Personalised medicine of fluoropyrimidines using DPYD pharmacogenetics

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Fluoropyrimidines, such as 5-fluorouracil (5-FU) and its oral pro-drug capecitabine, are widely used anti-cancer drugs in the treatment of several tumour types. Despite ample experience with these drugs, severe adverse drug reactions occur in up to 30% of patients treated with fluoropyrimidines. Over 80% of 5-FU is inactivated by the enzyme dihydropyrimidine dehydrogenase (DPD), which is encoded by the gene *DPYD*. Because of this, DPD plays an important role in the development of adverse drug reactions, mentioned here as toxicity. To prevent severe fluoropyrimidine-induced toxicity, it is important to identify patients who have an increased risk of toxicity and treat them in a personalised way. In other words, it is important to identify patients with a deficient DPD enzyme and treat them with reduced fluoropyrimidine dosages. Research has been executed on DPD deficiency, or variants in the *DPYD* gene, and the association with severe fluoropyrimidine-induced toxicity. This thesis focusses on reducing the risk of severe fluoropyrimidine-induced toxicity by optimising *DPYD* genotyping and improving implementation of *DPYD* genotyping in daily clinical care. In addition, we investigated DPD phenotyping and innovative genotyping techniques beyond current *DPYD* pharmacogenetics (PGx) to prevent severe fluoropyrimidine-induced toxicity.

***DPYD* genotyping: proof of principle and implementation in clinical practice**

Despite substantial evidence on the association between *DPYD* variants and the onset of severe fluoropyrimidine-induced toxicity, implementation of prospective *DPYD* genotyping in clinical practice remained limited. Therefore, an opinion review was written (chapter 2). In this review we summarize the available evidence on the association with severe fluoropyrimidine-induced toxicity for four variants in the *DPYD* gene. We discuss several advantages and disadvantages of *DPYD* genotyping. We substantiate why arguments against genotyping are unfounded and advocate implementation of prospective *DPYD* genotyping. In chapter 3 literature was extensively checked to discuss the functional effect of four *DPYD* variants on the DPD enzyme activity. This is converted into a gene activity score for each *DPYD* variant, which represents an expected remaining DPD enzyme activity, and which will be used in PGx guidelines to translate the *DPYD* genotype into a DPD phenotype. PGx guidelines by the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Pharmacists Association (KNMP) were already present in the Netherlands for *DPYD* and fluoropyrimidines. This guideline is made available outside of the KNMP network in the Netherlands in chapter 4, and provides a dose reduction advice for heterozygous *DPYD* variant allele carriers of the following four *DPYD* variants: *DPYD**2A, rs3918290, c.1905+1G>A, IVS14+1G>A; *DPYD**13, c.1679T>G, rs55886062, I560S; c.1236G>A/HapB3, rs56038477, E412E; and c.2846A>T, rs67376798, D949V. In addition to dosing guidelines, the DPWG also described an implication score in which *DPYD* genotyping is considered 'essential', directing *DPYD* genotyping prior to treatment with fluoropyrimidines.

DPYD genotyping was applied prospectively in a nationwide clinical trial in chapter 5. Patients with an intention to treatment with fluoropyrimidines were genotyped for *DPYD**2A, *DPYD**13, c.2846A>T and c.1236G>A. Heterozygous carriers of a *DPYD* variant were treated with an initially reduced dose of fluoropyrimidine according to the DPWG PGx guidelines at the start of the study. This study showed that prospective *DPYD* genotyping followed by individualised dose adjustments improved patient safety by reducing the risk

of severe fluoropyrimidine-induced toxicity. No treatment-related deaths occurred in *DPYD* variant allele carriers who were treated with a reduced dose. Despite the low frequency of *DPYD* variant allele carriers, executing prospective *DPYD* genotyping did not increase costs, but reduced average costs slightly with €50 per patient, as was shown in the cost analysis of the trial (chapter 6).

Current PGx guidelines do not distinguish fluoropyrimidine dosing recommendations between treatment regimens. Fluoropyrimidine dosages in chemoradiation therapy are substantially lower compared to fluoropyrimidine dosages in other treatment regimens. Therefore, it was unclear if further fluoropyrimidine dose reductions could result in underdosing in *DPYD* variant allele carriers treated with chemoradiation therapy. In chapter 7 we compared severe toxicity between wild-type patients and *DPYD* variant allele carriers who received chemoradiation therapy, the latter group either treated with standard or reduced fluoropyrimidine dosages. *DPYD* variant allele carriers treated with regular fluoropyrimidine doses in chemoradiation therapy experienced severe toxicity more often compared to *DPYD* variant allele carriers treated with reduced fluoropyrimidine doses in chemoradiation therapy, showing dose reductions are required as well in this treatment regimen.

The feasibility of implementing prospective *DPYD* genotyping in daily clinical care was shown in chapter 8 of this thesis. The first 21 months of *DPYD* genotyping at the Leiden University Medical Center (LUMC) were investigated, starting with the introduction as routine care in April 2013 until the end of the observation period in December 2014. This study showed that the implementation of *DPYD* genotyping was first characterised by a learning or acceptance curve, but was feasible in a real world clinical setting with 90–100% of the patients treated with fluoropyrimidines being genotyped. This study also showed 90% dose adherence.

Another aspect of (*DPYD*) genotyping is the certainty of a test result, and the consequences of an erroneous result. In chapter 9 we describe the dilemma of confirmation practice as a quality control aspect of PGx testing. We discuss if it should be required to have two independent genotyping assays to correctly determine a genotype. In this study we discovered that, even after extensive validation, erroneous results can still occur due to misclassification of a genotype, e.g. caused by allele dropout. Despite the increase in costs and labour, a confirmation method is useful for genetic tests with high clinical impact, such as *DPYD* testing. Clear guidelines will help to align confirmatory laboratory practices for pharmacogenetics, which may need to be specified per gene and test.

Beyond current *DPYD* pharmacogenetics

In the first part of this thesis we describe how to reduce severe fluoropyrimidine-induced toxicity by *DPYD* genotyping of *DPYD**2A, *DPYD**13, c.2846A>T and c.1236G>A. Yet, it is known not all severe fluoropyrimidine-induced toxicity can be predicted by these four variants alone. Therefore, we investigated other options, beyond genotyping of the current four *DPYD* variants, to reduce severe fluoropyrimidine-induced toxicity. This is shown in the second part of this thesis, entitled “beyond current *DPYD* pharmacogenetics”.

In chapter 10, we present a first-time head-to-head comparison study of four DPD

phenotyping assays in a patient cohort which was not selected based on –or enriched for– (severe) toxicity. The goal was to determine the clinical value of each DPD phenotyping assay, by assessing clinical validity parameters (e.g. sensitivity and specificity) for DPD deficiency and the onset of severe fluoropyrimidine-induced toxicity. We could not show associations with DPD deficiency or the onset of severe fluoropyrimidine-induced toxicity. To determine the clinical value of DPD phenotyping assays additional research is required.

In chapter 11 we investigated a special subgroup of *DPYD* variant allele carriers, i.e. the compound heterozygous patients. These patients carry multiple *DPYD* variants and the effect of the *DPYD* variants on the DPD enzyme activity cannot be predicted using the gene activity score. Without dose reductions, these patients have an increased risk to develop severe toxicity. We describe seven cases and examine diagnostic and therapeutic strategies for fluoropyrimidine treatment of patients carrying multiple *DPYD* variants. The additional genotyping methods investigated in this study are still in early phases of development or currently too expensive to implement in clinical care, compared to a well-established DPD-phenotyping test. Therefore, we concluded to execute a phenotype test in these patients to determine a safe starting dose.

It is expected that other enzymes besides DPD, and thus other genes besides *DPYD,* are involved in the onset of severe fluoropyrimidine-induced toxicity. With the genome-wide approach in chapter 12 we aimed to discover other variants, mainly outside the *DPYD* gene, which are associated to the onset of severe fluoropyrimidine-induced toxicity. Approximately 700,000 single nucleotide polymorphisms (SNPs) in different genes were genotyped and imputed to over four million SNPs. We identified six variants suggestive of association to the onset of severe fluoropyrimidine-induced toxicity. In addition, we present an optimistic polygenic risk score analysis, suggesting highly polygenic nature of toxicity predisposition.

With the execution of the clinical trial described in chapter 5, an increasing number of hospitals in the Netherlands applied *DPYD* genotyping prior to start of therapy. An increased uptake in implementation of *DPYD* genotyping was thus visible, especially in the Netherlands. Outside of the Netherlands, great differences exist in the uptake of *DPYD* genotyping, whether or not including DPD phenotyping. In some countries initiatives to implement prospective testing for DPD deficiency are effective, where in other countries great differences in execution of tests exist between centres within that country. Uptake of *DPYD* genotyping will benefit from clear guidelines, i.e. recommendations whom and when to genotype, and dosing recommendations for *DPYD* variant allele carriers.

Currently four *DPYD* variants are included in the genotyping panel, yet it is known these four variants cannot predict all patients who will develop severe toxicity. It is likely other variants are associated to the onset of severe fluoropyrimidine-induced toxicity. To further improve the predictive power of the genotyping panel DPD phenotyping tests can be used, or novel variants can be added to the genotyping panel. Novel variants can be e.g. rare variants in the *DPYD* gene or variants in other genes.

The future of fluoropyrimidines

5-FU has been used to treat cancer for decades. Now, capecitabine is the preferred drug of use over 5-FU in various tumour types in several countries, including the Netherlands.

To improve efficacy of cancer therapy, fluoropyrimidines are combined with several other anticancer drugs, yet they remain the backbone of therapy for a substantial number of tumour types. Ample research on fluoropyrimidines and DPD (deficiency) has been executed. Right now, prospective *DPYD* genotyping should be executed for all patients starting treatment with fluoropyrimidines. Additional research will be executed to continue the search for other factors which could predict the onset of severe fluoropyrimidine-induced toxicity.