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Personalised medicine of fluoropyrimidines using DPYD pharmacogenetics

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CHAPTER 13

General discussion and future perspectives

Introduction

Severe (grade ≥ 3) toxicity remains a significant problem in treatment with fluoropyrimidines such as 5-fluorouracil (5-FU) and capecitabine. Personalised medicine, specifically *DPYD* genotyping, is a promising strategy to predict and prevent severe fluoropyrimidine-induced toxicity. This thesis focusses on reducing the risk of severe fluoropyrimidine-induced toxicity by optimizing *DPYD* genotyping and improving implementation of *DPYD* genotyping in daily clinical care. In addition, we investigate DPD phenotyping and innovative genotyping techniques beyond current *DPYD* pharmacogenetics (PGx) to prevent severe fluoropyrimidine-induced toxicity.

Personalised medicine: why choose pharmacogenetics (PGx)?

Up to 30% of patients treated with fluoropyrimidines experience severe treatment-related toxicity. Besides the direct consequences of severe fluoropyrimidine-induced toxicity, it additionally can affect patients' quality of life and efficacy of the therapy can be reduced when treatment cannot be resumed due to toxicity. A major contributor to the onset of severe fluoropyrimidine-induced toxicity is a reduced activity of the enzyme dihydropyrimidine dehydrogenase (DPD), as has been described since the eighties in several case reports.¹⁻³ Patients with a complete deficiency for DPD are rare (~0.1%) and have shown neurological disorders, such as convulsion, seizures and epileptic attacks.⁴⁻⁷ Yet, there is great variation between patients. Also, patients who are partially DPD deficient generally do not show any phenotypic features. In order to predict and prevent severe fluoropyrimidine-induced toxicity, DPD deficient patients must be identified prospectively and treated individually (personalised medicine).

One way to identify DPD deficient patients, is to measure the DPD enzyme activity in peripheral blood mononuclear cells (PBMCs).^{2,8,9} However, the method is not widely used since feasibility in clinical practice is difficult due to substantial costs, complex sample logistics and specific equipment required for the radio assay. In addition, there is substantial intra patient variability (up to 25%) in DPD enzyme activity, possibly caused by circadian rhythm.^{10,11} An estimated 3–8% of the patients is DPD deficient. Therefore it is important to have inexpensive diagnostics for DPD deficiency, as all patients receiving fluoropyrimidines need to be tested while the majority of the tested patients does not require an adjusted dose or therapy. When a treatment plan has been decided, it is important to start the chemotherapy as soon as possible, thus short turn-around times of a test are essential as well.

Multiple genetic variants in *DPYD*, the gene encoding for DPD, lead to altered DPD enzyme activity.¹² Identifying such *DPYD* variants can indirectly identify DPD deficient patients. There are relatively quick, easy and inexpensive methods available to perform genotyping, therefore upfront *DPYD* genotyping can be used successfully to apply personalised medicine of fluoropyrimidines (pharmacogenetics, PGx).¹³ This was shown in a prospective clinical trial by Deenen *et al.*¹⁴ Prospective genotyping of the variant *DPYD**2A, followed by initial dose reductions in heterozygous carriers, reduced the risk of severe fluoropyrimidine-induced toxicity in these patients significantly. Also, this study showed that the genotyping approach did not increase costs, despite the fact that only 1.1% of tested patients was a carrier of the

*DPYD**2A variant. In chapter 5 and chapter 6, we have shown similar results, i.e. increasing patient safety without increasing treatment costs, for prospective genotyping of four *DPYD* variants (*DPYD**2A, rs3918290, c.1905+1G>A, IVS14+1G>A; c.1679T>G, *DPYD**13, rs55886062, I560S; c.1236G>A/HapB3, rs56038477, E412E; and c.2846A>T, rs67376798, D949V).^{15,16}

Feasibility of *DPYD* genotyping in daily clinical care was shown in chapter 8 of this thesis.¹⁷ *DPYD* genotyping at the Leiden University Medical Center (LUMC) was 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 thereafter in a real world clinical setting with 90-100% of the patients treated with fluoropyrimidines being genotyped. The dose adherence in this study was 90% instead of 100%, due to concerns of oncologists to reduce the dose in a *DPYD* variant allele carrier about to start chemoradiation therapy. The doubt was caused by the fact that fluoropyrimidine dosages in chemoradiation therapy are already lower compared to fluoropyrimidine dosages in other treatment regimens, and further reduction of the fluoropyrimidine dose could result in underdosing. To remove the uncertainty on fluoropyrimidine dose reductions in *DPYD* variant allele carriers who will receive chemoradiation therapy, we investigated this specific group in chapter 7.¹⁸ *DPYD* variant allele carriers treated with regular fluoropyrimidine doses in chemoradiation therapy experienced more severe toxicity 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 abovementioned studies show that *DPYD* genotyping to reduce severe fluoropyrimidine-induced toxicity is a useful strategy for all patients starting treatment with fluoropyrimidines. Both implementation of *DPYD* genotyping and adherence to a dose advice is feasible in a real world clinical setting.

Resistance and acceptance in implementation of *DPYD* genotyping

Despite substantial evidence on the association between *DPYD* variants and the onset of severe fluoropyrimidine-induced toxicity,¹⁹⁻²⁶ implementation of *DPYD* genotyping in clinical practice remained limited.^{27,28} To improve uptake of genotyping an opinion review (chapter 2) was written, in which arguments for and against genotyping were discussed.²⁹ One of these arguments against genotyping was that a randomized clinical trial (RCT) is necessary to obtain the required evidence on *DPYD* genotyping prior to implementation. As described in chapter 2, there was one attempt to perform such an RCT. Dose adjustments were applied based on the prospectively determined *DPYD* genotype and DPD phenotype of patients in arm A, compared to patients in arm B who were retrospectively analysed and treated with full dose. This trial was stopped prematurely due to ethical reasons, and was later published in 2017.³⁰ Patients were in fact not randomized, as inclusion in either study arm was dependent on current practice of each participating institution and some patients were thus predestined to receive treatment in the control arm. However, this large trial of the group of Boisdron-Celle *et al.* was closest to the set-up of an RCT thus far performed and results were long awaited for. Unfortunately, significant differences in the frequency of DPD

deficient patients between study arms at baseline were detected, with more DPD deficient patients in the retrospectively screened study arm. This results in bias and could lead to the expectation of lower toxicity in the prospectively screened study arm, regardless of applying their multi-parametric approach.³¹ Due to the available evidence on the increased risk of toxicity in DPD deficient patients or *DPYD* variant allele carriers, most researchers consider it unethical to perform an RCT and no further attempts are to be expected. Therefore, evidence from an RCT will never be gathered. In addition to this, it was debated that adequate (pharmacogenetic) evidence can also be provided by small-scale, innovative, prospective interventional studies,³² and indeed, some other predictive biomarkers were previously implemented in clinical care without evidence from an RCT.²⁹ In the study of Deenen *et al*, a historic cohort of patients who appeared to be carrier of *DPYD**2A after treatment with fluoropyrimidines, was used to compare severe toxicity between groups.¹⁴ The use of a historic cohort was applied as well in the clinical trial presented in this thesis (chapter 5).¹⁵ Considering ethics, this study set-up is the best possible method to collect evidence in a prospective way, since an RCT is not possible.

Besides the lack of evidence from an RCT, there are other arguments against *DPYD* genotyping. The fear of underdosing patients is an often used argument not to implement *DPYD* genotyping. However, both the study of Deenen *et al*. and our study (chapter 5) show that *DPYD* variant allele carriers who received initial dose reductions have comparable 5-FU levels or 5-FU metabolite levels to *DPYD* wild-type patients treated with a standard dose,^{14,15} therefore differences in efficacy are less likely. Secondly, treating physicians could increase fluoropyrimidine dosages in *DPYD* variant allele carriers during treatment based on the onset of severe toxicity (dose titration). In 55% of the *DPYD* variant allele carriers in whom the dose was increased during treatment, treatment had to be stopped or the dose had to be reduced again due to toxicity. Lastly, a recently published matched pair analysis by Henricks *et al*. showed no differences in efficacy, measured as overall survival and progression-free survival, between carriers of *DPYD**2A treated with a reduced dose and *DPYD**2A wild-type patients.³³ These results indicate that the fear of underdosing is unjustified.

Many of the arguments against *DPYD* genotyping can be refuted with the current evidence in favour of *DPYD* genotyping. Unfortunately, negative opinions on *DPYD* genotyping will always exist and maybe not everyone can be convinced. In 2010, Ciccolini *et al*. already pointed out that it was time to mandate the integration of systematic prospective testing for *DPYD* as part of routine clinical practice in oncology.²⁷ Yet, in order to align patient care, guidelines of health care authorities should be available.

Recommendations and guidelines

The Food and Drug Administration (FDA) state warnings or contraindications for the use of 5-FU or capecitabine in DPD deficient patients, however does not recommend to test for DPD deficiency.³⁴ No formal recommendations on DPD deficiency testing prior to treatment are given by health authorities, regulatory agencies or guideline committees from the National Comprehensive Cancer Network (NCCN) or American Society of Clinical Oncology (ASCO). In March 2018, the European Medicine Agency (EMA) has asked the involved pharmaceutical companies to update the Summary of Product Characteristics (SPC) of capecitabine by

including information on *DPYD* genotyping and the associated risk of severe fluoropyrimidine-induced toxicity.³⁵ An updated SPC, including a paragraph on *DPYD* genotyping, is attached to the European Public Assessment Report (EPAR) for capecitabine on the EMA website.³⁶ They state that genotyping of four variants is recommended, and variant carriers should be treated with extreme caution. Yet, it cannot be excluded that patients with a negative result can experience severe toxicity. The European Society for Medical Oncology (ESMO) explicitly states that they do not recommend upfront routine testing for DPD deficiency,³⁷ which was publicly questioned.^{38,39} In October 2018, the results of chapter 5 were presented at the ESMO conference and the presenter suggested to ESMO to update their guidelines. In the Netherlands, updated guidelines (September 2017) for colorectal carcinoma from the Dutch Society of Medical Oncology clearly state that *DPYD* genotyping is recommended prior to treatment with fluoropyrimidines.⁴⁰ These updated guidelines were of assistance in the uptake of prospective *DPYD* genotyping in the Netherlands, which implies that the lack of official recommendations on pre-therapeutic genotyping is limiting the process of implementation of *DPYD* genotyping in other countries.

Dosing recommendations for *DPYD* genotyping

There are several pharmacogenetic dosing guidelines available for the use of fluoropyrimidines in *DPYD* variant allele carriers published by the Clinical Pharmacogenetics Implementation Consortium (CPIC), the Dutch Pharmacogenetics Working Group (DPWG) established by the Royal Dutch Pharmacists Association (KNMP), the French Network of Pharmacogenetics (RNPGe) and the Italian Association of Medical Oncology (AIOM-SIF, *unpublished guidelines, edited by the AIOM-SIF Working Group*).⁴¹⁻⁴³ In addition to dosing guidelines, the DPWG also describes an implication score in which *DPYD* genotyping is considered 'essential', directing *DPYD* genotyping prior to treatment with fluoropyrimidines (chapter 4). Both CPIC and DPWG guidelines recommend to treat carriers of the *DPYD**2A and *DPYD**13 variants with a 50% dose reduction. CPIC recommended to treat carriers of the c.2846A>T and c.1236G>A variants with a 25–50% dose reduction due to limited evidence for these variants, compared to the DPWG who recommended a 25% dose reduction. These dose reductions are based on the functional effect of a variant on the DPD enzyme activity and represent an expected remaining DPD enzyme activity, as described in chapter 3.⁴⁴ However, after publication of chapter 5, both groups discussed the results of this study and the possibility to adjust the recommendation from a 25% dose reduction to a 50% dose reduction for variants c.2846A>T and c.1236G>A/HapB3. This has resulted in an update from CPIC published online November 2018, in which dose reductions of 50% are recommended for all four *DPYD* variants.⁴⁵ An update from the DPWG is expected soon and will be implemented in the guideline.

In chapter 5 we indeed describe that a 25% dose reduction seems inadequate to reduce the risk of severe toxicity in carriers of c.2846A>T and c.1236G>A to the risk of severe toxicity for *DPYD* wild-type patients. We could not provide evidence that a 50% dose reduction is the best option for these patients. In fact, for carriers of c.2846A>T a 35% dose reduction seems more logical, which is based on the median DPD enzyme activity (67% of *DPYD* wild-type patients) and the additional dose reductions made by physicians in carriers of c.2846A>T (average dose titration from 73 to 64% during treatment) in our clinical trial (*unpublished*

data). On the other hand, a 35% dose reduction for carriers of c.2846A>T is not proven to be more adequate compared to a 50% dose reduction. In addition, a 50% dose reduction would be more feasible in clinical practice. The c.1236G>A variant has a large variation in DPD enzyme activity with a median of 74% activity of *DPYD* wild-type patients in our study. However, our study showed that a 25% dose reduction in carriers of c.1236G>A did not result in a reduction of the relative risk for these patients, as some patients require a larger dose reduction.¹⁵ As was commented by Amstutz and Largiader, our study would support a 50% dose reduction in carriers of both c.2846A>T and c.1236G>A, provided that this should be used as a starting dose.⁴⁶ Further dose adaptations guided by the onset of toxicity (dose titration) are possible and should be applied slowly, as fluoropyrimidine-induced toxicity can occur with a certain delay.

Currently, there are no specific recommendations available on how to apply these additional dose adaptations. Recently, Kleinjan *et al.* retrospectively investigated dose escalations in *DPYD* variant allele carriers according to a local pre-specified protocol.⁴⁷ Eleven *DPYD* variant allele carriers were identified, of which six patients (55%) received a dose escalation of 15%. In two patients, the dose had to be reduced again due to toxicity, resulting in a median dose escalation of 9%. In two *DPYD* variant allele carriers (18%) the initially lower dose was further reduced. In the clinical trial (chapter 5) no pre-specified protocol was available for dose adjustments. We identified 85 *DPYD* variant allele carriers. In eleven patients (13%) the dose was increased by 21% on average, yet in five patients the dose had to be reduced again and one patient had to stop treatment, resulting in a mean dose escalation of 13%. In ten patients (12%) initially lower dosages were further reduced by 20% on average. Without a pre-defined protocol, the dose was increased in fewer patients, yet the dose adjustment steps were larger. The dose reductions applied after a dose escalation point out the importance of slowly applying dose escalations in relatively small steps. The additional dose reductions required after the low initial dose, again point out the variation in DPD enzyme activity in *DPYD* variant allele carriers, and could explain the higher overall severe toxicity rates in *DPYD* variant allele carriers of the clinical trial (39% versus 23% for wild-type patients).¹⁵

Dose adjustments after exposure to 5-FU or capecitabine

Therapeutic drug monitoring (TDM) is a useful method to guide dose adaptations after start of therapy. Unfortunately, the use of TDM for fluoropyrimidines in the Netherlands is limited as the wide majority of patients (approximately 90%) are prescribed capecitabine over 5-FU. For TDM of 5-FU defined target ranges and dosing algorithms are available.⁴⁸⁻⁵⁰ Yet, the intracellular conversion of capecitabine into 5-FU and its metabolites result in low plasma concentrations of capecitabine and its metabolites, which makes it more difficult to develop TDM protocols for capecitabine.⁵¹ Until such protocols have been established, TDM of fluoropyrimidines in the Netherlands will be used sparingly. Furthermore, TDM can be used to monitor drug levels after start of treatment, not to determine initial dose reductions in order to prevent quick-onset severe fluoropyrimidine-induced toxicity.

A method to determine if initial dose adaptations in patients are required, is to expose the patient prior to treatment to a 5-FU test dose of 250 mg/m².^{52,53} After the test dose, 5-FU

and 5-fluoro-5,6-dihydrouracil (5-FDHU) plasma levels are used to calculate pharmacokinetic parameters. In a study setting, three patients had marked alterations in pharmacokinetic parameters and possibly severe toxicity was avoided by changing the 5-FU treatment into irinotecan treatment.⁵² The 5-FU test dose did not result in side effects in any of the patients in this study, which questions the suitability of this test dose, as the metabolizing enzyme DPD has a certain overcapacity. As was stated by van Staveren *et al.*, a test dose of uracil of 500 mg/m² fully saturates the DPD enzyme.⁵⁴

Implementation of *DPYD* genotyping in the Netherlands

Three Dutch hospitals participated in the study of Deenen *et al.*, applying *DPYD**2A genotyping in over 2,000 recruited study patients between May 2007 and October 2011. Thereafter, more studies on *DPYD* variants and their association with the onset of severe fluoropyrimidine-induced toxicity became available. Within this period, some hospitals in the Netherlands implemented routine *DPYD* genotyping of all patients starting fluoropyrimidines, e.g. the LUMC in April 2013 and the Maastricht University Medical Center in 2013 as well.^{9,17} In April 2015 we started recruiting patients in our prospective study (chapter 5).¹⁵ Seventeen hospitals in the Netherlands participated in this study and implemented or outsourced *DPYD* genotyping either for study patients only or for all patients starting fluoropyrimidine treatment. In 2016, a survey was published in the Dutch Medical Oncology Journal.⁵⁵ This survey was sent to oncologists in the Netherlands. Some remarkable results were found. First, 65% of the responders answered that DPD status was determined as standard for all patients starting treatment with fluoropyrimidines. Second, 80% of the oncologists used *DPYD* genotyping to determine DPD deficiency, compared to 15% of responders who used a DPD phenotyping test. Possibly these results were a little overestimated, as physicians who had experience with requesting these tests were more likely to reply to the survey compared to physicians who did not order DPD deficiency tests. Also, the results of the survey were not adjusted based on the number of respondents per hospital, which could give a misleading image on the status of *DPYD* genotyping in the Netherlands in 2016. Yet, it is clear that the use of *DPYD* genotyping in the Netherlands is ahead of the use in many other countries. Some research groups in France, the UK, Italy, Germany and the USA were able to implement *DPYD* genotyping, whether or not combined with DPD phenotyping, in their hospital or clinical institute and surrounding centres.

Other aspects of implementation

Treatment costs for patients did not increase when applying prospective genotyping of *DPYD**2A, or *DPYD**2A, *DPYD**13, c.2846A>T and c.1236G>A, as was shown by Deenen *et al.* and in chapter 6 of this thesis.^{14,16} Expanding the genotyping panel from one variant to four variants did not increase the costs of genotyping much, while more patients at risk could be identified, and thus more (costs of) severe toxicity could be prevented. Currently, most hospitals can offer *DPYD* genotyping tests for approximately €100. Genotyping assays are becoming less expensive despite the addition of more variants to a genotyping panel, therefore it is expected that *DPYD* genotyping will probably remain cost-neutral. However, this holds to a current extend. If the panel of predictive variants becomes too large to be

genotyped with current genotyping techniques, and more expensive genotyping techniques need to be used, it is uncertain if *DPYD* genotyping remains cost-neutral. For example, at this moment sequencing the entire *DPYD* gene is too expensive to be used in a daily clinical care setting. Also, reimbursement for *DPYD* genotyping costs in the Netherlands is not (yet) covered by nationwide health care insurances. Therefore, hospitals in the Netherlands will cover costs in different ways, which leads to differences in health care between patients.

In chapter 9 we describe the dilemma of required confirmation practice as a quality control aspect of PGx testing.⁵⁶ Implementation of *DPYD* genotyping will benefit from the inexpensiveness of current genotyping arrays. Yet, as PGx tests are usually only executed once in a lifetime, it is of utmost importance to have a correct genotyping result. When applying the most adequate, but comprehensive, confirmation method, i.e. executing a second, independent genotyping assay, erroneous results can be discovered. 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 a high clinical impact, such as *DPYD* testing. We also showed substantial variability between laboratories in the use of a second, independent technique for PGx testing. As is the case for applying *DPYD* genotyping in the first place, clear guidelines are required to align confirmatory laboratory practices for PGx as well.

Currently, mostly assays testing single variants are used to genotype *DPYD*. In case of a compound heterozygous *DPYD* variant carrier, a patient who carries multiple different *DPYD* variants, the genotyping result cannot be translated into a dose recommendation when phasing information (the allelic location of variants) is missing. Compound heterozygous *DPYD* variant allele carriers are at increased risk of severe fluoropyrimidine-induced toxicity when dose reductions cannot be applied. In chapter 11, we describe seven cases and examine diagnostic and therapeutic strategies for fluoropyrimidine treatment of patients carrying multiple *DPYD* variants.⁵⁷ 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 in order to determine a safe starting dose. When genotyping techniques which can determine the phasing of variants, such as long-read sequencing, will become less expensive in the future and are implemented in clinical care, phasing of variants of compound heterozygous *DPYD* variant allele carriers will be known directly and these patients can be treated according to dosing guidelines.

The probability of identifying a compound heterozygous *DPYD* variant carrier is low, yet while completing this chapter, five other patients were discovered in several genotyping facilities in the Netherlands, showing that this is a clinically relevant issue. Some of these patients were identified prospectively, after which the advice was given to determine the DPD enzyme activity. One patient was a carrier of three *DPYD* variants (*DPYD**2A, c.2846A>T and c.1236G>A) and was treated safely with a 40% dose based on the results of an executed DPD enzyme activity measurement. The other patients were carriers of two *DPYD* variants in different combinations (*DPYD**2A + c.2846A>T, c.2846A>T + c.1236G>A and *DPYD**13 + c.2846A>T).

Beyond current *DPYD* genotyping

It is known that *DPYD* variants are not the only risk factor for DPD deficiency, and DPD deficiency is not the only risk factor for severe fluoropyrimidine-induced toxicity. Approximately 17% of patients experiencing severe fluoropyrimidine-induced toxicity can be identified as carriers of one of the four currently genotyped *DPYD* variants. 39-61% of the patients who experienced severe toxicity were identified as DPD deficient patients, thus it was estimated that less than half of the DPD deficient patients could be identified by the four currently genotyped *DPYD* variants.⁵⁸ In order to increase the predictability of severe fluoropyrimidine-induced toxicity, we must better predict risk factors for DPD deficiency, and additionally look into factors outside of DPD. Recently, a study was published in which eight years of combining genotyping and phenotyping tests were described.⁹ This study showed that only 25.3% of the DPD deficient patients was a carrier of one of the four currently genotyped *DPYD* variants. Patients with a DPD deficiency, but who did not carry the *DPYD**2A variant, were genotyped for the entire coding region of *DPYD*. DPD deficiency could be explained by *DPYD* variants in 23% of these patients. This results in an expected approximately 42% of DPD deficiency related to *DPYD* variants. Variants in other regions, which have not been sequenced before, could still contribute to DPD deficiency. Unfortunately, the abovementioned study had no toxicity data of the patients, thus the prediction of *DPYD* variants for DPD deficiency could be made, but not the prediction for severe toxicity.

It is clear that not only *DPYD* variants are involved in the onset of severe fluoropyrimidine-induced toxicity. Therefore the DNA of the patients participating in chapter 5 was analysed by genome-wide association study (GWAS), in order to discover novel variants related to the onset of severe fluoropyrimidine-induced toxicity. This study was described in chapter 12. Approximately 700,000 single nucleotide polymorphisms (SNPs) in different genes were genotyped, and imputed to over four million SNPs. While no genome-wide significant SNPs could be identified, six variants were suggestive for the onset of severe toxicity. These variants warrant replication in an independent cohort. After validation, variants can be added to the prospective genotyping panel. In addition to the variants in chapter 12, validation is required for all newly identified variants. For example, some newly identified variants were recently presented in a series of patients who experienced severe toxicity,⁵⁹ yet it is unclear if these variants could also be identified in patients who did not experience severe toxicity, and thus the clinical value of these variants needs to be determined. As described by Ciccolini *et al.* in 2010, both genetic and epigenetic factors, such as promotor hyper methylation or variations in transcriptional factor expression, play a role in *DPYD* dysregulations,⁶⁰ and should be a focus of future research in *DPYD* genotyping.

Phenotyping assays

DPD phenotyping could also be used to predict severe fluoropyrimidine-induced toxicity. As described before, the DPD enzyme activity measurement in PBMCs is a well-established method to determine DPD activity.^{2,8,9} Additionally, DPD phenotyping assays were developed, such as the 2-¹³C uracil breath test,⁶¹⁻⁶³ the uracil loading dose,^{54,64} endogenous dihydrouracil/uracil (DHU/U) ratio and endogenous uracil concentrations.^{65,66} The status of each DPD

phenotyping assay was summarized in two reviews.^{58,67} Advantages and disadvantages per assay were discussed, such as the limited feasibility of an assay in clinical practice, lack of calculated test parameters (i.e. sensitivity, specificity), or lack of clear threshold values for patients who are prone to develop severe fluoropyrimidine-induced toxicity. In chapter 10, we executed a first-time head-to-head comparison of four DPD phenotyping assays in a patient cohort which was not selected based on –or enriched for– (severe) toxicity, but represents a daily clinical care patient cohort. We could not show associations with DPD deficiency or the onset of severe fluoropyrimidine-induced toxicity. The latter is possibly due to the fact that only ~30–50% of severe fluoropyrimidine-induced toxicity can initially be explained by DPD deficiency.⁶⁸ Previously it was described that clinical validity and utility were not yet determined for all phenotyping assays,⁵⁸ yet with this study we were unable to fully complement this lack of evidence. In order to determine the clinical value of DPD phenotyping assays additional research is required. DPD phenotyping assays, whether or not combined with *DPYD* genotyping, are already used in clinical care in some centres to predict and prevent toxicity. Yet, it is clear that additional research should be performed in order to determine and compare the clinical value of DPD phenotyping assays.

FUTURE PERSPECTIVES

Dosing algorithms

It is clear that toxicity is not caused by a single factor, but is due to a combination of multiple risk factors. In order to be able to predict and prevent severe fluoropyrimidine-induced toxicity in a larger number of patients, multiple risk factors should be taken into account. An algorithm in which multiple factors are included, can be used to calculate the total risk of severe toxicity and potentially required dose adjustments. This algorithm should include the abovementioned four *DPYD* variants, as they are proven to be associated to the onset of severe toxicity. However, the algorithm should be expanded by including other factors.

In an ongoing study, we investigate rare variants in *DPYD* by means of sequencing, as they might be predictive for the onset of severe fluoropyrimidine-induced toxicity. Besides the current four *DPYD* variants, identified rare *DPYD* variants, variants outside of the *DPYD* gene, or variants in modifier gene regions, could be added to the algorithm in the future when their association with toxicity has been validated. Possibly, a large panel of genetic variants could be used to calculate the ‘genetic’ risk, so-called polygenic risk score, which is increasingly being applied in research. Depending on which variants from the panel are identified in the patient, the patient has a different risk to develop severe toxicity.

The algorithm could also be supplemented by non-genetic factors, as they can play a role in the onset of (severe) toxicity. For example, results of phenotyping assays for DPD or other enzymes involved in the metabolism of 5-FU related to severe toxicity,⁶⁹ could be included in the algorithm. In addition, baseline characteristics of patients, such as age, gender, performance status or renal dysfunction, were described as risk factors for toxicity.⁷⁰⁻⁷⁴ Also therapy-related factors, such as dosing schedule or co-medication, could influence the risk of toxicity.⁷⁵ Not all of the abovementioned risk factors have a similar effect on (severe) toxicity, therefore each risk factor included in the algorithm should have a corresponding

weighing factor, depending on the severity of the risk.

In addition to analysing *DPYD* genetics, baseline characteristics of patients and therapy-related factors to develop dosing algorithms, ethnicity should also be taken into account. The current four *DPYD* variants associated to the onset of severe fluoropyrimidine-induced toxicity are mainly identified in Caucasian patients. *DPYD**2A and c.2846A>T have been identified in ~0.1% in African-Americans, compared to a frequency of ~1% in Caucasians.⁷⁶⁻⁷⁸ Novel deleterious *DPYD* variants can be identified in different ethnic populations, as was recently shown for an East African population.⁷⁹ Dosing algorithms might not predict DPD activity correctly in patients who are not Caucasian, depending on the variants included in the algorithm.

Current genotyping techniques are mostly single SNP-based assays or chip-based assays. In the near future extensive sequencing techniques will become less expensive and more available for daily practice in the laboratories of hospitals, or hospitals can outsource genotyping to special genotyping facilities. An increasing amount of genotyping data of patients will be known in a shorter period of time, and should be linked to clinical patient data in order to first translate the genotype into a prediction for toxicity, and second, the data can be used to complement and perfect the algorithm. The question that remains is, can we build an algorithm which can predict the majority of severe fluoropyrimidine-induced toxicity? When all previously reported risk factors for toxicity are validated and included, and when the complete genotype of patients is taken into account, what risk factors will remain to be discovered?

The future of fluoropyrimidines

5-FU has been used to treat cancer for decades and the first studies on DPD deficiency were published in the eighties.¹⁻³ 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.

To conclude with the following quote by Hamzic *et al.*: “While additional genetic factors or phenotyping approaches may complement pharmacogenetic testing in the future, *DPYD* genotyping provides an important tool that is available today to identify patients at increased risk of severe adverse effects from fluoropyrimidine-based therapies”.⁸⁰

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