Short Report

Capecitabine-based treatment of a patient with a novel *DPYD* genotype and complete dihydropyrimidine dehydrogenase deficiency

Linda M. Henricks [1], Ester J.M. Siemerink[2], Hilde Rosing[3], Judith Meijer[4], Susan M.I. Goorden[4], Abeltje M. Polstra[4,5], Lida Zoetekouw[4], Annemieke Cats[6], Jan H.M. Schellens[1,7] and André B.P. van Kuilenburg[4]

[1] Division of Pharmacology and Division of Clinical Pharmacology, Department of Medical Oncology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

[2] Department of Internal Medicine, Ziekenhuis Groep Twente (ZGT), Hengelo, The Netherlands

[3] Department of Pharmacy and Pharmacology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

[4] Laboratory Genetic Metabolic Diseases, Departments of Clinical Chemistry and Clinical Genetics, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

[5] Department of Clinical Genetics, VU University Medical Center, Amsterdam, The Netherlands

[6] Department of Gastrointestinal Oncology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

[7] Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands

Fluoropyrimidines are frequently used anti-cancer drugs. It is known that patients with reduced activity of dihydropyrimidine dehydrogenase (DPD), the key metabolic enzyme in fluoropyrimidine inactivation, are at increased risk of developing severe fluoropyrimidine-related toxicity. Upfront screening for DPD deficiency and dose reduction in patients with partial DPD deficiency is recommended and improves patient safety. For patients with complete DPD deficiency, fluoropyrimidine-treatment has generally been discouraged. During routine pretreatment screening, we identified a 59-year-old patient with a sigmoid adenocarcinoma who proved to have a complete DPD deficiency. Genetic analyses showed that this complete absence of DPD activity was likely to be caused by a novel *DPYD* genotype, consisting of a combination of amplification of exons 17 and 18 of *DPYD* and heterozygosity for *DPYD**2A. Despite absence of DPD activity, the patient was treated with capecitabine-based chemotherapy, but capecitabine dose was drastically reduced to 150 mg once every 5 days (0.8% of original dose). Pharmacokinetic analyses showed that the area under the concentration-time curve (AUC) and half-life of 5-fluorouracil were respectively tenfold and fourfold higher than control values of patients receiving capecitabine 850 mg/m^2. When extrapolating from the dosing schedule of once every 5 days to twice daily, the AUC of 5-fluorouracil was comparable to controls. Treatment was tolerated well for eight cycles by the patient without occurrence of capecitabine-related toxicity. This case report demonstrates that a more comprehensive genotyping and phenotyping approach, combined with pharmacokinetically-guided dose administration, enables save fluoropyrimidine-treatment with adequate drug exposure in completely DPD deficient patients.

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What's new?

Patients with reduced activity of dihydropyrimidine dehydrogenase (DPD) are at increased risk of developing severe fluoropyrimidine drug-related toxicity. Here, the authors describe a case where a patient was identified with a complete DPD deficiency caused by a novel *DPYD* genotype, i.e. amplification of exons 17 and 18 in combination with *DPYD**2A. Pharmacokinetic analyses showed that the chosen dose reduction of capecitabine to 150 mg per 5 days resulted in adequate drug exposure. This case report demonstrates that a more comprehensive genotyping and phenotyping approach, combined with pharmacokinetically-guided dose administration, enables the safe treatment of completely DPD deficient patients with fluoropyrimidines.

Introduction

The fluoropyrimidine anti-cancer drugs 5-fluorouracil (5-FU) and its oral prodrug capecitabine are widely used for the treatment of several solid tumor types. After oral administration, capecitabine is rapidly converted into 5-FU through a three-step conversion. Approximately 80–90% of 5-FU is inactivated in the liver by the enzyme dihydropyrimidine dehydrogenase (DPD) and DPD is, therefore, considered to be the key enzyme in the catabolism of 5-FU.[1] DPD activity has shown to be highly variable in the population, with an estimated 3% to 5% of the population being partially DPD deficient.[2,3] Patients with reduced DPD activity have an increased risk of developing severe and potentially fatal fluoropyrimidine-associated toxicity, when treated with a full dose of capecitabine or 5-FU.[4] Reduced DPD activity can often be attributed to the presence of pathogenic single nucleotide polymorphisms (SNPs) in *DPYD*, the gene encoding for the DPD enzyme. Four *DPYD* SNPs that are currently considered clinically relevant are *DPYD**2A (c.1905 + 1G > A, IVS14 + 1G > A), c.1679T > G, c.2846A > T and c.1236G > A/HaplotypeB3.[4] Dose reduction of capecitabine and 5-FU is recommended in heterozygous carriers of these variants.[5] Upfront screening for *DPYD**2A and dose reduction in heterozygous carriers has shown to improve patient safety.[6] For patients with complete DPD deficiency, such as patients homozygous for *DPYD**2A, fluoropyrimidine-containing regimens have been discouraged and, therefore, potentially effective anti-cancer treatment is withheld.

The combined sensitivity of these four risk variants to predict severe fluoropyrimidine-associated toxicity remains low and there is increasing awareness that additional rare variants may collectively explain an appreciable fraction of DPD deficient patients.[7] Therefore, other approaches to detect DPD deficiency, including more extensive *DPYD* genotyping or DPD phenotyping methods are gaining attention. A DPD phenotyping approach that is often used is *ex vivo* quantification of DPD activity in peripheral blood mononuclear cells (PBMCs).[8]

Here we describe a patient with a novel *DPYD* genotype and complete DPD deficiency, that was safely treated with a pharmacokinetically-guided administration of capecitabine. Our study demonstrates that a more comprehensive genotyping and phenotyping approach, combined with pharmacokinetically-guided dose administration, enables the save treatment of completely DPD deficient patients with fluoropyrimidines.

Material and Methods

Patient

The patient was identified during routine pretreatment screening and was treated as part of individualized standard medical care, not part of a clinical trial. Toxicity was scored according to the Common Terminology Criteria for Adverse Events (CTC-AE) version 4.03. Blood and urine samples for genetic, DPD phenotyping and pharmacokinetic analyses were collected with the aim of supporting clinical decision making. The patient gave written informed consent for use of data for scientific publication.

DPD enzyme activity assay and pyrimidine metabolites

PBMCs were isolated as described before from peripheral blood collected in an EDTA tube.[8] The activity of DPD was determined in a reaction mixture containing 35 mM potassium phosphate (pH 7.4), 2.5 mM MgCl$_2$, 1 mM dithiothreitol, 250 μM NADPH and 25 μM [4-^{14}C]-thymine. Separation of radiolabeled thymine from radiolabeled dihydrothymine was performed by reversed-phase high-performance liquid chromatography (HPLC) with online detection of the radioactivity.[8] Concentrations of uracil and thymine (endogenous substrates of DPD) in plasma and urine were determined using reversed-phase HPLC hyphenated with electrospray tandem mass spectrometry (MS/MS).[9,10]

PCR amplification and sequence analysis of coding exons of *DPYD*

DNA was isolated from whole blood using the Nucleospin Tissue kit (Macherey-Nagel, Dünen, Germany). Polymerase chain reaction (PCR) amplification of all 23 coding exons and flanking intronic regions of *DPYD* was carried out using intronic primer sets, as described before.[7] Sequence analysis of genomic fragments amplified by PCR was carried out on an Applied Biosystems model 3730 automated DNA sequencer using the dye-terminator method (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). The *DPYD* sequence of the DPD deficient patient was compared to those observed in controls and the reference sequence of *DPYD* (Ref Seq NM_000110.3; Ensembl ENST00000370192).

MLPA and SNP array analysis

The multiplex ligation-dependent probe amplification (MLPA) test for *DPYD* (P103, MRC-Holland, Amsterdam, The Netherlands) contains 38 probes for *DPYD*, including one probe to detect the *DPYD**2A variant, and nine control probes specific
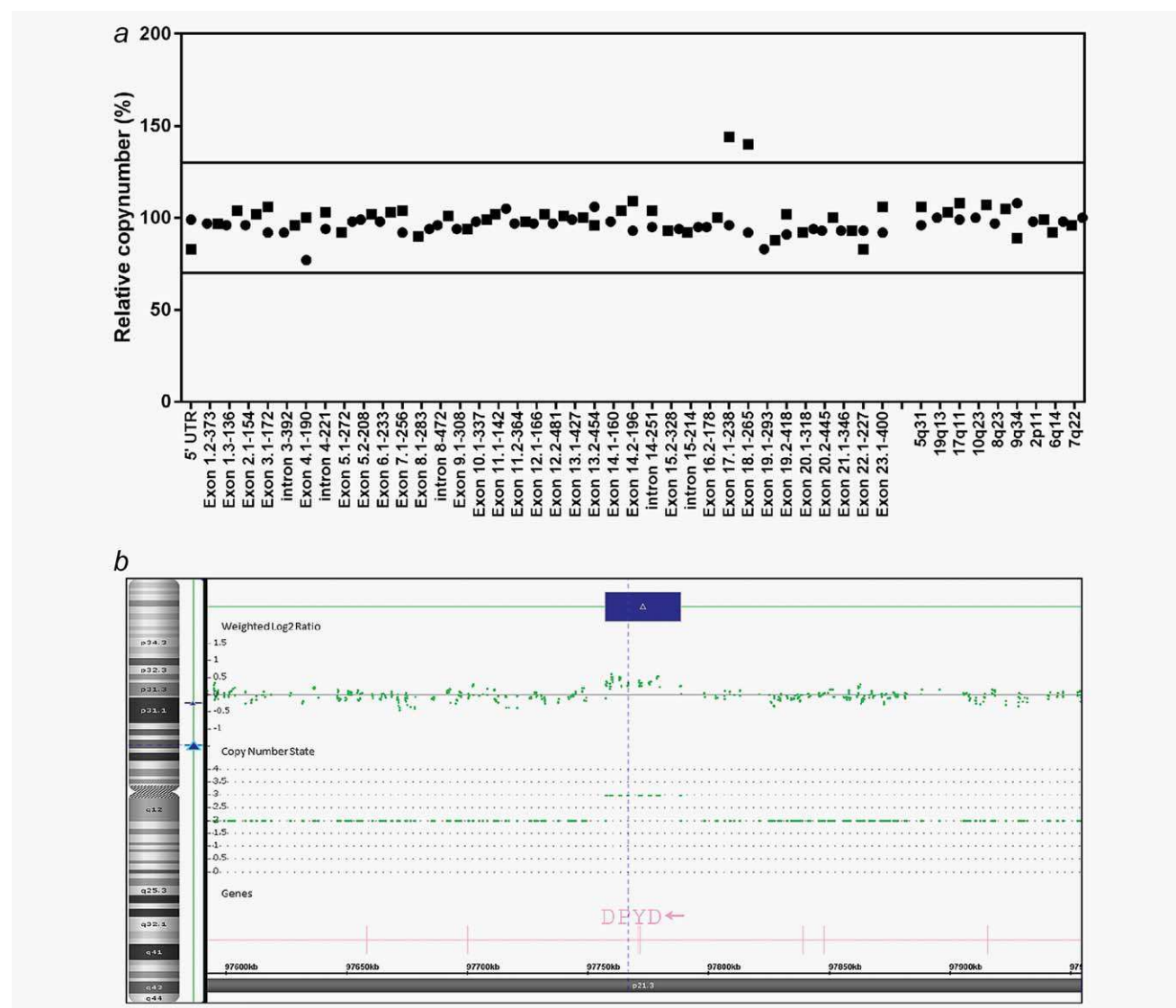
Figure 1. Analysis of copy number changes in *DPYD* using MLPA. Panel *a* shows the MLPA analysis of the patient (■) and a control (●). The solid lines represent the cut-off values indicative for amplification (relative copy number >1.3) or deletion (relative copy number <0.7) of that particular sequence. Panel *b* shows detection of copy number changes by SNP array for the patient. The *y*-axis represents the weighted log2 ratio of the intensities of patient and the copy number state. On the *x*-axis SNPs are ordered by kB position. The panel shows a view for the probes located in the *DPYD* region (hg19). The box represents the minimal amplified region for the patient. [Color figure can be viewed at wileyonlinelibrary.com]

for DNA sequences outside *DPYD*. MLPA was performed as described before.[7,11] Data analysis was performed using Gene Mapper software (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands).[7,11]

An Affymetrix Cytoscan HD SNP array was performed using standard protocols. The relative DNA copy numbers at the copy number variation (CNV) loci were determined by comparison of the normalized array signal intensity data for the DNA sample of the DPD deficient patient against the HapMap270 reference file provided by Affymetrix, using ChAS software (v 3.1.0.15, Affymetrix, Thermo Scientific, Waltham, MA, USA).

Pharmacokinetic analyses
At the first intake of capecitabine, peripheral blood samples were collected in heparin tubes on ten pre-defined time points,

up to 10 hr after capecitabine intake; isolated plasma was stored at $-80°C$ until analysis. Urine was collected as well during these 10 hr, and was collected per portion and stored at $-80°C$. Plasma and urine samples were used for measurement of capecitabine and its metabolites 5′-deoxy-5-fluorocytidine (5′-dFCR), 5′-deoxy-5-fluorouridine (5′-dFUR), 5-fluorouracil (5-FU), dihydro-5-fluorouracil (FUH_2), α-fluoro-ureidopropionic acid (FUPA) and fluoro-β-alanine (FBAL). Levels were quantified with validated methods using HPLC-MS/MS.[12]

Results
Clinical course
In December 2016, a 59-year-old female patient was diagnosed with a sigmoid adenocarcinoma and underwent a

Table 1. Endogenous and pharmacokinetic parameters in plasma and urine and comparison to control values

Endogenous parameters	Patient values	Control values (mean ± SD)	Patient/control ratio
DPD activity (nmol/(mg*hr))	0.05	9.9 ± 2.8[1]	0.0050
Plasma uracil level (µM)	15.5	0.3 ± 1.0[2]	52
Plasma thymine level (µM)	7.9	0.01 ± 0.03[2]	790
Urine uracil level (µmol/mmol creatinine)	124	7.1 ± 5.5[3]	17
Urine thymine level (µmol/mmol creatinine)	66	0.1 ± 0.3[3]	660

Metabolites in plasma: $AUC_{0\text{-last time point}}$ (ng*hr/ml)[4]	Patient values	Control values (mean, CV%)[5]	Patient values normalized for administered dose[8]
Capecitabine	358	4,281 (31%)	3,952
5′-dFCR	2,364	8,192 (30%)	29,077
5′-dFUR	1,072	7,673 (29%)	11,834
5-FU	3,890	381 (40%)	42,942
5-FU relative exposure[6]	*1.02*	*1 (reference value)*	–
FUPA	<LLOQ[7]	ND	NA
FUH$_2$	<LLOQ[7]	ND	NA
FBAL	<LLOQ[7]	14,177 (31%)	NA

Metabolites in plasma: $T_{1/2}$ (hr)[4]	Patient values	Control values (mean, CV%)[5]	Patient/control ratio
Capecitabine	0.41	0.76 (55%)	0.54
5′-dFCR	1.00	1.0 (35%)	1.0
5′-dFUR	1.18	0.9 (34%)	1.3
5-FU	4.26	1.0 (57%)	4.3
FUPA	<LLOQ[7]	ND	NA
FUH$_2$	<LLOQ[7]	ND	NA
FBAL	<LLOQ[7]	2.6 (33%)	NA

Abbreviations: 5′-dFCR, 5′-deoxy-5-fluorocytidine; 5′-dFUR, 5′-deoxy-5-fluorouridine; 5-FU, 5-fluorouracil; AUC, area under the concentration-time curve; FBAL, fluoro-β-alanine; FUH$_2$, dihydro-5-fluorouracil; FUPA, α-fluoro-ureidopropionic acid; LLOQ, lower limit of quantification; NA, not applicable; ND, not determined; SD, standard deviation; $T_{1/2}$, half-life.
[1]Control values are derived from Van Kuilenburg *et al.*[13] ($N = 54$).
[2]Control values are determined in a group of $N = 57$ patients.
[3]Control values are determined in a group of $N = 112$ patients.
[4]$AUC_{0\text{-last time point}}$ and $T_{1/2}$ are calculated using non-compartmental analysis based on plasma levels measured up to 10 hr after the first capecitabine intake (150 mg, 77 mg/m^2).
[5]Control values are derived from Deenen *et al.*[15] and are the mean values for 22 patients, after administration of 850 mg/m^2 capecitabine.
[6]For the 5-FU AUC, the relative exposure after extrapolation for the dosing interval is depicted. 5-FU relative exposure = 5-FU AUC patient value/(factor * 5-FU AUC from Deenen *et al.*[15]). Factor = 10 (as dosing 1× in the 5 days, compared to twice daily in Deenen *et al.*[15]).
[7]LLOQ of FUPA, FUH$_2$ and FBAL is 50 ng/ml.
[8]Patient values for AUC, normalized for the administered dose. Dose for Deenen *et al.*[15] was 850 mg/m^2, the patient received a dose of 77 mg/m^2, so normalized AUC = 5-FU AUC patient value * (850/77).

sigmoid resection (pT4N2M0). She was subsequently scheduled for adjuvant chemotherapy treatment (capecitabine 1,000 mg/m^2 twice daily for 14 days and oxaliplatin 130 mg/m^2 on Day 1, given in a three-weekly cycle, eight cycles in total). Before start of this fluoropyrimidine-containing chemotherapy, *DPYD* screening for four *DPYD* variants was performed (*DPYD**2A, c.1679T > G, c.2846A > T, c.1236G > A), which is standard procedure in the hospital. The patient was then found to be heterozygous for the *DPYD**2A variant and as an additional investigation, analysis of the DPD activity in PBMCs was performed, before determining the individualized starting dose of capecitabine. This

revealed a complete DPD deficiency [DPD enzyme activity in PBMCs = 0.05 nmol/mg/hr, reference activity: 9.9 ± 2.8 nmol/(mg*hr)[13]]. Based on these DPD phenotyping results and on previous experience with another patient with complete DPD deficiency,[14] it was decided to start with capecitabine- and oxaliplatin-based treatment with a drastically lowered capecitabine dose. An absolute dose of 150 mg (77 mg/m^2) on Days 1 and 6 for the first two cycles was chosen (approximately 0.8% of originally planned dose). Oxaliplatin was given in the originally planned dose. After the first intake of capecitabine (Day 1), pharmacokinetic results were awaited before continuing with the second dose (Day 6) as a safety precaution. From the
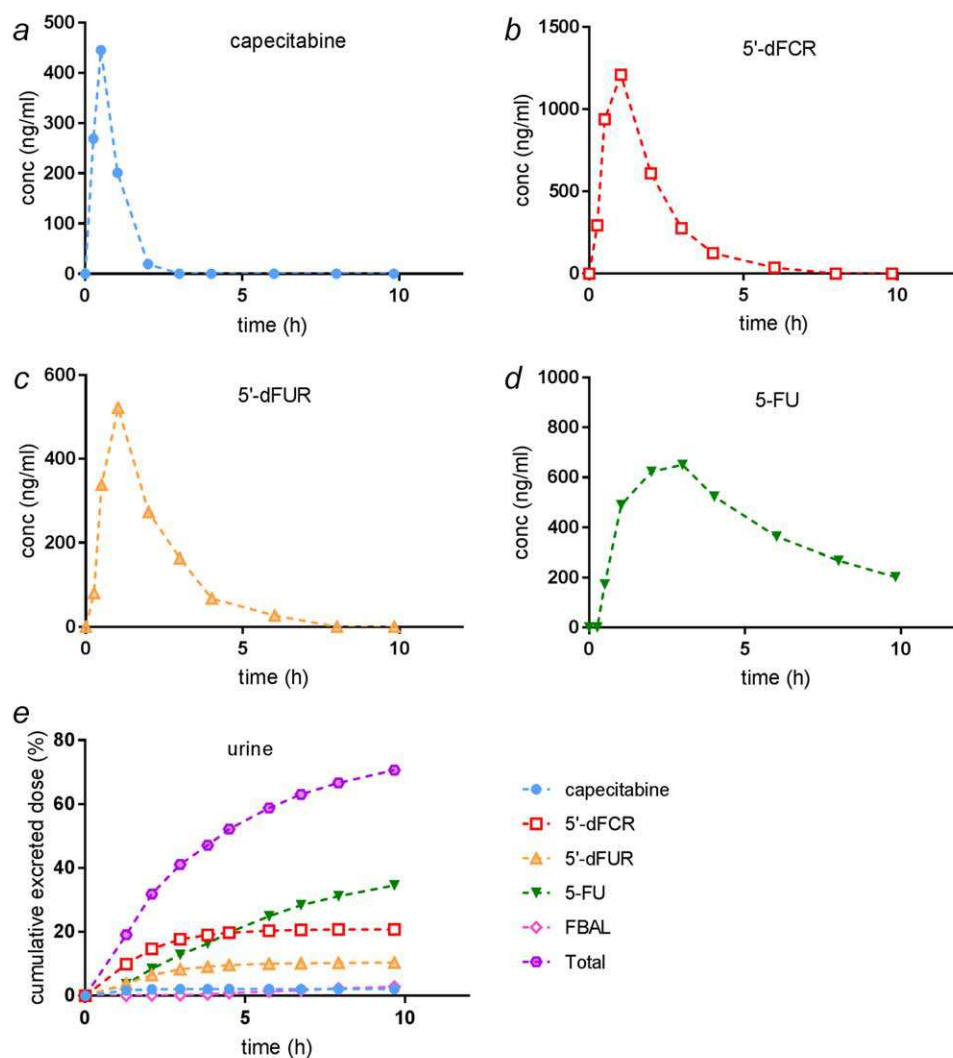
Figure 2. Plasma and urine levels of capecitabine and metabolites. Results of plasma levels of capecitabine (*a*) and the metabolites 5′-deoxy-5-fluorocytidine (5′-dFCR, *b*), 5′-deoxy-5-fluorouridine (5′-dFUR, *c*) and 5-fluorouracil (5-FU, *d*), after the first intake of capecitabine. Panel *e* depicts results of urine excretion of capecitabine and 5′-dFCR, 5′-dFUR, 5-FU, fluoro-β-alanine (FBAL) and the total excretion, after the first intake of capecitabine. Excretion is calculated as percentage of the administered dose of capecitabine (150 mg). [Color figure can be viewed at wileyonlinelibrary.com]

third cycle onwards, capecitabine was administered on Days 1, 6 and 11, as treatment during the first two cycles was considered safe. The capecitabine treatment was tolerated well, without occurrence of capecitabine-related toxicity (e.g., no diarrhea, hand-foot syndrome or leukopenia occurred), and eight cycles were completed as planned. However, the patient experienced severe neurological toxicity, most likely caused by the oxaliplatin. Sensory neuropathy developed during the first cycle, and became more severe (grade 3) during the second cycle. Therefore, the oxaliplatin dose was decreased to 75% from the third cycle onwards and discontinued after cycle six.

DPYD genetic results

Since initial upfront screening for four *DPYD* variants (*DPYD**2A, c.1679T > G, c.2846A > T, c.1236G > A) revealed heterozygosity for *DPYD**2A (thus expecting only a partial DPD deficiency), whereas analysis of the DPD activity in PBMCs showed the presence of a complete DPD deficiency, additional genetic *DPYD* analyses were performed. Sequence analysis of all 23 coding exons and flanking intronic regions of *DPYD* showed that the patient was heterozygous for the *DPYD**2 A variant only. However, subsequent MLPA analysis showed amplification of exons 17 and 18 of *DPYD* (Fig. 1*a*). To delineate the boundaries and size of the amplification, SNP array analysis was performed. Detailed analysis of the chromosome 1p21.3 region showed a minimal amplified region of 31kB ranging from base pair 97757459 to 97788493 (hg19) encompassing exons 17 and 18 of *DPYD* (Fig. 1*b*).

Pharmacokinetic and pyrimidine metabolite results

Strongly elevated concentrations of endogenous uracil and thymine were detected in plasma and urine of the patient

which is in line with the presence of a complete DPD deficiency (Table 1). When calculating a patient/control ratio, it was noted that the ratio for thymine was markedly higher than the ratio for uracil, in both plasma and urine. Pharmacokinetic analyses showed that only capecitabine, 5′-dFCR and 5′-dFUR and 5-FU could be quantified in plasma, the metabolites FUH$_2$, FUPA and FBAL were below the lower limit of quantification (Table 1 and Figs. 2ad). 5-FU exposure (area under the concentration-time curve; AUC) and half-life were respectively tenfold and fourfold higher than control values.[15] When extrapolating from the dosing schedule of once every 5 days to twice daily (tenfold difference), the AUC of 5-FU was comparable to the control value. When calculating a patient/control ratio for which values were normalized for the administered dose in mg/m^2, the 5-FU AUC of the patient is around 113 times higher than observed in patients receiving capecitabine 850 mg/m^2 (42,942 ng*hr/ml vs 381 ng*hr/ml).

In urine the same metabolites as in plasma were detectable, and additionally, a very small proportion was detected as FBAL (Fig. 2e). Approximately 70% of the administered dose was recovered in the urine after 10 hr, of which approximately half as 5-FU.

Discussion

DPD deficiency is now generally accepted as a major determinant of severe fluoropyrimidine-associated toxicity. This case report describes a patient who, if not identified before treatment as being completely DPD deficient and treated with a full capecitabine dose, may well have experienced fatal fluoropyrimidine-related toxicity. This emphasizes the importance of prospective screening for DPD deficiency. Ample evidence has been provided that carriers of the *DPYD**2A, c.1679T > G, c.2846 A > T and c.1236G > A/HaplotypeB3 variants have an increased risk of developing toxicity.[4] In addition, dose adaptation for these *DPYD* variants is recommended by the Clinical Pharmacogenetics Implementation Consortium (CPIC).[5] However, standard screening for these four *DPYD* variants only, as is most often performed, would not have been sufficient to prevent severe and most likely fatal toxicity for this patient, as she would have received a 50% dose reduction only. Implementation of a more extensive genetic *DPYD* screening and/or a DPD phenotyping approach, is expected to identify a larger proportion of the patients with DPD deficiency who are at risk of severe fluoropyrimidine-related toxicity.[7]

Genetic analysis of this patient showed an amplification of exons 17 and 18 of the *DPYD* gene. In addition to the observed heterozygosity for the *DPYD**2A variant, a conclusive genotype was obtained that was likely to underlie the complete DPD deficiency. To our knowledge, amplification of exons 17 and 18 of the *DPYD* gene has not been described before. Recently, heterozygosity of an amplification of exons 9–12 in *DPYD* was shown to result in a profoundly decreased DPD activity.[7] Previously, we have shown that large deletions in *DPYD* occurred in 7% of pediatric patients with a complete DPD deficiency.[11] Thus, genomic rearrangements in *DPYD* can provide a molecular basis for a DPD deficiency in patients with a phenotypically-established reduced DPD activity.

In literature several examples are described of patients experiencing fatal toxicity who were retrospectively identified as completely DPD deficient.[16,17] Therefore, fluoropyrimidine treatment in completely DPD deficient patients has been generally discouraged. As there was a high medical need to treat this patient, based on poor tumor characteristics and no appropriate alternative chemotherapeutic regimens, it was still decided to start with a capecitabine-containing treatment. A dose of 150 mg once every 5 days was chosen, based on previous experience in our institute, where another patient with complete DPD deficiency (due to homozygosity for *DPYD**2A) tolerated this dose well and resulted in adequate drug exposure.[14] Applying very low doses of capecitabine is hampered by the available formulations of capecitabine (i.e., 150 and 500 mg), which we resolved by dosing intermittently once every 5 days.

One tablet of 150 mg resulted in a very high plasma exposure of 5-FU, with an AUC value around ten times higher than in pharmacokinetic studies with capecitabine in standard dosage in non-DPD deficient patients.[15,18–20] When correcting for the dosing interval of once every 5 days, which is ten times less than standard twice-daily dosing, 5-FU exposure in our patient was comparable to reference levels associated with efficacy and acceptable toxicity.[15,18–20]

Complete DPD deficiency has not only be linked with severely increased risk for fluoropyrimidine-related toxicity, but also with neurological or developmental abnormalities in several cases.[21–25] Our patient, however, did not present with any physical or psychomotor abnormalities.

In conclusion, this case report shows the clinical need of an appropriate prospective screening approach for DPD deficiency. Since screening for the most common *DPYD* variants will not identify all patients at risk of severe toxicity; it is recommended to investigate the feasibility of more extensive genetic screening and/or DPD phenotyping methods. Furthermore, we showed that a patient with a complete DPD deficiency can be safely treated with a very low dose of a fluoropyrimidine drug.

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