Dihydropyrimidine dehydrogenase (DPD) testing and fluorouracil toxicity

Status: September 2020

Diagnostic recommendation

Method	Anticoagulant	Recommendation	Consent according to GenDG
Molecular genetics	EDTA	mandatory	mandatory

Dihydropyrimidine dehydrogenase and fluorouracil toxicity

Fluorouracil (FU)-containing drugs are very frequently used cytostatic drugs in systemic tumor therapy. Serious and life-threatening side effects can occur in 10 - 40% of patients, and the therapy-associated lethality is 0.2 - 1.0% (Hoff et al. 2001, van Cutsem et al. 2001). A major cause of severe FU toxicity is the genetic deficiency of dihydropyrimidine dehydrogenase (DPD), the enzyme mainly responsible for FU degradation. The DPD deficiency is caused by variants in the dihydropyrimidine dehydrogenase (*DPYD*) gene, which are associated with an increased risk of severe, specific side effects in carriers (Meulendijks et al. 2015). In total, up to 9% of the population carries a *DPYD* gene variant that leads to reduced enzyme activity, and 0.1% to 0.5% show complete DPD deficiency (**EMA Recommendations 2020**; Amstutz et al. 2018). Therefore, pharmacogenetic testing for the most common and clinically significant *DPYD* gene variants is recommended prior to systemic therapy with FU-containing drugs (Lunenburg et al. 2020, **DGHO Position Paper 2020**).

About 30% of severe FU toxicity reactions can be explained by a genetic DPD deficiency, but there are also numerous other factors that influence the risk of severe side effects of FU-containing therapies (Amstutz et al. 2018, Froehlich et al. 2015, Schwab et al. 2008). The DGHO therefore recommends that patients with increased toxicity under FU-containing therapy not caused by the *DPYD* genotype should also be evaluated for other causes and, in the case of 5-FU, therapeutic drug monitoring should be performed if necessary (Wilhelm et al. 2016, **DGHO Position Paper 2020**).

Pharmacogenetic diagnostics of DPYD gene variants

Molecular genetics

Molecular genetic testing for *DPYD* variants is a diagnostic test within the meaning of § 3 No. 7 c of the German Genetic Diagnostics Act (GenDG), which requires medical education and patient consent (GEKO Guideline 2017). Therefore, the analysis can only be carried out when the declaration of consent according to GenDG signed by the patient or his legal representative is available in the laboratory.

To clarify a genetic deficiency of the FU-degrading enzyme DPD, the four most common and clinically significant variants of the *DPYD* gene are tested for which a clear effect on DPD enzyme function has been described and for which testing is recommended according to the position paper of the German Society for Hematology and Medical Oncology (DGHO) before therapy with an FU-containing drug (Table 1) (Henricks et al. 2017, Lunenburg et al. 2020, **DGHO Position Paper 2020**).

Table 1: Overview of the investigated DPYD variants (modified according to DGHO Position Paper 2020, Amstutz et al. 2018 and Meulendijks et al. 2015)

Designation	Investigated variant [1]	RefSNP ID [2]	Enzyme activity	Allele-frequency [3]	Toxicity [4]
*DPYD**2A	c.1905+1G>A Exon 14 Skipping	rs3918290	none (0)	0.006	2.9 (1.8-4.6)
*DPYD**13	c.1679T>G (p.Ile560Ser)	rs55886062	none (0)	0.001	4.4 (2.1-9.3)
	c.2846A>T (p.Asp949Val)	rs67376798	reduced (0.5)	0.007	3.0 (2.2-4.1)
Haplotype B3	c.1236G>A	rs56038477	reduced (0.5)	0.022	1.6 (1.3-2.0)

[1] related to *DPYD* transcript 1 (NM_000110.4)
[2] referring to SNP (Single Nucleotide Polymorphism) database
[3] for Caucasians
[4] Relative risk for severe toxicity under FU-containing therapy, confidence interval in brackets according to Meulendijks et al. 2015

- The *DPYD* variant c.1905+1G>A - also called *DPYD**2A - leads to a splicing defect with skipping of exon 14 of the *DPYD* gene and consequently to a truncated protein with loss of DPD enzyme activity (van Kuilenburg et al. 1997).
- The *DPYD* variant c.1679T>G (p.Ile560Ser) - also known as *DPYD**13 - leads to an amino acid exchange and is associated with loss of DPD enzyme activity (van Kuilenburg et al. 2002).
- The *DPYD* variant c.2846A>T (p.Asp949Val) leads to an amino acid exchange and is associated with a decreased DPD enzyme activity (van Kuilenburg et al. 2002).
- The *DPYD* variant c.1236G>A is in complete linkage disequilibrium with the c.1129-5923C>G variant, with which it jointly defines the haplotype B3. Haplotype B3 is associated with alternative *DPYD* splicing and reduced DPD enzyme activity (Froehlich et al. 2015, Nie et al. 2017).

Molecular genetic testing for *DPYD* variants in MLL is performed by means of "Loop-mediated isothermal amplification (LAMP)" and subsequent melting curve analysis. The average processing time is 2 days. A distinction is made as to whether the tested variant is heterozygous (on one of the two alleles) or homozygous (on both alleles) or whether it is not present and thus a homozygous expression of the wild-type allele (most common form with normal enzyme activity) exists. The absence of all variants in *DPYD* gene is also called *DPYD**1. However, very rare functionally relevant variants outside the investigated regions of the *DPYD* gene cannot be excluded by the study (Amstutz et al. 2018). However, their clinical relevance has not been conclusively clarified at present, so that currently no investigation of the complete coding sequence of *DPYD* is recommended (Lunenburg et al. 2020, **DGHO Position Paper 2020**).

Complete DPD deficiency (homozygosity or combined heterozygosity of *DPYD* variants with lack of enzyme function) has also been associated with the variable clinical picture of hereditary thymine uraciluria or familial pyrimidinemia, although the genotype-phenotype relationship has not been clearly established (**OMIM #274270**, Fernandez-Salguero et al. 1997, van Gennip et al. 1997, van Kuilenburg et al. 1999).

Prediction of DPD enzyme activity based on the DPYD genotype

The prediction of the DPD phenotype on the basis of the *DPYD* genotype is performed according to the DGHO guidelines, taking into account guidelines of the Clinical Pharmacogenetics Implementation Consortium (CPIC; Amstutz et al. 2018) and the Dutch Pharmacogenetics Working Group (DPWG; Lunenburg et al. 2020). Essentially, a sum score of the two weakest variant activities is formed. A score of 2 corresponds to a normal DPD enzyme activity and a score of 0 to a complete DPD deficiency (Table 2).

Table 2: Prediction of the DPD phenotype based on the two weakest variant activities (modified according to DGHO Position Paper 2020)

***DPYD*-Genotype**	**Activity score**
No carrier of a *DPYD* variant with reduced or lost function (*1/*1) – normal enzyme activity	2
Heterozygous carrier of a *DPYD* variant with reduced function (*1/c.1236G>A or *1/c.2846A>T)	1.5
Heterozygous carrier of a *DPYD* variant with loss of funtion (*1/*2A or *1/*13)	1
Carrier of two *DPYD* variants with reduced function (e. g. c.1236G>A and c.2846A>T)	0.5 [1,2]
Carrier of a *DPYD* variant with reduced function and a variant with loss of funtion (combination of c.1236G>A or c.2846A>T with *2A or *13)	0.5 [1]
Homozygous carrier of a *DPYD* variant with loss of funtion (*2A/*2A; *13/*13) or heterozygous carrier of two *DPYD* variants with loss of funtion (*2A/*13)	0

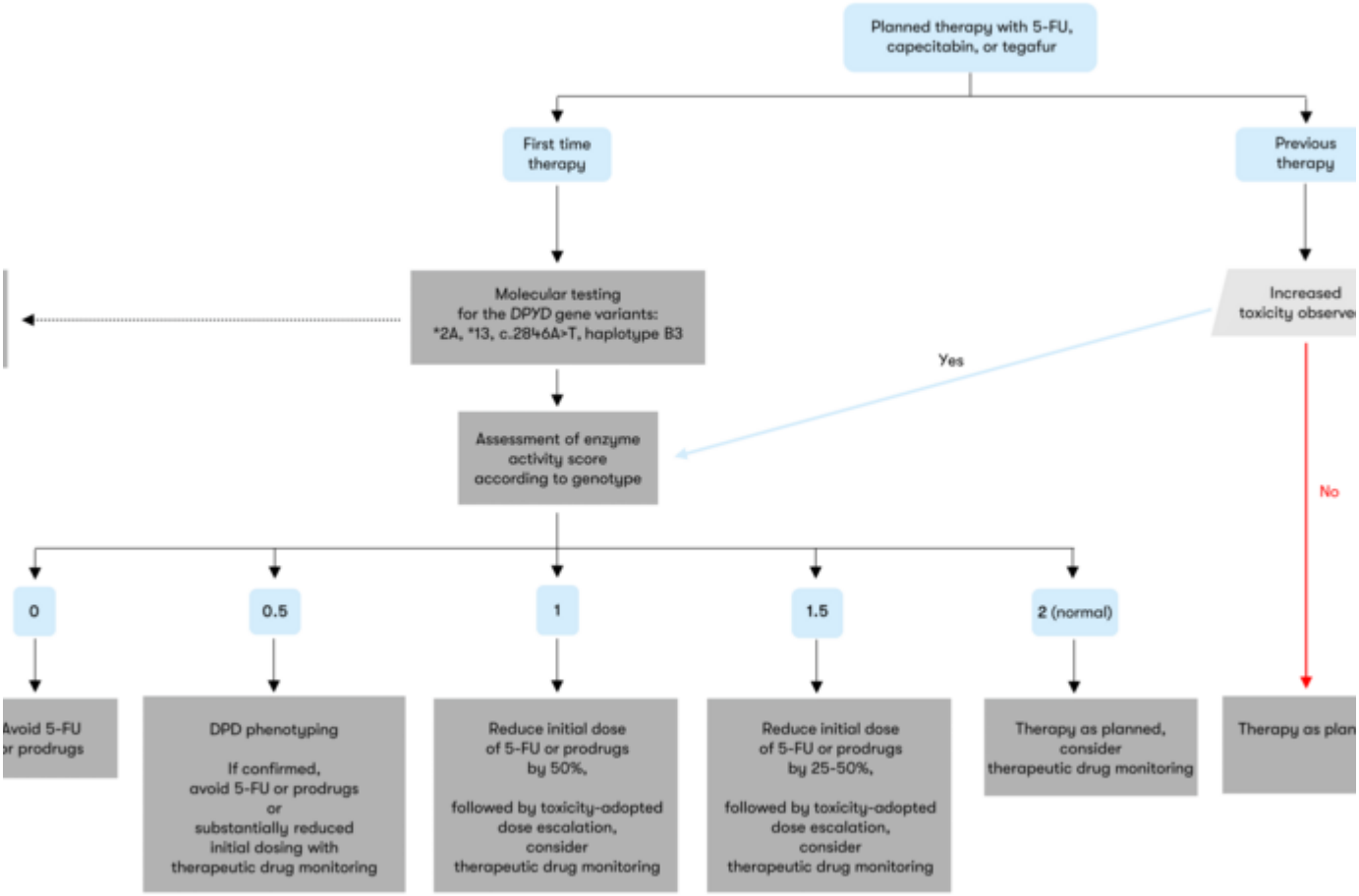
[1] Additional phenotyping is recommended for the reliable determination of enzyme activity (Lunenburg et al. 2020)
[2] Different classification with an activity score of 1 according to CPIC (Amstutz et al. 2018)

There are deviations in the recommendations of the professional associations for individual constellations. For example, carriers of two *DPYD* variants with reduced function are classified by DGHO with a DPD activity score of 0.5, while CPIC assigns an activity score of 1 (Amstutz et al. 2018). In this constellation, the DPWG recommends additional phenotypic testing to determine DPD activity (Lunenburg et al. 2020).

Phenotypic alternatives or additions to the genetic analysis of the *DPYD* gene are the measurement of uracil in plasma or the physiological ratio of dihydrouracil to uracil as well as the determination of DPD activity in leucocytes (Meulendijks et al. 2016). However, the data basis for this procedure is narrower than for *DPYD* genetic diagnostics and the analyses are not yet part of the standard procedure in Germany before a therapy with FU-containing drugs (**DGHO Position Paper 2020**). In individual cases, however, such phenotypic testing may be indicated in addition to genotyping. In particular, additional phenotypic testing is recommended in the presence of two *DPYD* variants with reduced function or the combination of a *DPYD* variant with reduced function and a variant with no function (Henricks et al. 2017, Lunenburg et al. 2020).

Recommendation for dosage according to DPYD genotype

Die Europäische Arzneimittel-Agentur (EMA) empfiehlt, alle Patienten vor einer systemischen Therapie mit den FU-haltigen Arzneimitteln 5-Fluorouracil (5-FU), Capecitabin und Tegafur auf einen DPD-Mangel zu testen (**EMA Recommendations 2020**). Diese Empfehlung wurde auch vom Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM) aufgegriffen und in die Fachinformationen der betroffenen Arzneimittel aufgenommen. Die DGHO empfiehlt zur Umsetzung dieser Vorgaben eine Testung auf die vier häufigsten genetischen *DPYD*-Varianten und eine Therapie auf Basis eines differenzierten, risiko-adaptierten Algorithmus nach Ergebnis der genetischen Analyse unter Berücksichtigung der individuellen Erkrankungssituation und der möglicherweise vorhandenen Therapiealternativen (Abbildung 1) (Henricks et al. 2018, **DGHO Positionspapier 2020**). Die genetische Analyse kann durch ein therapeutisches Drug Monitoring bzw. eine phänotypische Testung ergänzt werden (Gamelin et al. 2008, Lunenburg et al. 2020).



and dose modification before FU-containing therapy (modified according to DHGO position paper 2020).

References

You can find the corresponding references here:
https://www.mll.com/en/diagnostic-offer/pharmakogenetik/dihydropyrimidine-dehydrogenase-dpd-testing-and-fluorouracil-toxicity.html#referenzen