

Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Dihydropyrimidine Dehydrogenase Genotype and Fluoropyrimidine Dosing: 2017 Update

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The purpose of this guideline is to provide information for the interpretation of clinical dihydropyrimidine dehydrogenase (*DPYD*) genotype tests so that the results can be used to guide dosing of fluoropyrimidines (5-fluorouracil and capecitabine). Detailed guidelines for the use of fluoropyrimidines, their clinical pharmacology,¹ as well as analyses of cost-effectiveness are beyond the scope of this document. The Clinical Pharmacogenetics Implementation Consortium (CPIC[®]) guidelines consider the situation of patients for which genotype data are already available² (updates available at <https://cpicpgx.org/guidelines/guideline-for-fluoropyrimidines-and-dpyd/>).

FOCUSED LITERATURE REVIEW

A systematic literature review focused on *DPYD* genotype and 5-fluorouracil, capecitabine, and tegafur was conducted (see **Supplement**), with reviews used as summaries of earlier literature.

GENE: *DPYD*

Background

DPYD, the gene encoding dihydropyrimidine dehydrogenase (DPD), the rate-limiting enzyme for fluoropyrimidine catabolism, spans 950 kb on chromosome 1p22 with 4,399 nucleotides in 23 coding exons.³ Numerous genetic variants in *DPYD* are

known that alter the protein sequence or mRNA splicing (see *DPYD* Allele Frequency Table available at [ref. 4]). Some of these variants, based on current knowledge, do not affect DPD activity in a clinically relevant manner (e.g., c.85T>C, *9A, rs1801265, p.C29R; c.1627A>G, *5, rs1801159, p.I543V; c.2194G>A, *6, rs1801160, p.V732I), whereas others result in reduced enzyme function. In the context of 5-fluorouracil, four decreased function *DPYD* variants are of primary relevance due to their population frequency and established impact on enzyme function and toxicity risk: c.1905+1G>A (rs3918290, also known as *DPYD**2A, *DPYD*:IVS14 + 1G>A), c.1679T>G (rs55886062, *DPYD* *13, p.I560S), c.2846A>T (rs67376798, p.D949V), and c.1129-5923C>G (rs75017182, HapB3). Of these variants, c.1905+1G>A and c.1679T>G have the most deleterious impact on DPD activity, whereas c.2846A>T and c.1129-5923C>G result in moderately reduced DPD activity (see further details below in *Linking genetic variability to variability in drug-related phenotypes*).

The most well-studied *DPYD* variant, c.1905+1G>A (*2A), is located at the intron boundary of exon 14 and results in skipping of the entire exon and a nonfunctional protein.⁵ The variant c.1129-5923C>G, located deep in intron 10, introduces a cryptic splice site and the partial production of a nonfunctional transcript.⁶ This single nucleotide polymorphism (SNP) is the likely

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Table 1 Assignment of likely DPD phenotypes based on *DPYD* genotypes

Likely phenotype	Activity score ^a	Genotypes ^b	Examples of genotypes ^c
<i>DPYD</i> normal metabolizer	2	An individual carrying two normal function alleles.	c.[=];[=], c.[85T>C];[=], c.[1627A>G];[=]
<i>DPYD</i> intermediate metabolizer	1 or 1.5	An individual carrying one normal function allele plus one no function allele or one decreased function allele, or an individual carrying two decreased function alleles.	c.[1905+1G>A];[=], c.[1679T>G];[=], c.[2846A>T];[=]; c.[1129-5923C>G];[=] ^d ; c.[1129-5923C>G];[1129-5923C>G] ^d ; c.[2846A>T];[2846A>T]
<i>DPYD</i> poor metabolizer	0 or 0.5	An individual carrying two no function alleles or an individual carrying one no function plus one decreased function allele.	c.[1905+1G>A];[1905+1G>A], c.[1679T>G];[1679T>G], c.[1905+1G>A];[2846A>T] c.[1905+1G>A]; [1129-5923C>G]

^aCalculated as the sum of the two lowest individual variant activity scores. See text for further information. ^bAllele definitions, assignment of allele function and references can be found on the CPIC website (*DPYD* Allele Functionality Table available at [ref 4]) ^cHGVS nomenclature using the reference sequence NM_000110.3 ^dLikely HapB3 causal variant. See *DPYD* Allele Functionality Table available at [ref 4] for other HapB3 proxy SNPs.

underlying causal variant of a *DPYD* haplotype (HapB3) spanning intron 5 to exon 11.⁷ The synonymous variant c.1236G>A (rs56038477) is in perfect linkage disequilibrium with c.1129-5923C>G ($r^2 = 1.0$, $D' = 1.0$ in 1000 Genomes Project), and thus a proxy for this variant in Europeans. The variants c.1679T>G and c.2846A>T are missense mutations that affect protein function.⁸

In Europeans, HapB3 with c.1129-5923C>G is the most common decreased function *DPYD* variant (see *DPYD* Allele Frequency Table available at [ref 4]) with carrier frequencies of 4.7%, followed by c.1905+1G>A (carrier frequency: 1.6%) and c.2846A>T (carrier frequency: 0.7%). Considering all four variants combined, ~7% of Europeans carry at least one decreased function *DPYD* variant. In individuals with African ancestry, the decreased function variant c.557A>G (rs115232898, p.Y186C) is relatively common (3–5% carrier frequency). Most other *DPYD* variants of phenotypic consequence are very rare (summarized in the *DPYD* Allele Frequency Table available at [ref 4]) and were not observed even in large cohort studies.^{9–11}

Nomenclature. While some *DPYD* variants have been assigned a star (*) allele, only a minority of known variants has such a designation. Furthermore, the (*) allele nomenclature is used for other drug-metabolizing enzymes to designate haplotypes consisting of more than one variant. Due to the size of *DPYD* and the low frequency of most variants, reliable haplotype inference across the entire gene is not possible. Therefore, the preferred nomenclature for *DPYD* variants is the use of Human Genome Variation Society (HGVS) nomenclature or rsID (see **Supplement** for further details).

Genetic test interpretation

Evidence supporting DPD function associated with known *DPYD* variants is summarized in the *DPYD* Allele Functionality Table available at [ref 4]. The relationship between *DPYD* genotype and phenotype has only been clearly established for a few variants, whereas the functional impact of many rare variants has been only assessed *in vitro*. Thus, the *DPYD* Allele Functionality Table available at [ref 4] was divided into sections according to the strength of evidence supporting the assigned allele function:

Strong evidence supporting function (from both *in vitro* and clinical studies); moderate evidence supporting function (from *in vitro* and clinical/ *ex vivo* studies); *in vitro* data only and/or limited clinical/ *ex vivo* data supporting function; uncertain function (conflicting or insufficient evidence supporting function, currently not considered actionable). For each variant, an activity score similar to that described in Ref. 12 was applied: 1 for normal function, 0.5 for decreased function, and 0 for no function variants (including variants with minimal DPD activity).

Table 1 summarizes the likely DPD phenotype based on genotype. The DPD phenotype is assigned using a gene activity score (*DPYD*-AS), calculated as the sum of the activity scores of the two *DPYD* variants with the lowest variant activity score (based on the *DPYD* Allele Functionality Table available at [ref 4]). Briefly, carriers of two no function variants are classified as *DPYD* poor metabolizers (*DPYD*-AS: 0); carriers of one no function or decreased function variant are considered *DPYD* intermediate metabolizers (*DPYD*-AS: 1 or 1.5), and those with only normal function variants are classified as *DPYD* normal metabolizers (*DPYD*-AS: 2). If two different decreased/no function variants are present, they are presumed to be on different gene copies. Irrespective of the presence of decreased/no function variants, patients may carry multiple normal function variants. Common normal function variants may be located on the same gene copy as other normal function variants or decreased/no function variants (see **Supplement** for further details). For example genotype to phenotype interpretations see the Genotype-Phenotype Table available at [ref 4].

To ensure correct test interpretation for the transversion variants c.1129-5923C>G and c.2846A>T, the strand to which alleles are assigned needs to be considered. In this guideline, allele designations are relative to the coding DNA reference sequence (NM_000110.3) and thus the decreased function (i.e., minor) alleles are c.1129-5923G and c.2846T, respectively.

Available genetic test options

Testing options for *DPYD* genotype range from targeted analysis of selected variants to resequencing of the complete coding

regions. In the context of 5-fluorouracil toxicity, at present most tests focus on the four most common and well-established risk variants (c.1905+1G>A, c.1679T>G, c.2846A>T, c.1129–5923C>G) or a subset thereof. Additional information about commercially available genetic testing options can be found at the Genetic Testing Registry website (<http://www.ncbi.nlm.nih.gov/gtr/>).

Incidental findings

Individuals who harbor one copy of a no function *DPYD* variant can be considered to have carrier status for an inborn error of metabolism and consideration should be given to its potential effects on offspring. Patients homozygous for inactivating variants of *DPYD* have complete dihydropyrimidine dehydrogenase deficiency, a clinically heterogeneous autosomal recessive disorder of pyrimidine metabolism that shows wide variability of clinical presentations, ranging from no symptoms to severe convulsive disorders with motor and mental retardation.^{13,14}

Other considerations

Some of the testing options for 5-fluorouracil toxicity also include testing for other gene variants in *TYMS* and *MTHFR*. To date, however, the clinical utility of these genotypes is unclear (see further details in **Supplement**), and predictive dosing strategies have yet to be successfully applied. For a summary of pharmacogenomic studies of 5-fluorouracil, see the PGx Research tab at <http://www.pharmgkb.org/drug/PA128406956>.

There are alternative or complementary tests to *DPYD* genotyping that assess DPD activity directly in peripheral mononuclear cells or indirectly through the endogenous dihydropyrimidin/uracil ratio (UH2/U) in plasma, or using a uracil loading test.¹⁵ See Ref. 16 for a review of these methods. The application of a combined genotype/phenotype approach including selected *DPYD* risk variants has been shown to reduce toxicity in a prospective study.¹⁷ However, such tests are not widely available. Furthermore, the mean and range of the pretherapeutic endogenous UH2/U ratio varied widely between studies, limiting its practical use, and several studies did not observe a strong correlation between the UH2/U ratio and 5-fluorouracil plasma concentrations.¹⁸

DRUGS: FLUOROPYRIMIDINES

Background

The fluoropyrimidines 5-fluorouracil and capecitabine are widely used in the treatment of solid tumors including colorectal and breast cancer, and cancers of the aerodigestive tract. Each year, over 2 million patients are newly diagnosed with tumors that are commonly treated with fluoropyrimidines, mostly in combination with other antineoplastic drugs.¹⁹ Approximately 10–40% of fluoropyrimidine-treated patients develop severe and sometimes life-threatening toxicity (neutropenia, nausea, vomiting, severe diarrhea, stomatitis, mucositis, hand-foot syndrome).^{7,11,20}

5-Fluorouracil has a narrow therapeutic window, resulting in a small difference between minimum efficacious and maximum tolerable dose. Only 1–3% of the administered 5-fluorouracil is metabolized to cytotoxic metabolites, with ~80% of the

administered dose being degraded and the rest excreted in the urine. DPD is the first and rate-limiting step in the catabolic pathway converting 5-fluorouracil to dihydrofluorouracil (DHFU) (for further details, see the 5-fluorouracil pathway at <http://www.pharmgkb.org/pathway/PA150653776>). DPD levels show high inter- and intraindividual variation, which influences 5-fluorouracil exposure.²¹ Reduced activity of DPD results in reduced clearance and increased half-life of 5-fluorouracil, and can cause profound dose-related toxicities.^{22,23} Capecitabine is a prodrug of 5-fluorouracil, being converted to 5-fluorouracil and also metabolized by DPD. Therefore, toxic effects are similar in patients with decreased/no function *DPYD* variants.^{9,24}

Linking genetic variability to variability in drug-related phenotypes

There is substantial evidence linking *DPYD* genotype with variability in DPD enzyme activity, 5-fluorouracil clearance, and 5-fluorouracil toxicity (summarized in **Supplemental Table S1**), which provides the basis for the dosing recommendations (**Table 2**).

In a meta-analysis combining data from eight cohort studies ($n = 7,365$ patients), the association of four *DPYD* variants with severe fluoropyrimidine-related toxicity was demonstrated²⁰: c.1905+1G>A (*2A), c.2846A>T, c.1679T>G (*13), and c.1129–5923C>G (HapB3) with relative risks for toxicity of 2.9 (95% confidence interval (CI): 1.8–4.6), 3.0 (2.2–4.1), 4.4 (2.1–9.3), and 1.6 (1.3–2.0), respectively. For all of these variants, an impact on DPD activity (assessed in PBMCs or using the UH2/U ratio) has been shown⁶ (**Supplemental Table S1**). The strongest impact on DPD activity was observed for c.1905+1G>A and c.1679T>G, with a 50% and 68% reduction in heterozygous carriers, respectively.⁶ A moderate reduction in DPD activity was observed in heterozygous carriers of c.2846A>T and c.1129–5923C>G (30% and 35% reduced activity, respectively).⁶ Two homozygous carriers of c.1129–5923C>G had 41% and 55% DPD activity compared to controls, consistent with a partial DPD deficiency.²⁵ Homozygous expression *in vitro* resulted in dramatically reduced DPD activity (<25% of wildtype activity) for c.1905+1G>A and c.1679T>G, and in reduced DPD activity (39–59% of wildtype activity) for c.2846A>T.^{26,27} In heterozygous carriers of c.1905+1G>A, c.2846A>T, and c.1679T>G, 5-fluorouracil clearance was reduced by 40–80% compared to noncarriers.^{23,28} For heterozygous carriers of c.557A>G (p.Y186C), commonly observed in individuals of African ancestry, a 46% reduction in PBMC DPD activity compared to noncarriers was observed.²⁹

Prescribing recommendations

Table 2 summarizes the genetics-based dosing recommendations for fluoropyrimidines using the calculated *DPYD* activity score (*DPYD*-AS). The strength of the prescribing recommendations is based on the known impact of some variants (c.1905+1G>A, c.1679T>G, c.2846A>T, c.1129–5923C>G) on DPD activity, the demonstrated relationship between DPD activity and 5-fluorouracil clearance, and between 5-fluorouracil exposure and its toxic effects. Patients who are heterozygous for *DPYD* decreased/no function variants demonstrate partial DPD

Table 2 Recommended dosing of fluoropyrimidines^a by DPD phenotype

Phenotype	Implications for phenotypic measures	Dosing recommendations	Classification of recommendations ^b
<i>DPYD</i> normal metabolizer	Normal DPD activity and “normal” risk for fluoropyrimidine toxicity.	Based on genotype, there is no indication to change dose or therapy. Use label-recommended dosage and administration.	Strong
<i>DPYD</i> intermediate metabolizer	Decreased DPD activity (leukocyte DPD activity at 30% to 70% that of the normal population) and increased risk for severe or even fatal drug toxicity when treated with fluoropyrimidine drugs.	Reduce starting dose based on activity score followed by titration of dose based on toxicity ^c or therapeutic drug monitoring (if available). Activity score 1: Reduce dose by 50% Activity score 1.5: Reduce dose by 25% to 50%	Activity score 1: Strong Activity score 1.5: Moderate
<i>DPYD</i> poor metabolizer	Complete DPD deficiency and increased risk for severe or even fatal drug toxicity when treated with fluoropyrimidine drugs.	Activity score 0.5: Avoid use of 5-fluorouracil or 5-fluorouracil prodrug-based regimens. In the event, based on clinical advice, alternative agents are not considered a suitable therapeutic option, 5-fluorouracil should be administered at a strongly reduced dose ^d with early therapeutic drug monitoring. ^e Activity score 0: Avoid use of 5-fluorouracil or 5-fluorouracil prodrug-based regimens.	Strong

^a5-fluorouracil or capecitabine. ^bRating scheme described in Supplement. ^cIncrease the dose in patients experiencing no or clinically tolerable toxicity in the first two cycles to maintain efficacy; decrease the dose in patients who do not tolerate the starting dose to minimize toxicities. ^dIf available, a phenotyping test (see main text for further details) should be considered to estimate the starting dose. In the absence of phenotyping data, a dose of <25% of the normal starting dose is estimated assuming additive effects of alleles on 5-FU clearance. ^eTherapeutic drug monitoring should be done at the earliest timepoint possible (e.g., minimum timepoint in steady state) in order to immediately discontinue therapy if the drug level is too high.

deficiency and should receive reduced starting doses. Prospective genotyping of c.1905+1G>A followed by a 50% dose reduction in heterozygous carriers resulted in a rate of severe toxicity comparable to noncarriers.³⁰ This study thus demonstrated that *DPYD* genetic testing can reduce the occurrence of severe fluoropyrimidine-related toxicity, and that a dose reduction of 50% is suitable for heterozygous carriers of no function variants (*DPYD*-AS: 1). For decreased function variants, evidence is limited regarding the optimal degree of dose reduction. For c.2846A>T, a small retrospective study observed that the average capecitabine dose in heterozygous carriers was reduced by 25% compared to noncarriers.²⁴ In a small prospective study, five patients carrying c.1236G>A (proxy for c.1129–5923C>G) were safely treated with a 25% reduced capecitabine starting dose.³¹ This suggests that heterozygous carriers of decreased function variants (*DPYD*-AS: 1.5) may tolerate higher doses compared to carriers of no function variants (*DPYD*-AS: 1). In patients with *DPYD*-AS of 1.5, the individual circumstances of a given patient should therefore be considered to determine if a more cautious approach (50% starting dose followed by dose titration), or an approach maximizing potential effectiveness with a potentially higher toxicity risk (25% dose reduction) is preferable. Of note, both studies indicating the suitability of a 25% dose reduction in decreased function variant carriers included only patients receiving capecitabine and no data are currently available for infusional 5-fluorouracil.

Given that some patients carrying decreased or no function variants tolerate normal doses of 5-fluorouracil, to maintain effectiveness, doses should be increased in subsequent cycles in patients experiencing no or clinically tolerable toxicity in the first two chemotherapy cycles or with subtherapeutic plasma

concentrations. Similarly, doses should be decreased in patients who do not tolerate the starting dose.

In *DPYD* poor metabolizers (*DPYD*-AS: 0.5 or 0), it is strongly recommended to avoid use of 5-fluorouracil-containing regimens. However, if no fluoropyrimidine-free regimens are considered a suitable therapeutic option, 5-fluorouracil administration at a strongly reduced dose combined with early therapeutic drug monitoring may be considered for patients with *DPYD*-AS of 0.5. It should be noted, however, that no reports of the successful administration of low-dose 5-fluorouracil in *DPYD* poor metabolizers are available to date. Assuming additive effects of decreased and no function alleles (*DPYD*-AS: 0.5), it is estimated that a dose reduction of at least 75% would be required (i.e., starting dose <25% of normal dose). Furthermore, in such cases a phenotyping test (see Gene: *DPYD*: Other Considerations) is advisable to estimate DPD activity and a starting dose.

The US Food and Drug Administration (FDA) and the Health Canada Santé Canada (HCSC) have added statements to the drug labels for 5-fluorouracil and capecitabine that warn against use in patients with DPD deficiency, and prescribing recommendations for 5-fluorouracil, capecitabine, and tegafur are also available from the Dutch Pharmacogenetics Working Group.³²

Tegafur. Tegafur (not available in the United States), is a pro-drug of 5-fluorouracil administered in combination with uracil (UFT) or with gimeracil and oteracil (S-1, Teysono). For these therapies, evidence regarding the impact of *DPYD* variants on toxicity risk is very limited. Given the inhibition of DPD by the coadministered uracil or gimeracil, dose requirements of patients carrying decreased/no function *DPYD* variants are currently

unknown. The dosing recommendations provided here currently apply only to 5-fluorouracil and capecitabine. As such, tegafur is rated as a CPIC “no recommendation” (see **Supplement** for definition).

Pediatrics. At the time of this writing, data on the possible role of *DPYD* genetic variation in 5-fluorouracil toxicity in pediatric patient populations are extremely scarce; however, there is no evidence to suggest that 5-fluorouracil pharmacokinetics differ from adult patients,³³ and thus no evidence that *DPYD* variants would affect 5-fluorouracil metabolism differently in children.

Recommendations for incidental findings

Symptoms of DPD deficiency generally present in childhood and, in the majority of patients, within the first year of life. Currently, a correlation between symptom severity and DPD function and/or genetics has not been established. However, early phenotypic (e.g., urine screening of uracil and its degradation products) and/or genetic testing (pre- or postnatal) of offspring of *DPYD* no function variant carriers could aid in early diagnosis¹⁴ to avoid a lengthy diagnostic odyssey.

Other considerations

Recently, a common polymorphism (rs895819A>G) in the *DPYD*-regulatory microRNA miR-27a was associated with lower DPD activity³⁴ and with fluoropyrimidine-related toxicity in patients carrying decreased function *DPYD* variants.^{35,36} This suggests that this *MIR27A* variant may allow further stratification of *DPYD* risk variant carriers. However, pharmacokinetic studies combining *DPYD* and *MIR27A* genotype are needed before dosing recommendations that incorporate *MIR27A* genotype can be made.

Other genetic variation and patient characteristics such as sex and age have also been associated with 5-fluorouracil toxicity; however, the clinical utility of these associations are not fully understood (see **Supplement** for more information). Disease and treatment regimens may influence the overall risk of toxicity, and thus also the absolute risk of toxicity in carriers of *DPYD* decreased/no function variants. However, the association of *DPYD* variants with 5-fluorouracil-related toxicity has been found to be fairly consistent across treatment regimens.^{9,20}

Pharmacokinetically guided dosing of 5-fluorouracil has been shown to result in an increase in the proportion of patients with 5-fluorouracil exposure (AUC) within the targeted therapeutic range and a reduced number of 5-fluorouracil-related adverse effects.^{37–39} In particular, to avoid underdosing of patients with genotype-based dose reductions who tolerate higher 5-fluorouracil doses, follow-up therapeutic drug monitoring is recommended.

Implementation of this guideline. The guideline supplement contains resources that can be used within electronic health records (EHRs) to assist clinicians in applying genetic information to patient care for the purpose of drug therapy optimization (see *Resources to incorporate pharmacogenetics into an electronic health record with clinical decision support* sections of the **Supplement**).

POTENTIAL BENEFITS AND RISKS FOR THE PATIENT

The benefit of *DPYD* genotyping has been demonstrated in a prospective study, which showed a reduced occurrence of severe 5-fluorouracil-related toxicity and no toxicity-related deaths in carriers of c.1905+1G>A after genotype-guided dose reduction.³⁰ Conversely, not all carriers of *DPYD* decreased/no function variants develop severe toxicity at standard doses.^{20,28} As a consequence, some carriers of such variants may not receive the full benefit of fluoropyrimidine therapy with the recommended dose reductions. To maintain efficacy, it is important to increase the dose in patients experiencing no or clinically tolerable toxicity or with subtherapeutic 5-fluorouracil plasma concentrations. Patients who proceed with 5-fluorouracil therapy may still experience acceptable lower-grade toxicity that may even be necessary in order to achieve efficacy. A possible risk is the misreporting or misinterpretation of genetic test results.

CAVEATS: APPROPRIATE USE AND/OR POTENTIAL MISUSE OF GENETIC TESTS

The presence of decreased or no function variants does not always result in toxicity. Overall, ~50% of decreased function *DPYD* variant carriers develop severe 5-fluorouracil-related toxicity with standard doses,^{20,28,40} with estimates varying depending on the overall frequency of toxicity for a given treatment regimen and the number of treatment cycles evaluated.^{7,11,28,40,41} At the same time, patients without a *DPYD* decreased/no function variant may still experience severe toxicity due to other genetic, environmental, or other factors.

The sensitivity of *DPYD* genetic testing depends on the number of variants investigated. By combining the *DPYD* variants c.1905+1G>A, c.2846A>T, c.1679T>G, c.1129–5923C>G, 20–30% of early-onset 5-fluorouracil toxicities can be explained.⁷ However, a test that includes only a subset of those *DPYD* variants (e.g., only c.1905+1G>A) has a reduced sensitivity. Finally, given the existence of many additional rare deleterious *DPYD* variants, a genetic test investigating only selected decreased/no function variants does not fully rule out DPD defects.

Additional Supporting Information may be found in the online version of this article.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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