

Tailoring treatment with fluoropyrimidines (5 fluorouracil): towards personalised medicine

Fluoropyrimidines are molecules widely used in oncology, and are part of nearly 60% of chemotherapy protocols in the treatment of many cancers such as colorectal, oesophageal, stomach, breast, upper digestive tract or pancreatic cancer. They are used not only in metastatic situations but also, to an increasing extent, in adjuvant therapy, in patients treated for a localized tumour, with a risk of relapse.

These molecules (5-fluorouracil (5-FU), and also the oral prodrugs: capecitabine-Xeloda®, ftorafur-UFT®), although usually well tolerated are sometimes the cause of severe toxicities, essentially of digestive, hematopoietic and mucocutaneous types.

The early onset acute toxicities related to fluoropyrimidines are due to interindividual variability in their metabolism, mainly determined by the activity of dihydropyrimidine dehydrogenase (DPD), the major rate-limiting enzyme in the catabolism of fluoropyrimidines.

This very significant interindividual variability is not taken into account at present, which puts the patient at serious risk of toxicity or therapeutic inefficacy.

As a result, a new multiparametric approach has been developed which takes into account the pharmacogenetics, (polymorphisms of the gene involved in the metabolism of 5-FU), the metabolisation index (uracil, dihydrouracil) and the patient's physiological characteristics used in combination with treatment monitoring. This approach can be used to avoid severe toxic effects while ensuring therapeutic efficacy.

1. Metabolism of 5-FU

The metabolism of 5-FU is basically governed by the activity of an enzyme, DPD, whose activity is characterised by high interindividual variability (up to a factor of 6).

5-fluorouracil (5-FU) is principally eliminated after metabolisation, mainly via the liver, but also via the lungs. Only 5-10% of the administered dose is excreted in the urine in an unchanged form.

DPD is the rate-limiting enzyme in the metabolism of pyrimidines. This ubiquitous enzyme is responsible for the

catabolism of 85% of 5-FU. DPD allows the reduction of 5-FU to 5-fluoro-5,6-dihydrouracil (FUH2) and is also responsible for the transformation of natural pyrimidine bases (uracil and thymine) into their dihydrogenous derivatives. The second stage of catabolism involves another enzyme, dihydropyrimidinase.

DPD is subject to a genetic polymorphism, which is autosomal codominant. This polymorphism is responsible for major enzyme deficiencies (3 to 5%) or even total deficiencies (0.2-0.5%), which can have serious clinical complications.

This enzyme deficiency is multifactorial, however ; genetic polymorphisms are found in only 33 % of patients who present with early-onset severe toxicity, which does not explain the majority of these Grade 3 and 4 toxicities. The genotype is established by searching for single nucleotide polymorphisms (SNPs) on the DPYD gene which are known to play a major role in the inter-individual variability of patients' tolerance of fluoro-pyrimidines. The other factors governing the variability of DPD activity from patient to patient are not well established at this time, but the phenotype established by testing for the metabolites uracil and dihydrouracil provides a significant reflection of the patient's metabolic activity which is critical in confirming the risk of toxicity to fluoropyrimidines before treatment begins.

2. Screening for DPD deficient patients

Various approaches have been developed. Screening methods for patients with deficient DPD-activity can be divided into two broad classes: phenotypic and genotypic.

2.1 Phenotypic approach

Various tests have been evaluated; the best performing test combines pretreatment plasma levels of the endogenous natural uracil substrate (U) and its metabolite dihydrouracil (UH2). Both compounds are assayed in plasma by UPLC (*Ultra Performance Liquid Chromatography*) with a UV detector before treatment. This assay is used to determine, among other things, the metabolisation index (UH2/U).

2.2 Genotypic approach

This approach enables detection of SNPs on the *DPYD* gene. Various specific techniques can be applied: denaturing HPLC, pyrosequencing, *restriction length polymorphism* (RFLP) or the PCR technique with allelic discrimination.

More than 13,000 SNP's have been reported on the *DPYD* gene: most are inert, others, (a dozen or so) are located at important sites for enzyme activity. The most studied deleterious mutation (but not the most common: IVS14+1G>A) is located on the splice site near exon 14. It has been shown that the activity of the mutated protein is zero in homozygous subjects. In the heterozygous state, its activity is reduced by more than half compared to an unmutated subject, which is sufficient to induce severe toxicity to 5-FU. The most common mutations (in order of frequency) are:

- D949V - rs67376798 - exon 22: **1.8 %**;
- IVS14+1G>A - *DPYD**2 - rs3918290, intron 14: **1.2 %**;
- I560S - *DPYD**13 - rs55886062 - exon 13: **0.3 %**;
- Del TCAT - *DPYD**7 - exon 4: **0.3 %**.

2.3 Discussion

Currently, this multiparametric approach (5FU^{ODPMTox™}) for detecting DPD deficient patients is the most specific and sensitive. Phenotyping is highly sensitive but less specific, while the genotyping has an excellent specificity but a poor sensitivity. The two approaches, associated with physiological and pathophysiological characteristics of the patient are complementary and when combined (multiparametric approach), make it possible to detect 99% of DPD deficient patients. As a result, screening for high-risk patients for toxicity is made practical in clinical practice (results in 10 working days).

3. Therapeutic monitoring of 5-FU

After intravenous injection of 5-FU, plasma concentrations in 'extensive metabolizers' decrease rapidly with a 10 minute half-life. The plasma protein binding is low, of the order of 10%. Many authors have shown that the pharmacokinetics of 5-FU is non-linear and that there is substantial interindividual variation with an interindividual variation safety coefficient of equilibrium concentrations of 15 to 20%. This, coupled with the great variability of fluoropyrimidine metabolism means that plasma concentrations of 5-FU measured in treated patients vary significantly.

In a randomized study published in 2008 in the *Journal of Clinical Oncology*, the authors showed that for the same dose calculated according to body surface area, 40% of patients were under-dosed (risk of treatment failure) while 20% were overdosed (risk of toxicity).

Combined with prior screening for DPD deficiency, monitoring based on plasma levels of 5-FU can be offered to patients throughout their treatment in order to optimise treatment regimens.

4. Therapeutic applications

Screening for DPD deficiency does not limit itself to a simple result: deficient or non-deficient. The diagnosis of deficiency does not usually contraindicate treatment with fluoropyrimidines but requires a dose reduction and above all close pharmacological surveillance. Therapeutic advice is therefore essential to help the clinician to find the appropriate dosage of 5-FU.

With this combined approach of pretreatment screening for DPD deficits and pharmacokinetic monitoring (if the deficit is established), the percentage of patients suffering severe side effects falls from 20 - 25% to 0.6% and treatment efficacy is considerably improved. A retrospective health economics study presented at ASCO GI in January 2012 clearly demonstrated the economic benefits of screening.

5. Conclusion

Advances in pharmacology now make it possible to offer a pretreatment screening test for DPD deficiency and a therapeutic pharmacological monitoring for fluoropyrimidine therapy.

Prior DPD deficiency screening prevents severe toxicities by reducing the initial dose; subsequent therapeutic monitoring allows the dosage to be adjusted upwards if plasma concentrations are found to be insufficient. Algorithms are now available that combine several parameters related to the patient and treatment to offer therapeutic guidance.

In practice, a multiparametric toxicity assessment is carried out prior to fluoropyrimidine therapy (5-FU^{ODPMTox™}) combining phenotypic (U and UH₂) and genotypic approaches (test for the 4 most frequent mutations of the gene encoding DPD) as well as the various physiopathological parameters (age, weight, height, origin of the tumour...). During treatment by continuous infusion of 5-FU, 5-FU levels are determined (5-FU^{ODPMTox™}), the results of these doses are integrated into an algorithm and providing, in accordance with the therapeutic regimen, interpretation of results and dose adjustment guidance.

6. Contact Us

For more information about these tests or any of our other tests/services please contact Biomnis Ireland at:

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