Genotyping versus phenotyping: the case of fluoropyrimidines

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Fluoropyrimidines 5-fluorouracil (5-FU) and its oral pro-drug capecitabine are widely used in the treatment of solid tumors, including colorectal and breast cancer and cancers of the aerodigestive tract. Only a very small fraction of these drugs is transformed into active cytotoxic metabolites, while more than 80% of the administered dose is detoxified and excreted as metabolites (mainly fluoro-beta-alanine, FBAL) in urine. The first and rate-limiting step in this catabolic pathway is catalysed by an enzyme called dihydropyrimidine dehydrogenase or DPD which is coded by the *DPYD* gene. Of course, any decrease in DPD activity will increase the toxicity of these drugs. In relation to drug safety, it is generally accepted that about 20 to 25% of all patients treated with 5-FU or capecitabine will experience severe toxicity (grade ≥3) in relation to the treatment. In rare cases, estimated between 0.1 and 1%, such toxicity could be fatal for the patient. Since 1985 and the first description in NEJM of a fatal outcome in a patient treated with 5-FU and presenting with a DPD deficiency (1), it is now estimated that about 50% of all toxicity cases with 5-FU or capecitabine treatments are related to a DPD deficiency. In such cases, toxicity appears quite early on, during the first treatment cycles. In Caucasians, partial DPD deficiency is found in up to 8% of the patients while total DPD deficiency is found in up to 0,5% of them. This safety warning about fluoropyrimidines has already been shown on drug labels for a long time and EMA recommends, for instance, not using capecitabine in patients with total DPD deficiency. Similar warnings have been published by the FDA in relation to the use of 5-FU and the increased risk of serious or fatal ADRs in patients with low or absent DPD activity.

Considering these warnings by the EMA and FDA, several questions could be raised. What is the best strategy to identify patients with total or partial DPD deficiency? What is the performance of available screening tests (mainly in term of sensitivity and positive predictive value (PPV)) to identify total and/or partial DPD deficiency?

A first option to screen for DPD deficiency is to use a **genotyping approach**. In patients of Caucasian origin, the main variant associated with a total loss of DPD activity is *DPYD*2A* (rs3918290, c.1905+1G>A) resulting in exon 14 skipping. Therefore, a 50% dose reduction in 5-FU based-treatments has been proposed in heterozygous patients. However, as minor allelic frequency in Caucasians is estimated at 0.008, around 1.6% and less than 0.01% of the patients are expected to be heterozygous and homozygous, respectively, explaining only a small fraction of partial and total DPD deficiency. The very first PGx prospective study specifically designed to test the clinical utility of a 50% dose reduction in patients heterozygous for *DPYD*2A* allele was published in 2016 (2). The primary endpoint of this study was patient safety, assessed by the incidence of grade≥ 3 adverse effects and only the *DPYD*2A* variant was tested. In this prospective study, wild-type patients received the standard-dose treatment while heterozygous patients (representing just over one

percent in this population) received the 50% adjusted dose. Of course, as a true randomized controlled trial was considered as not ethical, the patients from the genotype-guided dosing arm were first compared with a group of heterozygous patients who received the full dose treatment in a historical cohort. The authors observed that severe toxicity could be decreased from 73% in heterozygous patients receiving a standard dose (n=48) to 28% by genotype-guided dosing (*DPYD*2A* carriers receiving a 50% dose reduction) (n=18). Moreover, the observed toxicity was short in duration, in contrast to the long-lasting toxicity usually observed in variant allele carriers receiving the full dose. This was also clearly demonstrated by absolute risk reduction in the incidence of drug-induced death with genotype-guided dosing approach (0 versus 10%). With this study design, a second comparator was carried out between heterozygous patients receiving a 50% dose reduction and wild-type patients receiving the standard-dose treatment. The study showed that when reducing the dose by 50% in heterozygous patients, severe toxicity was reduced to a frequency (28%) comparable to that in wild-type patients treated with a standard dose (23%), suggesting that heterozygous patients were not underexposed when treated with lower starting doses. Also, interestingly, this point was confirmed in the study done by a drug PK analysis showing a two-fold higher 5-FU dose-normalized AUC in heterozygous compared with wild-type patients, meaning that they could reach the same blood concentration even with the 50% reduced dose. Without going into much detail, the authors also demonstrated that their approach, based on the screening of only one variant, was cost-effective, taking into consideration a genotyping cost of 75 €. A recent update from the Clinical Pharmacogenetics Implementation Consortium (CPIC) now recommends to search for at least 4 *DPYD* variants associated with either a total loss (*DPYD*2A* and *DPYD*13*, rs55886062, c.1679T>G) or a reduced function (rs67376798, c.2846A>T, p.D949V and HaplotypeB3, rs75017182, c.1129-5923C>G/rs56038477, c.1236G>A, p.E412E/rs56276561, c.483+18G>A) of the DPD enzyme (3). Briefly, dosing recommendations published for capecitabine and 5-FU are based on a scoring system where alleles with a total loss of activity are attributed a score of 0, alleles with reduced activity a score of 0.5 and alleles with normal activity a score of 1. Then, the activity score for the genotype is calculated as the sum of the scores obtained from maternal and paternal alleles. In terms of dosing recommendations, for an activity score of 1.5, it is recommended to reduce the dose by 25 to 50%, for an activity score of 1, to reduce the dose by 50% and for activity scores of 0.5 and 0, to avoid, when possible, treatment using 5-FU or its pro-drug capecitabine (3). These new dosing guidelines recommended by the CPIC have been tested in a second prospective PGx study whose results were published at the end of 2018 in Lancet Oncology (4). Briefly, the authors showed that a 50% dose reduction in *DPYD*2A* and *DPYD*13* heterozygous patients was adequate in terms of drug safety while a larger dose reduction of 50% (instead of 25%) would probably be also advised in c.2846A>T and c.1236G>A carriers. They concluded that prospective *DPYD* genotyping was feasible in routine clinical practice and that implementation of DPYD genotype-guided individualized dosing should be a new standard of care.

In summary, the main advantages of the genotyping approach are (i) its simplicity of implementation in terms of pre-analytical and analytical conditions and (ii) the high positive predictive value of the four main variants towards severe toxicity (grade≥3). However, the main limitations of this approach are (i) the very low sensitivity to detect total (and partial) DPD deficiency and (ii) the fact that this approach has only been validated in Caucasians so far.

To overcome the main limitations of the genotyping test, a **phenotyping approach** based on the measurement of uracil (U), the natural substrate of the DPD enzyme, and its metabolite dihydrouracil

(UH2) in plasma before treatment has been proposed. Plasma concentrations of U and UH2 are commonly measured by high performance liquid chromatography (HPLC) with different possible detection methods (UV spectrophotometry, mass spectrometry).

Three prospective observational studies assessed the performance of DPD phenotyping (5, 6, 7). The original study was conducted in 252 colorectal cancers treated with intravenous 5-FU. The authors reported fairly similar results between UH2/U and plasma U regarding grade 3-4 toxicity (sensitivity = 82% and 88%; specificity = 78% and 69%, respectively). This study showed that the clearance of 5-FU was significantly correlated with U plasma concentration (inverse correlation) whereas it was not correlated with the UH2/U ratio (5). The two other studies concluded that U is more effective than UH2/U in predicting the toxicity of capecitabine (6, 7).

Both UH2/U ratio and U are continuous variables. Interpretation thus requires the determination and the validation of threshold values to distinguish patients with DPD deficiency from non-deficient patients. The literature available regarding UH2/U threshold is scarce and suggests large heterogeneity between laboratories. This is primarily due to the variability in the analytical methods used (HPLC with UV detection, diode array or MS-MS) and to analytical interference regarding UH2 determination. In contrast, the three independent prospective studies previously cited converge remarkably on the threshold value of U determining a risk of toxicity: greater than 15 ng/mL for the historical study (5) and 16 ng/mL for the other two (6, 7). It is commonly admitted that U > 100 ng/mL is associated with DPD total deficiency (although this could not be validated prospectively, given the rarity of this phenotype).

However, it must be stressed that the main limitation of the phenotyping test is the very strict pre-analytical requirements. Indeed, U level rapidly increases in whole blood mainly when the sample is kept at room temperature and the maximum delay for centrifugation and plasma freezing is 1h30 after blood collection.

Few studies have documented the predictive performance of approaches combining phenotyping and genotyping. These approaches are very heterogeneous depending on the studies, the number of DPYD variants considered, etc.

In summary, DPD phenotyping based on plasma U determination appears to be a very relevant approach to identify patients with DPD deficiency prior to treatment with fluoropyrimidines. This approach has been officially recommended in France since January 2019. DPD genotyping seems to be complementary. It has a much lower sensitivity, in particular to detect total deficiency but it is useful to document the case of deficiency and to define the dose to administer in case of partial deficiency. EMA is currently evaluating the clinical utility of both approaches and a conclusion is expected within the coming months. Time will tell.

References

(1) Tuchman et al. N. Engl. J. Med. 1985; 313: 245-9.
(2) Deenen et al. J. Clin. Oncol. 2016; 34: 227-34.
(3) Amstutz et al. Clin. Pharmacol. Ther. 2018; 103: 210-216.
(4) Henricks et al. Lancet Oncol. 2018; 19: 1459-1467.

(5) Boisdron-Celle et al. Cancer lett. 2007; 249: 271-82.
(6) Meulendijks et al. Br J Cancer. 2017; 116:1415-1424.
(7) Etienne-Grimaldi et al. PLoS One. 2017; 12(5):e0175998.