



## **Topical update: The Hong Kong College of Pathologists, Incorporated in Hong Kong with Limited Liability**

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### **Screening of DPD Deficiency before Fluoropyrimidine Chemotherapy**

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#### **Editorial Note:**

Screening of dihydropyrimidine dehydrogenase (DPD) deficiency before systemic fluoropyrimidine chemotherapy can improve safety and prevent the occurrence of the associated toxicity. Both genotyping and phenotyping approaches have been advocated. In this review, Dr Felix Wong compares and contrasts both approaches and explains the upcoming situation in Hong Kong in the near future. We welcome any feedback or suggestions. Please direct them to [Dr Esther Hung](#) of Education Committee, the Hong Kong College of Pathologists. Opinions expressed are those of the authors or named individuals, and are not necessarily those of the Hong Kong College of Pathologists.

#### **Introduction:**

Pre-emptive testing for dihydropyrimidine dehydrogenase (DPD) deficiency is currently recommended for the prevention of fluoropyrimidine toxicity. Dihydropyrimidine dehydrogenase (DPD), which is encoded by the *DPYD* gene on chromosome 1, is the major catabolic enzyme of fluoropyrimidines responsible for 80% of their metabolism. Fluoropyrimidines include the chemotherapeutic agents 5-fluorouracil (5-FU) and its prodrugs (capecitabine and tegafur). DPD converts 5-FU to dihydrofluorouracil, which is non-cytotoxic. Genetic variants of the *DPYD* gene are associated with decreased DPD activity and increased fluoropyrimidine-related toxicity (bone-marrow and gastrointestinal toxicity, even death due to severe toxicity) due to an accumulation of 5-FU and its downstream active cytotoxic metabolites. In recent years, multiple guidelines recommending preemptive *DPYD* testing before fluoropyrimidine chemotherapy from different countries or regions have been published<sup>1-6</sup>. These guidelines are focused on targeted genotyping of the following variants based on studies done in the Caucasian population: 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 – corresponds to a combination of five genetically linked polymorphisms. It includes four intronic variants and one exon variant, two of which are in complete linkage disequilibrium: the c.1129-5923C>G variant (intronic variant) and the c.1236G>A variant (exon variant). The c.1129-5923C>G variant introduces a cryptic splice site and the partial production of a non-functional transcript.<sup>5,7</sup>). Nevertheless, other rarer decreased- or no-function *DPYD* variants exist. Notably, based on

multiethnic population databases, e.g. Genome Aggregation Database (gnomAD, <https://gnomad.broadinstitute.org/>) and multiple studies performed in the East Asian population, it is known that these 4 variants are rare or absent in the East Asian population. For Hong Kong, an international city with a predominantly Chinese population, DPYD genotyping has been mostly performed in a retrospective manner to ascertain the cause in patients who had suffered from severe fluoropyrimidine toxicity<sup>8</sup>. Obviously, this approach does not prevent fluoropyrimidine toxicity or improve patient outcomes. It is uncertain how preemptive *DPYD* testing should be conducted in this locality. Whole DPYD gene sequencing with investigation of DPD enzyme activity for patients with novel variants has been proposed by some local experts<sup>9</sup>.

An alternative approach to DPYD genotyping is DPD phenotyping. 5-FU is a synthetic analog of uracil, which is the native substrate of the DPD enzyme located in the liver as part of the pyrimidine catabolic pathway. DPD deficiency is associated with an increase in uracil and decrease in dihydrouracil, the product of the DPD enzymatic reaction. Therefore, a high plasma uracil (U) concentration or a low dihydrouracil (UH2) to uracil ratio (UH2/U ratio) indicates DPD deficiency. DPD phenotyping by plasma uracil measurement is recommended by the European Medicines Agency<sup>10</sup> for the detection of DPD deficiency and has been adopted in France<sup>11</sup> since 2018 using a cutoff of  $\geq 16$  ng/mL, above which DPD deficiency is diagnosed. France is the first country in the world adopting DPD phenotyping by plasma uracil measurement for the screening of DPD deficiency and there has been an increase in the number of tests and European countries offering the test from 2019 to 2021<sup>12</sup>.

#### Genotyping

The Activity Score system, proposed by Henricks et al<sup>13</sup> and endorsed by the Clinical Pharmacogenetics Implementation Consortium (CPIC)<sup>5</sup>, translates DPYD genotype to a predicted DPD phenotype. An Activity Score of 2 represents normal DPD activity and is a sum of a score of 1 for each normal allele that an individual carries. A decreased-function allele is assigned a score of 0.5 and a no-function allele is assigned a score of 0. *DPYD*\*2A and \*13 alleles are assigned a score of 0 (no-function variant) while c.2846A>T and c.1129–5923C>G alleles are assigned a score of 0.5 (decreased-function variant). Based on this system, an Activity Score of 2 translates into DPYD normal metabolizer, while an Activity Score of 1 or 1.5 translates into DPYD intermediate metabolizer and an Activity Score of 0 or 0.5 translates into DPYD poor metabolizer. For example, homozygous DPYD c.1129-5923C>G has an Activity Score of  $0.5 + 0.5 = 1$ , which is equal to the Activity Score of heterozygous DPYD\*2A. Dosage recommendations based on the metabolizer status are available, ranging from a reduced starting dose of 50% in intermediate metabolizers to a complete avoidance of fluoropyrimidines for poor metabolizers with an Activity Score of 0. Furthermore, therapeutic drug monitoring of 5-FU is recommended for guiding dosage adjustment following initial dosing. Prospective clinical trials involving upfront testing for these variants with genotype-based dosage adjustments have demonstrated improved patient safety<sup>14,15</sup> and cost-effectiveness<sup>15,16</sup>, with no adverse impact on treatment response rates for the reduced fluoropyrimidine dose given to DPYD variant carriers<sup>17</sup>. Nevertheless, it is known that this approach has limited sensitivity (17%) for severe fluoropyrimidine-related toxicity [ $\geq$  grade 3 according to the Common Terminology Criteria for Adverse Events (CTCAE)] because only 39 – 61% of patients having severe fluoropyrimidine-related toxicity are due to DPD deficiency, and even if DPD deficiency is present, it cannot be always traced back to a genetic alteration in the DPYD gene despite full gene sequencing<sup>18</sup>.

On the other hand, multiple studies performed in the East Asian population (Chinese<sup>19–21</sup>, Korean<sup>21,22</sup>, Japanese<sup>21,23</sup>) have

demonstrated the scarcity of the \*2A, I560S, D949V and c.1129–5923C>G in the studied populations. Indeed, fluoropyrimidine-induced toxicity appears to be less common in the East Asian population than other populations<sup>24,25</sup>. From the DPYD allele frequencies based on population databases together with a comprehensive evaluation of variants by functional data/in silico predictions, it has been deduced that the East Asian population may have a lower incidence of DPD deficiency than the European population (3.4% and 0.02% of intermediate metabolizer and poor metabolizer, respectively, in East Asians, in contrast to 7.6% and 0.09%, respectively, in non-Finnish Europeans).<sup>26</sup> Table 1 shows a comparison of minor allele frequencies (MAF) in Genome Aggregation Database (gnomAD) of the 4 variants in the European and East Asian populations. It can be deduced that targeted genotyping of these 4 variants in the local Chinese population will result in poor sensitivity of detecting DPD deficiency, and whole DPYD gene sequencing is required for the detection of other deleterious DPYD alleles. Based on the literature review performed by Zhou et al, 49 DPYD variants (\*2A, I560S and D949V included) are considered to be pathogenic<sup>26</sup>, and by in silico predictions of all observed DPYD variants in the global population in the same study, it was estimated that a large number of DPYD variants (174 variations, which corresponds to only 34.3% of deleterious DPYD variants) have to be tested in order to explain 95% of genetically encoded functional DPD variability in the population, underscoring the presence of significant allelic heterogeneity. DPYD variants with unknown functional impact may be analyzed by in silico prediction tools, which are computational algorithms developed to predict the functional impact of DPYD variants, e.g. DPYD-Varifier<sup>27</sup>, Absorption, Distribution, Metabolism and Excretion (ADME) Prediction Framework<sup>26,28</sup>, or evaluated using in vitro functional assays of DPD enzyme activity, which involves expression of DPYD variants in mammalian cells and incubation of radiolabelled 5-FU coupled with the measurement of rate of formation of dihydrofluorouracil from 5-FU<sup>29–32</sup>. It has been suggested that computation algorithms specifically developed for pharmacogenes (e.g. ADME Prediction Framework) or in a gene-specific manner (e.g. DPYD-Varifier) may be preferable to conventional prediction methods for non-pharmacogenes because conventional methods are primarily based on evolutionary conservation while pharmacogenes are poorly conserved<sup>33</sup>. Given the rarity of each individual DPYD variant, in vivo data from clinical studies is mostly unavailable or limited to isolated case reports. Nevertheless, the approach of including additional DPYD deficiency variants with evidence of a deleterious impact on protein function equivalent to the CPIC high level evidence variants has been shown to improve the sensitivity of the prediction of grade 3/4 haematological toxicity while retaining excellent specificity.<sup>34</sup>

DPYD Variant	MAF (European, non-Finnish)(%)	MAF (East Asian)(%)
<b>c.1905+1G&gt;A (rs3918290, *2A)</b>	2.385	0
<b>c.1679T&gt;G (rs55886062, I560S)</b>	0.06220	0
<b>c.2846A&gt;T (rs67376798, D949V)</b>	0.5163	0.005013
<b>c.1129–5923C&gt;G (rs75017182)</b>	2.102	0.1926

Table 1. Minor allele frequencies (MAF) for \*2A (c.1905+1G>A), I560S (c.1679T>G), D949V(c.2846A>T) and c.1129–5923C>G. Source: Genome Aggregation Database, accessed 20 December 2023.

A prospective study was performed from November 2020 to December 2021 in the author’s hospital recruiting patients requiring fluoropyrimidine-containing chemotherapy. Polymerase chain reaction followed by Sanger sequencing of the 23 coding exons, their flanking intronic regions and HapB3 (c.1129-5923C>G included) of the *DPYD* gene was performed. 103 patients (97 Chinese, 94%) requiring chemotherapy for colorectal, stomach and other cancers were recruited. 4

heterozygous decreased-function/no-function *DPYD* variants were detected in 4 Chinese patients (heterozygous c.2210C>T, p.Thr737Ile: 2 patients; heterozygous c.220C>T, p.Arg74Ter and heterozygous c.1314T>G, p.Phe438Leu: 1 patient each). In addition, two heterozygous VUS were detected in three patients (heterozygous c.2303C>A, p.Thr768Lys: two Chinese patients; heterozygous c.2528T>C, p.Ile843Thr in a Filipino patient). None of the well-established alleles affecting *DPYD* function were detected in the study population.

### Phenotyping

The DPD enzyme activity is mainly located in the liver in vivo. It is also present in peripheral blood mononuclear cells (PBMCs) which can be measured ex vivo and acts as a surrogate marker of the hepatic enzymatic activity<sup>35</sup>. Nevertheless, the assay is not widely available because it is time-consuming, requires a large volume of blood and the use of radiolabeled materials<sup>36</sup>. Of note, only 25% of patients with a decreased DPD enzyme activity carries one of the four *DPYD* variants<sup>37</sup>. Alternatively, plasma uracil and dihydrouracil measurements by High Performance Liquid Chromatography – Ultraviolet detection (HPLC-UV) or Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) have been studied as surrogate markers of DPD enzyme activity. The theoretical advantage of DPD phenotyping resides in the possibility of identifying more patients with DPD deficiency beyond which can be explained by *DPYD* genetic variants alone. In a study of 550 patients requiring fluoropyrimidine chemotherapy with pretreatment serum uracil measured, a concentration of  $\geq 16$  ng/mL strongly associated with global severe toxicity (odds ratio 5.3,  $p = 0.009$ ). Serum uracil was shown to correlate better with DPD activity in PBMCs than UH2/U ratio and be superior to UH2/U ratio as a predictor of severe toxicity<sup>38</sup>. The same cutoff in another study demonstrated a sensitivity of 67% and specificity of 92% in the prediction of grade 4 capecitabine toxicity with a relative risk of 20.6 ( $p = 0.021$ )<sup>39</sup>. The distribution of plasma uracil in the pre-treatment population across studies is shown in Table 2. One of these studies was performed in the Chinese population<sup>40</sup>. Plasma uracil  $\geq 16$  ng/mL was observed in approximately 10 – 15% of the population<sup>39,41</sup>. Plasma uracil measurement has been endorsed by the European Medicines Agency (EMA) in 2020 for the detection of DPD deficiency<sup>10</sup>. Detailed guidelines on the detection of DPD deficiency by plasma uracil measurement are available in France ( $\geq 16$  ng/mL to 150 ng/mL: partial DPD deficiency;  $\geq 150$  ng/mL: complete DPD deficiency)<sup>42</sup> and Belgium ( $\geq 14$  ng/mL to 100 ng/mL: partial DPD deficiency;  $\geq 100$  ng/mL: complete DPD deficiency)<sup>43</sup>. Dosage adjustment based on plasma uracil leads to a decreased 5FU toxicity in patients with partial DPD deficiency, while the resulting lower dose of 5FU received by these patients suggests the need for therapeutic drug monitoring of 5FU to uphold treatment efficacy<sup>44</sup>. Plasma uracil is unstable in whole blood samples and strict preanalytical requirements are required (maximum delay between sample and centrifugation of 1h30 mins if the sample is stored at ambient temperature, and 4h if it is stored at + 4°C, centrifugation preferably at + 4°C then immediate freezing of the resulting plasma, transport respecting the cold chain)<sup>42</sup>, otherwise, artefactual elevations may occur<sup>45</sup>. There are known effects of food and circadian rhythm affecting plasma uracil concentration<sup>45,46</sup>. Plasma uracil concentration may be elevated in renal impairment<sup>47,48</sup>, abnormal liver function<sup>48</sup> and tumor lysis syndrome<sup>49</sup>. Patients with end stage renal disease on haemodialysis were shown to have a higher uracil concentration pre-analysis (mean = 14 ng/mL) than post-dialysis (mean = 8 ng/mL)<sup>50</sup>. Plasma uracil concentration is also elevated if fluoropyrimidine has already been administered because of the competition between uracil and 5-FU for metabolism by DPD<sup>51</sup>.

In the author's laboratory, residual plasma from 74 specimens delivered on ice for plasma renin analysis was used for plasma uracil analysis. The average, median, 5th and 95th percentile of plasma uracil were: 9.8, 9.6, 5.9 and 16.2 ug/L. Therefore,

around 5% of samples is expected to show a plasma uracil  $\geq 16$  ug/L (“Flagging rate”), indicating a positive result for DPD screen. Uracil  $\geq 150$  ug/L is expected to be rare (occurrence rate 0.08% in France)<sup>11</sup> and there were no such cases in our 74 specimens.

Study	Median (ng/mL)	Range (ng/mL)	No. of subjects (n)	Methodology	Diagnosis	Ethnicity
Coudoré et al, 2012 <sup>52</sup>	8.4	0.6 – 43.3	26	LC-MS/MS	advanced or recurrent cancer	French
Etienne-Grimaldi et al, 2017 <sup>39</sup>	9.6	3.9 – 75.3	205	HPLC-UV	Advanced breast cancer patients	French
Pan et al, 2017 <sup>40</sup>	11.2	5.16 – 120.6	68	HPLC-UV	Colorectal cancer patients	Chinese
Dolat et al, 2020 <sup>53</sup>	10.8	3 – 37.6	169	LC-MS/MS	Colorectal, pancreas and stomach cancer	French
Tafzi et al, 2020 <sup>41</sup>	10.6	3.9 – 81.6	526	LC-MS/MS	Patients before 5FU treatment	French

Table 2. Distribution of plasma uracil concentrations across different studies. LC-MS/MS: liquid chromatography tandem mass spectrometry; HPLC-UV: high performance liquid chromatography – ultraviolet spectroscopy.

#### Genotyping and Phenotyping

Plasma uracil concentration correlates with the presence of known deleterious DPYD variants \*2A, I560S and D949V – such variants were associated with a higher plasma uracil concentration, though not necessarily higher than the cutoff of 16 ng/mL, while this association appears to be weaker for HapB3<sup>38,39,54</sup>. The presence of any of the 4 variants has a sensitivity and specificity of 11% and 95%, respectively, for the detection of DPD deficiency defined as a uracil concentration of  $> 16$  ng/mL<sup>54</sup>. A retrospective study of 472 patients with DPYD targeted genotyping (the 4 DPYD variants recommended by CPIC, plus DPYD\*7, a frameshift variant classified to be a no-function allele by CPIC) and phenotyping (plasma uracil and UH2/U ratio) done without fluoropyrimidine dosage being adjusted according to such results (either because the patient has already suffered from toxicity of fluoropyrimidine or because such testing was done in parallel with treatment initiation) demonstrated sensitivities and specificities of targeted genotyping and plasma uracil to be 33%/59% and 95%/84%, respectively, for the prediction of grade 3 or higher fluoropyrimidine toxicity<sup>55</sup>.

For the local population who is predominantly Chinese, it was demonstrated that none of the 4 variants recommended for testing by CPIC (c.1905+1G>A, c.1679T>G, c.2846A>T and c.1129–5923C>G) were detected in the author’s laboratory, while

sequencing of whole *DPYD* gene enabled the detection of other *DPYD* variants enriched in the East Asian population which may lead to DPD deficiency and confer susceptibility to fluoropyrimidine toxicity. Nevertheless, these variants are less well characterized and their interpretation in the local population as decreased or no function variants relies on in vitro or in silico data, i.e. human data is lacking. According to the CPIC guideline, when two different decreased-/no -function variants are detected in a patient, it is assumed that the two variants are in trans, i.e. located on different alleles, while this may not be true if one of the two detected variants is novel. In the absence of haplotyping techniques, phenotyping may be helpful to differentiate partial from complete DPD deficiency.<sup>5</sup> This approach has been highlighted in a review by Knikman et al. which outlined the possibility of combining phenotyping with genotyping in the same patient. They proposed that while dose modification may be based on genotyping result if one known variant is detected, dose modification should be based on phenotyping results in the event of two variants detected or no variants detected<sup>56</sup>. In fact, even if two deleterious alleles are proven to be in trans by haplotyping techniques, the Dutch Pharmacogenetics Working Group (DPWG) guideline recommends the use of phenotyping to determine the residual DPD activity for guiding the fluoropyrimidine use at significantly reduced doses<sup>2</sup>.

#### Screening of DPD deficiency in Hong Kong

While screening of DPD deficiency by plasma uracil has not been formally studied in the Chinese population, it is assumed to be applicable to all populations because the phenotype of uracilemia is reasoned to a biomarker of DPD deficiency independent of ethnicity<sup>57</sup>. A decision was made to go for the phenotyping rather than genotyping approach and Queen Mary Hospital Chemical Pathology will launch preemptive screening of DPD deficiency by plasma uracil measurement to all patients requiring systemic fluoropyrimidine chemotherapy in the Hospital Authority in 2024 Q1. Plasma uracil is measured by liquid chromatography tandem mass spectrometry, which requires in-house method development and evaluation. We have subscribed to an external quality assurance program based in France (ASQUALAB). Uracil is an unstable analyte in plasma and an unbroken cold chain is required from specimen collection to centrifugation and storage. A turnaround time of less than 10 calendar days, which fulfils the French standard<sup>42</sup>, will be implemented to minimize the delay before treatment initiation. Under this service, genotyping is not required and will not be performed, even for patients screened positive for partial or complete DPD deficiency by plasma uracil. While fluoropyrimidines are contraindicated in patients with complete DPD deficiency, patients with partial DPD deficiency may be started on a reduced dose and the option of therapeutic drug monitoring of intravenous infusion of 5-fluorouracil (the topic of which is beyond the scope of this article) will be available as a part of the service to improve the drug safety of fluoropyrimidines. The service of measuring plasma 5FU level will be launched simultaneously with that of plasma uracil. According to an article published in 2023, no preventable toxicity-related death has been declared to the French Pharmacovigilance Network in patients with complete or partial DPD deficiency since 2020<sup>57</sup>. We hope that the same benefits will soon become a reality in Hong Kong.

#### References

1. Hamzic S, Aebi S, Joerger M, Montemurro M, Ansari M, Amstutz U, et al. Fluoropyrimidine chemotherapy: recommendations for *DPYD* genotyping and therapeutic drug monitoring of the Swiss Group of Pharmacogenomics and Personalised Therapy. *Swiss Med Wkly* 2020;150:w20375
2. Lunenburg CATC, van der Wouden CH, Nijenhuis M, Crommentuijn-van Rhenen MH, de Boer-Veger NJ, Buunk AM, et

- al. Dutch Pharmacogenetics Working Group (DPWG) guideline for the gene-drug interaction of DPYD and fluoropyrimidines. *Eur J Hum Genet EJHG*. 2020 Apr;28(4):508–17.
3. Wörmann B, Bokemeyer C, Burmeister T, Köhne CH, Schwab M, Arnold D, et al. Dihydropyrimidine Dehydrogenase Testing prior to Treatment with 5-Fluorouracil, Capecitabine, and Tegafur: A Consensus Paper. *Oncol Res Treat*. 2020;43(11):628–36.
  4. 5-fluorouracil (intravenous), capecitabine, tegafur: DPD testing recommended before initiation to identify patients at increased risk of severe and fatal toxicity [Internet]. GOV.UK. [cited 2023 Dec 21]. Available from: <https://www.gov.uk/drug-safety-update/5-fluorouracil-intravenous-capecitabine-tegafur-dpd-testing-recommended-before-initiation-to-identify-patients-at-increased-risk-of-severe-and-fatal-toxicity>
  5. Amstutz U, Henricks LM, Offer SM, Barbarino J, Schellens JHM, Swen JJ, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Dihydropyrimidine Dehydrogenase Genotype and Fluoropyrimidine Dosing: 2017 Update. *Clin Pharmacol Ther*. 2018 Feb;103(2):210–6.
  6. García-Alfonso P, Saiz-Rodríguez M, Mondéjar R, Salazar J, Páez D, Borobia AM, et al. Consensus of experts from the Spanish Pharmacogenetics and Pharmacogenomics Society and the Spanish Society of Medical Oncology for the genotyping of DPYD in cancer patients who are candidates for treatment with fluoropyrimidines. *Clin Transl Oncol*. 2022 Mar 1;24(3):483–94.
  7. Amstutz U, Farese S, Aebi S, Largiadèr CR. Dihydropyrimidine dehydrogenase gene variation and severe 5-fluorouracil toxicity: a haplotype assessment. *Pharmacogenomics*. 2009 Jun;10(6):931–44.
  8. Tong CC, Lam CW, Lam KO, Lee VHF, Luk MY. A Novel DPYD Variant Associated With Severe Toxicity of Fluoropyrimidines: Role of Pre-emptive DPYD Genotype Screening. *Front Oncol*. 2018;8:279.
  9. Lam KO, Tong CC, Lee VHF, Luk MY, Lam CW. DPYD genotype-guided dose individualisation of fluoropyrimidine therapy: who and how? *Lancet Oncol*. 2019 Feb;20(2):e66.
  10. EMA recommendations on DPD testing prior to treatment with fluorouracil, capecitabine, tegafur and flucytosine | European Medicines Agency [Internet]. [cited 2023 Dec 21]. Available from: <https://www.ema.europa.eu/en/news/ema-recommendations-dpd-testing-prior-treatment-fluorouracil-capecitabine-tegafur-flucytosine>
  11. Méthodes de recherche d'un déficit en dihydropyrimidine deshydrogénase visant à prévenir certaines toxicités sévères associées aux traitements incluant une fluoropyrimidine (5-fluorouracile ou capécitabine) [Internet]. Haute Autorité de Santé. [cited 2023 Dec 21]. Available from: [https://www.has-sante.fr/jcms/c\\_2891090/fr/methodes-de-recherche-d-un-deficit-en-dihydropyrimidine-deshydrogenase-visant-a-prevenir-certaines-toxicites-severes-associees-aux-traitements-incluant-une-fluoropyrimidine-5-fluorouracile-ou-capecitabine](https://www.has-sante.fr/jcms/c_2891090/fr/methodes-de-recherche-d-un-deficit-en-dihydropyrimidine-deshydrogenase-visant-a-prevenir-certaines-toxicites-severes-associees-aux-traitements-incluant-une-fluoropyrimidine-5-fluorouracile-ou-capecitabine)
  12. De With M, Sadlon A, Cecchin E, Haufroid V, Thomas F, Joerger M, et al. Implementation of dihydropyrimidine

dehydrogenase deficiency testing in Europe. *ESMO Open*. 2023 Apr;8(2):101197.

13. Henricks LM, Lunenburg CATC, Meulendijks D, Gelderblom H, Cats A, Swen JJ, et al. Translating DPYD genotype into DPD phenotype: using the DPYD gene activity score. *Pharmacogenomics*. 2015;16(11):1277–86.
14. Henricks LM, Lunenburg CATC, de Man FM, Meulendijks D, Frederix GWJ, Kienhuis E, et al. DPYD genotype-guided dose individualisation of fluoropyrimidine therapy in patients with cancer: a prospective safety analysis. *Lancet Oncol*. 2018 Nov;19(11):1459–67.
15. Deenen MJ, Meulendijks D, Cats A, Sechterberger MK, Severens JL, Boot H, et al. Upfront Genotyping of DPYD\*2A to Individualize Fluoropyrimidine Therapy: A Safety and Cost Analysis. *J Clin Oncol Off J Am Soc Clin Oncol*. 2016 Jan 20;34(3):227–34.
16. Henricks LM, Lunenburg CATC, de Man FM, Meulendijks D, Frederix GWJ, Kienhuis E, et al. A cost analysis of upfront DPYD genotype-guided dose individualisation in fluoropyrimidine-based anticancer therapy. *Eur J Cancer Oxf Engl* 1990. 2019 Jan;107:60–7.
17. Glewis S, Alexander M, Khabib MNH, Brennan A, Lazarakis S, Martin J, et al. A systematic review and meta-analysis of toxicity and treatment outcomes with pharmacogenetic-guided dosing compared to standard of care BSA-based fluoropyrimidine dosing. *Br J Cancer*. 2022 Mar 19;1–11.
18. Meulendijks D, Cats A, Beijnen JH, Schellens JHM. Improving safety of fluoropyrimidine chemotherapy by individualizing treatment based on dihydropyrimidine dehydrogenase activity - Ready for clinical practice? *Cancer Treat Rev*. 2016 Nov;50:23–34.
19. Li GY, Duan JF, Li WJ, Liu T. DPYD\*2A/\*5A/\*9A and UGT1A1\*6/\*28 polymorphisms in Chinese colorectal cancer patients. *J Cancer Res Ther*. 2016 Jun;12(2):782–6.
20. He YF, Wei W, Zhang X, Li YH, Li S, Wang FH, et al. Analysis of the DPYD gene implicated in 5-fluorouracil catabolism in Chinese cancer patients. *J Clin Pharm Ther*. 2008;33(3):307–14.
21. Shin JG, Cheong HS, Kim JY, Kim LH, Han CS, Kim JO, et al. Screening of Dihydropyrimidine Dehydrogenase Genetic Variants by Direct Sequencing in Different Ethnic Groups. *J Korean Med Sci*. 2013 Aug;28(8):1129–33.
22. Cho HJ, Park YS, Kang WK, Kim JW, Lee SY. Thymidylate synthase (TYMS) and dihydropyrimidine dehydrogenase (DPYD) polymorphisms in the Korean population for prediction of 5-fluorouracil-associated toxicity. *Ther Drug Monit*. 2007 Apr;29(2):190–6.
23. Maekawa K, Saeki M, Saito Y, Ozawa S, Kurose K, Kaniwa N, et al. Genetic variations and haplotype structures of the DPYD gene encoding dihydropyrimidine dehydrogenase in Japanese and their ethnic differences. *J Hum Genet*. 2007 Oct;52(10):804–19.
24. Haller DG, Cassidy J, Clarke SJ, Cunningham D, Van Cutsem E, Hoff PM, et al. Potential regional differences for the



tolerability profiles of fluoropyrimidines. *J Clin Oncol Off J Am Soc Clin Oncol*. 2008 May 1;26(13):2118–23.

25. Loh M, Chua D, Yao Y, Soo RA, Garrett K, Zeps N, et al. Can population differences in chemotherapy outcomes be inferred from differences in pharmacogenetic frequencies? *Pharmacogenomics J*. 2013 Oct;13(5):423–9.
26. Zhou Y, Dagi Hernandez C, Lauschke VM. Population-scale predictions of DPD and TPMT phenotypes using a quantitative pharmacogene-specific ensemble classifier. *Br J Cancer*. 2020 Dec;123(12):1782–9.
27. Shrestha S, Zhang C, Jerde CR, Nie Q, Li H, Offer SM, et al. Gene-Specific Variant Classifier (DPYD-Varifier) to Identify Deleterious Alleles of Dihydropyrimidine Dehydrogenase. *Clin Pharmacol Ther*. 2018 Oct;104(4):709–18.
28. Zhou Y, Mkrtychian S, Kumondai M, Hiratsuka M, Lauschke V. An optimized prediction framework to assess the functional impact of pharmacogenetic variants. *Pharmacogenomics J*. 2019 Apr 1;19.
29. Offer SM, Wegner NJ, Fossum C, Wang K, Diasio RB. Phenotypic Profiling of *DPYD* Variations Relevant to 5-Fluorouracil Sensitivity Using Real-time Cellular Analysis and *In Vitro* Measurement of Enzyme Activity. *Cancer Res*. 2013 Mar 15;73(6):1958–68.
30. Offer SM, Fossum CC, Wegner NJ, Stuflessen AJ, Butterfield GL, Diasio RB. Comparative functional analysis of *DPYD* variants of potential clinical relevance to dihydropyrimidine dehydrogenase activity. *Cancer Res*. 2014 May 1;74(9):2545–54.
31. Elraiyah T, Jerde C, Shrestha S, Wu R, Nie Q, Giama N, et al. Novel Deleterious Dihydropyrimidine Dehydrogenase Variants May Contribute to 5-Fluorouracil Sensitivity in an East African Population. *Clin Pharmacol Ther*. 2017;101(3):382–90.
32. Ly RC, Schmidt RE, Kiel PJ, Pratt VM, Schneider BP, Radovich M, et al. Severe Capecitabine Toxicity Associated With a Rare *DPYD* Variant Identified Through Whole-Genome Sequencing. *JCO Precis Oncol*. 2020 Jun 12;4:PO.20.00067.
33. Zhou Y, Lauschke VM. Computational Tools to Assess the Functional Consequences of Rare and Noncoding Pharmacogenetic Variability. *Clin Pharmacol Ther*. 2021;110(3):626–36.
34. Palles C, Fotheringham S, Chegwidan L, Lucas M, Kerr R, Mozolowski G, et al. An Evaluation of the Diagnostic Accuracy of a Panel of Variants in *DPYD* and a Single Variant in *ENOSF1* for Predicting Common Capecitabine Related Toxicities. *Cancers*. 2021 Jan;13(7):1497.
35. Lu Z, Zhang R, Diasio RB. Dihydropyrimidine dehydrogenase activity in human peripheral blood mononuclear cells and liver: population characteristics, newly identified deficient patients, and clinical implication in 5-fluorouracil chemotherapy. *Cancer Res*. 1993 Nov 15;53(22):5433–8.
36. Hodroj K, Barthelemy D, Lega JC, Grenet G, Gagnieu MC, Walter T, et al. Issues and limitations of available biomarkers for fluoropyrimidine-based chemotherapy toxicity, a narrative review of the literature. *ESMO Open*. 2021 Jun;6(3):100125.

37. Coenen MJH, Paulussen ADC, Breuer M, Lindhout M, Tserpelis DCJ, Steyls A, et al. Evolution of Dihydropyrimidine Dehydrogenase Diagnostic Testing in a Single Center during an 8-Year Period of Time. *Curr Ther Res.* 2019 Jan 1;90:1–7.
38. Meulendijks D, Henricks LM, Jacobs BAW, Aliev A, Deenen MJ, de Vries N, et al. Pretreatment serum uracil concentration as a predictor of severe and fatal fluoropyrimidine-associated toxicity. *Br J Cancer.* 2017 May;116(11):1415–24.
39. Etienne-Grimaldi MC, Boyer JC, Beroud C, Mbatchi L, van Kuilenburg A, Bobin-Dubigeon C, et al. New advances in DPYD genotype and risk of severe toxicity under capecitabine. *PLoS One.* 2017;12(5):e0175998.
40. Pan W, Li Y, Feng Y, Yang F, Liu H. A new sample preparation and separation combination for the precise, accurate, and simultaneous determination of uracil and dihydrouracil in human plasma by reversed-phase HPLC. *J Sep Sci.* 2017 Oct;40(19):3763–70.
41. Tafzi N, Woillard JB, Fleytoux A, Picard N, Marquet P. Phenotyping of Uracil and 5-Fluorouracil Metabolism Using LC-MS/MS for Prevention of Toxicity and Dose Adjustment of Fluoropyrimidines. *Ther Drug Monit.* 2020 Aug;42(4):540–7.
42. INAHTA Brief - Screening for dihydropyrimidine dehydrogenase deficiency to decrease the risk of severe toxicities related to fluoropyrimidines (5-fluorouracil or capecitabine) [Internet]. [cited 2023 Dec 21]. Available from: [https://www.inahta.org/upload/2019/19011\\_Screening%20for%20dihydropyrimidine%20dehydrogenase%20deficiency.pdf](https://www.inahta.org/upload/2019/19011_Screening%20for%20dihydropyrimidine%20dehydrogenase%20deficiency.pdf)
43. Casneuf V, Borbath I, Van den Eynde M, Verheezzen Y, Demey W, Verstraete AG, et al. Joint Belgian recommendation on screening for DPD-deficiency in patients treated with 5-FU, capecitabine (and tegafur). *Acta Clin Belg.* 2021 Jan 11;1–7.
44. Laures N, Konecki C, Brugel M, Giffard AL, Abdelli N, Botsen D, et al. Impact of Guidelines Regarding Dihydropyrimidine Dehydrogenase (DPD) Deficiency Screening Using Uracil-Based Phenotyping on the Reduction of Severe Side Effect of 5-Fluorouracil-Based Chemotherapy: A Propension Score Analysis. *Pharmaceutics.* 2022 Oct 6;14(10):2119.
45. Capiou S, Van Landschoot A, Reyns T, Stepman H. Pre-analytical considerations for the analysis of uracil and 5,6-dihydrouracil in heparin plasma. *Clin Chem Lab Med CCLM.* 2022 Apr 26;60(5):e112–5.
46. Henricks LM, Jacobs BAW, Meulendijks D, Pluim D, van den Broek D, de Vries N, et al. Food-effect study on uracil and dihydrouracil plasma levels as marker for dihydropyrimidine dehydrogenase activity in human volunteers. *Br J Clin Pharmacol.* 2018;84(12):2761–9.
47. Burns KE, Chavani O, Jeong SH, Duley JA, Porter D, Findlay M, et al. Comparison of a thymine challenge test and endogenous uracil–dihydrouracil levels for assessment of fluoropyrimidine toxicity risk. *Cancer Chemother Pharmacol.* 2021 May 1;87(5):711–6.
48. Callon S, Brugel M, Botsen D, Royer B, Slimano F, Feliu C, et al. Renal impairment and abnormal liver function tests in pre-therapeutic phenotype-based DPD deficiency screening using uracilemia: a comprehensive population-based study

in 1138 patients. *Ther Adv Med Oncol.* 2023 Jan 1;15:17588359221148536.

49. Launay M, Guitton J, Balluet R, Moreau A, Phelip JM, Tholance Y, et al. Clinical considerations for DPD deficiency testing in advanced cancer patients: tumor lysis syndrome should be considered as a major interference. *Ann Oncol.* 2022 Aug;33(8):850-852.
50. Gaible C, Narjoz C, Lorient MA, Roueff S, Pallet N. Pretherapeutic screening for Dihydropyrimidine dehydrogenase deficiency in measuring uracilemia in dialysis patients leads to a high rate of falsely positive results. *Cancer Chemother Pharmacol.* 2021 Dec 1;88(6):1049–53.
51. Thomas F, Maillard M, Launay M, Tron C, Etienne-Grimaldi MC, Gautier-Veyret E, et al. Artificial increase of uracilemia during fluoropyrimidine treatment can lead to DPD deficiency misinterpretation. *Ann Oncol.* 2021 Jun;32(6):810–1.
52. Coudoré F, Roche D, Lefeuvre S, Fausot D, Billaud EM, Lorient MA, et al. Validation of an Ultra-High Performance Liquid Chromatography Tandem Mass Spectrometric Method for Quantifying Uracil and 5,6-Dihydrouracil in Human Plasma. *J Chromatogr Sci.* 2012 Nov 1;50(10):877–84.
53. Dolat M, Macaire P, Goirand F, Vincent J, Hennequin A, Palmier R, et al. Association of 5-FU Therapeutic Drug Monitoring to DPD Phenotype Assessment May Reduce 5-FU Under-Exposure. *Pharmaceuticals.* 2020 Nov 23;13(11):416.
54. Pallet N, Hamdane S, Garinet S, Blons H, Zaanani A, Paillaud E, et al. A comprehensive population-based study comparing the phenotype and genotype in a pretherapeutic screen of dihydropyrimidine dehydrogenase deficiency. *Br J Cancer.* 2020 Sep;123(5):811–8.
55. Capitain O, Seegers V, Metges JP, Faroux R, Stampfli C, Ferec M, et al. Comparison of 4 Screening Methods for Detecting Fluoropyrimidine Toxicity Risk: Identification of the Most Effective, Cost-Efficient Method to Save Lives. *Dose-Response.* 2020 Jul 1;18(3):1559325820951367.
56. Knikman JE, Gelderblom H, Beijnen JH, Cats A, Guchelaar HJ, Henricks LM. Individualized Dosing of Fluoropyrimidine-Based Chemotherapy to Prevent Severe Fluoropyrimidine-Related Toxicity: What Are the Options? *Clin Pharmacol Ther.* 2021;109(3):591–604.
57. Etienne-Grimaldi MC, Pallet N, Boige V, Ciccolini J, Chouchana L, Barin-Le Guellec C, et al. Current diagnostic and clinical issues of screening for dihydropyrimidine dehydrogenase deficiency. *Eur J Cancer.* 2023 Mar;181:3–17.