, other GI, breast, or unknown primary | 683 | 2R/2R vs. 2R/3R + 3R/3R | 24 | 35 | 78 | 23 | 86 |
| Ichikawa et al. 2006 Prospective cohort study | Consecutive patients with histologically proven adenocarcinoma of the colon or rectum, treated with bolus 5-FU and LV adjuvant chemotherapy | 69 | 2R/2R vs. 2R/3R + 3R/3R | 4.6 (3 patients) | 18 | 98 | 67 | 85 |
| Largillier et al. 2006 Prospective cohort study | Consecutive advanced breast cancer patients receiving oral capecitabine alone | 105 | 2R/2R vs. 2R/3R + 3R/3R | 15 | 20 | 85 | 23 | 83 |
| Lecomte et al. 2004 Retrospective cohort study | Patients with colorectal cancer, stages II-IV, who received adjuvant or palliative 5-FU-based chemotherapy, with leucovorin +/- oxaliplatin or irinotecan | 87 | 2R/2R vs. 2R/3R + 3R/3R | 16 | 40 | 89 | 43 | 88 |
| Analysis of <i>TYMS</i> G>C SNP in Second Tandem Repeat | | | | | | | | |
| Gusella et al. 2009 | (As above) | 130 | 3RC/3RC vs. other | 55 | 56 | 45 | 35 | 66 |

Table 3. Summary of Published Studies Reporting Clinical Validity of *TYMS* Gene Variants for Severe 5-FU Toxicity

| Study | Patients | N | Genotype Comparison | Genotype Frequency (%) | Clinical Validity for All Grade 3–4 Toxicity | | | |
|---|--|-----|--|------------------------|--|-------------|-----|-----|
| | | | | | Sensitivity | Specificity | PPV | NPV |
| Analysis of <i>TYMS</i> Insertion/Deletion Variant in 3' UTR | | | | | | | | |
| Gusella et al. 2009 | (As above) | 130 | del/del or wt/del vs. wt/wt | 65 | 56 | 31 | 30 | 57 |
| Sharma et al. 2008 | Patients with locally advanced or metastatic colorectal cancer treated with capecitabine | 54 | del/del or wt/del vs. wt/wt | 66 | 66 | 36 | 54 | 47 |
| Multicenter prospective cohort study | | | | | | | | |
| Largillier et al. 2006 | (As above) | 105 | del/del or wt/del vs. wt/wt | 47 | 80 | 80 | 24 | 91 |
| Lecomte et al. 2004 | (As above) | 87 | del/del or wt/del vs. wt/wt | 59 | 47 | 38 | 14 | 77 |
| Zhu et al. 2004 | Patients with stage III/IV colorectal adenocarcinoma and no prior 5-FU treatment | 21 | del/del or wt/del vs. wt/wt | no information | No association with toxicity, $p=0.24$ | | | |
| Analysis of <i>TYMS</i> Variant Haplotypes | | | | | | | | |
| Gusella et al. 2009 | (As above) | 130 | low activity vs. high activity haplotypes ¹ | 26 | 24 | 73 | 32 | 65 |
| Sharma et al. 2008 | (As above) | 54 | low activity vs. high activity haplotypes ² | 43 | 45 | 58 | 57 | 47 |
| Largillier et al. 2006 | (As above) | 105 | low activity vs. high activity haplotypes ² | 55 | 40 | 41 | 13 | 76 |

¹Low activity: 2R/2R, 2R/3RC, 3RC/ 3RC with either 3'UTR ins/del or del/del; high activity: 2R/3RG, 3RC/3RG, 3RG/3RG with any 3'UTR genotype or low activity genotype plus 3'UTR ins/ins.

²Low activity: 2R/2R, 2R/3RC, 3RC/ 3RC; high activity: 2R/3RG, 3RC/3RG, 3RG/3RG.

result of alterations in DPD gene regulatory regions may be a patient such as the one reported by Ben Fredj et al. (2009), who experienced “excessive toxicity” for the first 2 treatment cycles and potentially lethal toxicity in the third cycle despite dose reduction, leading to cessation of treatment. This patient had no detectable genotypic variants to explain the toxicity, but did have a 5-FU half-life that was about 8 times longer than patients without genotypic variants or toxicity. The study by van Kuilenburg et al. (2008) observed highly significant differences in 5-FU half life between DPD-deficient patients and normal controls. Unfortunately, 5-FU half-life is a cumbersome measure, requiring several blood samples following a test 5-FU infusion.

What is the technical accuracy and reliability of the tests for *DPYD* and *TYMS* gene variants? (analytic validity)

There are no published studies that specifically address this question regarding commercially available assays. Of the example laboratories listed in Table 1, LabCorp and Specialty Laboratories list their method for detecting the most common *DPYD* gene variant as polymerase chain reaction (PCR), but provide no technical performance information on their website. Except for Myriad Genetics, the other laboratories, which also test for only the most common *DPYD* variant, provide no information on method or performance.

Myriad Genetics provides DPD and TS pharmacogenetic testing as a laboratory-developed service. A limited summary of technical specifications for their assays is provided on their website. For analyzing *DPYD* gene variants, the entire coding region is analyzed in at least 2 sequencing runs (includes 23 exons and approximately 690 noncoding adjacent base pairs for a total of about 3,768 base pairs). For detecting *TYMS* gene variants, the number of 28-base pair repeats within the 5' untranslated region is analyzed. False-positive results are estimated at less than 1% overall (including all aspects of the laboratory testing process) and none were seen in a set of 60 samples from unselected individuals. False-negative results are also estimated at less than 1% overall and none were seen in the same set of 60 samples. According to the technical specifications, “This test detects the majority of *DPYD* and *TYMS* variations leading to toxicity...This assay will not detect genomic rearrangements or some

types of errors in RNA transcript processing. The proportion of clinically significant defects in *DPYD* and *TYMS* attributable to undetected genomic rearrangements is unknown.” (Myriad Genetics, TheraGuide 5-FU™ Technical Specifications, available at <http://www.myriadtests.com/provider/doc/TheraGuide-5-FU-Technical-Specifications.pdf>)

It should be noted that while *DPYD* variant homozygotes are extremely rare (but the most important type of mutation to detect due to the certainty of serious toxicity), calculation of the accuracy of detection is based on the population prevalence of the variant allele (3–5%), not on the prevalence of the homozygote (0.1%). This is because the sequencing method provides the sequence of each *DPYD* allele the patient carries; 2 different sequences at the same locus of interest within the gene (e.g., a known inactivating mutation) indicate heterozygosity. Detecting the same mutation on both gene alleles (homozygosity, indicated by the absence of the wild-type sequence at that gene locus) is technically no different than detecting the mutation on just one gene allele. Similarly, methods that target specific mutations first amplify larger areas around regions of interest, then hybridize patient DNA with both mutant and wild-type-binding test sequences. Detection of both sequences indicates heterozygosity; detection of only mutant sequences indicates homozygosity for the specific mutation. Thus, at an allele prevalence of 3%, analytic sensitivity would range from 86 to 97% and analytic specificity holds at about 99% if false-positive and false-negative rates each vary from 0.1 to 0.5%.

Discussion

There are no published studies that prospectively tested whether using pharmacogenetic testing for *DPYD* or *TYMS* variants to identify patients at high risk for severe toxicity and to adjust their 5-FU starting dose improves toxicity outcomes without compromising tumor response compared to standard dosing and monitoring for toxicity reactions. In the absence of direct studies of clinical utility, reports of clinical validity were evaluated for data to estimate sensitivity, specificity, and predictive values of the genetic variants, individually or in combination.

Studies varied considerably in *DPYD* variants selected for testing; only 2 studies screened the entire coding and flanking regions of the gene.

Table 4. Example Toxicity Reactions of Patients with Homozygous or Biallelic *DPYD* Inactivating Variants

| Study | DPYD Variant(s) | Patient | Toxicity Reaction |
|-------------------------------------|--|---|--|
| Patients from Cohort Studies | | | |
| Morel et al. 2006 | *2A homozygous (results in no DPD activity) | Breast cancer; low-dose 5-FU adjuvant chemotherapy | Within a few days of the first 5-FU cycle, patient was hospitalized in ICU with grade 4 diarrhea, neutropenia, and mucositis, remaining there 15 d |
| Boisdron-Celle et al. 2007 | *2A, 2846A>T, both heterozygous | Colorectal cancer; 5-FU chemotherapy | Patient experienced grade 4 polyvisceral toxicity and died after 40 days in ICU; 5-FU plasma clearance was near 0 |
| Case Reports | | | |
| Kouwaki et al. 1998 | 62G>A, 1156 G>T on one allele, 1003G>T on the other allele; all heterozygous | Breast cancer; 5-FU chemotherapy | Patient presented with leukopenia, thrombocytopenia, and severe mucositis during 5-FU therapy; DPD activity was <5% of the normal control |
| Johnson et al. 1999 | *2A homozygous (results in no DPD activity) | Basal cell carcinoma (scalp lesions) treated with <u>topical</u> 5-FU | After 1 week of <u>topical</u> 5-FU treatment, the patient experienced severe stomatitis, an erythematous skin rash, and abdominal pain; he was neutropenic and thrombocytopenic. Colonoscopy revealed severe inflammatory colitis. Inflammatory changes were found in the esophagus, stomach, and small bowel. 5-FU treatment was stopped. Total parenteral nutrition was required for 3 weeks until full recovery. |

Table 5. Schwab et al. (2008): Effect of Various Factors on 5-FU Toxicity by Multivariable Logistic Regression Analysis

| Factor | Adjusted for Interaction | Comparison | OR | 95% CI | p value |
|--------|--------------------------|----------------|------|-----------|---------|
| DPYD | SEX = male | *2A:wt | 41.8 | 9.2–190 | <0.0001 |
| SEX | DPYD = *2A | male:female | 16.0 | 2.2–119 | 0.0067 |
| LV | | yes:no | 2.7 | 1.41–5.1 | 0.0028 |
| SEX | DPYD = wt | male:female | 0.5 | 0.45–0.71 | 0.0001 |
| TYMS | | 2/3+3/3:wt | 0.6 | 0.4–0.93 | 0.018 |
| ADMIN | | infusion:bolus | 0.5 | 0.33–0.65 | <0.0001 |
| MTHFR | | mt:wt | 0.7 | 0.50–0.96 | 0.029 |
| DPYD | SEX = female | 2A:wt | 1.3 | 0.34–5.2 | 0.68 |

Partly due to selection, but possibly also due to regional differences in the frequencies of particular variants (Amstutz et al. 2009), there were differing results as to which variants were most strongly associated with severe toxicity. Taken together, sensitivity and positive predictive value were poor while specificity and negative predictive value were much higher across studies. However, the majority of patients with severe toxicity did not have *DPYD* variants, which are uncommon. In addition, most patients with variant genotypes were heterozygous, and not all (50–60%) developed serious 5-FU toxicity (Schwab et al. 2008; Morel et al. 2006). Supporting these results in part is a series of 131 case reports of patients with severe 5-FU-related toxicity (Magne et al. 2007). Of these 13% had low DPD activity, and only 6% had severe DPD deficiency; 9 patients died from toxicity¹, among them 2 with normal DPD activity, but only 2 patients (2.2%) had the *DPYD**2A mutation (both heterozygous). Patients homozygous for inactivating mutations, who suffer from early, serious, and potentially lethal 5-FU toxicity and who should avoid 5-FU treatment entirely, were extremely rare in these studies.

Similarly, results for each of the 3 types of *TYMS* genetic variants considered individually were generally poor in terms of sensitivity, specificity, and predictive values. Three studies

that combined types of *TYMS* variants into haplotypes improved clinical validity descriptors somewhat, but not sufficiently for clinical use.

Data reported did not allow calculation of clinical validity descriptors for combined *DPYD* and *TYMS* genetic variants as predictors of toxicity. For those studies that evaluated both, authors primarily conducted regression analyses to establish their independent effect on toxicity. For example, Schwab et al. (2008) is considered one of the best studies to address pharmacogenetic testing for 5-FU toxicity due primarily to its large patient enrollment and to its focus on 5-FU monotherapy, avoiding confounding toxicity from other chemotherapy drugs.² This study addressed not only *DPYD* and *TYMS* genetic variants, but several other possible factors influencing toxicity in a multivariable regression analysis (Table 5). Results indicated the strongest influences were from an interaction between *DPYD* variants and sex (such that *DPYD**2A variants were significant only in male patients), and from leucovorin administration (yes/no); there were also significant influences from method of 5-FU administration, and from variants of the *TYMS* and *MTHFR* genes. From these results the authors constructed a nomogram for estimating 5-FU toxicity risk; however, use of this nomogram was not tested prospectively in the reported study.

¹Although tested in patients administered a 5-FU overdose rather than in DPD-deficient patients, an antidote to 5-FU overexposure is now available. As of May 1, 2009, vistonuridine was granted orphan drug status by the FDA as an antidote in the treatment of 5-FU “overexposure.” Under an FDA emergency-use Investigational New Drug provision, vistonuridine was administered to 17 patients within 8–96 hours after 5-FU overdose. All patients recovered, even though a fatal outcome was predicted for at least 15 based on the dose and rate of administration (von Borstel et al. 2009).

²This study has also been criticized as unrealistic, since most patients administered 5-FU are treated with combination therapy and drug interactions may need to be addressed (Ezzeldin and Diasio 2008).

The interaction between *DPYD* variants and sex was surprising and was not explained by DPD enzyme activity or protein content in the liver, nor by sex-specific promoter methylation. The possibility that the result was random and due to small numbers of gene variant carriers cannot be ignored.

Most commercial laboratories only offer screening for most common DPD gene variant, *DPYD**2A, which would result in false-negative results for patients with other rare DPD-inactivating mutations as well those with a normal sequence but who will nevertheless have severe toxicity for other reasons, although one commercial laboratory does offer scanning of the complete coding and flanking sequences. Few commercial laboratories seem to offer screening for *TYMS* variants, and of those found, detect only 1 of the 3 types of variants, similarly limiting the validity of the results. In terms of technical performance, limited data from one commercial laboratory suggest few if any false-positive or false-negative results.

Because the development of 5-FU toxicity is dependent on molecular events in several key metabolic pathways, a more comprehensive and integrated pharmacogenetic approach may be needed before chemotherapeutic toxicity can be reliably predicted in advance of current, largely combination treatment regimens (Ezzeldin and Diasio 2008). Considering DPD alone, *DYPD* genotype is incompletely related to DPD activity due to possible epigenetic and mRNA processing modifications; thus, developing more direct measures of DPD activity may improve toxicity prediction more than scanning for all possible gene variants. But DPD activity alone, as already noted, does not account for the majority of patients with 5-FU toxicity (Magne et al. 2007) and would need to be incorporated into a larger pharmacogenetic/functional pathway approach that is not yet available for 5-FU or its combination with other chemotherapeutic agents.

Conclusion

It has been tempting to postulate alterations in activity of key enzymes such as DPD and TS in the 5-FU metabolic pathway as the causal basis for 5-FU toxicity, and specific genetic variants of the genes coding for those enzymes as the starting points in the causal chain. Indeed, patients with homozygous, DPD-inactivating

mutations in the DPD gene uniformly experience severe, potentially fatal toxicity reactions when administered standard 5-FU doses. However, homozygosity for DPD-inactivating mutations was rarely reported in the studies included in this Assessment and heterozygous *DPYD* variants were observed in relatively small proportions of patients with grade 3/4 toxicity. Moreover, not all patients with *DPYD* variants experience toxicity (even when variants assessed are limited to those with prior associations with toxicity), making *DPYD* variants poor predictors. The clinical validity evidence for each of the 3 types of TS gene variants is similarly poor in terms of the ability of *TYMS* variants to predict which patients are likely to experience severe 5-FU toxicity.

In summary, testing for genetic variants of the DPD and TS genes has poor predictive value for 5-FU toxicity and no studies have shown that it is useful in directing 5-FU dose alterations to reduce toxicity without adversely affecting tumor response.

Summary of Application of the Technology Evaluation Criteria

Based on the available evidence, the Blue Cross and Blue Shield Association Medical Advisory Panel made the following judgments about whether the use of pharmacogenetic testing to predict serious toxicity from 5-FU for patients administered 5-FU-based chemotherapy for cancer meets the Blue Cross and Blue Shield Association's Technology Evaluation Center (TEC) criteria.

1. The technology must have final approval from the appropriate governmental regulatory bodies.

No assay kits have been cleared by the U.S. Food and Drug Administration (FDA) for genetic testing for *DPYD* or *TYMS* genotypes, nor are any kits being actively manufactured and marketed for distribution. Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; such tests must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). The laboratory offering the service must be licensed by CLIA for high-complexity testing. The FDA is currently evaluating how to incorporate laboratory-developed tests into its current regulatory program.

2. The scientific evidence must permit conclusions concerning the effect of the technology on health outcomes.

The evidence for this Assessment consists of cohort studies that address the clinical validity of DPD and TS pharmacogenetic testing. In general, both assays have 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. No studies address the clinical utility of reducing the initial 5-FU dose in patients with inactivating mutations and maximizing subsequent doses while avoiding toxicity. The evidence is insufficient to permit conclusions regarding the effect of DPD and TS pharmacogenetic testing on benefits (reduced toxicity) and potential harms (poorer response to treatment).

3. The technology must improve the net health outcome; and

4. The technology must be as beneficial as any established alternatives.

There is insufficient evidence to permit conclusions regarding the use of pharmacogenetic testing to predict serious toxicity from 5-FU for patients administered 5-FU-based chemotherapy for cancer.

5. The improvement must be attainable outside the investigational settings.

Whether or not the use of use of pharmacogenetic testing to predict serious toxicity from 5-FU for patients administered 5-FU-based chemotherapy for cancer improves health outcomes has not been demonstrated in the investigational setting.

For the above reasons, the use of pharmacogenetic testing to predict serious toxicity from 5-FU for patients administered 5-FU-based chemotherapy for cancer does not meet the TEC criteria.

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