Personalised medicine of fluoropyrimidines using DPYD pharmacogenetics

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CHAPTER 2

Prospective *DPYD* genotyping to reduce the risk of fluoropyrimidine-induced severe toxicity: ready for prime time

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Abstract
5-Fluorouracil (5-FU) and capecitabine are among the most frequently prescribed anticancer drugs. They are inactivated in the liver by the enzyme dihydropyrimidine dehydrogenase (DPD). Up to 5% of the population is DPD deficient and these patients have a significantly increased risk of severe and potentially lethal toxicity when treated with regular doses of 5-FU or capecitabine. DPD is encoded by the gene *DPYD* and variants in *DPYD* can lead to a decreased DPD activity. Although prospective *DPYD* genotyping is a valuable tool to identify patients with DPD deficiency, and thus those at risk for severe and potential life-threatening toxicity, prospective genotyping has not yet been implemented in daily clinical care. Our goal was to present the available evidence in favour of prospective genotyping, including discussion of unjustified worries on cost-effectiveness, and potential underdosing. We conclude that there is convincing evidence to implement prospective *DPYD* genotyping with an upfront dose adjustment in DPD deficient patients. Immediate benefit in patient care can be expected through decreasing toxicity, while maintaining efficacy.

Case: fatal toxicity following treatment with capecitabine

A 52-year-old woman with human epidermal growth factor receptor 2 (HER2)-positive metastasised breast cancer was treated with capecitabine 1,250 mg/m^2 twice daily, for 14 days every three weeks, plus intravenous trastuzumab on day 1. The first cycle was fully completed; at day 18 of treatment mild diarrhoea and a herpes zoster infection located at her mouth were noticed during routine outpatient visit. Due to low haematological laboratory values (leucocytes, neutrophils CTC-AE grade 2, and thrombocytes CTC-AE grade 3), the second cycle was planned to be deferred by one week. However, three days later she returned to the hospital with now severe diarrhoea (CTC-AE grade 4), sepsis, neutropenic fever, severe leucopenia and life-threatening thrombocytopenia and mucositis, for which she was admitted to the intensive care unit. A long and intensive hospitalisation period followed, but despite optimal treatment and supportive care, the patient did not recover from severe toxicity and deteriorated even further. At day 34 of admission the patient deceased as a result of this severe toxicity. Genetic testing revealed that the patient was heterozygous for *DPYD**2A, a variant allele known to result in dihydropyrimidine dehydrogenase deficiency.[1] In case screening would have been performed prior to start of therapy, capecitabine dosage could have been reduced by 50%, thereby possibly preventing fatal capecitabine-induced toxicity.[2]

Introduction

5-Fluorouracil (5-FU) and its oral pro-drug capecitabine belong to the group of the fluoropyrimidine drugs, and are among the most frequently used anticancer drugs in the treatment of common cancer types such as colorectal, stomach, breast, head and neck and skin cancer.[3-7] 5-FU has a relatively narrow therapeutic index and, depending on type of treatment regimen, around 15–30% of patients suffer from severe toxicity such as diarrhoea, nausea, mucositis, stomatitis, myelosuppression, neurotoxicity and hand-foot syndrome.[4,8-12] These side-effects lead to mortality in approximately 0.5–1% of patients using 5-FU and capecitabine.[4,13]

The enzyme dihydropyrimidine dehydrogenase (DPD) plays a key role in the catabolism of 5-FU. It is the rate limiting enzyme degrading over 80% of the drug to its inactive metabolite 5-fluoro-5,6-dihydrouracil.[9,14,15] Because of this, DPD is an important factor for efficacy,[16,17] as well as the development of toxicity.[10] DPD is encoded by the gene *DPYD*, which consists of 23 exons on chromosome 1p22.[18] More than 160 single nucleotide polymorphisms (SNPs) are known within this gene, some resulting in altered enzyme activity.[19] Eighty *DPYD* variants were experimentally tested for their enzyme activity[20] and *DPYD* variants may result in an absolute or a partial DPD-deficiency (0.5% versus 3–5% of the population, respectively).[21,22] About 30–50% of the patients treated with a fluoropyrimidine drug who suffer from severe or life-threatening toxicity (grade 3–5) have no or decreased DPD enzyme activity, and 50–88% of patients carrying a variant in *DPYD* suffer from grade ≥3 fluoropyrimidine-related toxicity.[6,10,11,21,23-25]

Although pharmacogenomic tests in general have the potential to improve clinical outcome by increasing efficacy and decreasing toxicity, and the potential to decrease the cost of healthcare, their use in routine clinical practice is still limited.[26] This also holds true

for the use of *DPYD* genotyping prior to start of treatment with fluoropyrimidines.[27,28] Other DPD deficiency screening methods (e.g. phenotyping) have been described,[29] and are currently being investigated (NCT02324452), but we feel are not ready yet for clinical application. In the current paper, we present an overview on the evidence for prospective *DPYD* genotyping and discuss critical questions related to its implementation. Associations of *DPYD* variants with fluoropyrimidine-induced toxicity, prevention of severe toxicity upon *DPYD* testing, cost consequences and existing guidelines will be discussed.

Available evidence for the association of *DPYD* variants and 5-FU-induced severe toxicity

The relationship between *DPYD* variants and 5-FU-induced severe toxicity is widely acknowledged. Recently, data have been summarised in three separate meta-analyses.[8,9,30] Terrazzino *et al.* evaluated 4,094 patients (15 studies) for *DPYD**2A (IVS14+1G>A; rs3918290) and 2,308 patients for c.2846A>T (D949V, rs67376798). They confirmed the clinical validity of these SNPs as risk factors for the development of fluoropyrimidine-associated severe toxicities (details in Table 1).[9] The second meta-analysis, performed by Rosmarin *et al.*, included data of 4,855 patients (17 studies). They describe eight *DPYD* variants of which *DPYD**2A and c.2846A>T also showed convincing evidence of an association with toxicity (Table 1).[8] The third meta-analysis of Meulendijks *et al.*, included data of 7,365 patients (eight studies) and confirmed the association between severe toxicity and the variants *DPYD**2A and c.2846A>T, but also for *DPYD**13 (I560S; c.1679T>G; rs55886062) and c.1236G>A/HapB3 (E412E; rs56038477) (Table 1). Very recently, three additional papers, not part of the three meta-analyses, have confirmed significant associations between *DPYD* variants and toxicity (Table 1).[4,31,32] Although multiple variants of *DPYD* have been described, *DPYD**2A, *DPYD**13, c.2846A>T and c.1236G>A/HapB3 are the variants that are most extensively studied and convincingly associated with fluoropyrimidine-related severe toxicity.[8,9,30]

The HuGE risk translator[33] is an online tool to calculate test characteristics for the evaluation of the predictive ability of genetic markers. Data (e.g. odds ratio) from two of three meta-analyses described above could be entered as a 'two-risk genotype' for *DPYD**2A and c.2846A>T, resulted in low (~10 to ~25%) sensitivity and positive predictive values and high (>96%) specificity and negative predictive values (NPV). The number needed to screen (i.e. genotype) appears to be 210–250 patients and the number needed to treat (i.e. apply dose adjustments) is five or six patients (Table 2). Important to note is that values for diagnostic test criteria of a pharmacogenomic test based on SNPs in *DPYD* can never reach 100%, because not all DPD deficiencies and toxicity can be explained by variants in *DPYD*.[34] It must also be said that the high specificity (±98%) and high NPV (±96.5%) in this setting are most important, when the goal is to treat all patients with a variant (including false-positives). The consequence of a (false) positive result is a relatively low-risk dose-reduction for the first of many cycles, which can be adjusted in safe conditions in the second cycle and onwards if no toxicity occurs. The consequence of a false negative result may be much larger since it could result in a too high systemic drug exposure that subsequently leads to severe, potentially lethal toxicity, which is associated with long-lasting hospital and/or intensive care unit (ICU) admissions.

In a previous study approximately 10% of the *DPYD**2A variant allele carriers treated with the standard fluoropyrimidine dose deceased as a result of drug-induced severe toxicity.[35] The approach of pre-treatment genotyping followed by a reduced starting dose plus tolerance-guided dose titration could prevent the occurrence of severe toxicities in *DPYD* variant allele carriers, resulting in a direct safer use with minimum risk of underdosing. The above mentioned test characteristics are reached using the two most investigated SNPs and these values will probably improve when a larger panel of *DPYD* SNPs is probed. Costs are not likely to increase substantially when adding SNPs because genotyping costs continue to decrease.[36,37] Although more *DPYD* variants that alter DPD enzyme activity are continuously discovered and studied, the perfect set of SNPs has not been defined yet. Currently we feel there is substantial evidence to support dose recommendations for at least four variants (*DPYD**2A, c.2846A>T, *DPYD**13 and c.1236G>A/HapB3).[38] Another possibility for prospective screening could be the more informative, but hugely more expensive genotyping of the entire coding region of *DPYD*. However we have focused on genotyping SNPs. To date, SNP genotyping has been most extensively studied, is technically feasible in a general hospital setting and multiple guidelines providing SNP-based dose recommendations are available.

What is needed for implementation of *DPYD* genotyping in daily routine clinical care?

Clinical implementation of a biomarker test such as *DPYD* pharmacogenomics is hampered due to the on-going discussion on whether a randomised clinical trial (RCT) is considered necessary to provide the required evidence before clinical implementation.[26,29,37,39-45] Despite the fact that RCTs are considered the gold standard study design to prove effectiveness, adequate evidence can also be provided by small-scale, innovative, prospective interventional studies.[40] However, with the available evidence favouring upfront genotyping, it may not be ethically feasible to randomise patients, and patients may not be willing to be included in the control arm with an increased risk for severe toxicity. Indeed, the only attempt at a prospective randomised study was performed in France. Boisdron-Celle *et al.* presented a multicentre prospective cohort study of upfront DPD deficiency screening executed from 2008 until 2012.[46] The purpose of the study was to confirm the medical and economic aspect of upfront DPD deficiency screening in a prospective way as was done retrospectively by Traoré *et al*.[47] Patients using 5-FU based chemotherapy were included in one of two parallel patient cohorts (arm A and arm B). Patients in arm A were prospectively screened for DPD-deficiency (a combined genotyping and phenotyping approach), and patients in arm B were retrospectively tested. A total of 1,130 patients were included (arm A: 720 patients, arm B: 410 patients). One patient died due to 5-FU early-onset toxicity and it was retrospectively confirmed that this patient was DPD deficient (arm B). The enrolment of patients was prematurely closed for ethical reasons, because of the proven 5-FU-induced toxic death of this patient.[46,48] Against this background, we conclude that evidence from a randomised prospective clinical trial on *DPYD* genotyping will never be acquired for ethical reasons. In addition, some predictive biomarkers were previously implemented without evidence from an RCT. Clinical use of (K)RAS selection for EGFR therapy was influenced by updated registration texts for epidermal growth factor receptor (EGFR) inhibitors from

the Food and Drug Administration (FDA)[49] and European Medicines Agency (EMA) after retrospective analyses of three studies (CRYSTAL trial, OPUS trial and CA225025).[50-52] Also hormone receptor status for hormone therapy in breast cancer has never been proven in a prospective randomised study.

Table 1. Toxicity associations of *DPYD* variants

Group	*DPYD* variant	Association with 5-FU and/or capecitabine grade ≥3 toxicity (OR/*RR [95% CI], p-value)
Terrazzino *et al.* 2013[9]	*DPYD**2A (rs3918290)	Overall toxicity (5.42 [2.79–10.52], p<0.001) Diarrhoea (5.54 [2.31–13.29], p<0.001) Haematological toxicity (15.77 [6.36–39.06], p<0.001) Mucositis (7.48 [3.03–18.47], p<0.001)
	c.2846A>T (rs67376798)	Overall toxicity (8.18 [2.65–25.25], p<0.001) Diarrhoea (6.04 [1.77–20.66], p=0.004)
Rosmarin *et al.* 2014[8]	*DPYD**2A (rs3918290)	Overall toxicity (6.71 [1.66-27.1], p=0.0075) (5-FU in.) Diarrhoea (7.71 [1.61–36.9], p=0.011) (5-FU in.) Mucositis/stomatitis (7.15 [1.75–29.1], p=0.0061) (5-FU bo.) Neutropenia (12.90 [3.13–53.3], p=0.00040) (5-FU bo.)
	c.2846A>T (rs67376798)	Overall toxicity (9.35 [2.01–43.4], p=0.0043) (cap) Diarrhoea (3.14 [0.82–11.9], p=0.093) (cap) Hand-foot syndrome (1.31 [0.35–4.96], p=0.69) (cap)
	*DPYD**2A (rs3918290) c.2846A>T (rs67376798)	Overall toxicity (5.51 [1.95–15.51], p=0.0013) (cap)
Meulendijks *et al.* 2015[30]	*DPYD**2A (rs3918290)	Overall toxicity (*2.85 [1.75–4.62], p<0.0001)
	c.2846A>T (rs67376798)	Overall toxicity (*3.02 [2.22–4.10], p<0.0001)
	*DPYD**13 (rs55886062)	Overall toxicity (*4.40 [2.08–9.30], p<0.0001) Gastrointestinal toxicity (*5.72 [1.40–23.33], p=0.015) Haematological toxicity (*9.76 [3.03–31.48], p=0.00014)
	c.1236G>A/HapB3 (rs56038477)	Overall toxicity (*1.59 [1.29–1.97], p<0.0001) Gastrointestinal toxicity (*2.04 [1.49–2.78], p<0.0001) Haematological toxicity (*2.07 [1.17–3.68], p=0.013)
Rosmarin *et al.* 2015[4]	rs12132152 (AF: 0.03)	Overall toxicity (3.83 [3.26–4.40], $p=4.31*10^{-6}$) (cap) Hand-foot syndrome (6.12 [5.48–6.76], $p=3.29*10^{-8}$) (cap) Diarrhoea (0.44 [0–1.32], p=0.065) (cap)
	rs12022243 (AF: 0.22)	Overall toxicity (1.69 [1.45–1.94], $p=2.55*10^{-5}$) (cap) Hand-foot syndrome (1.43 [1.16–1.7], p=0.0096) (cap) Diarrhoea (1.79 [1.54–2.05], $p=9.86*10^{-6}$) (cap)
Rosmarin *et al.* 2015[4]	rs76387818	Overall toxicity (4.05 [3.47–4.62], $p=2.11*10^{-6}$) (cap) Hand-foot syndrome (6.44 [5.79–7.09], $p=1.75*10^{-8}$) (cap) Diarrhoea (0.44 [0–1.33], p=0.071) (cap)
	rs7548189	Overall toxicity (1.67 [1.43–1.91], $p=3.79*10^{-5}$) (cap) Hand-foot syndrome (1.42 [1.15–1.69], p=0.011) (cap) Diarrhoea (1.21 [0.84–1.58], p=0.0015) (cap)

table continues

Group	*DPYD* variant	Association with 5-FU and/or capecitabine grade ≥3 toxicity (OR/*RR [95% CI], p-value)
Falvella *et al.* 2015[32]	c.496A>G (rs2297595)	Overall toxicity (5.94 [1.29–27.22], p=0.022) (cap)
	c.1896T>C (rs17376848)	Overall toxicity (14.53 [1.36–155.20], p=0.027) (cap)
Joerger *et al.* 2015[31]	c.1896T>C (rs17376848) c.85T>C (rs1801265) c.2846A>T (rs67376798)	Diarrhoea (p<0.05) (cap) Hand-foot syndrome (p<0.02) (cap)

Brief summary of a few selected studies showing the results of *DPYD* variants and their associations with 5-FU and/or capecitabine induced severe toxicity. Included are three meta-analyses and three more recent papers. Results originating with only 5-FU or only capecitabine are explicitly marked. Rosmarin *et al.* have also tested 5-FU infusion and 5-FU bolus separately. Meulendijks *et al.* have described RR values, not OR values, as shown by *.

Abbreviations: 5-FU: 5-fluorouracil; in: infusion; bo: bolus; cap: capecitabine; CI: confidence interval; OR: odds ratio; RR: relative risk; AF: allele frequency.

Table 2. Test characteristics of genotyping for *DPYD2A and c.2846A>T**

Test characteristics	Terrazzino *et al.*[9]	Rosmarin *et al.*[8]
Sensitivity	14.5%	11.8%
Specificity	97.6%	98.4%
Positive predictive value	19.8%	23.6%
Negative predictive value	96.5%	96.4%
Number needed to screen (i.e. genotype)	210 patients	251 patients
Number needed to treat (i.e. apply dose adjustments)	6 patients	5 patients

Clinical utility test characteristics of genotyping for *DPYD**2A and c.2846A>T, calculated using "The HuGE Risk translator"[33] for Terrazzino *et al*. and Rosmarin *et al*.

Clinical implementation of DPD deficiency testing

Advantages and disadvantages of phenotyping and genotyping as possible DPD deficiency screening methods were described previously[29] and several institutes[53-59] have executed (prospective) screening of *DPYD* variants or DPD deficiency in a study context. Unfortunately, available literature of clinical implementation remains limited to only a few centres in France, Germany, the Netherlands, Ireland and the United States of America (USA).[44,53,60,61] An established and well-recognised *DPYD* clinical implementation program is that of the 'Institut de Cancerologie de l'Ouest' in Angers (France) where screening for DPD deficiency has been a regular procedure for over 10 years. Besides this institute, over 100 centres in France use the 'Onco Drug Personalized Medicine' or ODPM Tox™ and 2,000 patients are being screened with this approach every year.[62,63] Boisdron-Celle *et al.* describe a large trial in which 11,104 patients were prospectively screened (combining genotyping and phenotyping) and patients with a *DPYD* variant or decreased DPD activity received an individual dose adjustment. Genotyping in the trial consisted of 24 mutations in *DPYD*

and phenotyping included the DHU/U ratio. Two hundred forty seven patients with grade 3–5 toxicity were retrospectively tested. In total, 3% of all patients carried one or more mutations. Twenty seven out of 247 retrospectively tested patients died of whom 16 (59%) and 24 (89%) were identified with genotyping or phenotyping, respectively. The combined approach would have identified 98% of grade 3–5 toxicity patients and 100% of mortalities.[63]

(Cost) Effectiveness of DPD deficiency testing

A prospective, multicentre study was conducted by Deenen *et al.,* in which 2,038 patients were screened for *DPYD**2A prior to start with 5-FU or capecitabine.[64] Twenty-two patients (1.1%) were heterozygous carriers of *DPYD**2A and patients received an initial dose reduction of 50% when starting therapy, followed by dose titration based on clinical tolerance. Toxicity results showed that the risk of grade ≥3 toxicity was significantly reduced to 28% compared to 73% in historical controls ($p<0.001$). Drug-induced death reduced from 10% to 0%. This study convincingly shows that pre-treatment genotyping of *DPYD**2A followed by dose adjustment in carrier patients improves patient safety. A cost analysis was executed using a decision analytic model from a health care payer perspective, including only direct medical costs. Genotyping costs were €75 per test. The average total treatment cost per patient was slightly lower for screening (€2,772) than for non-screening (€2,817). The approach was shown to be feasible in routine clinical practice.[64] Ahmed *et al.* presented a cost analysis of a retrospective screening for four *DPYD* variants in 31 patients who experienced grade 3–5 toxicity. Five patients carried a variant and were admitted to the ICU due to toxicity. The costs of hospital admission (€155,083) were much higher than the screening costs of all patients starting with fluoropyrimidine therapy for CRC during the study period (€26,800).[53] Another retrospective study of 48 patients shows cost effectiveness with *DPYD* screening costs for four variants being almost nine times lower than hospital admissions of four patients (£1,776 versus £15,525; approximately €2,500 versus €21,500).[58] We must bear in mind that genotyping technology is developing fast and prices continue to decline.[37] Phenotyping tests have been recently reviewed by van Staveren *et al.*, and to our knowledge, to date no additional cost-effectiveness analysis for a phenotyping test has been published.[29]

Recommendations and guidelines of *DPYD* pharmacogenomics

Warnings or contraindications for using 5-FU/capecitabine in DPD deficient patients are stated by the FDA and EMA.[65,66] This is meaningless without knowing, and thus testing a patient for DPD deficiency. No formal recommendations on pre-therapeutic (upfront) screening for DPD deficiency are given by health authorities, regulatory agencies or guideline committees from the National Comprehensive Cancer Network or American Society of Clinical Oncology. The European Society for Medical Oncology explicitly states that they do not recommend upfront routine testing for DPD deficiency despite the risk of severe and potential lethal toxicity.[67] It is unknown to us what arguments underlie this recommendation. Only in cases of severe toxicity due to 5-FU treatment DPD deficiency screening is strongly recommended, and exposure to standard dose of 5-FU is contraindicated in proven DPD deficiency patients, according to guidelines published in 2012.[67] The lack of official recommendations on pre-therapeutic genotyping is limiting the process of implementation. One of the reasons may

be that such a recommendation is drug-specific and not tumour-type specific while oncology guidelines are traditionally tumour-type specific (e.g. KRAS mutation, human epidermal growth factor receptor 2 (HER2) expression).

The Clinical Pharmacogenetics Implementation Consortium and the Dutch Pharmacogenetics Working Group of the Royal Dutch Pharmacists Association provide evidence-based guidelines and recommendations what dose adjustments to apply in *DPYD* variant allele carriers.[37,68,69] Recommendations depend on the *DPYD* allele and carrier status (heterozygous, homozygous), and are guided by the gene activity score. After initial reduction dosages can be further titrated based on clinical tolerance. Dose reductions are 75, 50 or 25% for gene activity scores of 0.5, 1 and 1.5, respectively. The gene activity score varies from 0 (no DPD activity) to 2 (normal DPD activity).[38,69]

Barriers for clinical implementation

Potential barriers hampering the clinical implementation of prospective *DPYD* testing are:

'Perceived lack of scientific evidence';

The evidence for the association of *DPYD* variants and severe fluoropyrimidine-induced toxicity has been discussed and is considered convincing. Furthermore, an RCT is considered unethical and unnecessary.

'There is a lack of laboratory facilities and there is no reimbursement';

The number of laboratories that offer genetic testing for *DPYD* is continuously increasing, techniques are easier to operate and prices for genetic testing will continue to decrease.[37] The cost of a *DPYD* genetic test is currently in the range of €50 to €100. These amounts are negligible compared to the costs of treatment that could easily reach €10,000 or more.[70] This genetic test (which is a once-in-a-lifetime test when no additional SNPs are added) should be as normal as testing for other contraindications for drugs such as liver enzymes, renal function or physical condition. Laboratories usually offer the test with a turnaround time of 2–3 days which is acceptable and does not result in treatment delay, which is a serious concern of clinicians and patients.

'There is not enough guidance on how to use the test';

Peer reviewed guidance on how to use the outcomes of the genetic test is well covered.[37,38,68,69]

'There is a risk of underdosing patients';

Guidelines advise to reduce the dose of fluoropyrimidines in the first cycle in patients carrying *DPYD* variants associated with decreased DPD activity to create similar systemic drug levels compared to wild-type patients. In the following cycles tolerance-guided dose titration is used to create the most optimal treatment. This strategy minimises the risk for underdosing. In addition, 5-FU and capecitabine are often used in combination with other anti-cancer drugs, so only a fraction of the total therapy is reduced.

'Phenotyping tests are more specific';

Phenotyping tests measuring DPD enzyme activity directly are more closely predicting DPD deficiency as compared to *DPYD* genotyping. However, DPD enzyme measurements are also more expensive, more time consuming, have dreadful logistics (can be time-dependent), high turnaround-times (>1 week) and only a very limited number of laboratories provide the tests. For these reasons DPD enzyme activity measurements are less likely to be

implemented as a routine clinical test compared to the genotyping test.

'Genetic screening does not predict DPD deficiency perfectly';

Patients who do not carry a *DPYD* variant can still develop severe side-effects and patients carrying a *DPYD* variant do not necessarily develop toxicity. Clearly, as with other drugs, other patient and treatment characteristics also influence the risk of severe toxicity. The sensitivity and specificity shall for this reason never reach 100% as discussed above. In the USA, with a population of 300 million, there are 1,300 deaths each year due to 5-FU induced toxicity.[71] More than half of the deceased patients could have been identified using genotyping according to Boisdron-Celle *et al*.[63]

Summary

Although pharmacogenomics in general has the potential to result in safer use of drugs by supporting individualised therapy, this unfortunately has not resulted in clinical implementation of *DPYD* screening in the oncology field. Based on the available evidence, we argue that upfront *DPYD* screening using a pharmacogenomic test in patients planned to be treated with a fluoropyrimidine should become the standard of care. Treatment with fluoropyrimidines has been the cornerstone chemotherapy for several oncological indications for more than 50 years, and will probably continue to stay so. With the increasing incidence of cancer the number of patients who are likely to be treated with a fluoropyrimidine drug will increase, as well as the number of patients that would be saved from 5-FU or capecitabine induced severe toxicity when using pre-treatment genetic screening. 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.[10] Based on the arguments given above we truly believe it is time to add upfront *DPYD* genotyping to the current guidelines and to start implementation of *DPYD* screening without further delay. When upfront testing followed by dose adjustments is fully functional as part of routine clinical practice we can expect that grade ≥3 fluoropyrimidine-related toxicity substantially decreases without the risk of underdosing.

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