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Fluorouracil Therapy and *DPYD* Genotype

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Introduction

Fluorouracil is a chemotherapy agent that belongs to the drug class of fluoropyrimidines. When given as an IV solution, fluorouracil is used in the palliative management of carcinoma of the colon, rectum, breast, stomach, and pancreas (1). When prescribed as a cream for topical use, fluorouracil is used to treat multiple actinic or solar keratoses of the face and scalp (2).

The *DPYD* gene encodes dihydropyrimidine dehydrogenase (DPD), an enzyme that catalyzes the rate-limiting step in fluorouracil metabolism. Individuals who carry at least one copy of no function *DPYD* variants, such as *DPYD*2A*, may not be able to metabolize fluorouracil at normal rates, and are at risk of potentially life-threatening fluorouracil toxicity, such as bone marrow suppression and neurotoxicity. The prevalence of DPD deficiency in Caucasians is approximately 3%-5%.

The FDA-approved drug label for fluorouracil states that "rarely, unexpected, severe toxicity (e.g., stomatitis, diarrhea, neutropenia and neurotoxicity) associated with 5-fluorouracil has been attributed to deficiency of dipyrimidine dehydrogenase activity" (1).

The Clinical Pharmacogenetics Implementation Consortium (CPIC) has published dosing recommendations for fluoropyrimidines (capecitabine, fluorouracil, and tegafur) based on *DPYD* genotype (3) (Table 1). CPIC recommends using an alternative drug for patients who are "poor metabolizers." These individuals carry two copies of no function *DPYD* variants and typically have complete DPD deficiency. CPIC also recommends considering a 50% reduction in starting dose for "intermediate metabolizers." These individuals carry a combination of a normal function and a no function variant and typically have reduced DPD activity (approximately 50% reduced) (3, 4).

Drug Class: Fluoropyrimidines

Fluoropyrimidines are a class of antimetabolite drugs that are widely used in the treatment of cancer. Currently, there are three types of fluoropyrimidines in clinical use: capecitabine, fluorouracil, and tegafur. Capecitabine and tegafur are both active precursors of fluorouracil.

Fluoropyrimidines are thought to exert their chemotherapeutic effects in a number of ways, through several active metabolites. The main mechanism of action is thought to be the inhibition of thymidylate synthase, which plays an important part in the folate-homocysteine cycle, and purine and pyrimidine synthesis pathways. Also, active metabolites can be incorporated into RNA and DNA, ultimately leading to cell death (5).

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Approximately 10-40% of patients develop severe and potentially life-threatening toxicity early during treatment with fluoropyrimidines (6). This toxicity typically leads to an interruption or discontinuation of potentially effective anticancer therapy, and often requires hospitalization (7).

The inter-individual variation in the occurrence and severity of adverse events in patients receiving fluoropyrimidines can be partly explained by clinical factors, such as age and sex. However, much of the variability in adverse events remains unexplained (8).

Of the genetic factors thought to contribute to fluoropyrimidine intolerance, the *DPYD* gene has been the most studied. This gene encodes the primary enzyme involved in breaking down fluoropyrimidines to inactive metabolites. Individuals who have a deficiency of the DPD enzyme have a significantly increased risk of suffering from severe fluoropyrimidine toxicity, and the stratification of patients on the basis of the *DPYD* genotype may help to prevent such adverse events (9-14).

The Clinical Pharmacogenetics Implementation Consortium (CPIC) has published genetics-based dosing recommendations for fluoropyrimidines based on *DPYD* genotype (Table 1).

Table 1. 2013 Recommended dosing of Fluoropyrimidines by DPD phenotype, from Clinical Pharmacogenetics Implementation Consortium (CPIC)

Phenotype	Implications for phenotypic measures	Dosing recommendations	Classification of recommendations[a]
Normal metabolizer	Normal DPD activity and "normal" risk for fluoropyrimidine toxicity	Use label-recommended dosage and administration	Moderate
Intermediate metabolizer	Decreased DPD activity (leukocyte DPD activity at 30–70% that of the normal population) and increased risk for severe or even fatal drug toxicity when treated with fluoropyrimidine drugs	Start with at least a 50% reduction in starting dose, followed by titration of dose based on toxicity[b] or pharmacokinetic test (if available)	Moderate
Poor metabolizer	Complete DPD deficiency and increased risk for severe or even fatal drug toxicity when treated with fluoropyrimidine drugs	Select alternative drug	Strong

Fluoropyrimidines: 5-fluorouracil, capecitabine, and tegafur.
DPD, dihydropyrimidine dehydrogenase.
[a] Rating scheme is described here (3)
[b] Increase the dose in patients experiencing no or clinically tolerable toxicity to maintain efficacy; decrease the dose in patients who do not tolerate the starting dose to minimize toxicities.
Table is adapted from Caudle KE, Thorn CF, Klein TE, Swen JJ, McLeod HL, Diasio RB, Schwab M. Clinical Pharmacogenetics Implementation Consortium guidelines for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing. Clinical pharmacology and therapeutics.2013:94(6):640-5 (3)
Note: The nomenclature used in this table reflects the standardized nomenclature for pharmacogenetic terms proposed by CPIC in a 2016 paper, "Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC)" (15).

Drug: Fluorouracil

Fluorouracil is a form of chemotherapy that when given as an IV solution, is used in the palliative management of carcinoma of the colon, rectum, breast, stomach, and pancreas. Fluorouracil may also be used topically as a cream, for the treatment of multiple actinic or solar keratoses of the face and anterior scalp.

Fluorouracil is structurally similar to pyrimidines, and the enzyme that catalyzes the rate-limiting step in the breakdown of pyrimidines (DPD, dihydropyrimidine dehydrogenase) also catalyzes the rate-limiting step in 5-

fluorouracil catabolism. DPD catalyzes the conversion of fluorouracil to the non-cytotoxic dihydrofluorouracil (DHFU) (16).

Fluorouracil is a highly toxic drug with a narrow margin of safety. The FDA-approved label contains the following boxed warning: "It is recommended that Fluorouracil Injection, USP be given only by or under the supervision of a qualified physician who is experienced in cancer chemotherapy and who is well versed in the use of potent antimetabolites. Because of the possibility of severe toxic reactions, it is recommended that patients be hospitalized at least during the initial course of therapy. These instructions should be thoroughly reviewed before administration of Fluorouracil Injection, USP."

The FDA also states that fluorouracil therapy should be discontinued promptly whenever one of the following signs of toxicity appears:

- Stomatitis or esophageal pharyngitis, at the first visible sign
- Leukopenia (WBC under 3500) or a rapidly falling white blood count
- Vomiting, intractable
- Diarrhea, frequent bowel movements, or watery stools
- Gastrointestinal ulceration and bleeding
- Thrombocytopenia (platelets under 100,000)
- Hemorrhage from any site

Symptomatic DPD deficiency is a rare autosomal recessive disorder with a wide range of symptoms, ranging from no symptoms or signs, to severe neurological problems. In affected individuals, the absent or greatly reduced DPD activity results in uracil and thymine accumulating in the blood, urine, and cerebrospinal fluid. Neurological symptoms typically manifest in early childhood and include seizures, small head size, and delayed cognitive and motor development (17).

Symptomatic DPD deficiency is typically caused by homozygous inactivation of *DPYD*; whereas individuals who are heterozygotes tend to be asymptomatic. However, all patients with less than 70% DPD activity are considered at risk for the development of severe drug toxicity when treated with fluoropyrimidines (18). Signs of fluorouracil toxicity include severe diarrhea, severe mucositis, neutropenia, neurotoxicity, and hand-foot syndrome (redness, swelling, and blisters on the palms of the hands an soles of the feet) (1).

Approximately 3-5% of Caucasians have partial DPD deficiency and 0.2% have complete DPD deficiency (19). Currently, most patients are not screened for DPD deficiency before starting capecitabine therapy (20).

Gene: *DPYD*

The *DPYD* gene encodes the enzyme dihydropyrimidine dehydrogenase (DPD), which catalyzes the first and the rate-limiting step in the breakdown of the pyrimidine nucleotides thymine and uracil. DPD also catalyzes the rate-limiting step in the breakdown of fluoropyrimidines.

Many *DPYD* variants have been described, although only a few have been demonstrated to influence DPD enzyme activity. *DPYD*1* is the wild-type allele and is associated with normal enzyme activity. Individuals who carry two copies of *DPYD* alleles with normal activity are known as "normal metabolizers" and have fully functional DPD enzyme activity (Table 2 and Table 3). Next to *DPYD*1*, the *DPYD* alleles **4*, **5*, **6*, and **9A* are also considered to have normal activity (21).

Table 2. Activity Status of Selected *DPYD* Alleles

Allele type	Alleles
Functional	**1, *4, *5, *6, *9A*

Table 2. continued from previous page.

Allele type	Alleles
No function	**2A*, **13*, rs67376798

Table is adapted from (13, 16) For the nomenclature of human DPYD alleles, please see (22)

The no function *DPYD* variants which have been associated with low DPD activity and an increased risk of toxicity with fluoropyrimidines include **2A*, **13*, and rs67376798 (16). The most well studied variant is *DPYD*2A*, in which a single nucleotide substitution at the invariant splice donor site of intron 14 leads to translation skipping exon 14, resulting in the production of a truncated protein with virtually no enzyme activity.

Individuals who carry combinations of normal function, decreased function, and/or no function *DPYD* alleles are known as "intermediate metabolizers." They have partial DPD deficiency and are at increased risk of capecitabine toxicity. And individuals who carry a combination of no function *DPYD* alleles and/or decreased function *DPYD* alleles are known as "poor metabolizers." They have complete DPD deficiency and are at an even higher risk of capecitabine toxicity. Overall, the prevalence of individuals who are heterozygous for no function variant *DPYD* alleles (partially DPD deficient) that place them at risk of severe drug reactions is estimated to be as high as 3-5%, but this varies in different populations (6, 23-27). For example, in the Dutch population, the *DPYD*2A* had an allele frequency of 0.91% in Caucasians (18).

Table 3 Assignment of likely phenotype based on *DPYD* genotypes

Likely phenotype	Functional definition	Genetic definition	Example diplotypes
Normal metabolizer	Fully functional DPD enzyme activity	Combinations of normal function and decreased function alleles	*DPYD*1/*1*
Intermediate metabolizer (~3–5% of patients)	Decreased DPD enzyme activity (activity between normal and poor metabolizer)	Combinations of normal function, decreased function, and/or no function alleles	**1/*2A*; **1/*13*; or *1/ rs67376798
Poor metabolizer (~0.2% of patients)	Little to no DPD enzyme activity	Combination of no function alleles and/ or decreased function alleles	*2A/*2A; 13/*13; **2/*13*; or rs67376798/ rs67376798

Table is adapted from Caudle KE, Thorn CF, Klein TE, Swen JJ, McLeod HL, Diasio RB, Schwab M. Clinical Pharmacogenetics Implementation Consortium guidelines for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing. Clinical pharmacology and therapeutics.2013:94(6):640-5 (3)
Note: The nomenclature used in this table reflects the standardized nomenclature for pharmacogenetic terms proposed by CPIC in the 2016 paper, "Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC)" (15).

A recent study proposed distinguishing between the various *DPYD* alleles and their functionality by assigning gene activity scores. The use of such scores could result in differentiated individualized dosing advice for fluororpyrimidines, which is essential for reducing toxic side effects while maintaining efficacy (13).

Genetic Testing

The NIH Genetic Testing Registry, GTR, displays genetic tests that are currently available for the *DPYD* gene and the fluorouracil drug response. The *DPYD*2A* variant is the most commonly tested.

Biochemical genetic tests may also be used, which assess the level of activity of the DPD enzyme. These tests include biochemical assays such as analyte testing (e.g., measuring the amount of thymine and uracil in the urine or blood) or an enzyme assay (e.g., directly measuring the activity of DPD using RNA extracted from blood cells and measuring the DPD mRNA copy number) (3, 28, 29).

GTR provides a list of biochemical tests that assess the levels of thymine and uracil analytes, and the activity of the enzyme dihydropyrimidine dehydrogenase.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2016 Statement from the US Food and Drug Administration (FDA): Rarely, unexpected, severe toxicity (e.g., stomatitis, diarrhea, neutropenia and neurotoxicity) associated with 5-fluorouracil has been attributed to deficiency of dipyrimidine dehydrogenase activity. A few patients have been rechallenged with 5-fluorouracil and despite 5-fluorouracil dose lowering, toxicity recurred and progressed with worse morbidity. Absence of this catabolic enzyme appears to result in prolonged clearance of 5-fluorouracil.

Please review the complete therapeutic recommendations that are located here: (1).

2013 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC): [...] Furthermore, patients who are heterozygous for the nonfunctional *DPYD* variants mostly demonstrate partial DPD deficiency (leukocyte DPD activity at 30–70% that of the normal population). Thus, our recommendation is to start with at least a 50% reduction of the starting dose; followed by an increase in dose in patients experiencing no or clinically tolerable toxicity, to maintain efficacy; and a decrease in dose in patients who do not tolerate the starting dose, to minimize toxicities. An alternative is pharmacokinetic-guided dose adjustment (if available). Patients who are homozygous for *DPYD*2A*, **13*, or rs67376798 may demonstrate complete DPD deficiency, and the use of 5-fluouracil or capecitabine is not recommended in these patients. Because capecitabine and tegafur are converted to 5-fluorouracil and then metabolized by DPD, the clearance of and exposure to 5-fluorouracil, in addition to its toxic effects, are similar in patients with these variants.

Please review the complete therapeutic recommendations that are located here: (3).

Nomenclature

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*DPYD*2A*	IVS14+1G>A c.1905+1G>A	NM_000110.3:c.1905+1G>A	Not applicable—deletion of exon 14 leads to the production of a truncated protein	rs3918290
*DPYD*13*	1679T>G Ile560Ser	NM_000110.3:c.1679T>G	NP_000101.2:p.Ile560Ser	rs55886062
rs67376798	2846A>T Asp949Val	NM_000110.3:c.2846A>T	NP_000101.2:p.Asp949Val	rs67376798

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

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[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

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