Supplemental Material

Clinical Pharmacogenetics Implementation Consortium Guidelines for Dihydropyrimidine Dehydrogenase Genotype and Fluoropyrimidine Dosing

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Literature Review
A literature search of the PubMed database (1966 to March 2013) using the keywords ((DPD OR DPYD OR Dihydropyrimidine Dehydrogenase) AND (fluorouracil OR 5-FU OR fluoropyrimidines OR capecitabine OR tegafur) AND genotype) was performed and results were limited to those available in English. Further articles were found via the reference sections of reviews.

Available Genetic Test Options
Commercially available genetic testing options change over time. An updated and fully linked table is available at http://www.pharmgkb.org/gene/PA145. Furthermore, the Genetic Testing Registry (GTR) provides a central location for voluntary submission of genetic test information by providers and is available at http://www.ncbi.nlm.nih.gov/gtr/conditions/C2720286/.

Genetic Test Interpretation
The haplotype, or star (*) allele name, is determined by a specific SNP or a combination of SNPs that are interrogated in the genotyping analysis. The genotypes that constitute the haplotype, or star (*) alleles for *DYPD*, and the rs# for each of the specific genomic nucleotide alterations that define the alleles, are described in Supplemental Table S1. However, at least one SNP, rs67376798, is associated with low activity but has not been given a * allele designation.

The genotype results are generally reported as a diplotype, which include one maternal and one paternal star allele (e.g. *1/*2A). The functional status associated with each of the common * alleles is summarized in Supplemental Table S2.

DPD function associated with the known *DPYD* allelic variants is summarized in Supplemental Table S2. The dosing recommendations in this guideline are specific for variant alleles in which there are clear data linking the *DPYD* genotype to fluoropyrimidine toxicity (*2A, *13, and rs67376798) (Supplemental Table S5). However, several other variants have been reported to be associated with reduced enzyme activity and/or linked to fluoropyrimidine toxicity, albeit with somewhat weaker evidence. These variants have been categorized as “probable reduced-function” or “unknown/unclear/contradictory” based on the lack of evidence linking these genotypes to fluoropyrimidine toxicity or conflicting evidence, respectively. All of the variants listed in the “probable reduced-function” category (*3, *8, *9B, *10, *11, and *12) are very rare (0-0.15% allele frequency in populations studied) and thus, reports describing DPD activity and the effects on fluoropyrimidine toxicity for these variants are rare. For example, *DPYD*7*, **8*, and **10* have only been reported in one study analyzing *DPYD* in 17 families (22 individuals) with complete dihydropyrimidine dehydrogenase deficiency (no detectable DPD activity in fibroblasts, <0.2% of controls).(1) To date, there are no studies linking these variant alleles (*7, *8, and *10) to toxicities related to fluoropyrimidines. Although the variants listed in the “unknown/unclear/contradictory” category have been observed in patients experiencing fluoropyrimidine toxicities, there is a lack of studies making a clear association between these variants and fluoropyrimidine

toxicity and/or decreased DPD activity. These alleles would therefore be informative in any *DPYD* testing.

Other considerations

Several other genes may influence responses to 5-fluorouracil (2, 3). The most well-studied of these are *ABCB1*, *MTHFR* and *TYMS*, although to date results have been inconsistent and predictive dosing strategies have yet to be successfully applied. Some of the testing options for 5-fluorouracil toxicity and *DPYD* also include testing for other gene variants in *TYMS* and *MTHFR*. Furthermore, Fernandez-Rozadilla *et al* performed a genome-wide association study on 221 colorectal cancer patients (including a validation set of 791 patients) that had been treated with a 5-fluorouracil-based regimen. Seven SNPs (rs16857540 (*NLGN1*), rs2465403 (*COLEC10*), rs10876844 (*OR10AE3P*, *PSMB2*P), rs10784749, rs17626122 (*PARD3B*), rs7325568 and rs4243761) showed evidence of association with adverse drug reactions. However, they also evaluated the association signals for seven SNP variants that had been linked to 5-fluorouracil-related toxicity in the literature (*DPYD*5* and **9A*, rs18010919 (*UMPS*), rs1801133 (*MTHFR*), rs34743033, rs34489327 (*TYMS*), rs1695 (*GSTP1*)). Four of these variants had good proxy SNPs in the study, although none of them showed a statistically significant association. These associations underscore the potential importance of other genes that may contribute increased risk of toxicity of 5-fluorouracil (4).

Level of Evidence

The evidence summarized in Supplemental Table S5 is graded using a scaled modified slightly from Valdes *et al* (5).

> High: Evidence includes consistent results from well-designed, well-conducted studies.
> Moderate: Evidence is sufficient to determine effects, but the strength of the evidence is limited by the number, quality, or consistency of the individual studies; generalizability to routine practice; or indirect nature of the evidence.
> Weak: Evidence is insufficient to assess the effects on health outcomes because of limited number or power of studies, important flaws in their design or conduct, gaps in the chain of evidence, or lack of information

Strength of Recommendation

CPIC's dosing recommendations (Table 2, main manuscript) are based weighting the evidence from a combination of preclinical functional and clinical data, as well as on some existing disease-specific consensus guidelines. Some of the factors that are taken into account include in vivo clinical outcome for reference drug, in vivo PK/PD for reference drug, and in vitro enzyme activity with probe substrate only.

Overall, the dosing recommendations are simplified to allow rapid interpretation by clinicians. We chose to use a slight modification of a transparent and simple system for just three categories for recommendations adopted from the rating scale for evidence-based recommendations on the use of retroviral agents

(http://aidsinfo.nih.gov/contentfiles/AdultandAdolescentGL.pdf): strong, where “the evidence is high quality and the desirable effects clearly outweigh the undesirable effects”; moderate, in which “there is a close or uncertain balance” as to whether the evidence is high quality and the desirable clearly outweigh the undesirable effects; and optional, in which the desirable effects are closely balanced with undesirable effects and there is room for differences in opinion as to the need for the recommended course of action.

Strong recommendation for the statement
Moderate recommendation for the statement
Optional recommendation for the statement

The strength of the 5-fluorouracil dosing recommendations (Table 2; main manuscript) is based on the fact that some variants (*DPYD*2A*, **13*, and rs67376798) clearly affect DPD activity, and DPD activity is clearly related to 5-fluorouracil clearance, and 5-fluorouracil exposure is associated with its toxic effects. Therefore, reduction of 5-fluorouracil dosage in patients with these variants may prevent severe and possibly life-threatening toxicities. The strength of the capecitabine and tegafur dosing recommendations is based on the fact that both of these drugs are prodrugs of 5-fluorouracil and metabolized by DPD in the same manner.

Supplemental Table S1. Genotypes[1] that constitute the * alleles for *DPYD*			
Allele	**Constituted by genotypes at:**	**Position on NM_000110.3**	**Amino acid change**
*1			
*2A	rs3918290 C>T	c.1905+1G>A	n/a
*2B	rs3918290 C>T and rs1801159 T>C	c.1905+1G>A and c.1627A>G	n/a and Ile543Val
*3	rs72549303 C>del	c.1898delC	Pro633fx
*4	rs1801158 C>T	c.1601G>A	Ser534Asn
*5	rs1801159 T>C	c.1627A>G	Ile543Val
*6	rs1801160 C>T	c.2194G>A	Val732Ile
*7	rs72549309 TCAT>del	c.302delTinsTCAT	Ile101fx
*8	rs1801266 G>A	c.703C>T	Arg235Trp
*9A	rs1801265 A>G	c.85T>C	Cys29Arg
*9B	rs1801265 A>G and rs1801267 C>T	c.85T>C and c.2657G>A	Cys29Arg and Arg886His
*10	rs1801268 C>A	c.2983G>T	Val995Phe
*11	rs72549306 C>A	c.1003G>T	Val335Leu
*12	rs80081766 C>T and rs78060119 C>A	c.62G>A and c.1156G>T	Arg21Gln and Glu386Ter
*13[§]	rs55886062 A>C	c.1679T>G	Ile560Ser
None[¶]	rs67376798 T>A	c.2846A>T	Asp949Val

1: **Bases reported on the positive chromosomal strand from Golden Path**. See www.pharmgkb.org for updates on *DPYD* gene alleles and nomenclature

Nomenclature from *1-10 from McLeod et al (6), *11 and *12 from Mattison et al, (7), *13 from Johnson et al, (8).

Supplemental Table S2. Association between allelic variants and DPD function		
Functional Status	**Alleles**	**Phenotype**
Functional / normal activity/ wild-type[1]	*1	
Non-functional, variant, or mutant / no activity	*2A	Associated with toxicity in most (3, 9-12), but not all (13, 14). Observed in patients with low DPD activity (11, 14-21) and DPD deficiency (1). Observed in patients with severe or fatal toxicity (15, 22-27). Associated with reduced 5-fluorouracil clearance (12, 17, 21) and inactive catalytic activity (28).
	*13	Associated with toxicity (12). Observed in patients with low DPD activity (8, 29). Observed in patients with severe or fatal toxicity (20, 27). Associated with reduced 5-fluorouracil clearance (12).
	rs67376798	Observed in individuals with low DPD activity (20, 30). Associated with toxicity (3, 10-12, 23, 27). Associated with reduced 5-fluorouracil clearance (12, 21).
Probable Reduced-function / decreased activity (these alleles are mostly very rare and so reports have been rare)	*3	Observed in individuals with DPD deficiency (1, 31).
	*7	Observed in individuals with DPD deficiency (1).
	*8	Observed in individuals with low DPD activity (1).
	*9B	Observed in individuals with low DPD activity (31, 32).
	*10	Observed in individuals with DPD deficiency (1).
	*11	Observed in individuals with low DPD activity (33).
	*12	Observed in individuals with low DPD activity (33).

Unknown/unclear/contradictory evidence	*4†	Observed in individuals with low DPD activity (29, 34), but not in another study (35). Associated with toxicity (27, 36, 37).
	*5†	Associated with toxicity in some (38) but not other studies (39). Not associated with low DPD activity (35). Not associated with DPD protein levels (40).
	*6†	Associated with toxicity (10, 41). Not associated with low DPD activity (1)
	*9A†	Associated with reduced DPD activity (31) but not associated with reduced DPD in other study (29). Associated with toxicity (42)
1: an important caveat for all genotyping tests is that the decision to assign an allele a "wild-type" status is based upon a genotyping test that interrogates only the most common and already-proven sites of functional variation. In human DNA, it is always possible that a new, previously undiscovered (and therefore un-interrogated) site of variation may confer loss-of-function in an individual, and thus lead to the rare possibility of a non-functional allele being erroneously called as "wild-type."		

†Designated as fully functional by the Dutch Pharmacogenetics Working Group based on the lack of an association to toxicity reported in studies and/or decreased clearance or activity (43).

Supplemental Table S3. Frequencies[1] of alleles in major race[2] groups				
Allele	Caucasian	Asian	African-American or Black	Middle Eastern
*2A	0.00862	0.0015	0	0
*3	0	0	0	0
*4	0.0194	0.001	0.00237	0.0293
*5	0.147	0.268	0.177	0.119
*6	0.0412	0.015	0.0451	0.092
*7	0.00122	0	n/a	n/a
*9A	0.182	0.0315	0.137	n/a
*11	n/a	0.0015	n/a	n/a
*12	0	0	n/a	n/a
*13	0.001	0	n/a	n/a
IVS10-15T>C	n/a	0.018	0.042	n/a
rs75017182	0.0155	n/a	n/a	n/a
rs67376798	0.0111	n/a	n/a	n/a

[1]Average frequencies are reported based on the average from the actual numbers of subjects with each allele reported in multiple studies. See Supplemental Table S4 for details and references.

[2]Race/ethnic group designations correspond to those indicated in Supplemental Table S4

Supplemental Table S4. *DPYD* minor allele frequency															
Pooled grouping	**Ethnicity**	***DPYD* minor allele frequency (%)**													**Total Alleles**
		***2A**	***3**	***4**	***5**	***6**	***7**	***9A**	***11**	***12**	***13**	**IVS10-15T>C**	**rs75017182**	**rs67376798**	
Caucasian	Finnish (15)	2.2	-	-	-	-	-	-	-	-	-	-	-	-	90
Caucasian	Finnish (34)	-	-	3.3	7.2	6.7	-	-	-	-	-	-	-	-	180
Caucasian	Scottish (44)	0	0	0.8	28	5.8	-	-	-	-	-	-	-	-	120
Caucasian	Dutch (45)	0.91	-	-	-	-	-	-	-	-	-	-	-	-	2714
Caucasian	German (22)	0.5	-	-	-	-	-	-	-	-	-	-	-	-	1702
Caucasian	German (30)	-	-	1.6	14	2.0	0.3	19	-	-	-	-	-	0.6	314
Caucasian	French (21)	0.6	-	-	-	-	0	17.7	-	0	-	-	-	-	504
Caucasian	French (12)	1.1	-	-	-	-	-	-	-	0	0.1	-	-	1.0	974
Caucasian	Swedish (46)	1.6	-	-	-	-	-	-	-	-	-	-	-	-	1284
Caucasian	Bosnian (47)	2.0	-	-	-	-	-	-	-	-	-	-	-	-	100
Caucasian	Dutch (48)	-	-	-	-	-	-	-	-	-	-	-	1.3	-	382
Caucasian	German (48)	-	-	-	-	-	-	-	-	-	-	-	1.7	-	906
Caucasian	Turkish (49)	0	-	-	-	-	-	-	-	-	-	-	-	-	500

Supplemental Table S4. *DPYD* minor allele frequency															
Pooled grouping	**Ethnicity**	***DPYD* minor allele frequency (%)**													**Total Alleles**
		***2A**	***3**	***4**	***5**	***6**	***7**	***9A**	***11**	***12**	***13**	**IVS10-15T>C**	**rs75017182**	**rs67376798**	
Caucasian	Turkish (50)	0.46	-	-	-	-	-	-	-	-	-	-	-	-	436
African	Afro-American (15)	0	-	-	-	-	-	-	-	-	-	-	-	-	40
African	Afro-American (34)	-	-	0.5	22.7	1.9	-	-	-	-	-	-	-	-	210
African	Tunisian (51)	0	0	0	12.7	7.1	-	13.7	-	-	-	4.2	-	-	212
Asian	Japanese (15)	0	-	-	-	-	-	-	-	-	-	-	-	-	70
Asian	Taiwanese (15)	2.7	-	-	-	-	-	-	-	-	-	-	-	-	72
Asian	Taiwanese (34)	-	-	0	21	1.4	-	-	-	-	-	-	-	-	262
Asian	Japanese (34)	-	-	1.1	35.2	4.4	-	-	-	-	-	-	-	-	100
Asian	Taiwanese (40)	-	0	28.3	1.2	-	-	2.2	-	-	-	-	-	-	600
Asian	Japanese (52)	-	-	-	-	-	-	2.9	-	-	-	-	-	-	2692

Supplemental Table S4. *DPYD* minor allele frequency															
Pooled grouping	**Ethnicity**	***DPYD* minor allele frequency (%)**													**Total Alleles**
		***2A**	***3**	***4**	***5**	***6**	***7**	***9A**	***11**	***12**	***13**	**IVS10-15T>C**	**rs75017182**	**rs67376798**	
Asian	Japanese (53)	0	-	-	28.3	1.5	0	2.9	0.15	0	0	1.8	-	-	682
Asian	Chinese (38)	0	0	0	30	-	-	4.7	-	0	-	-	-	-	150
Asian	Chinese (54)	0	0	0	20.8	0.7	-	7.0	-	-	0	-	-	-	284
Middle Eastern	Egyptian (55))	0	0	2.9	11.9	9.2	-	-	-	-	-	-	-	-	478

Supplemental Table S5. Evidence linking *DPYD* genotype with DPD phenotype and dihydropyrimidine toxicity			
Type of experimental model (in vitro, in vivo preclinical, or clinical)	**Major findings**	**References**	**Level of evidence[a,b]**
In vitro	*DPYD*2A* was expressed in mammalian cells and the DPD enzymatic activity was determined relative to wild-type. The *DPYD*2A* was found to be catalytically inactive.	Offer et al. (2013) (28)	High
Clinical	Severe neurotoxicity due to 5-fluorouracil and complete DPD deficiency in a patient with familial pyrimidinemia was observed. A markedly prolonged 5-fluorouracil elimination half-life was observed after administration of a test dose of 5-fluorouracil in this patient.	Diasio et al. (1988) (56)	Moderate
Clinical	In a study of patients with severe 5-fluorouracil-related toxicity and low DPD activity in peripheral blood mononuclear cells isolated from patients (n=14), six were heterozygous for the ***DPYD*2A*** variant and one heterozygous for the **rs67376798** variant.	van Kuilenburg *et al*. (2000) (16)	Moderate
Clinical	In a study of cancer patients with reduced (n=23) or normal (n=14) DPD activity, 1 patient with reduced activity and 5-fluororuracil toxicity was heterozygous for ***DPYD*13*** and one patient with normal activity was heterozygous for ***DPYD*2A***.	Collie-Duguid *et al*. (2000) (29)	Moderate
Clinical	In a study of patients with severe 5-FU-related toxicity (n=25), 5 were heterozygous and 1 was homozygous for the ***DPYD*2A*** variant. Lethal outcome was seen in the homozygous and two of the heterozygous cases.	Raida *et al*. (2001)(22)	Moderate
Clinical	Severe toxicity observed in a single patient with colorectal cancer treated with 5-fluorouracil heterozygous for the ***DPYD*2A*.** Further analysis revealed low DPD activity in peripheral blood mononuclear cells and reduced clearance in this patient as compared to control patients with normal 5-fluorouracil symptoms.	Maring *et al*. (2002)(17)	Moderate

Clinical	Severe toxicity and profound DPD deficiency (in peripheral blood mononuclear cells) in a breast cancer patient heterozygous for ***DPYD*2A*** and ***DPYD*13*** treated with a 5-fluorouracil-based regimen.	Johnson *et al*. (2002) (8)	Moderate
Clinical	In a study of cancer patients (n=60) suffering from severe grade 3-4 toxicity after the administration of 5-fluorouracil, 17 patients were heterozygous and 1 patient was homozygous for ***DPYD*2A*** variant (28%). All patients but one with the ***DPYD*2A*** variant had reduced DPD activity in their peripheral blood mononuclear cells.	van Kuilenburg *et al*. (2002)(18)	Moderate
Clinical	In a study of cancer patients (n=25) suffering from severe grade 4 toxicity after the administration of 5-fluorouracil, 9 patients were heterozygous and 1 patient was homozygous for ***DPYD*2A*** variant and decreased DPD activity could be detected in peripheral blood mononuclear cells.	Van Kuilenburg *et al*. (2002) (19)	Moderate
Clinical	In a study of Portuguese cancer patients receiving fluorouracil-based chemotherapy (n=73), 8 had grade 3-4 toxicity, one of which was heterozygous for the ***DPYD*2A*** variant.	Salgueiro *et al*. (2004) (57)	Moderate
Clinical	In a large study of colorectal cancer patients (n=252), where ten patients were heterozygous for either the ***DPYD*2A*** variant (n=3) or the ***DPYD*** **rs67376798** (n=8) variant, seven had toxicity (grade 3-4), one of which was fatal (heterozygous for both variants). Two patients had grade I toxicity and the dose was immediately reduced. Only one patient with the **rs67376798** had no toxic side effects. ***DPYD*2A*** and **rs67376798** resulted in statistically significant reduced clearance of 5-fluorouracil and lower DPD activity (as determined by dihydrouracil/uracil ratio) as compared to patients with wild-type *DPYD*.	Boisdron-Cellier *et al*. (2007) (21)	High
Clinical	In a large study of cancer patients treated with various fluorouracil-based regimens (n=487) 60% of patients with either the ***DPYD*2A*** (n=6 of 10) and **rs67376798** (n=6 of 10) variants experienced grade 3 or 4 toxicity. Four of those with no toxicity were receiving reduced doses. One patient with ***DPYD*13*** was identified and experienced grade 3 or 4 toxicity. Patients with these variants had significantly reduced 5-fluorouracil plasma clearance.	Morel *et al*. (2006) (12)	High

Clinical	Fatal toxicity in a single patient heterozygous for the ***DPYD*2A*** variant with metastatic pancreatic adenocarcinoma treated with bolus fluorouracil.	Saif *et al*. (2007) (24)	Moderate
Clinical	In a retrospective study of case reports of patients with various cancers treated with fluorouracil-based regimens who experienced severe toxicity (n=93), two heterozygotes for ***DPYD*2A*** were found. Both patients had low DPD activity in their peripheral blood mononuclear cells.	Magne *et al*. (2007) (14)	Weak
Clinical	In a study of colorectal cancer patients treated with fluorouracil regimens (n=76), nine patients had reduced clearance and increased toxicity, one of whom was heterozygous for the ***DPYD*2A*** variant and two of whom were heterozygous for the **rs67376798** variant.	Capitain *et al*. (2008) (11)	Moderate
Clinical	In a large prospective study of fluorouracil monotherapy for various cancers (n=683), ***DPYD*2A*** was associated with increased risk of mucositis and leukopenia. Six out of thirteen patients with the ***DPYD*2A*** variant and 3 out of the 5 patients with the **rs67376798** had grade 3-4 toxicity.	Schwab *et al*. (2008) (3)	High
Clinical	In a large study of Polish colorectal cancer patients treated with fluorouracil (n=252), four patients had grade 3-4 neutropenia one of whom was heterozygous for ***DPYD*2A***.	Sulzyc-Bielicka *et al*. (2008) (58)	Moderate
Clinical	In a study of patients with various cancers treated with either fluorouracil or capecitabine (n=181), five patients with ***DPYD*2*** variant and one patient with **rs67376798** variant all experienced grade 3-4 toxicity.	Gross *et al*. (2008)(59)	Moderate
Clinical	In a study of colorectal cancer patients treated with 5-fluorouracil- or capecitabine-based regimens (n=76 grade 3-4 toxicity; n=48 tolerant), ***DPYD*2A*** was associated with increased risk of mucositis. Five out of five patients with ***DPYD*2A*** variant had grade 3-4 toxicity (no patients with this variant were detected in the tolerant group).	Kleibl *et al*. (2009) (41)	Moderate
Clinical	In a study of patients with various cancers treated with fluorouracil-based regimens (n=111), the ***DPYD*2A*** variant was not significantly associated with increased risk of toxicity however only a single heterozygote was observed; this patient experienced grade 0-2 toxicity. One patient with the **DPYD*13** variant experienced grade 0-2 toxicity.	Amstutz *et al*. (2009) (13)	Weak

Clinical	In a large prospective clinical trial of various fluorouracil-based regimens for colorectal cancer (n=750), the ***DPYD*2A*** was not significantly associated with increased risk of toxicity. Seven patients were heterozygous for ***DPYD*2A*** of whom 4 experienced grade 3-4 toxicity.	Braun *et al*. (2009) (42)	Moderate
Clinical	In a study of patients with various cancers treated with a fluorouracil-based regimen and experiencing grade 3 or 4 toxicities (n=47), 4 patients were heterozygous for the ***DPYD*2A*** variant, 4 were heterozygous for the **rs67376798** variant and one was heterozygous for the ***DPYD*13*** variant accounting for 19% of all toxicity cases.	Loganayagam *et al*. (2010) (20)	Moderate
Clinical	In a large study of metastatic colorectal cancer patients treated with fluorouracil regimens (n=349), only two heterozygotes for ***DPYD*2A*** were observed, both of whom had grade 4 neutropenia.	Boige *et al*. (2010) (60)	Moderate
Clinical	In a study of cancer patients receiving capecitabine or fluorouracil (n=50), one patient was heterozygous for ***DPYD*2A*** and experienced grade 4 toxicity which was fatal.	Ceric *et al*. (2010) (47)	Moderate
Clinical	In a study of patients with gastrointestinal cancers who experienced severe fluorouracil toxicity (n=45), two patients were heterozygous for the ***DPYD*2A*** variant.	Savva-Bordalo *et al*, (2010) (26)	Moderate
Clinical	In a study of patients with colorectal cancer treated with fluorouracil or capecitabine regimens (n=68), ***DPYD*2A*** was associated with increased risk of toxicity. Two ***DPYD*2A*** heterozygotes were observed in this cohort. One **rs67376798** heterozygote was observed but did not experience toxicity.	Kristensen *et al*. (2010) (9)	Moderate
Clinical	In a study of French breast cancer patients receiving capecitabine (n=105), a single patient who was heterozygous for the ***DPYD*2A*** variant had fatal toxicity.	Largillier *et al*. (2006) (61)	Moderate
Clinical	In a study of patients with colorectal cancer treated with capecitabine regimens (n=568), ***DPYD*2A*** and **rs67376798** were associated with increased risk of severe toxicity. Seven out of seven patients heterozygous for ***DPYD*2A*** and seven out of eight patients heterozygous for ***DPYD*** **rs67376798** had grade 3-4 toxicity.	Deenen *et al*, (2011) (10)	High

Clinical	In a study of patients with rectal cancer receiving tegafur (n=63), a single patient who was heterozygous for variant ***DPYD*2A*** experienced very early grade 4 neutropenia and a patient who was heterozygous for variant **rs67376798** experienced grade four diarrhea.	Cellier *et al*, (2011) (25)	Moderate
Clinical	In a study of cancer patients receiving 5-fluorouracil, patients who were heterozygous for variant *DPYD*2A* (n=3) were treated with a dose reduction of 50% and still experienced severe toxicities that resulted in hospitalization of patient and premature discontinuation of treatment.	Magnani *et al*, (2013) (62)	High

[a]Rating Scheme for Quality of Evidence as per (5)
[b]Some of the small case series, although not strong individually, collectively do support a strong recommendation.

Supplemental Table S6. Summary of the effects of *DPYD* variants on 5-fluorouracil clearance

Reference	***DPYD*2A***	**rs67376798**	***DPYD*13***	**Wild-type or no *DPYD* variant noted**
Deenen *et al*, (2011) (10)	Mean dose intensity after 6 cycles = 56% (n=5)	Mean dose intensity after 6 cycles = 76% (n=5)	No included	Mean dose intensity after 6 cycles =90% (n=410)
Morel *et al*. (2006) (12)	5-FU clearance NCI grade 0-2 tox = 72.11 L h^{-1} m^{-2} (n=4) 5-FU clearance for pts with NCI grade 3-4 = 54.32 L h^{-1} m^{-2} (n=6)	5-FU clearance for pts with NCI grade 0-2 tox = 89.68 L h^{-1} m^{-2} (n=4) 5-FU clearance for pts with NCI grade 3-4 = 60.76 L h^{-1} m^{-2} (n=6)	5-FU clearance for pts with NCI grade 3-4 = 41.06 L h^{-1} m^{-2} (n=1)	5-FU clearance for pts with NCI grade 0-2 tox = 136.33 L h^{-1} m^{-2} (n=264) 5-FU clearance for pts with NCI grade 3-4 = 78.67 L h^{-1} m^{-2} (n=19)
Boisdron-Cellier *et al*. (2007) (21)	5-FU clearance FuFol 21.22 L h^{-1} m^{-2} (n=2) UH2/U mean (n=2) 5.16±0.07 μg/L	5-FU clearance FuFol 43.9 L h^{-1} m^{-2} (n=7) UH2/U mean (n=7) 4.4±1.6 μg/L	Not included	5-FU clearance FuFol 104.7 L h^{-1} m^{-2} (n=163) UH2/U mean (n=163) 8.1±2.5 μg/L

5-FU, 5-fluorouracil; FuFol, Weekly 5-FU 1200 mg/m^2, 4 h infusion plus 200 mg/m^2 folinic acid

References

(1) Van Kuilenburg, A.B. *et al.* Genotype and phenotype in patients with dihydropyrimidine dehydrogenase deficiency. *Human genetics* **104**, 1-9 (1999).

(2) Thorn, C.F., Marsh, S., Carrillo, M.W., McLeod, H.L., Klein, T.E. & Altman, R.B. PharmGKB summary: fluoropyrimidine pathways. *Pharmacogenet Genomics* **21**, 237-42 (2011).

(3) Schwab, M. *et al.* Role of genetic and nongenetic factors for fluorouracil treatment-related severe toxicity: a prospective clinical trial by the German 5-FU Toxicity Study Group. *J Clin Oncol* **26**, 2131-8 (2008).

(4) Fernandez-Rozadilla, C. *et al.* Pharmacogenomics in colorectal cancer: a genome-wide association study to predict toxicity after 5-fluorouracil or FOLFOX administration. *The pharmacogenomics journal* **13**, 209-17 (2013).

(5) Valdes, R., Payne, D.A. & Linder, M.W. Laboratory analysis and application of pharmacogenetics to clinical practice. (Washington, DC, NACB, 2010).

(6) McLeod, H.L. *et al.* Nomenclature for human DPYD alleles. *Pharmacogenetics* **8**, 455-9 (1998).

(7) Mattison, L.K., Johnson, M.R. & Diasio, R.B. A comparative analysis of translated dihydropyrimidine dehydrogenase cDNA; conservation of functional domains and relevance to genetic polymorphisms. *Pharmacogenetics* **12**, 133-44 (2002).

(8) Johnson, M.R., Wang, K. & Diasio, R.B. Profound dihydropyrimidine dehydrogenase deficiency resulting from a novel compound heterozygote genotype. *Clin Cancer Res* **8**, 768-74 (2002).

(9) Kristensen, M.H., Pedersen, P.L., Melsen, G.V., Ellehauge, J. & Mejer, J. Variants in the dihydropyrimidine dehydrogenase, methylenetetrahydrofolate reductase and thymidylate synthase genes predict early toxicity of 5-fluorouracil in colorectal cancer patients. *The Journal of international medical research* **38**, 870-83 (2010).

(10) Deenen, M.J. *et al.* Relationship between single nucleotide polymorphisms and haplotypes in DPYD and toxicity and efficacy of capecitabine in advanced colorectal cancer. *Clin Cancer Res* **17**, 3455-68 (2011).

(11) Capitain, O., Boisdron-Celle, M., Poirier, A.L., Abadie-Lacourtoisie, S., Morel, A. & Gamelin, E. The influence of fluorouracil outcome parameters on tolerance and efficacy in patients with advanced colorectal cancer. *The pharmacogenomics journal* **8**, 256-67 (2008).

(12) Morel, A. *et al.* Clinical relevance of different dihydropyrimidine dehydrogenase gene single nucleotide polymorphisms on 5-fluorouracil tolerance. *Molecular cancer therapeutics* **5**, 2895-904 (2006).

(13) Amstutz, U., Farese, S., Aebi, S. & Largiader, C.R. Dihydropyrimidine dehydrogenase gene variation and severe 5-fluorouracil toxicity: a haplotype assessment. *Pharmacogenomics* **10**, 931-44 (2009).

(14) Magne, N. *et al.* Dihydropyrimidine dehydrogenase activity and the IVS14+1G>A mutation in patients developing 5FU-related toxicity. *Br J Clin Pharmacol* **64**, 237-40 (2007).
(15) Wei, X., McLeod, H.L., McMurrough, J., Gonzalez, F.J. & Fernandez-Salguero, P. Molecular basis of the human dihydropyrimidine dehydrogenase deficiency and 5-fluorouracil toxicity. *The Journal of clinical investigation* **98**, 610-5 (1996).
(16) van Kuilenburg, A.B. *et al.* Clinical implications of dihydropyrimidine dehydrogenase (DPD) deficiency in patients with severe 5-fluorouracil-associated toxicity: identification of new mutations in the DPD gene. *Clin Cancer Res* **6**, 4705-12 (2000).
(17) Maring, J.G. *et al.* Reduced 5-FU clearance in a patient with low DPD activity due to heterozygosity for a mutant allele of the DPYD gene. *Br J Cancer* **86**, 1028-33 (2002).
(18) Van Kuilenburg, A.B., Meinsma, R., Zoetekouw, L. & Van Gennip, A.H. High prevalence of the IVS14 + 1G>A mutation in the dihydropyrimidine dehydrogenase gene of patients with severe 5-fluorouracil-associated toxicity. *Pharmacogenetics* **12**, 555-8 (2002).
(19) Van Kuilenburg, A.B., Meinsma, R., Zoetekouw, L. & Van Gennip, A.H. Increased risk of grade IV neutropenia after administration of 5-fluorouracil due to a dihydropyrimidine dehydrogenase deficiency: high prevalence of the IVS14+1g>a mutation. *Int J Cancer* **101**, 253-8 (2002).
(20) Loganayagam, A., Arenas-Hernandez, M., Fairbanks, L., Ross, P., Sanderson, J.D. & Marinaki, A.M. The contribution of deleterious DPYD gene sequence variants to fluoropyrimidine toxicity in British cancer patients. *Cancer chemotherapy and pharmacology* **65**, 403-6 (2010).
(21) Boisdron-Celle, M. *et al.* 5-Fluorouracil-related severe toxicity: a comparison of different methods for the pretherapeutic detection of dihydropyrimidine dehydrogenase deficiency. *Cancer letters* **249**, 271-82 (2007).
(22) Raida, M. *et al.* Prevalence of a common point mutation in the dihydropyrimidine dehydrogenase (DPD) gene within the 5'-splice donor site of intron 14 in patients with severe 5-fluorouracil (5-FU)- related toxicity compared with controls. *Clinical cancer research : an official journal of the American Association for Cancer Research* **7**, 2832-9 (2001).
(23) Salgado, J., Zabalegui, N., Gil, C., Monreal, I., Rodriguez, J. & Garcia-Foncillas, J. Polymorphisms in the thymidylate synthase and dihydropyrimidine dehydrogenase genes predict response and toxicity to capecitabine-raltitrexed in colorectal cancer. *Oncol Rep* **17**, 325-8 (2007).
(24) Saif, M.W., Ezzeldin, H., Vance, K., Sellers, S. & Diasio, R.B. DPYD*2A mutation: the most common mutation associated with DPD deficiency. *Cancer chemotherapy and pharmacology* **60**, 503-7 (2007).
(25) Cellier, P. *et al.* Phase II study of preoperative radiation plus concurrent daily tegafur-uracil (UFT) with leucovorin for locally advanced rectal cancer. *BMC Cancer* **11**, 98 (2011).
(26) Savva-Bordalo, J. *et al.* Promoter methylation and large intragenic rearrangements of DPYD are not implicated in severe toxicity to 5-

fluorouracil-based chemotherapy in gastrointestinal cancer patients. *BMC cancer* **10**, 470 (2010).
(27) Loganayagam, A. *et al.* Pharmacogenetic variants in the DPYD, TYMS, CDA and MTHFR genes are clinically significant predictors of fluoropyrimidine toxicity. *Br J Cancer* **108**, 2505-15 (2013).
(28) Offer, S.M., Wegner, N.J., Fossum, C., Wang, K. & Diasio, R.B. Phenotypic Profiling of DPYD Variations Relevant to 5-Fluorouracil Sensitivity Using Real-time Cellular Analysis and In Vitro Measurement of Enzyme Activity. *Cancer Res* **73**, 1958-68 (2013).
(29) Collie-Duguid, E.S., Etienne, M.C., Milano, G. & McLeod, H.L. Known variant DPYD alleles do not explain DPD deficiency in cancer patients. *Pharmacogenetics* **10**, 217-23 (2000).
(30) Seck, K. *et al.* Analysis of the DPYD gene implicated in 5-fluorouracil catabolism in a cohort of Caucasian individuals. *Clin Cancer Res* **11**, 5886-92 (2005).
(31) Vreken, P., Van Kuilenburg, A.B., Meinsma, R. & van Gennip, A.H. Dihydropyrimidine dehydrogenase (DPD) deficiency: identification and expression of missense mutations C29R, R886H and R235W. *Human genetics* **101**, 333-8 (1997).
(32) Vreken, P., Van Kuilenburg, A.B., Meinsma, R. & van Gennip, A.H. Identification of novel point mutations in the dihydropyrimidine dehydrogenase gene. *J Inherit Metab Dis* **20**, 335-8 (1997).
(33) Kouwaki, M. *et al.* Identification of novel mutations in the dihydropyrimidine dehydrogenase gene in a Japanese patient with 5-fluorouracil toxicity. *Clin Cancer Res* **4**, 2999-3004 (1998).
(34) Wei, X. *et al.* Characterization of the human dihydropyrimidine dehydrogenase gene. *Genomics* **51**, 391-400 (1998).
(35) Ridge, S.A. *et al.* Dihydropyrimidine dehydrogenase pharmacogenetics in patients with colorectal cancer. *Br J Cancer* **77**, 497-500 (1998).
(36) Gross, E. *et al.* Detailed analysis of five mutations in dihydropyrimidine dehydrogenase detected in cancer patients with 5-fluorouracil-related side effects. *Human mutation* **22**, 498 (2003).
(37) Lazar, A., Mau-Holzmann, U.A., Kolb, H., Reichenmiller, H.E., Riess, O. & Schomig, E. Multiple organ failure due to 5-fluorouracil chemotherapy in a patient with a rare dihydropyrimidine dehydrogenase gene variant. *Onkologie* **27**, 559-62 (2004).
(38) Zhang, H., Li, Y.M. & Jin, X. DPYD*5 gene mutation contributes to the reduced DPYD enzyme activity and chemotherapeutic toxicity of 5-FU: results from genotyping study on 75 gastric carcinoma and colon carcinoma patients. *Med Oncol* **24**, 251-8 (2007).
(39) Cho, H.J., Park, Y.S., Kang, W.K., Kim, J.W. & Lee, S.Y. Thymidylate synthase (TYMS) and dihydropyrimidine dehydrogenase (DPYD) polymorphisms in the Korean population for prediction of 5-fluorouracil-associated toxicity. *Ther Drug Monit* **29**, 190-6 (2007).

(40) Hsiao, H.H. *et al.* Dihydropyrimidine dehydrogenase pharmacogenetics in the Taiwanese population. *Cancer chemotherapy and pharmacology* **53**, 445-51 (2004).
(41) Kleibl, Z. *et al.* Influence of dihydropyrimidine dehydrogenase gene (DPYD) coding sequence variants on the development of fluoropyrimidine-related toxicity in patients with high-grade toxicity and patients with excellent tolerance of fluoropyrimidine-based chemotherapy. *Neoplasma* **56**, 303-16 (2009).
(42) Braun, M.S. *et al.* Association of molecular markers with toxicity outcomes in a randomized trial of chemotherapy for advanced colorectal cancer: the FOCUS trial. *J Clin Oncol* **27**, 5519-28 (2009).
(43) Swen, J.J. *et al.* Pharmacogenetics: from bench to byte--an update of guidelines. *Clin Pharmacol Ther* **89**, 662-73 (2011).
(44) Ridge, S.A. *et al.* Dihydropyrimidine dehydrogenase pharmacogenetics in Caucasian subjects. *Br J Clin Pharmacol* **46**, 151-6 (1998).
(45) van Kuilenburg, A.B. *et al.* Lethal outcome of a patient with a complete dihydropyrimidine dehydrogenase (DPD) deficiency after administration of 5-fluorouracil: frequency of the common IVS14+1G>A mutation causing DPD deficiency. *Clin Cancer Res* **7**, 1149-53 (2001).
(46) Ofverholm, A., Arkblad, E., Skrtic, S., Albertsson, P., Shubbar, E. & Enerback, C. Two cases of 5-fluorouracil toxicity linked with gene variants in the DPYD gene. *Clin Biochem* **43**, 331-4 (2010).
(47) Ceric, T. *et al.* Investigation of IVS14 + 1G > A polymorphism of DPYD gene in a group of Bosnian patients treated with 5-Fluorouracil and capecitabine. *Bosnian journal of basic medical sciences / Udruzenje basicnih mediciniskih znanosti = Association of Basic Medical Sciences* **10**, 133-9 (2010).
(48) van Kuilenburg, A.B. *et al.* Intragenic deletions and a deep intronic mutation affecting pre-mRNA splicing in the dihydropyrimidine dehydrogenase gene as novel mechanisms causing 5-fluorouracil toxicity. *Human genetics* **128**, 529-38 (2010).
(49) Suzen, H.S., Yuce, N., Guvenc, G., Duydu, Y. & Erke, T. TYMS and DPYD polymorphisms in a Turkish population. *Eur J Clin Pharmacol* **61**, 881-5 (2005).
(50) Uzunkoy, A., Dilmec, F., Ozgonul, A., van Kuilenburg, A.B. & Akkafa, F. Investigation of IVS14+ 1G > A polymorphism of DPYD gene in a group of Turkish patients with colorectal cancer. *Anticancer research* **27**, 3899-902 (2007).
(51) Ben Fredj, R. *et al.* Mutational spectrum of dihydropyrimidine dehydrogenase gene (DPYD) in the Tunisian population. *C R Biol* **330**, 764-9 (2007).
(52) Tanaka, D. *et al.* Polymorphism of dihydropyrimidine dehydrogenase (DPYD) Cys29Arg and risk of six malignancies in Japanese. *Nagoya journal of medical science* **67**, 117-24 (2005).
(53) Maekawa, K. *et al.* Genetic variations and haplotype structures of the DPYD gene encoding dihydropyrimidine dehydrogenase in Japanese and their ethnic differences. *J Hum Genet* **52**, 804-19 (2007).

(54) He, Y.F. *et al.* Analysis of the DPYD gene implicated in 5-fluorouracil catabolism in Chinese cancer patients. *J Clin Pharm Ther* **33**, 307-14 (2008).
(55) Hamdy, S.I. *et al.* Allele and genotype frequencies of polymorphic cytochromes P450 (CYP2C9, CYP2C19, CYP2E1) and dihydropyrimidine dehydrogenase (DPYD) in the Egyptian population. *Br J Clin Pharmacol* **53**, 596-603 (2002).
(56) Diasio, R.B., Beavers, T.L. & Carpenter, J.T. Familial deficiency of dihydropyrimidine dehydrogenase. Biochemical basis for familial pyrimidinemia and severe 5-fluorouracil-induced toxicity. *J Clin Invest* **81**, 47-51 (1988).
(57) Salgueiro, N. *et al.* Mutations in exon 14 of dihydropyrimidine dehydrogenase and 5-Fluorouracil toxicity in Portuguese colorectal cancer patients. *Genetics in medicine : official journal of the American College of Medical Genetics* **6**, 102-7 (2004).
(58) Sulzyc-Bielicka, V. *et al.* 5-Fluorouracil toxicity-attributable IVS14 + 1G > A mutation of the dihydropyrimidine dehydrogenase gene in Polish colorectal cancer patients. *Pharmacological reports : PR* **60**, 238-42 (2008).
(59) Gross, E. *et al.* Strong association of a common dihydropyrimidine dehydrogenase gene polymorphism with fluoropyrimidine-related toxicity in cancer patients. *PloS one* **3**, e4003 (2008).
(60) Boige, V. *et al.* Pharmacogenetic assessment of toxicity and outcome in patients with metastatic colorectal cancer treated with LV5FU2, FOLFOX, and FOLFIRI: FFCD 2000-05. *J Clin Oncol* **28**, 2556-64 (2010).
(61) Largillier, R. *et al.* Pharmacogenetics of capecitabine in advanced breast cancer patients. *Clin Cancer Res* **12**, 5496-502 (2006).
(62) Magnani, E. *et al.* Fluoropyrimidine toxicity in patients with dihydropyrimidine dehydrogenase splice site variant: the need for further revision of dose and schedule. *Internal and emergency medicine* **8**, 417-23 (2013).