Personalised medicine of fluoropyrimidines using DPYD pharmacogenetics

Lunenburg, C.A.T.C.

CHAPTER 4

Dutch Pharmacogenetics Working Group (DPWG) guideline for the gene-drug interaction of *DPYD* and fluoropyrimidines

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Carin A.T.C. Lunenburg[*], Cathelijne H. van der Wouden[*], Marga Nijenhuis, Mandy H. Crommentuijn-van Rhenen , Nienke J. de Boer-Veger, Anne Marie Buunk, Elisa J.F. Houwink, Hans Mulder, Gerard A. Rongen, Ron H.N. van Schaik, Jan van der Weide, Bob Wilffert, Vera H.M. Deneer, Jesse J. Swen[#], Henk-Jan Guchelaar[#]

[*]Contributed equally

[#]Contributed equally

Abstract
Despite advances in the field of pharmacogenetics (PGx), clinical acceptance has remained limited. The Dutch Pharmacogenetics Working Group (DPWG) aims to facilitate PGx implementation by developing evidence-based pharmacogenetics guidelines to optimize pharmacotherapy. This guideline describes the starting dose optimization of three anti-cancer drugs (fluoropyrimidines: 5-fluorouracil, capecitabine and tegafur) to decrease the risk of severe, potentially fatal, toxicity; such as diarrhoea, hand-foot syndrome, mucositis or myelosuppression. Dihydropyrimidine dehydrogenase enzyme (DPD) deficiency (encoded by the *DPYD* gene) increases risk of fluoropyrimidine-induced toxicity. The *DPYD*-gene activity score, determined by four *DPYD* variants, predicts DPD activity and can be used to optimize an individual's starting dose. The gene activity score ranges from 0 (no DPD activity) to 2 (normal DPD activity). Subjects with a gene activity score of 0 are recommended not to initiate fluoropyrimidines. Alternatively, DPD activity may be determined to adjust the dose accordingly. Subjects with a gene activity score of 0.5, 1 or 1.5 are recommended to initiate therapy with 25%, 50% or 75% of the normal dose of 5-fluorouracil or capecitabine, respectively. When initiating tegafur, an alternative chemotherapeutic agent, or a low dose is recommended. Dose may be increased in subsequent cycles in patients experiencing no or clinically tolerable toxicity. Subjects with a gene activity score of 2 (reference) should receive a normal dose. In case it is not possible to calculate the gene activity score based on *DPYD* genotype, we recommend to determine the DPD activity. Based on the DPWG clinical implication score, *DPYD* genotyping is considered "essential", therefore directing *DPYD* testing prior to initiating treatment with fluoropyrimidines.

Disclaimer
The Pharmacogenetics Working Group of the KNMP (DPWG) formulates the optimal recommendations for each phenotype group based on the available evidence. If this optimal recommendation cannot be followed due to practical restrictions, e.g. therapeutic drug monitoring or a lower dose is not available, then the health care professional should consider the next best option.

Introduction

The role of heritable genetic variation on drug response is referred to as pharmacogenetics (PGx). Germline mutations in pharmacogenetic loci can predict phenotypic differences in drug response and can be used to guide dose and drug selection to achieve safer and more (cost)effective pharmacotherapy. PGx guided pharmacotherapy is one of the first clinical applications of genomics in medicine. Despite scientific and clinical advances in the field of PGx, clinical adoption has remained limited. Barriers preventing implementation have been previously reported.[1] Some of these barriers have been overcome in the past years. One of these barriers was the lack of clear guidelines on how to interpret and apply PGx test results.

The Royal Dutch Pharmacists Association (KNMP) established the Dutch Pharmacogenetics Working Group (DPWG) in 2005 to overcome this barrier.[2] The main objectives of the DPWG are 1) to develop PGx informed therapeutic recommendations based on systematic literature review, and 2) to assist physicians and pharmacists by integrating the recommendations into computerized systems for drug prescription, dispensing, and automated medication surveillance. This manuscript thus provides both the content required for enabling local translation of assay results into the predicted phenotype (in this case the gene activity score) and for programming therapeutic recommendations into local clinical decision support systems. With the objective of implementing PGx into routine care, the DPWG has additionally developed the clinical implication score, which is given to every gene-drug interaction. The aim of this score is to direct clinicians on whether or not to order relevant PGx genotyping tests before initiating therapy. Recently, the DPWG guidelines were endorsed by the European Association of Clinical Pharmacology and Therapeutics (EACPT) and the European Association of Hospital Pharmacists (EAHP).[3,4]Other initiatives such as the Clinical Pharmacogenetics Implementation Consortium (CPIC) were also established to support clinical implementation.[5,6]

The DPWG is a multidisciplinary group in which (clinical) pharmacists, physicians, clinical pharmacologists, clinical chemists and epidemiologists are represented. From 2005 onwards, the DPWG has systematically executed 90 risk analyses for potential gene-drug interactions resulting in 49 guidelines providing therapeutic recommendations for one or more aberrant phenotypes.[7] Available DPWG guidelines and future updates will be published in an effort to provide transparency of their development and to fulfil the public demand for their publication.

This guideline describes the starting dose optimization of three anti-cancer drugs (fluoropyrimidines: 5-fluorouracil, capecitabine and tegafur) to decrease the risk of severe, potentially fatal, toxicity; such as diarrhoea, hand-foot syndrome, mucositis or myelosuppression. Dihydropyrimidine dehydrogenase enzyme (DPD) deficiency (which is encoded by the *DPYD* gene) increases the risk of fluoropyrimidine-induced toxicity. The gene activity score is currently based on the results of four *DPYD* variants, predicts DPD enzyme activity and is used to optimize an individual's starting dose. The gene activity score ranges from 0 (no DPD activity) to 2 (normal DPD activity). This manuscript provides an overview of the guideline development and summarizes the pharmacotherapeutic recommendations. Additionally, a comparison to alternative guidelines is presented. The *gene-drug interaction* section includes background on the pharmacological mechanism of the interaction. In

addition it also includes a list of the *DPYD* variants associated with toxicity and the method developed by DPWG for local translation of assay results into the gene activity score. This information may be useful for laboratories to select and design a *DPYD* genotyping assay and subsequently determine the patients' predicted phenotype based on the genotype results. Consequently, the literature review supporting the *DPYD*-fluoropyrimidine interaction is described and the DPWG guideline is presented. A summary of all references identified by the systematic review which were subsequently used to develop this guideline, can be found in Supplementary Tables 1 and 2. The recommendations provided in this manuscript can be used in combination with a patients' predicted phenotype to optimize starting dose of fluoropyrimidines, thereby decreasing the risk of severe and potentially fatal toxicity.

Drugs: fluoropyrimidines (5-fluorouracil, capecitabine and tegafur with DPD-inhibitors)

Fluoropyrimidines are antimetabolite drugs widely used in the treatment of colorectal, breast, stomach and skin cancer. Each year, over two million patients worldwide receive treatment with fluoropyrimidines. This includes 5-FU and its oral pro-drugs capecitabine and tegafur. Up to 30% of patients experience severe toxicity (common terminology criteria for adverse events, CTC-AE, grade ≥3), including diarrhoea, hand-foot syndrome, mucositis and myelosuppression. For ~1% of patients toxicity is fatal.[8,9] Toxicity may occur within the first treatment cycle (early onset), supporting the importance of optimizing the starting dose of fluoropyrimidine pharmacotherapy on a personalized basis, before initiating therapy.[10]

Capecitabine is metabolised into 5-FU in three consecutive steps. Capecitabine is firstly metabolised to 5'-deoxy-5-fluorocytidine (5'-DFCR) by carboxylesterase, subsequently, 5'-DFCR is converted into 5'-deoxy-5-fluorouridine (5'-DFUR) by cytidine deaminase, and to 5-FU by thymidine phosphorylase. 5-FU is metabolised in tissues to 5-fluoro-2'-deoxyuridine and then to 5-fluoro-2'-deoxyuridine-5'-monophosphate, the active metabolite of the drug. The active metabolite inhibits the enzyme thymidylate synthase, resulting in inhibition of DNA synthesis and repair, inducing cell apoptosis and thus, its effect. Additionally, toxic effects resulting from partial incorporation of 5-FU and its metabolites in DNA and RNA contribute to the drug's mechanism of action.[11]

Tegafur is metabolised into 5-FU and into the less cytotoxic metabolites 3-hydroxytegafur, 4-hydroxytegafur and dihydrotegafur by *CYP2A6*. The less toxic metabolites are renally cleared. Tegafur was combined with the DPD inhibitor uracil and is now combined with the DPD inhibitor gimeracil and the orotate phoshoribosyltransferase (OPRT) inhibitor oteracil. Oteracil diminishes the activity of 5-FU in normal gastrointestinal mucosa. The DPD inhibitors diminish the formation of functionally inactive metabolites of 5-FU that contribute to adverse events like stomatitis and mucositis. Both uracil and gimeracil inhibit DPD activity reversibly and have a shorter elimination half-life and thus shorter period of action than tegafur. For this reason, genetic variants influencing DPD enzyme activity are clinically relevant for tegafur in combination with DPD inhibitors.

Gene: dihydropyrimidine dehydrogenase (*DPYD*)

The *DPYD* gene encodes the enzyme DPD. *DPYD* is located on chromosome 1p21.3, and transcription variant 1 (NM_000110.3) has 26 exons, spanning approximately 900 kb.[12]

Over 160 different allele variants in *DPYD* have been identified and described in literature.[13] According to the gnomAD browser,[14] which contains whole exome data of almost 140,000 individuals, *DPYD* contains 2,190 known variants. The prevalence of individual variants is low. The effect of genetic variation on DPD enzyme activity is not fully established for the majority of variants and the size of the effect can differ between variants.

The frequency of the various *DPYD* variants and the associated phenotypes appears to vary significantly between nations and ethnic groups. For example, in the Caucasian population, approximately 3—5% has a partial DPD enzyme deficiency and 0.1—0.2% has a complete DPD enzyme deficiency. On the other hand, approximately 8% of the African American population has a partial DPD enzyme deficiency.[15,16]

Gene-drug interaction

Pharmacological mechanism

A schematic overview of fluoropyrimidine metabolism is shown in Figure 1. The DPD enzyme is mainly found in liver, but also intestinal mucosa, leucocytes, tumour cells and other tissues. Over 80% of 5-FU is inactivated to 5-fluoro-5,6-dihydrouracil (DHFU) by DPD. The decreased metabolic activity of DPD leads to increased intracellular concentrations of active metabolites of 5-FU.[17] The increased intracellular concentration of 5-fluoro-2'-deoxyuridine-5'-monophosphate (FdUMP) increases the risk of toxicity such as diarrhoea, hand-foot syndrome, mucositis and myelosuppression. Variants in the *DPYD* gene can result in reduced or even absent DPD enzyme activity, increasing the risk of severe toxicity. For example, 73% of the patients with *DPYD**2A experienced severe toxicity when treated with a full dose, compared to 23% of *1 allele carriers (wild-type patients) who experienced toxicity.[18] Many enzymes are involved in fluoropyrimidine metabolism, however, this guideline is limited to the role of the DPD enzyme in causing toxicity.

Since the genetic variation in *DPYD* only partially determines DPD enzyme activity, these guidelines for dose adjustment based on the predicted phenotype are no more than a tool that can be used to achieve the desired intracellular concentration of the active metabolite, to minimize risk of toxicity. The absence of tested variants does not eliminate the risk of toxicity. Pharmacokinetic dose adjustment (guided by steady-state plasma concentrations or AUC) may also be useful to optimize the dose of 5-FU. This is, however, currently not routinely used for capecitabine and tegafur, as they are mainly converted into 5-FU within tissue.

DPYD variants associated with toxicity

The variants known or suspected to have an effect on DPD enzyme activity, are listed in Table 1. These variants are mapped by the level of evidence for which association with toxicity has been established (columns) and the variant's effect on DPD enzyme activity (rows). Novel variants in *DPYD* will continue to be identified with the introduction of next generation sequencing techniques to clinical practice. However, in order for these variants to be included in Table 1, sufficient evidence regarding the effect on enzyme function or the onset of toxicity must be investigated, possibly by using the *DPYD*-Varifier[19] or by phenotyping patients who carry a novel variant. An update of this guideline will be published when a

renewed recommendation is given following newly published articles.

Translation of genotype to predicted phenotype

The DPWG has concluded that four variants have sufficient evidence to be implemented into clinical care: *DPYD**2A (IVS14+1G>A), *DPYD**13 (c.1679T>G), c.2846A>T and c.1236G>A (in linkage disequillibrium with c.1129-5923C>G). The current guideline only reports recommendations for these four variants; no recommendations are provided for other variants in *DPYD* or other genes. The results of this genotyping panel can be used to predict a patient's phenotype, i.e. the DPD enzyme activity. This predicted DPD activity can be expressed as the *DPYD*-gene activity score, which ranges from 0 (no or virtually no DPD enzyme activity) to 2 (normal DPD enzyme activity due to homozygosity for fully functional alleles, both assigned an activity score 1). The gene activity score is a sum of the two activities of protein isoforms expressed from both alleles. The development of the gene activity score is published elsewhere.[20]

The included variants are those for which substantial and sufficient evidence on the relation to severe toxicity has been established. It is a limitation to restrict to these four variants, as other variants may influence DPD activity as well. However, not all variants having a possible effect on DPD enzyme activity may have been identified yet or evidence for identified variants is insufficient. Therefore, this may result in the incorrect prediction of the DPD enzyme activity. Another limitation is that currently used genotyping methods are unable to determine the allelic location of the variants, but only the dichotomous presence or absence of the variant. This becomes a limitation when two or more different genetic variants are identified in a patient. In this case, either both genetic variants may be on the same allele, resulting in a genotype with one fully functional allele and one reduced functionality allele, or alternatively, both genetic variants may reside on different alleles, resulting in two alleles with inactive or reduced functionality. The latter is more likely to occur. The total gene activity score, however, differs between these cases. When the DPD enzyme activity cannot be predicted correctly, an additional phenotyping test is required to determine the DPD enzyme activity. The relationship between genotype result and predicted phenotype in patients carrying no variants or one or more variants leading to decreased DPD enzyme activity are shown in Supplementary Table 3. The frequency of individuals carrying two or more of four variants considered in the current guideline is rare, but can be assigned a gene activity score. A complete genotype to predicted phenotype translation table can be found in Supplementary Table 4, which can be used to program the translation of genotype results into predicted phenotypes in laboratory information systems.

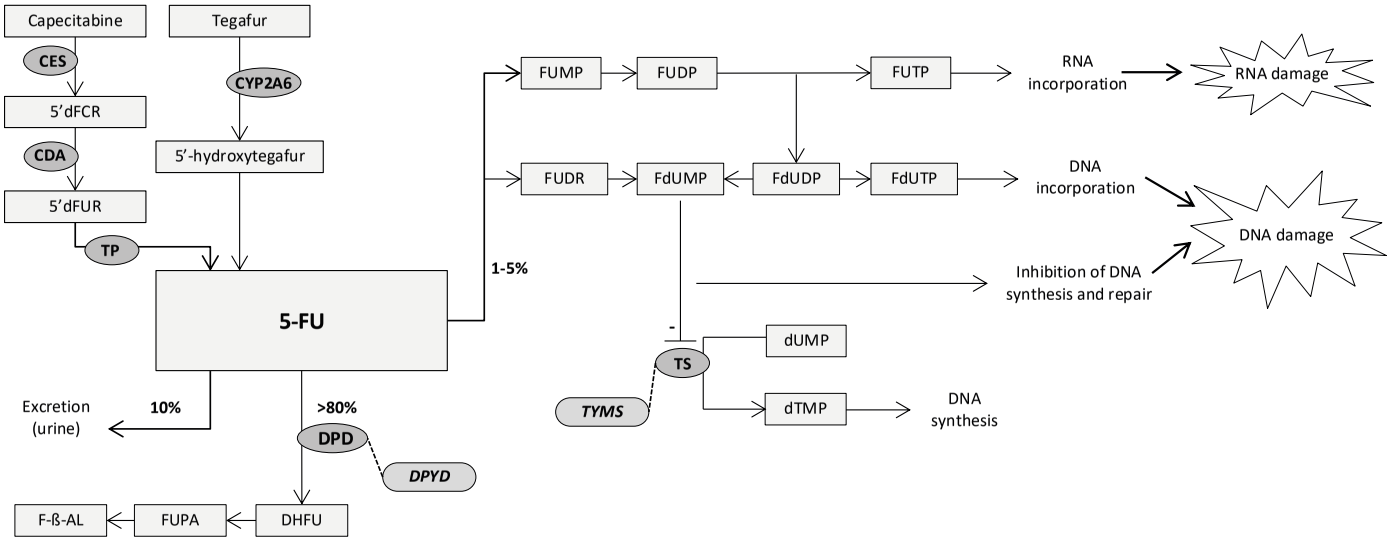

Figure 1. Schematic overview of fluoropyrimidine metabolism
In brief: tegafur, 5FU, capecitabine are metabolised into three major metabolites. FdUMP, which inhibits TS and prevents conversion of dUMP to dTMP, which is necessary for pyrimidine and DNA synthesis. FdUTP is incorporated in DNA, FdUTP is incorporated in RNA, both resulting in cell death.
Abbreviations: CES: carboxylesterase; 5'dFCR: 5'-deoxy-5-fluorocytidine; CDA: cytidine deaminase; 5'dFUR: 5'-deoxy-5-fluorouridine; TP: thymidine phosphorylase; 5-FU: 5-fluorouracil; FUMP: fluorouridine monophosphate; FUDP: fluorouridine diphosphate; FUTP: fluorouridine triphosphate; RNA: ribonucleic acid; FUDR: fluorodeoxyuridine; FdUMP: fluorodeoxyuridine monophosphate; FdUDP: fluorodeoxyuridine diphosphate; FdUTP: fluorodeoxyuridine triphosphate; DNA: deoxyribonucleic acid; TS: thymidylate synthase; *TYMS*: gene encoding TS; dUMP: deoxyuridine monophosphate; dTMP: deoxythymidine monophosphate; DPD: dihydropyrimidine dehydrogenase; *DPYD*: gene encoding DPD; DHFU: 5,6-dihydrofluorouracil; FUPA: fluoro-ß-ureidopropionate; F-ß-AL: fluoro-ß-alanine.

Table 1. Known *DPYD* variants stratified by level of evidence on the association with toxicity and predicted DPD enzyme activity

The variants in this table were selected based on literature in Supplementary Table 1 and 2. However, high allele frequency variants reported only in case reports with fluoropyrimidine toxicity were excluded. For these variants the association with DPD enzyme activity, and resulting severe fluoropyrimidine-induced toxicity, cannot be determined.

Level of evidence / DPD enzyme activity	Sufficient evidence[a]	Insufficient evidence[b]
Fully functional[c]	*DPYD* *4 = c.1601G>A *DPYD* *5 = c.1627A>G *DPYD* *9A = c.85T>C	
Reduced functionality[d]	c.2846A>T c.[1236G>A;1129-5923C>G] (hapB3)[e]	c.496A>G c.1129-15T>C (IVS10-15T>C) *DPYD*6 = c.2194G>A c.1896T>C *DPYD*3 = c.1897delC *DPYD*7 = c.299_302del *DPYD*8 = c.703C>T *DPYD* *9B = c.85T>C(;)c.2657G>A *DPYD* *10 = c.2983G>T *DPYD* *11 = c.1003G>T *DPYD* *12 = c.62G>A c.1156G>T c.1651G>A c.1845G>T
Fully dysfunctional[f]	*DPYD*2A = c.1905+1G>A (IVS14+1G>A) *DPYD*13 = c.1679T>G	c.300C>A[g] c.1024G>A[g] c.1025A>G[g] c.1475C>T[g] c.1774C>T[g] c.(2058_2059)_(2299_2300)dup

[a] DPWG has concluded an association between fully functional variants and no resulting toxicity, and an association between reduced functionality variants or fully dysfunctional variants and association with the onset of severe fluoropyrimidine-induced toxicity;

[b] DPWG has concluded there is insufficient evidence to associate a predicted DPD enzyme activity for these variants and the onset of severe fluoropyrimidine-induced toxicity;

[c] These variants are not included in the prospective *DPYD* genotyping panel, as there is no effect on predicted DPD enzyme activity, and therefore there is no association with the onset of severe fluoropyrimidine-induced toxicity;

[d] The effect of the variant on the protein sequence suggests that the protein may still be partially functional. Therefore residual metabolic DPD capacity may be present;

[e] Variant c.1236G>A, which does not lead to an alternative amino acid, is in complete linkage disequilibrium with variant c.1129-5923C>G, which leads to aberrant splicing in mRNA, which leads to a premature stop codon as a result. The resulting DPD enzyme activity is 50% of the normal activity.

Both variants are part of haplotype B3;
[f] The effect of the variant on the protein sequence suggests that the protein may be fully dysfunctional;
[g] These variants have decreased *in vitro* enzyme activity.
Variants from the table according to multiple nomenclatures (HGVS: NM_000110.3, NP_000101.2 and NC_000001.10):
(rs67376798, c.2846A>T, p.(Asp949Val), g.97547947T>A), (rs56038477, c.1236G>A, p.(Glu412=), g.98039419C>T, in haplotype B3), (rs75017182, c.1129-5923C>G, g.98045449G>C, in haplotype B3), (rs3918290, *2A, c.1905+1G>A, IVS14+1G>A, g.97915614C>G), (rs55886062, *13, c.1679T>G, p.(Ile560Ser), g.97981343A>C), (rs2297595, c.496A>G, p.(Met166Val), g.98165091T>C), (rs56293913, c.1129-15T>C, IVS10-15T>C, g:98039541A>G), (rs1801160, *6, c.2194G>A, p.(Val732Ile), g.97770920C>T), (rs17376848, c.1896T>C, p.(Phe632=), g.97915624A>G), (rs72549303, *3, c.1897delC/c.1898delC, p.(Pro633Glnfs), g.97915622delG), (rs72549309, *7, c.299_298delTCAT, p.(Phe100Serfs), g.98205971_98205974delATGA), (rs1801266, *8, c.703C>T, p.(Arg235Trp), g.98157332G>A), (rs1801265 + rs1801267, *9B, c.85T>C + c.2657G>A, p.(Cys29Arg) + p.(Arg886His), g.98348885G>A+ g.97564154C>T), (rs1801268, *10, c.2983G>T, p.(Val995Phe), g.97544627C>A), (rs72549306, *11, c.1003G>T, p.(Val335Leu), g.98058899C>A), (rs80081766, *12, c.62G>A, p.(Arg21Gln), g.98348908C>T), (rs78060119, c.1156G>T, p.(Glu386Ter), g.98039499C>A), (rs777425216, c.1651G>A, p.(Ala551Ser), g.97981371C>A), (c.1845G>T, p.(Glu615Asp)), (98205969, c.300C>A, (p.Phe100Leu)), (rs183385770, c.1024G>A, p.Asp342Asn, g.98058878C>T), (rs183385770, c.1025A>G, p.Asp342Asn, g.98058878C>T), (rs72549304, c.1475C>T, p.Ser492Leu, g.98015165G>A), (rs59086055, c.1774C>T, p.(Arg592Trp), g.97915746G>A), (g.(619762_619763)_(620801_620802) dup), (rs1801158, *4, c.1601G>A, p.(Ser534Asn), g.97981421C>T), (rs1801159, *5, c.1627A>G, p.(Ile543Val), g.97981395T>C), (rs1801265, *9A, c.85T>C, p.(Cys29Arg), g.98348885G>A).

Additional phenotyping test when genotype is unable to predict phenotype
In contrast to the *DPYD* genotyping test, which aims to predict DPD enzyme activity, a DPD phenotyping test can be performed to measure the actual DPD enzyme activity. Possible methods to perform phenotyping are to measure the DPD enzyme activity in peripheral blood mononuclear cells (PBMCs) or to measure the uracil concentrations in plasma or urine.[21] The average Caucasian DPD enzyme activity is 9.9±0.95 nmol/hour per mg protein.[22] Less commonly performed methods include: 1) the 2-^{13}C-uracil breath test,[23] where $^{13}C0_2$ is measured, which is a product of 2-C^{13}-uracil degradation by DPD and other enzymes involved in the katabolic route of pyrimidines; 2) the quantification of the uracil/dihydrouracil ratio in plasma, where endogenous substrates uracil and dihydrouracil are measured,[24,25] although recently it was shown that uracil levels were superior to the dihydrouracil/uracil ratio as a predictor of severe toxicity;[26] 3) measurement the metabolism of a single dose of uracil.[27] However, all DPD phenotyping tests have their limitations. Currently, the DPD enzyme activity measurements from PBMCs are considered the best developed DPD phenotyping test in The Netherlands.[27,28]

Supporting body of evidence
A detailed description of the methods used for literature collection, assessment and preparation of the gene-drug monograph has previously been published elsewhere.[2,7] In brief,

a systematic review of literature was performed and relevant articles were summarized by a scientist of the Royal Dutch Pharmacists Association (MN). The performed search strategy can be found in Supplementary Material 1. Each article was provided with two scores: 1) quality of evidence and 2) clinical impact. The quality of evidence was scored on a 5-point scale ranging from 0 (lowest; data on file) to 4 (highest; well performed controlled studies or meta-analysis) and the clinical impact of clinical effect was scored on a 7-point scale ranging from AA# (positive effect) to F (highest negative effect). The criteria used to develop these scores have been published in detail previously.[2,7] This clinical impact scale (AA#—F) runs parallel to the common terminology criteria for adverse events (CTC-AE); where CTC-AE grade 5 severity is equal to clinical relevance score F (death) and CTC-AE grade 1 severity is equal to clinical relevance score B. The clinical relevance score additionally includes the scores AA#, AA and A, since these do not exist in the CTC-AE. These regard "Positive clinical effect", "No clinical or kinetic effect", and "Significant kinetic effect or not clinically relevant effect", respectively. The summaries of articles, and their respective scores, reviewed to devise this guideline can be found in the Supplementary Table 1 and 2. The summaries of each article and their respective scores were checked by two independent DPWG members.

For 5-FU/capecitabine, the initial literature search was performed on March 24th 2009, followed by a second search on July 9th 2014. To update this guideline to the current date, an additional literature search was performed on October 19th 2017, resulting in eleven additional papers. Case reports concerning systemic 5-FU or capecitabine therapy were excluded in this literature review, due to a large number of case reports and other available publications of greater evidentiary quality. Kinetic studies from 2009 onwards were only included if the kinetic parameters were given per genotype. Clinical studies were only included if the patient numbers exceeded 500 (from 2009 onwards) or 1,000 (from May 2014 onwards) and the patient numbers with partially functional activity were at least ten or if the study investigated a variant for which no studies were as yet included or if the study investigated the effect of dose adjustment. From 2009, articles investigating the effect of a group containing both polymorphisms known to increase the risk of toxicity and polymorphisms not known to increase the risk of toxicity were not included. If more than one article described data of the same patient group and the same polymorphisms, only the article with data from the largest amount of patients was included.

For tegafur, the initial literature search was performed on August 20th 2009, followed by a second and third search on October 2nd 2012 and July 27th 2015. To update this guideline to current date, an additional literature search was performed on October 19th 2017, resulting in no additional papers.

General conclusion of evidence

In the systematic review performed for 5-FU/capecitabine, 16 of 18 studies and all three meta-analyses found an increased risk of grade ≥3 toxicity (either overall toxicity or at least one specified type of toxicity) for patients carrying variants resulting in reduced DPD enzyme activity (ranging from gene activity score 0 to 1.5). This increased risk was shown separately for patients assigned *DPYD*-gene activity scores 1 and 1.5, but gene activity scores 0 and 0.5 were only investigated when grouped with patients assigned other gene activity

scores. However, the increased risk of toxicity for patients assigned gene activity scores 0 and 0.5 can be concluded based on the confirmed association for gene activity scores 1 and 1.5, where deficiency is less, and is further supported by cases of patients assigned gene activity scores 0 and 0.5 who developed severe toxicity. Only one study investigating clinical outcome concluded there was no effect of variants on risk of toxicity. Based on the systematic review, the DPWG concludes that a gene-drug interaction is present and that DPD enzyme deficiency increases risk of severe toxicity in patients using capecitabine/5-FU. The highest quality of evidence concluding a gene-drug interaction was scored 4.

In the systematic review performed for tegafur with the DPD inhibitor uracil, one case report described four patients who used standard doses and developed severe toxicity. These patients were assigned *DPYD*-gene activity scores 1 and 1.5. Toxicity (CTC-AE grade 4) was similar to that reported in patients treated with 5-FU or capecitabine, both of which are given without a DPD inhibitor. There were no data available for patients assigned *DPYD*-gene activity score 0 or 0.5, however the increased risk of toxicity among these patients can be concluded based on the confirmed association with toxicity for gene activity scores 1 and 1.5, where deficiency is less. Based on the systematic review, the DPWG concludes that there is a clinically relevant gene-drug interaction present and that DPD enzyme deficiency increases risk of severe toxicity in patients using tegafur with DPD inhibitors. The highest quality of evidence concluding a gene-drug interaction was scored 2.

Pharmacotherapeutic recommendations

The DPWG therapeutic recommendation using a patient's pre-therapeutic PGx test result to optimize starting dose of 5-FU/capecitabine and tegafur with DPD inhibitors is summarized in Supplementary Table 5 and 6, respectively.

In brief, when initiating 5-FU, capecitabine or tegafur pharmacotherapy, a gene activity score of 0 recommends choosing an alternative chemotherapy or determining the residual DPD enzyme activity and adjusting the fluoropyrimidine starting dose accordingly. When initiating 5-FU or capecitabine, a gene activity score of 0.5, 1 or 1.5 recommends a starting dose of 25%, 50% or 75%, respectively. Further titration of the dose is possible, guided by toxicity. When initiating tegafur, a gene activity score of 0.5, 1 or 1.5 recommends choosing an alternative chemotherapy or starting with a lower dose and titrating dose based upon toxicity. A gene activity score of 2 (reference value) does not result in a recommendation for dose adaptation for 5-FU, capecitabine or tegafur. If genotype results cannot predict the gene activity score correctly, for example due to multiple identified variants, it is advised to determine the DPD enzyme activity to define an initial starting dose.

Where possible, dose adjustments have been calculated based on 5-FU clearance or AUC after administration of 5-FU or capecitabine. Data were also extrapolated to tegafur with DPD inhibitor, as this compound also follows the same catabolic and anabolic routes after conversion to 5-FU after clearance of the DPD inhibitor from the body. Data on 5-FU clearance are only available for patients carrying *DPYD**1/*DPYD**2A, *DPYD**1/c.2846A>T and *DPYD**2A/c.2846A>T. There are data from one patient with *DPYD**1/*DPYD**13 who developed severe toxicity after 5-FU use, from one patient with c.2846A>T/c.2846A>T and from one patient with c.1236G>A/c.2846A>T.

See Supplementary Table 7 and 8 for an overview of suggested pop-up texts for electronic prescribing systems for pharmacists and physicians. These can be used to program alerts into the clinical decision support system (CDSS). Spanish, Greek, Italian, German, Slovenian and Dutch translations of both the guidelines and background information are available on PharmGKB.org.

Implications for clinical practice

There is currently an ongoing debate regarding whether and which single-drug gene pairs should be implemented into routine care. Points of debate include the amount of evidence that is necessary supporting effectiveness of pre-emptive genotyping, the cost-effectiveness of the intervention and reimbursement of PGx testing.[29,30] This inconclusive debate seems to have hampered implementation of drug-gene pairs which seem ready for implementation.[1,31] In an effort to overcome this inconclusiveness and to direct clinicians on whether or not to order relevant PGx genotyping tests before initiating therapy, the DPWG has developed the clinical implication score. The pre-emptive PGx results for a certain drug-gene pair can be scored as: essential, beneficial, potentially beneficial or not required. The development of these categories and the systematic scoring criteria are discussed elsewhere.[32] In brief, the implications for clinical practice are based on a list of four criteria regarding the following: the clinical effect associated with the gene-drug interaction, the level of evidence supporting the clinical effect, the effectiveness of the intervention in preventing the clinical effect (which includes the number needed to genotype) and the PGx information included in the drug-label. The scores provided for each of these criteria by the DPWG can be found in Supplementary Table 9.

As a result, the DPWG has concluded the clinical implication score of *DPYD*-fluoropyrimidines to be "essential". This score dictates that *DPYD* genotyping prior to treatment must be performed for all patients initially being prescribed therapy with 5-FU, capecitabine or tegafur with DPD inhibitors, to optimize the initial dose and to prevent potentially fatal toxicity.

Differences between available guidelines

Other guidelines regarding the gene-drug interaction of *DPYD* and fluoropyrimidines have been developed. To the best of our knowledge, guidelines are available from CPIC,[11,33] French (French Network of Pharmacogenetics, RNPGx)[34] and Italian (Associazione Italiana di Oncologia Medica, AIOM-SIF) [unpublished guidelines, *edited by the AIOM-SIF Working Group*] initiatives. We have compared the DPWG guidelines to other available guidelines published in English. This regards only the CPIC guideline, since the French and Italian guidelines are unpublished or not in English.

CPIC

Differences between CPIC and DPWG methodology, genotype to phenotype conversion and recommendations have previously been described in detail.[6] However, both guidelines have been updated.[33,35] The current DPWG and CPIC guidelines[5] for *DPYD*/fluoropyrimidines differ regarding the therapeutic recommendations. In contrast to CPIC, DPWG distinguishes

between 5-FU/capecitabine and tegafur within the therapeutic recommendations for fluoropyrimidines, where the CPIC guideline does not provide any dosing recommendations for tegafur due to the limited available evidence. DPWG also further distinguishes between systemic and cutaneous routes of administration within the 5-FU/capecitabine recommendations. The therapeutic recommendations for 5-FU/capecitabine also differ regarding the following: 1) For patients with gene activity score 0: DPWG recommends phenotyping while CPIC does not when no alternative is available. 2) For patients with gene activity score 0.5: DPWG recommends initiating therapy with 25% of standard dose or an alternative whereas CPIC recommends an alternative or a strongly reduced dose with therapeutic drug monitoring, but does not provide an absolute percentage. 3) For patients with gene activity score 1.5 the DPWG recommends a 75% standard dose whereas CPIC recommends a 50% of standard dose.

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SUPPLEMENT CHAPTER 4

Dutch Pharmacogenetics Working Group (DPWG) guideline for the gene-drug interaction of *DPYD* and fluoropyrimidines

Submitted (under review)

Carin A.T.C. Lunenburg[*], Cathelijne H. van der Wouden[*], Marga Nijenhuis, Mandy H. Crommentuijn-van Rhenen , Nienke J. de Boer-Veger, Anne Marie Buunk, Elisa J.F. Houwink, Hans Mulder, Gerard A. Rongen, Ron H.N. van Schaik, Jan van der Weide, Bob Wilffert, Vera H.M. Deneer, Jesse J. Swen[#], Henk-Jan Guchelaar[#]

[*]Contributed equally

[#]Contributed equally

Supplementary Material
Search terms used to perform the literature review of *DPYD*-[5-FU/capecitabine/tegafur] interactions.

Search strategy
Pubmed was used to search English, Dutch, German articles were accepted. Keywords were the drugs of interest (fluorouracil, capecitabine and tegafur/S1), the gene and variations (*DPYD*, DPD, dihydropyrimidine dehydrogenase), and others (e.g. metabolizer, pharmacogenetics, polymorphism). The complete search string was;

Fluorouracil and capecitabine
Search performed in 2009: ("Fluorouracil"[Mesh] OR fluorouracil) AND ("Dihydropyrimidine Dehydrogenase Deficiency"[Mesh] OR metabolizer OR metaboliser OR polymorph* OR "Polymorphism, Genetic"[MeSH] OR "Pharmacogenetics"[MeSH]) AND (English[lang] OR German[lang] OR Dutch[lang])

("capecitabine "[Substance Name] OR capecitabine) AND ("Dihydropyrimidine Dehydrogenase Deficiency"[Mesh] OR metabolizer OR metaboliser OR polymorph* OR "Polymorphism, Genetic"[MeSH] OR "Pharmacogenetics"[MeSH]) AND (English[lang] OR German[lang] OR Dutch[lang])

("Fluorouracil"[Mesh] OR fluorouracil OR "capecitabine "[Substance Name] OR capecitabine) AND (("Dihydrouracil Dehydrogenase (NADP)"[Mesh] OR (dihydropyrimidine dehydrogenase)) AND mutation) AND (English[lang] OR German[lang] OR Dutch[lang])

Search performed in 2014: ("Fluorouracil"[Mesh] OR fluorouracil OR "capecitabine" [Supplementary Concept] OR capecitabine) AND ("Dihydropyrimidine Dehydrogenase Deficiency"[Mesh] OR "Dihydropyrimidine Dehydrogenase Deficiency" OR metabolizer OR metaboliser OR polymorph* OR "Polymorphism, Genetic"[MeSH] OR "Pharmacogenetics"[MeSH]) AND (English[lang] OR German[lang] OR Dutch[lang])

Search performed in 2017: ("Fluorouracil"[Mesh] OR fluorouracil OR "Capecitabine"[Mesh] OR capecitabine OR fluoropyrimidines) AND ("Dihydrouracil Dehydrogenase (NADP)"[Mesh] OR "Dihydropyrimidine Dehydrogenase Deficiency"[Mesh] OR "Dihydropyrimidine Dehydrogenase Deficiency" OR "Dihydropyrimidine Dehydrogenase" OR *DPYD* OR DPD) AND (English[lang] OR German[lang] OR Dutch[lang])

Tegafur
Search performed in 2009 and 2012: ("Tegafur"[Mesh] OR tegafur[Text Word]) AND ("Dihydropyrimidine Dehydrogenase Deficiency"[Mesh] OR metabolizer OR metaboliser OR polymorph* OR "Polymorphism, Genetic"[MeSH] OR "Pharmacogenetics"[MeSH]) AND (English[lang] OR German[lang] OR Dutch[lang])

Search performed in 2015: ("Tegafur"[Mesh] OR "S 1 (combination)" [Supplementary Concept] OR "tegafur-gimeracil-oteracil" [Supplementary Concept] OR tegafur[Text Word] OR S1 OR S-1 OR Teysuno) AND ("Dihydropyrimidine Dehydrogenase Deficiency"[Mesh] OR Dihydropyrimidine Dehydrogenase OR DPD OR *DPYD*) AND (English[lang] OR German[lang] OR Dutch[lang])

Search performed in 2017: ("Tegafur"[Mesh] OR "S 1 (combination)" [Supplementary Concept] OR "tegafur-gimeracil-oteracil" [Supplementary Concept] OR tegafur OR S1 OR S-1 OR "S 1" OR Teysuno) AND ("Dihydrouracil Dehydrogenase (NADP)"[Mesh] OR "Dihydropyrimidine Dehydrogenase Deficiency"[Mesh] OR "Dihydropyrimidine Dehydrogenase Deficiency" OR "Dihydropyrimidine Dehydrogenase" OR *DPYD* OR DPD) AND (English[lang] OR German[lang] OR Dutch[lang])

Supplementary Table 1. Literature review of *DPYD*-[5-FU/capecitabine] interactions to support the therapeutic dose guidelines to optimize dose

Reference	Code	Effect	Comments
ref. 1 – CAP/FU, mono/comb Henricks LM et al. Treatment algorithm for homozygous or compound heterozygous *DPYD* variant allele carriers with low-dose capecitabine. JCO Precis Oncol 2017 Oct 8.	Level of evidence score: 2 gene act. 1: Clinical Relevanc e Score A gene act. 0: Clinical Relevanc e Score A	5 patients, being either homozygous for a gene variant or having two different gene variants, received capecitabine or 5-FU treatment with doses based on the pre-treatment DPD activity in peripheral blood mononuclear cells. Pre-treatment DPD activity was also determined in a patient with genotype c.2846A>T/c.2846A>T, who did not receive treatment, because she was disease free after surgery. For 3 patients, the AUC of 5-FU after the first dose of capecitabine was determined, normalised to a dose of 850 mg/m^2 and compared to 22 patients from another study receiving combined chemotherapy with capecitabine 850 mg/m^2. Genotyping: - 2x c.1236A>G/c.1236A>G - 2x c.2846A>T/c.2846A>T - 1x *2A/*2A - 1 carrier of both c.1236A>G and c.2846A>T (either c.1236A>G/c.2846A>T (on separate alleles) or *1/(c.1236A>G+c.2846A>T) (variants on the same allele)) Results: - Of the four patients with gene activity score 1, the two patients with genotype c.1236A>G/c.1236A>G had respectively 79% and 42% of the normal DPD activity. The first was treated with 75% of the normal capecitabine dose in cycle 1 and with 100% in cycle 2. The second was treated with 50% of the normal 5-FU dose. The patients did not have severe toxicity on the reduced doses. The two patients with genotype c.2846A>T/c.2846A>T had respectively 29% and 10% of the normal DPD activity. The first was treated with 17% of the normal capecitabine dose (278 mg/m^2 once daily in combination with radiotherapy as neoadjuvant treatment) and the second was the patient who did not need treatment. The first patient tolerated treatment	Authors' conclusion: 'We showed that fluoropyrimidine treatment in homozygous or compound heterozygous *DPYD* variant allele carriers is feasible and that therapy does not have to be withheld. Additional DPD phenotyping tests, such as measurement of DPD activity in PBMCs, are recommended to compose an individualized treatment. After an initial dose reduction, tolerability in patients should be monitored closely, and the dose should be individually titrated according to tolerance.' Dose-corrected AUC versus gene activity 2: gene act. 1: 546% gene act. 0: 13812% Tolerated dose compared to gene activity 2: gene act. 1: 55% gene act. 0: 0.43%

table continues

		well without occurrence of severe toxicity and surgery was performed after treatment. The dose-corrected AUC of 5-FU in this patient was 866% of that of control patients. The mean DPD activity in these patients was 40%. There was a large variance in DPD activity between these patients (10-79%). - The patient with genotype *2A/*2A had undetectable DPD activity and tolerated monotherapy with 0.65% of the normal capecitabine dose (65 mg/m^2 every 5 days) for 1 month after which grade 2 diarrhoea developed. After a rest period of 3 weeks, treatment was restarted with the same dose, but every third gift was skipped (0.43% of the normal dose). The patient tolerated this dose also after addition of oxaliplatin and bevacizumab as originally planned and had stable metastatic colorectal carcinoma as best treatment response. The dose-corrected AUC of 5-FU in this patient was 13.812% of that of control patients. - The carrier of both c.1236A>G and c.2846A>T had 45% of the normal DPD activity, corresponding to gene activity score 1 (variants on different alleles). He was treated with 51% of the normal capecitabine dose in cycle 1 (daily dose of 900 mg/m^2 in combination with oxaliplatin), which was tolerated without toxicity. Increase to 71% of the planned dose (daily dose of 1250 mg/m^2) in cycle 2 resulted in grade 3 thrombocytopenia. The dose was reduced to 57% of the normal dose (1000 mg/m^2 daily), which was continued during cycle 3. However, because grade 2 thrombocytopenia developed after 8 days, the dose was reduced to 29% of the normal dose (500 mg/m^2 daily) for the rest of the cycle, resulting in platelets to increase to normal values. Progression of metastatic colorectal cancer was established after 3 cycles and capecitabine treatment was discontinued. The dose corrected AUC of 5-FU in this patient was 227% of that of control patients. NOTE: Patients were genotyped for *2A, *13, c.2846A>T and c.1236G>A.	DPD activity compared to gene activity 2: gene act. 1: 41% gene act. 0: 0%
ref. 2 – CAP, comb Henricks LM et al. Capecitabine-based treatment of a patient with a novel *DPYD*	Level of evidence score: 2 gene act. 0: Clinical Relevance Score A	A 59-year-old women with 0.5% of the normal DPD activity tolerated adjuvant chemotherapy with 0.8% of the normal capecitabine dose (77 mg/m^2 on days 1 and 6 of the first cycle and on days 1, 6 and 11 of the following cycles) in combination with oxaliplatin for eight cycles. Capecitabine-related toxicity like diarrhoea, hand-foot syndrome or leukopenia did not occur. However, sensory neuropathy developed during the first cycle, and became more severe (grade	Authors' conclusion: 'This case report demonstrates that a more comprehensive genotyping and phenotyping approach, combined with

table continues

genotype and complete dihydropyrimidine dehydrogenase deficiency. Int J Cancer. 2018 Jan 15;142(2):424-430.PubMed PMID: 28929491.		3) during the second cycle. Because this was most likely caused by oxaliplatin, the oxaliplatin dose was decreased to 75% from the third cycle onwards and discontinued after the sixth cycle. The dose-corrected AUC of 5-FU in this patient was 11.271% of that of control patients. Her genotype was *2A/(duplication of exon 17 and 18). NOTE: The patient was initially genotyped for *2A, *13, c.2846A>T and c.1236G>A. Additional gene variants were not found by sequencing of all 23 coding exons and flanking intronic regions, after which copy numbers of sequences were analysed.	pharmacokinetically-guided dose administration, enables save fluoropyrimidine treatment with adequate drug exposure in completely DPD deficient patients.' Dose-corrected AUC versus gene activity 2: gene act. 0: 11271%
ref. 3 – FU, mono/comb Meulendijks D et al. Pretreatment serum uracil concentration as a predictor of severe and fatal fluoropyrimidine-associated toxicity. Br J Cancer 2017;116:1415-24. PubMed PMID: 28427087.	Level of evidence score: 4 gene act. 1-1.5: CTC-AE 4	1606 *2A-negative patients from Deenen 2016 were genotyped for other gene variants. Toxicity was defined as toxicity grade ≥ 3, global toxicity as any toxicity, hospitalisation as toxicity related hospitalisation. Only outcomes during the first cycle of chemotherapy were included. ORs were adjusted for age, sex and treatment regimen. Genotyping: - 19 carriers of c.2846A>T - 3 carriers of *13 - 58 carriers of c.1236A>G Results: Result for carriers compared to non-carriers of the gene variant: gene variant / outcome / OR_{adj} (95% CI) c.2846A>T / global toxicity / NS, trend for an increase (p=0.095) gastrointestinal toxicity / NS haematological toxicity / NS, trend for an increase (p=0.066) hospitalisation / NS *13 / global toxicity / NS gastrointestinal toxicity / NS, trend for an increase (p=0.090) haematological toxicity / 24.9 (1.74-354) (S) hospitalisation / NS, trend for an	Authors' conclusion: 'None of the individual *DPYD* variants were found to be associated with global severe toxicity. For c.2846A>T and c.1679T>G combined, there was evidence for an association with global severe toxicity. In addition, *DPYD* c.1679T>G alone was associated with haematological toxicity.'

table continues

				increase (p=0.094)
		c.2846A >T and *13	global toxicity	3.0 (1.05-8.77) (S)
		c.1236A >G	global toxicity	NS
			gastrointestinal toxicity	NS
			haematological toxicity	NS
			hospitalisation	NS, trend for an increase (p=0.069)
		For the 3 gene variants combined, sensitivity was 6%, specificity 95%, positive predictive value 13% and negative predictive value 88% for prediction of global toxicity grade ≥ 3 in the first cycle. NOTE: No association was found for the gene variants *4 (84 carriers), except for a trend for gastrointestinal toxicity. However, most studies including a meta-analysis (Meulendijks 2015) do not show an association of this gene variant with toxicity. In addition, results regarding the effect on DPD activity are inconsistent.		
ref. 4 – FU/CAP, mono/comb Kodali S et al. Capecitabine-induced severe toxicity secondary to DPD deficiency and successful treatment with low dose 5-fluorouracil. J Gastrointest Cancer 2017;48:66-69. PubMed PMID: 26744322.	Level of evidence score: 2 gene act. 1: CTC-AE 4	A 51-year old male developed severe colitis with mucous stools (grade 4 toxicity) and neutropenic fever (neutrophils 0.18x10^9/L) on day 21 of neoadjuvant treatment with standard dose capecitabine (825 mg/m^2 twice daily) and radiotherapy. His genotype was *1/*2A. The patient tolerated adjuvant therapy with 5-FU 300 mg/m^2 per day as a continuous intravenous infusion (25% of the standard dose) and without bolus injections of 5-fluorouracil very well. Higher doses were not attempted, because they were judged not to influence recurrence or survival.		Authors' conclusion: 'The utility of pharmacokinetic-based dosing remains questionable as patients experienced toxicity even with 50% dose reduction of 5-FU, as recommended by current consortium guidelines. We therefore suggest that dosing of 5-FU should be customized in patients with DPD deficiency based on clinical judgment taking into account the severity of toxicity from initial exposure.'
ref. 5 – CAP, mono/comb Meulendijks D et al. Patients homozygous for *DPYD*	Level of evidence score: 2 gene act. 1: CTC-AE 2	Three patients treated with capecitabine containing chemotherapy were retrospectively determined to have genotype c.1236A>G/c.1236A>G. Gene variants *2A, *13 and c.2846A>T were not present in these patients. More than 4 weeks after the last treatment with fluoropyrimidines, DPD enzyme activity in		Authors' conclusion: 'The presented functional and clinical data indicate that the c.1129-5923 C>G variant is both functionally and

table continues

c.1129-5923C>G/ haplotype B3 have partial DPD deficiency and require a dose reduction when treated with fluoropyrimidines. Cancer Chemother Pharmacol 2016;78:875-80. PubMed PMID: 27544765.	peripheral blood mononuclear cells was determined and cDNA was analysed. Results: - A 47-year old female developed leukocytopenia grade 2 ($2.3x10^9$/L), neutropenia grade 2 ($1.3x10^9$/L), hand-foot syndrome grade 1, diarrhoea grade 1 and fatigue grade 1 on day 9 of neoadjuvant treatment with standard dose capecitabine (825 mg/m^2 twice daily) and radiotherapy. Because the symptoms intensified, the capecitabine dose was reduced by 40% on day 15. After dose reduction, treatment was well tolerated. Five days after a dose increase by 10%, she again developed leukopenia grade 2 ($2.5x10^9$/L) and neutropenia grade 1 ($1.5x10^9$/L). Despite this, treatment could be finished at reduced dose. The patient received surgery and was disease-free four years after treatment. The DPD activity of the patient was 41% of the normal DPD activity. - A 67-year old male developed fatigue grade 2 on day 7 of treatment with capecitabine 850 mg/m^2 on day 1-14 of the three-week cycle, docetaxel, oxaliplatin and bevacizumab. On day 11, the patient was hospitalised with neutropenia grade 2 ($1.3x10^9$/L) and fever grade 1 (38.7^{o}C, without apparent focus). After release from hospital, he refused further treatment. Because of disease progression, capecitabine 800 mg/m2 twice daily (64% of the standard dose) was started four months later as monotherapy. The patient again developed fatigue grade 2 and refused further treatment after cycle 1. The DPD activity of the patient was 55% of the normal DPD activity. - A 69-year old male tolerated 4 weeks of neoadjuvant treatment with standard dose capecitabine (825 mg/m^2 twice daily) and radiotherapy well. Treatment was completed without dose reductions or delays, and without adverse events and haematological changes. The patient had a relapse one year after surgery and died as a result of progressive disease before determination of DPD activity could be performed. cDNA analysis of the first two patients showed that they produced roughly equal amounts of wild type mRNA and aberrantly spliced mRNA with a premature stop codon. The authors indicate that the starting dose of capecitabine was relatively low in these patients (compared to the monotherapy dose of 1250 mg/m^2 twice daily). So, higher doses might have resulted in more pronounced toxicity. Amstutz 2009 describes a	clinically relevant, and support an upfront dose reduction of the fluoropyrimidine starting dose in patients carrying c.1129-5923C>G homozygously.' Tolerated dose versus gene activity 2: gene activity 1: 60% DPD activity versus gene activity 2: gene activity 1: 48%

table continues

		patient with genotype c.1236A>G/c.1236A>G, who developed fatal toxicity during the first cycle with full dose 5-FU plus cisplatin. NOTE: Patients were genotyped for c.1129-5923C>G and checked for the presence of c.1236G>A and c.959-51T>G, which are in complete linkage disequilibrium with c.1129-5923C>G in haplotype B3.	
ref. 6 – FU/CAP, mono/comb Lunenburg CA et al. Evaluation of clinical implementation of prospective *DPYD* genotyping in 5-fluorouracil- or capecitabine-treated patients. Pharmacogenomics 2016;17:721-9. PubMed PMID: 27181275.	Level of evidence score: 3 gene act. 1.5:CTC-AE 4(2)# gene act. 1:CTC-AE 4(2)# gene act. 0.5:CTC-AE 4(2)#	The results of routine prospective genotyping and genotype-guided dosing were retrospectively evaluated in patients receiving capecitabine or 5-fluorouracil, either as combined chemotherapy (different combinations) or as monotherapy (with or without radiotherapy). Genotyping was originally only for *2A (275 patients), but from approximately 30% of the total study time genotyping for *13 and 2846A>T was added (214 patients) and from 65% of the total study time genotyping for c.1236G>A was added (n = 109). Recommended dosing reductions were 50% of the normal dose per *2A- and *13-variant and 25% per c.1236A>G-variant. Recommended dosing reduction per c.2846A>T-variant was 50% (change to a recommendation of 25% reduction was only after the study), but was not applied. 14 patients with gene variants were identified. Due to the low number of patients with DPD variants the study was not powered to formally test the effect of genotype-guided dosing on fluoropyrimidine-induced toxicity and only explorative analyses could be performed. Genotyping: - 8x *1/c.1236A>G - 5x *1/*2A - 1 carrier of both *2A and c.2846A>T (either *2A/c.2846A>T (on separate alleles) or *1/(*2A+c.2846A>T) (variants on the same allele)) Results: - 8 patients (5x *1/c.1236A>G and 3x *1/*2A) received the recommended initial dose reduction and did not develop toxicity grade 3-4 in cycle 1. The dose of 4 patients was subsequently increased. Two patients (1x *1/c.1236A>G with a dose increase to 100% of the normal dose and 1x *1/*2A with a dose increase to 60% of the normal dose) did not develop toxicity grade 3-4. A patient with genotype *1/*2A developed diarrhoea grade 3 and enteritis after dose increase to 80% of the normal dose. Another patient with this genotype developed hand-foot-syndrome grade 2-3 after multiple cycles with the normal dose.	Authors' conclusion: 'Prospective *DPYD* screening can be implemented successfully in a real world clinical setting, is well accepted by physicians and results in low toxicity.'

table continues

		- 3 patients (1x *1/c.1236A>G and 2x *1/*2A) did not receive an initial dose reduction and developed toxicity grade 3-4 in cycle 1. For two of these patients, therapy was started before the genotype was known. For the third patient, the oncologist did not reduce the dose, because the dose in the chemotherapy regimen was already relatively low (capecitabine plus radiotherapy). For 1 patient with genotype *1/*2A, the dose was subsequently reduced to 50% of the normal dose and the patient did not develop toxicity grade 3-4 anymore. The other 2 patients quitted fluoropyrimidine therapy. - For the carrier of both *2A and c.2846A>T, there was no dose recommendation, because it was not known whether the variants were on different alleles or on the same allele. Because therapy had to be started before the DPD-activity would have been determined, the physician decided to use a 50% dose reduction, taking into account the results of genotyping and that this patient had tolerated 5-FU containing regimens before. Fluoropyrimidine therapy was stopped in this patient after the first cycle due to toxicity (≤ grade 3). - 2 patients (both with genotype *1/c.1236A>G) did not start fluoropyrimidine therapy.	
ref. 7 – FU, comb Lee AM et al. Association between *DPYD* c.1129-5923 C>G/hapB3 and severe toxicity to 5-fluorouracil-based chemotherapy in stage III colon cancer patients: NCCTG N0147 (Alliance). Pharmacogenet Genomics 2016;26:133-7. PubMed PMID: 26658227.	Level of evidence score: 3 gene act. 1-1.5: CTC-AE 4	A subset of patients from Lee 2014 was reanalysed: 1953 patients, negative for *2A, *13 and c.2846A>T, and treated with 12 cycles of adjuvant FOLFOX therapy (5-FU, folinic acid and oxaliplatin) with or without cetuximab. 62.9% of patients had any grade ≥ 3 adverse event, with 32.7% having any grade ≥ 3 adverse event common to 5-FU treatment. Adverse events classified as common to 5-FU treatment were fatigue, anorexia, dehydration, diarrhoea, stomatitis/mucositis, nausea/vomiting, leukopenia, neutropenia, febrile neutropenia, thrombocytopenia, and pain. Most frequent 5-FU adverse events included diarrhoea (12.5%), neutropenia (10.3%), pain (5.4%), fatigue (5.2%), nausea/vomiting (4.7%), and mucositis (4.1%). Results were adjusted for clinicopathological factors like age, sex, treatment, total number of treatment cycles and dose modifications. The latter two outcomes (higher percentage of patients with premature continuation and with dose modification) might be results of 5-FU adverse events instead of causes. Cetuximab increased the risk of 5-FU adverse events. Results were adjusted for this, but this indicates that adverse events common to 5-FU are not the same as 5-fluorouracil-induced adverse events. Genotyping:	Authors' conclusion: 'No significant associations were identified between c.1129 -5923 C>G/hapB3 and overall grade≥3 adverse event rate. Our results suggest that c.1129-5923 C>G/hapB3 have limited predictive value for severe toxicity to 5-FU-based combination chemotherapy.'

table continues

		- 1875x *1/*1 - 77x *1/c.1236A>G - 1x c.1236A>G/c.1236A>G Results: Risk of grade ≥ 3 adverse event for c.1236A>G/c.1236A>G versus *1/c.1236A>G versus *1/*1: any adverse event: NS, trend for an increase (p=0.082); OR_{adj} for (*1/c.1236A>G + c.1236A>G/c.1236A>G) compared to *1/*1 also showed a trend for an increase (NS, p=0.127). diarrhoea: NS neutropenia: S for an increase pain: NS fatigue: NS nausea/vomiting: NS stomatis/mucositis: NS dehydration: NS leukopenia: NS NOTE: Results were reported for 1129-5923C>G, which was in complete linkage disequilibrium with the also genotyped c.1236G>A.	
ref. 8 – FU/CAP, mono/comb Deenen MJ et al. Upfront genotyping of *DPYD*2A to individualize fluoropyrimidine therapy: a safety and cost analysis. J Clin Oncol 2016;34:227-34. PubMed PMID: 26573078.	Level of evidence score: 3 gene act. 1: Clinical Relevance Score A	1631 patients received genotype-guided therapy with capecitabine (90% of patients) or 5-FU (10% of patients), either as combined chemotherapy (different combinations) or as monotherapy (with or without radiotherapy). Genotyping was for *2A. For *1/*2A, dose reduction in the first two cycles was ≥ 50% and was followed by dose titration based on tolerance. Initial dose was not reduced for *1/*1. Patients with the *1/*2A genotype were compared with 48 patients with this genotype, treated with the full initial dose in published cohorts studies without genotype-guided dosing. Of these 48 patients, 79% was treated with 5-fluorouracil, 19% with capecitabine and 2% with tegafur combined with uracil. In addition, patients with the *1/*2A genotype were compared to patients with the *1/*1 genotype. For 16 *1/*2A-patients, 5-fluorouracil AUC in blood plasma after the first capecitabine dose was compared with that of 25 unselected patients from two studies (n = 11 and n = 14 per study). For 15 *1/*2A-patients, DPD enzyme activity in peripheral mononuclear blood cells was determined and compared with the mean Caucasian DPD enzyme activity (mainly *1/*1-patients). The study had 100% power to detect a reduction of the incidence of grade ≥ 3 toxicity in *2A-carriers from 85% to 20%.	Authors' conclusion: '*DPYD*2A genotype-guided dosing results in adequate systemic drug exposure and significantly improves safety of fluoropyrimidine therapy for the individual patient. On a population level, upfront genotyping seemed cost saving.' AUC versus gene activity 2: gene activity 1: 203%

table continues

The risk of grade ≥ 3 toxicity was higher in combination therapy than in monotherapy and chemo-radiotherapy regimens.
Genotyping:
- 1613x *1/*1
- 18x *1/*2A

Results:
Treatment characteristics of *1/*2A-patients:
- The initial dose varied from 29% to 60% of the full dose (median 46%). The final dose varied from 17% to 91% of the full dose. The median dose per treatment cycle was 48% (range 17% to 91%). All patients were treated with capecitabine.
- 5 patients developed toxicity grade ≥ 3 (first cycle 29% to 60% of the normal dose, final cycle 17% to 60% and maximum 29% to 67%)
- 2 patients developed toxicity grade 0 (first of the two cycles with 29% and second cycle with 59% of the nor-mal dose and all five cycles 48% of the normal dose, respectively)
- 11 patients developed toxicity grade 1 to 2 (first cycle 30% to 50% of the normal dose, final cycle 24% to 91% and maximum 46% to 91%)
- Toxicity was short in duration and well controlled using standard supportive care.
- For 6 patients, the dose was increased during treatment (dose in first cycle 29% to 47% of the normal dose; maxi-mum dose 46% to 91%).
In two of these patients (dose increase from 47% to 53% and from 44% to 67%, respectively), the dose was later reduced to the initial dose again because of toxicity.
- For 3 patients, the initial dose was still too high and had to be reduced further (initial dose 29% to 44% of the normal dose, final dose 17% to 24%).
- Of 4 evaluable patients, 2 achieved a partial response and 2 had stable disease.
In 4 of 5 patients with rectal cancer treated with chemo-radiotherapy, down staging of the tumour from pT3-4 to ypT0-2 was reached.

Percentage of *1/*2A patients with toxicity for reduced dosing compared to full dosing:

		value for full dosing
any grade ≥ 3 toxicity	x 0.38 (S) In addition, the observed toxicity was short in duration with reduced dosing and usually long-lasting with full dosing.	73%

table continues

		grade ≥ 3 haematological toxicity	x 0.26 (S)	66%
		grade ≥ 3 gastrointestinal toxicity	x 0.20 (S)	56%
		fluoropyrimidine-induced death	NS	10%
		Percentage of patients with toxicity for *1/*2A on reduced dosing compared to *1/*1 on full dosing:		value for *1/*1
		any toxicity grade ≥ 3	NS	23%
		any toxicity grade 1-2	NS	54%
		haematological toxicity grade ≥ 3	NS	10%
		haematological toxicity grade 1-2	NS	35%
		diarrhoea grade ≥ 3	NS	8%
		diarrhoea grade 1-2	NS	29%
		hand-foot syndrome grade ≥ 3	NS	5%
		hand-foot syndrome grade 1-2	NS	28%
		The authors indicate that the comparable toxicity burden suggests that *1/*2A is not underexposed when treated with a median dose of 48%.		
		Dose-normalised pharmacokinetics and DPD enzyme activity for *1/*2A compared to *1/*1:		value for *1/*1
		5-FU AUC normalised to a capecitabine dose of 1250 mg/m^2	x 2.03 (NS)	602 ng.h/ml
		DPD enzyme activity in peripheral mononuclear blood cells	x 0.64 (S)	9.9 nmol/ hr per mg protein
ref. 9 – FU/CAP, mono/comb Meulendijks D et al. Clinical relevance of *DPYD* variants c.1679T>G, c.1236G>A/HapB3, and c.1601G>A as predictors of severe fluoropyrimidine-associated	Level of evidence score: 4 gene act. 1: CTC-AE 4 gene act. 1-1.5: CTC-AE 4	Meta-analysis of 8 cohort studies with in total 7365 patients treated with 5-FU or capecitabine, either as combined chemotherapy (different combinations) or as monotherapy (with or without radiotherapy). Data on *13 were derived from 5 studies including a total of 5,616 patients and 11 carriers of *13. Data on c.1236G>A were derived from 6 studies including a total of 4,261 patients and 174 heterozygous carriers and 3 homozygous carriers of c.1236A>G. Data on *2A were derived from 7 studies including a total of 5.737 patients and 60 carriers of *2A. Data on c.2846A>T were derived from all 8 studies including a total of 7,318 patients and 85 carriers of c.2846A>T. 1 of the 8 studies in this meta-analysis is also included in the meta-analysis of Rosmarin 2014 (Rosmarin 2014). 2 of the 8 studies in this meta-analysis are also	Authors' conclusion: '*DPYD* variants c.1679T>G and c.1236G>A/HapB3 are clinically relevant predictors of fluoropyrimidine-associated toxicity. Upfront screening for these variants, in addition to the established variants *DPYD*2A and c.2846A>T, is recommended to improve the safety of

table continues

toxicity: a systematic review and meta-analysis of individual patient data. Lancet Oncol 2015;16:1639-50. PubMed PMID: 26603945.	included in the meta-analysis of Terrazzino 2013 (Morel 2006 and Deenen 2011). 5 of the 8 studies in this meta-analysis are also included separately in this risk analysis: Morel 2006, Deenen 2011, Lee 2014, Rosmarin 2014 and Meulendijks 2017. If possible, a RR was calculated for each study based on individual patient data and adjusted for age, sex, and treatment regimen. For 2 of the 5 studies for *13, it was not possible to use individual patient data. A random-effects model was used for the meta-analysis. Haematological toxicity included thrombocytopenia, neutropenia, leukocytopenia, and anaemia. Gastro-intestinal toxicity included diarrhoea, mucositis/stomatitis, and nausea/vomiting. Short timeframe was defined as shorter than the complete treatment duration, long timeframe as the whole treatment duration. In addition, a meta-analysis of 3 case-control studies with in total 799 patients was performed for c.1236G>A. One of these case-control studies is also included in the meta-analysis of Rosmarin 2014 (Schwab 2008) and two in the meta-analysis of Terrazzino 2013 (Schwab 2008 and Kleibl 2009). One of these case-control studies is also included separately in this risk analysis (Schwab 2008). Results: Risk of grade ≥ 3 toxicity for *1/*13 compared to *1/*1:	patients with cancer treated with fluoropyrimidines.'

	RR_{adj} (95% CI)	incidence for *1/*1 (% of patients)
any toxicity	4.40 (2.08-9.30) (S)	22%
haematological toxicity	9.76 (3.03-31.48) (S)	
gastrointestinal toxicity	5.72 (1.40-23.33) (S)	
hand-foot syndrome	- (RR could not be calculated due to an incidence of 0% in *1/*13)	

The heterogeneity between the studies was significant and substantial, possibly because of the small number of *1/*13.
There was no indication of publication bias.
The results for any toxicity were similar when patients carrying *2A and/or c.2846A>T were excluded from the meta-analysis. The association remained significant with p< 0.0167 after exclusion of any study from the meta-analysis,

table continues

except for Loganayagam 2013. After exclusion of Loganayagam 2013, the p-value was 0.0433.
The effect of *13 on risk of severe toxicity seemed similar in studies with long and short timeframes.
The sensitivity of *13 in prediction of grade ≥ 3 toxicity was 0.3% and the positive predictive value 46%.

Risk of grade ≥ 3 toxicity for (*1/c.1236A>G + c.1236A>G/c.1236A>G) compared to *1/*1:

	RR_{adj} (95% CI)	incidence for *1/*1 (% of patients)
any toxicity	1.59 (1.29-1.97) (S)	22%
haematological toxicity	2.07 (1.17-3.68) (S)	
gastrointestinal toxicity	2.04 (1.49-2.78) (S)	
hand-foot syndrome	NS (also for the sub-group treated with capecitabine)	

There was no significant heterogeneity between the studies.
There was no indication of publication bias.
The results for any toxicity were similar when patients carrying *2A and/or c.2846A>T were excluded from the meta-analysis. The association remained significant after exclusion of any study from the meta-analysis.
The effect of c.1236A>G on risk of severe toxicity seemed similar in studies with long and short timeframes.
The sensitivity of c.1236A>G in prediction of grade ≥ 3 toxicity was 6.4% and the positive predictive value 41%.
The meta-analysis of the case-control studies did not show a significant result, probably due to the smaller number of patients.
The authors reported to have treated 3 patients with genotype c.1236A>G/c.1236A>G safely with low dose capecitabine (825 mg/m^2 twice a day).

Risk of grade ≥ 3 toxicity for *2A-carriers compared to *1/*1:

	RR_{adj} (95% CI)	incidence for *1/*1 (% of patients)
any toxicity	2.85 (1.75-4.62) (S)	29%

The heterogeneity between the studies was significant and strong.

table continues

		There was no indication of publication bias. Risk of grade ≥ 3 toxicity for c.2846A>T-carriers compared to *1/*1: RR_{adj} (95% CI) — incidence for *1/*1 (% of patients) any toxicity — 3.02 (2.22-4.10) (S) — 25% The heterogeneity between the studies was significant and strong. There was no indication of publication bias. NOTE: c.1236G>A is in complete linkage disequilibrium with c.1129-5923C>G in haplotype B3. Studies analysing both gene variants were pooled. NOTE: Meta-analysis of 5 studies with in total 3900 patients, 182x *1/*4 and 2x *4/*4, showed no significant association between *4 and grade ≥ 3 toxicity. The only study that found a significant effect (Loganayagam 2013) was the cause of strong heterogeneity between the studies. In addition, results regarding the effect of *4 on DPD activity are inconsistent.	
ref. 10 – FU, comb Lee AM et al. *DPYD* variants as predictors of 5-fluorouracil toxicity in adjuvant colon cancer treatment (NCCTG N0147). J Natl Cancer Inst 2014;106:dju298. PubMed PMID: 25381393.	Level of evidence score: 3 gene act. 0.5-1: CTC-AE 4 gene act. 0.5 + gene act. 1.5: CTC-AE 4 gene act. 0.5:CTC-AE 5(2)#	2594 patients were treated with 12 cycles of adjuvant FOLFOX therapy (5-fluorouracil, folinic acid and oxaliplatin; 91.9% of patients) or FOLFIRI therapy (5-fluorouracil, folinic acid and irinotecan; 8.1% of patients) with or without cetuximab. Part of the patients received 6 cycles of FOLFOX followed by six cycles of FOLFIRI with or without cetuximab. 62.0% of patients had any grade ≥ 3 adverse event, with 33.1% having any grade ≥ 3 adverse event common to 5-fluorouracil treatment. Adverse events classified as common to 5-fluorouracil treatment were fatigue, anorexia, dehydration, diarrhoea, stomatitis/mucositis, nausea/vomiting, leukopenia, neutropenia, febrile neutropenia, thrombocytopenia, and pain. Most frequent 5-fluorouracil adverse events included diarrhoea (12.0%), neutropenia (11.7 %), nausea/vomiting (5.0%), fatigue (4.9%), and mucositis (4.2%). Follow-up for disease free survival was for 5 years. Results were adjusted for clinicopathological factors like age, sex, treatment, total number of treatment cycles and dose modifications. The latter two outcomes (higher percentage of patients with premature continuation and with dose modification) might be results of 5-fluorouracil adverse events instead of causes. Cetuximab increased the risk of 5-fluorouracil adverse events. OR's were adjusted for this, but other	Authors' conclusion: 'Statistically significant associations were found between *DPYD* variants (*DPYD**2A and 2846A>T) and increased incidence of grade 3 or greater 5FU-adverse events in patients treated with adjuvant 5-FU-based combination chemotherapy.'

table continues

outcomes were not. In addition, this indicates that adverse events common to 5-fluorouracil are not the same as 5-FU-induced adverse events.

Genotyping:
- 2532x *1/*1
- 24x *1/*2A
- 26x *1/c.2846A>T
- 1x *2A/c.2846A>T
- 1x *1/274C
- 5x *2A-genotyping failed
- 5x c.2846A>T-genotyping failed

Results:

Risk of grade ≥ 3 toxicity, premature treatment termination and disease free survival for *2A-carriers compared to non-carriers:

		incidence for non-carriers
any toxicity	OR_{adj} = 3.58 (95% CI: 1.01-12.64) (S)	62%
any 5-FU toxicity	OR_{adj} = 14.91 (95% CI: 4.26-52.18) (S)	33%
diarrhoea	NS	12%
neutropenia	x 5.7 (S)	11%
nausea/vomiting	x 4.2 (S)	4.8%
fatigue	NS	4.8%
stomatitis/mucositis	NS, trend for an increase, p=0.09	4.2%
dehydration	NS	2.3%
leukopenia	NS, trend for an increase, p=0.08	1.8%
febrile neutropenia	NS, trend for an increase, p=0.07	1.6%
anorexia	NS	1.5%
pain	NS	0.8%
thrombocytopenia	NS, trend for an increase, p=0.08	0.3%
premature treatment termination	x 1.7 (S)	26%
dose modification	NS	74%
disease free survival after 3 year	NS	73%

When restricting the analysis to Caucasians, sex or treatment, the association between *2A and grade ≥ 3 5-FU toxicity remained significant, whereas the association between *2A and grade ≥ 3 overall toxicity did not.

table continues

Risk of grade ≥ 3 toxicity, premature treatment termination and disease free survival for *c.2846A>T-carriers compared to non-carriers:

		incidence for non-carriers
any toxicity	OR_{adj} = 5.43 (95% CI: 1.52-19.43) (S)	62%
any 5-FU toxicity	OR_{adj} = 10.24 (95% CI: 3.57-29.40) (S)	33%
diarrhoea	x 2.8 (S)	12%
neutropenia	x 4.9 (S)	11%
nausea/vomiting	NS	5.0%
fatigue	NS	4.8%
stomatitis/mucositis	NS	4.1%
dehydration	x 5.0 (S)	2.2%
leukopenia	x 8.2 (S)	1.8%
febrile neutropenia	NS, trend for an increase, p=0.08	1.6%
anorexia	NS	1.5%
pain	NS	0.8%
thrombocytopenia	x 55.5 (S)	0.2%
premature treatment termination	NS	26%
dose modification	NS	74%
disease free survival after 3 year	NS	73%

When restricting the analysis to Caucasians, sex or treatment, the association between c.2846A>T and grade ≥ 3 5-FU toxicity remained significant. The association between c.2846A>T and grade ≥ 3 overall toxicity remained significant in the subgroups of Caucasians and males, but not in the subgroups of females, FOLFOX only and FOLFOX + cetuximab.

Other results:
- Because of its low frequency, a statistically significant association could not be demonstrated between *13 and either 5-FU or overall grade ≥ 3 toxicity (NS).
- The *2A/c.2846A>T-patient had a grade 5 adverse event. The patient was only able to receive one cycle of FOLFOX + cetuximab.
- The *1/274C-patient had no grade ≥ 3 adverse events.

table continues

		- The gene variants *2A, *13 and c.2846A>T together predicted 5-FU grade ≥ 3 toxicity with a sensitivity of 5.3%, specificity of 99.4%, positive predictive value of 81.8% and negative predictive value of 68%. The low sensitivity and negative predictive value might be attributed to the combination chemotherapy, which may add to the 5-FU toxicity. NOTE: Genotyping was for 25 gene variants of which only 4 (*2A, *13, c.2846A>T and 274G>C) were found in this population from the USA.	
ref. 11 – CAP/FU, comb Rosmarin D et al. Genetic markers of toxicity from capecitabine and other fluorouracil-based regimens: investigation in the QUASAR2 study, systematic review, and meta-analysis. J Clin Oncol 2014;32:1031-9. PubMed PMID: 24590654.	Level of evidence score: 4 gene act. 0-1,5: CTC-AE 4	After colorectal cancer excision, 927 patients received adjuvant therapy with capecitabine 1250 mg/m^2 twice daily on days 1-14 of a 3-week cycle either as monotherapy (n = 436) or in combination with bevacizumab (n = 491). Grade III-V toxicity comprised hand-foot syndrome (n = 206), diarrhoea (n = 97) and neutropenia (n = 19). Variant c.2846A>T: - Associated with grade III-V toxicity (OR = 9.35; 95% CI: 2.01-43.4) (S) - No association with grade III-V diarrhoea and grade III-V hand-foot syndrome (NS). Given the allele frequency found, this is apparently based on 5 defect alleles. Variants *2A, 496A>G, c.1236G>A: - No association with grade III-V toxicity, grade III-V diarrhoea and grade III-V hand-foot syndrome (NS). Given the allele frequency found, this is apparently based on 4 defect alleles for *2A, 83 for 496A>G and 18 for c.1236G>A. Variant c.2846A>T and/or *2A: - Associated with grade III-V toxicity (OR = 5.51; 95% CI: 1.95-15.5) (S) - No association with grade III-V diarrhoea and grade III-V hand-foot syndrome (NS) - Both patients who died were carriers of *2A or c.2846A>T Meta-analysis of 6 studies during which Caucasian patients received capecitabine or 5-FU-based therapy. Of these 6 studies, the study covered in the paragraph above and Schwab, 2008, were also included separately in this risk analysis. Variant *2A: - No association with grade III-V toxicity for capecitabine (2 studies, n = 1035) (NS) - No significant association with grade III-V toxicity for 5-FU infusion, but there was a trend (2 studies, n =	Authors' conclusion: "Global capecitabine toxicity (grades 0/1/2 v grades 3/4/5) was associated with the rare, functional *DPYD* alleles c.2846A>T>A and *2A (combined odds ratio, 5.51)."

table continues

		732) (NS; p=0.0075, whilst this should be less than 0.0048 due to multiple testing) - No significant association with grade III-V toxicity for 5-FU bolus injection, but increased risk of grade III-V neutropenia (OR = 12.9; 95% CI: 3.13-53.3) (1 study, n = 338) (S) Variant c.2846A>T: - No meta-analysis for capecitabine, 5-FU infusion and 5-FU bolus injection (1 study each time) Variant 496G>A: - No meta-analysis for capecitabine and 5-FU infusion (in both cases only 1 study) - No association with grade III-V toxicity for 5-FU bolus injection (2 studies, n = 379) (NS) Variant c.1236G>A: - No meta-analysis for capecitabine, 5-FU infusion and 5-FU bolus injection (1 study each time) Variant c.2846A>T and/or *2A: - No meta-analysis for capecitabine (only 1 study) - There was a significant association (p=0.05) with grade III-V toxicity for 5-FU infusion and 5-FU bolus injection (S) NOTE: No association was found for the gene variants *4, *5, *6 and *9A. However, associations with severe toxicity have never been found in studies concerning these gene variants.	
ref. 12 – FU/CAP, mono/comb Terrazzino S et al. *DPYD* IVS14+1 G>A and c.2846A>T genotyping for the prediction of severe fluoropyrimidine-related toxicity: a meta-analysis. Pharmacogenomics 2013;14:1255-72. PubMed PMID: 23930673.	Level of evidence score: 4 gene act. 1: CTC-AE 4 gene act. 1,5: CTC-AE 4	Meta-analysis of 15 studies investigating patients treated with fluorouracil, capecitabine or tegafur-uracil (1 study). Data on *2A (IVS14+1G>A) were derived from 15 studies including a total of 4,094 patients and 60 carriers of *2A. Data on c.2846A>T were derived from 7 studies including a total of 2,308 patients and 34 carriers of c.2846A>T. These 15 studies include 8 studies that have also been included separately in this risk analysis: Salgueiro 2004, Morel 2006, Largillier 2006, Boisdron-Celle 2007, Schwab 2008, Sulzyc-Bielicka 2008, Kristensen 2010 and Deenen 2011. *2A versus (no *2A): Increased risk of grade III-V toxicity (OR = 5.42; 95% CI: 2.79-10.52; increase in the percentage of patients with grade III-V toxicity from 39% to 68%) (S) Exclusion of each of the studies from the meta-analysis did not lead to substantially different results (OR = 4.05 - 7.32 (S)). The risk was increased in studies in which the percentage of patients with grade III-V toxicity was less than 40% (OR = 8.31; 95% CI: 3.63-19.06) (S).	Authors' conclusion: "The results of this meta-analysis confirm clinical validity of *DPYD* IVS14+1 G>A and 2846A>T as risk factors for the development of severe toxicities following fluoropyrimidine treatment."

table continues

	However, the increase was non-significant in studies including ≥40% of patients with toxicity. The results were similar if only prospective studies, only higher quality studies or only studies including ≥ 200 patients were analysed. In prospective studies, the risk also increased as the incidence of grade III-V toxicity decreased in the study. The risk was also increased when only studies investigating 5-FU-based therapy or 5-FU monotherapy were analysed. Increased risk of grade III-V haematological toxicity (OR = 15.77; 95% CI: 6.36-39.06) (S) Increased risk of grade III-V diarrhoea (OR = 5.54; 95% CI: 2.31-13.29) (S) Increased risk of grade III-V mucositis (OR = 7.48; 95% CI: 3.03-18.47) (S) *2A had a sensitivity of 5.2% (95% CI: 3.0-8.9) and a specificity of 99.2% (95% CI: 98.8-99.4) for predicting grade III-V toxicity (S) The sensitivity was 9.0% for studies that showed less than 40% grade III-V toxicity (95% CI: 5.7-13.9) (S). There was study heterogeneity in the overall group, but not in the group with less than 40% toxicity. *2A had a sensitivity of 13% (95% CI: 6.6-24.1) for predicting grade III-V haematological toxicity (S) *2A had a sensitivity of 5.6% (95% CI: 3.2-9.7) for predicting grade III-V diarrhoea (S) *2A had a sensitivity of 11.5% (95% CI: 6.2-20.5) for predicting grade III-V mucositis (S) c.2846A>T versus (no c.2846A>T): Increased risk of grade III-V toxicity (OR = 8.18; 95% CI: 2.65-25.25; increase in the percentage of patients with grade III-V toxicity from 34% to 71%) (S) Exclusion of each of the studies from the meta-analysis did not lead to substantially different results (OR = 6.20 - 12.88 (S)). The risk was increased in studies in which the percentage of patients with grade III-V toxicity was less than 40% (OR = 16.59; 95% CI: 5.06-54.43) (S). However, the increase was non-significant in studies including ≥40% of patients with toxicity. The results were similar if higher only quality studies or only studies including ≥ 200 patients were analysed. The risk was also increased when only prospective studies were analysed (OR = 18.14; 95% CI: 6.26-52.58) (S) or only studies investigating 5-FU-based therapy (OR = 21.38; 95% CI: 6.71-68.15) (S). There was moderate study heterogeneity in the overall group, but not in the low or high toxicity subgroups, among prospective studies or among those investigating 5-fluorouracil-based therapy. There may have been publication bias.	

table continues

		Increased risk of grade III-V diarrhoea (OR = 6.04; 95% CI: 1.77-20.66) (S) c.2846A>T had a sensitivity of 5.4% (95% CI: 1.7-16.1) and a specificity of 99.1% (95% CI: 98.7-99.4) for predicting grade III-V toxicity (S) The sensitivity was 11.2% for studies that showed less than 40% grade III-V toxicity (95% CI: 2.8-35.1) (S). There was heterogeneity between the studies. c.2846A>T had a sensitivity of 4.6% (95% CI: 2.2-9.4) for predicting grade III-V diarrhoea (S)	
ref. 13 – FU/CAP, comb Magnani E et al. Fluoropyrimidine toxicity in patients with dihydropyrimidine dehydrogenase splice site variant: the need for further revision of dose and schedule. Intern Emerg Med 2013;8:417-23. PubMed PMID: 23585145.	Level of evidence score: 2 gene act. 1: CTC-AE 4	3 patients with genotype *1/*2A with gastrointestinal or head and neck tumours received 5-FU or capecitabine-based therapy (adjuvant or metastatic therapy). A 4th patient with genotype *1/*2A was not given adjuvant therapy. A 43-year-old colon cancer patient was given adjuvant therapy with capecitabine/oxaliplatin and a 50% dose of capecitabine (500 mg/m^2 twice daily for 14 days, followed by a week-long rest period). The patient developed diarrhoea, grade 4 neutropenia and grade 3 thrombocytopenia after 19 days. The adjuvant therapy was discontinued. A 71-year-old colon cancer patient received the same adjuvant therapy including 40% of the normal capecitabine dose (400 mg/m^2 twice daily). After 1 day, the patient started vomiting and developed grade 3 abdominal pain. The adjuvant therapy was discontinued. A 68-year-old patient with metastatic maxillary sinus cancer initially received 5-FU/carboplatin/folinic acid with standard-dose 5-FU (3000 mg/m^2 continuous infusion + 400 mg/m^2 bolus every 3 weeks). After 15 days, he developed grade 4 neutropenia and thrombocytopenia, and grade 3 sepsis and ulceration of the palate. After recovery, the treatment was restarted at 44% of the original dose (1500 mg/m^2 by continuous infusion) and prophylactic growth factors. There was no toxicity for 2 cycles. In the third cycle, the dose was increased to 59% of the standard dose (2000 mg/m^2 bolus) and no growth factors were given. After 14 days, the patient developed grade 4 febrile neutropenia and grade 2 anaemia. He was henceforth given non-fluoropyrimidine-based therapy. The authors indicated that a 50% dose decrease in gene activity score 1 is not always adequate.	Authors' conclusion: "Our data suggest that greater dose reductions or alternative therapies are needed for patients with DPD IVS14+1 G>A mutations."
ref. 14 – FU, comb Vulsteke C et al. Genetic variability in the multidrug	Level of evidence score: 4 gene act. 1: Clinical	1012 breast cancer patients received neoadjuvant/adjuvant therapy with 5-FU, epirubicin and cyclophosphamide. The 5-FU dose was 500 mg/m^2 every 3 weeks with a maximum of 1000 mg (n=902) or 600 mg/m^2 with a maximum of 1200 mg (n = 110).	Authors' conclusion: "In our study, we did not observe any association with toxicity and IVS14+1 G>A. The absence of

table continues

resistance associated protein-1 (ABCC1/ MRP1) predicts hematological toxicity in breast cancer patients receiving (neo-)adjuvant chemotherapy with 5-fluorouracil, epirubicin and cyclophosphamide (FEC). Ann Oncol 2013;24:1513-25. PubMed PMID: 23396606.	Relevance Score AA	Variant *2A (c.1905+1G>A, rs3918290): No significant association with serious adverse events (febrile neutropenia, prolonged grade III-IV neutropenia or severe neutropenia, grade III-IV anaemia, grade III-IV thrombocytopenia or grade III-IV non-haematological toxicity) (NS) The authors indicated that the lack of association is likely due to the fact that 5-FU toxicity is not common among breast cancer patients treated with this combination therapy. The 5-FU dose in this combination therapy is much lower than the dose in combination therapies used for colorectal cancer. NOTE: Associations were also not found for gene variants *5 (1627A>G), *6 (2194G>A) and *9A (85T>C). However, associations with severe toxicity have never been found in studies concerning these gene variants.	a significant association with IVS14+1 G>A probably relates to the fact that 5-FU toxicity is not frequent in breast cancer patients treated with FEC due to a much lower 5-FU dose in breast compared with colorectal cancer patients."
ref. 15 – FU, mono/ comb van Kuilenburg AB et al. Evaluation of 5-fluorouracil pharmacokinetics in cancer patients with a c.1905+1 G>A mutation in *DPYD* by means of a Bayesian limited sampling strategy. Clin Pharmacokinet 2012;51:163-74. PubMed PMID: 22339448.	Level of evidence score: 3 gene act. 1: CTC-AE 5	Clinical aspects were determined in 20 patients who had been genotyped as *1/*2A beforehand and were treated with 5-FU. Kinetics were determined in 30 *1/*2A (c.1905+1G>A) and 18 *1/*1, who received a 5-FU bolus injection of 300 mg/m^2 and/or 450 mg/m^2. Treatment regimens were not given. *Clinical* - All 7 *1/*2A receiving a standard dose of 5-FU showed grade III-V toxicity, of which 3 showed grade IV neutropenia The severe toxicity occurred in the first cycle each time and 1 patient died. - Among 13 *1/*2A receiving low-dose 5-FU, 4 had grade III toxicity and none had grade IV toxicity The patients with grade III toxicity received on average 74% of the standard dose, and those with grade II or lower toxicity received 61% of the dose. *Kinetics* *1/*2A versus *1/*1: - The 5-FU AUC increased by 52% for the 300 mg/m^2 dose (from 6.0 to 9.1 mg.hour/L) and by 32% for the 450 mg/m^2 dose (from 13.4 to 17.7 mg.hour/L) (S) The dose-corrected AUC increased by 32% (from 0.026 to 0.034 mg.hour/L per mg/m^2; 45 and 25 patient/dose combinations respectively) (S). The AUC seems to be predictive of the first 2 hours after the injection and may therefore cause an	Authors' conclusion: "Profound differences in the elimination of 5FU could be detected between DPD-deficient patients and control patients. Furthermore, treatment of DPD-deficient patients with standard 5FU-containing chemotherapy was associated with severe (lethal) toxicity." Maximum clearance (V_{max} for 300 mg/m^2) versus EM: gene activity 1: 54% AUC_t versus EM: gene activity 1: 132%

table continues

		underestimate for *1/ *2A. The 5-FU concentration 1 hour after injection was around the detection limit for *1/*1. - The terminal half-life of 5-FU increased by 109% for the 300 mg/m^2 dose (from 0.128 to 0.268 hours) and by 69% for the 450 mg/m^2 dose (from 0.181 to 0.306 hours) (S) - The maximum enzymatic metabolic capacity (V_{max}) calculated in a multi-compartment model decreased by 46% for the 300 mg/m^2 dose (from 1749 to 942 mg/hour) and by 34% for the 450 mg/m^2 dose (from 1370 to 900 mg/hour) (S)	
ref. 16 – CAP, comb Deenen MJ et al. Relationship between single nucleotide polymorphisms and haplotypes in *DPYD* and toxicity and efficacy of capecitabine in advanced colorectal cancer. Clin Cancer Res 2011;17:3455-68. PubMed PMID: 21498394.	Level of evidence score: 4 gene act. 1: CTC-AE 5 (gene act. 1 + gene act. 1,5): CTC-AE 4 gene act. 1,5: CTC-AE 4	568 patients with advanced colorectal cancer were treated with capecitabine 1000 mg/m^2 twice daily for 14 days every 3 weeks, in combination with oxaliplatin and bevacizumab, with or without cetuximab. Oxaliplatin was discontinued from cycle 7 and the capecitabine dose increased to 1250 mg/m^2. Grade III-IV toxicity occurred in 85% of the patients. *1/*2A versus *1/*1: - Factor 3.0 increase in the percentage of patients with grade III-IV diarrhoea (from 24% to 71%) (S; strong association: false discovery rate < 0.3) The sensitivity of *2A for predicting grade III-IV diarrhoea was 4% and the specificity 100%. - No increase in the percentage of patients with grade II-III hand-foot syndrome and no significant increase in the percentage of patients with grade III-IV toxicity (NS) All 7 *1/*2A developed grade III-IV toxicity (including 3 women), and 1 patient died during the 3rd cycle. - Decrease in the cumulative dose over the first 6 cycles (S): the average dose decrease increased from 10% to 51% in the lowest-dose cycle and from 10% to 44% in cycle 6. - No difference in mortality or progression-free survival (NS) (*1/c.1236A>G + c.1236A>G/c.1236A>G) versus *1/*1: - Factor 2.2 increase in the percentage of patients with grade III-IV diarrhoea (from 23% to 50%) (S; strong association: false discovery rate < 0.3) The sensitivity of c.1236G>A for predicting grade III-IV diarrhoea was 10% and the specificity 97%. - No significant increase in the percentage of patients with grade II-III hand-foot syndrome or with grade III-IV toxicity (NS). - No significant increase in dose decreases (NS) - No difference in mortality or progression-free survival (NS) *1/c.2846A>T versus *1/*1:	Authors' conclusion: "Of the patients polymorphic for *DPYD* IVS14+1G>A, c.2846A>T, and c.1236G>A, 71% (5 of 7), 63% (5 of 8), and 50% (14 of 28) developed grade 3 to 4 diarrhoea, respectively, compared with 24% in the overall population. …… *DPYD* IVS14+1G>A and 2846A>T predict for severe toxicity to capecitabine, for which patients require dose reductions. ….. The data suggest that initial dose reductions of 50% in IVS14+1 G>A and 25% in c.2846A>T variant allele carriers with further dose titration would significantly reduce the total number of severe toxicity events, thereby separate validation is indicated."

table continues

	- Factor 2.6 increase in the percentage of patients with grade III-IV diarrhoea (from 24% to 62%) (S; medium association: false discovery rate 0.3-0.4) The sensitivity of c.2846A>T for predicting grade III-IV diarrhoea was 4% and the specificity 99%. - No significant increase in the percentage of patients with grade II-III hand-foot syndrome or with grade III-IV toxicity (NS). - Decrease in the cumulative dose over the first 6 cycles (S): the average dose decrease increased from 10% to 27% in the lowest-dose cycle and from 10% to 24% in cycle 6. - No difference in mortality or progression-free survival (NS) (*1/*6 + *6/*6) versus *1/*1: - Factor 1.8 increase in the percentage of patients with grade III-IV diarrhoea (from 23% to 41%) (S; medium association: false discovery rate 0.3-0.4) The sensitivity of *6 (2194G>A) for predicting grade III-IV diarrhoea was 12% and the specificity 95%. - No significant increase in the percentage of patients with grade II-III hand-foot syndrome or with grade III-IV toxicity (NS). - No significant increase in dose decreases (NS) - No difference in mortality or progression-free survival (NS) (*1/496G + 496G/496G) versus *1/*1: - Factor 1.4 increase in the percentage of patients with grade III-IV diarrhoea (from 23% to 33%) (S; weak association: false discovery rate < 0.3) The sensitivity of 496A>G for predicting grade III-IV diarrhoea was 24% and the specificity 84%. - Factor 1.3 increase in the percentage of patients with grade II-III hand-foot syndrome (from 41% to 53%) (S; weak association: false discovery rate < 0.3) The sensitivity of 496A>G for predicting grade II-III hand-foot syndrome was 22% and the specificity 85%. - No significant increase in the percentage of patients with grade III-IV toxicity (NS). - No significant increase in dose decreases (NS) - No difference in mortality or progression-free survival (NS) *13: - The percentage *1/*13 was 0% among 43 patients with grade IV-V toxicity or two forms of grade III-V toxicity and 1% in 99 randomly selected patients (NS) The authors indicated that the lack of association with grade III-IV toxicity for each of the investigated SNPs is likely caused by the high risk in the overall population.	

table continues

		NOTE: No associations were found for gene variants *4 (1601 G>A), *5 (1627A>G) and *9A (85T>C). However, associations with severe toxicity have never been found in studies concerning these gene variants.	
ref. 17 – FU/CAP, mono/comb Kristensen MH et al. Variants in the dihydropyrimidine dehydrogenase, methylenetetrahydrofolate reductase and thymidylate synthase genes predict early toxicity of 5-fluorouracil in colorectal cancer patients. J Int Med Res 2010;38:870-83. PubMed PMID: 20819423.	Level of evidence score: 3 gene act. 1,5: CTC-AE 4	68 patients with advanced colorectal cancer were given adjuvant or palliative treatment with fluoropyrimidine-based therapy. Therapy consisted of either a 5-FU bolus injection 500 mg/m^2 every 2 weeks plus folinic acid (n=24) or fluorouracil (400 mg/m^2 bolus plus 600 mg/m^2 by infusion every 2 weeks) plus folinic acid and oxaliplatin (n=27) or capecitabine 1250 mg/m^2 twice daily for 14 days every 3 weeks (n=17). There was no significant difference between incidences of grade I-IV toxicity in the first 2 cycles caused by the different chemotherapies. However, the proportion of grade III-IV toxicity did differ (67%, 33% and 0% respectively). Results: - Higher frequency of 1896C>T in the group with grade I-IV toxicity than in the group without toxicity (13% versus 2% 1896T heterozygotes; there were no homozygotes; RR = 6) (S) - Of the 4 1896T heterozygotes, 2 developed grade III-IV toxicity, 1 developed grade I toxicity and 1 did not develop toxicity; the number of patients with toxicity was 24, the number of patients without was 44. This is equivalent to 8.3% 1896T heterozygotes in the group with grade III-IV toxicity and 4.5% in the group with < grade III toxicity. This is equivalent to an RR of 1.8 for grade III-IV toxicity.	Authors' conclusion: "Patients with the genetic variant IVS14+1 G/A or c1896 C/T in the *DPYD* gene had a statistically significant increased risk of experiencing toxicity (RR 2 and 6, respectively), both having a high specificity (0.97 and 0.98, respectively) and low sensitivity (0.04 and 0.13, respectively). It is concluded that pre-treatment detection of genetic variants can help to predict early toxicity experienced by patients receiving 5-FU-based chemotherapy."
ref. 18 – FU/CAP, comb Gross E et al. Strong association of a common dihydropyrimidine dehydrogenase gene polymorphism with fluoropyrimidine-related toxicity in cancer patients. PLoS ONE 2008;3:e4003.	Level of evidence score: 3 gene act. 1,5: CTC-AE 5 gene act. 1: CTC-AE 4	128 Caucasian patients including 39 with poor tolerance to FU combination therapy (grade III or IV toxicity). 2 of the patients with poor tolerance died as a result of FU-associated toxicity. Independent group of 53 patients with poor tolerance to FU (n=39) or capecitabine combination therapy (n=14). The presence of variants was investigated by fully sequencing the DPD alleles. Variant 496A>G: Strongest association with grade III and IV toxicity: OR = 4.42 [95% CI = 2.12-9.23] for 92 patients with toxicity. The polymorphism attributable risk was 56.9%. The association was significant in patients with breast and gastro-oesophageal cancer (n=56 and n=158), but was non-significant in colon cancer patients n=128). 1 of the fatalities was heterozygous. All 3 homozygotes had grade III or IV toxicity. Grade III and IV toxicity (especially diarrhoea and hand-foot syndrome) also occurred in carriers using	Authors' conclusion: "Our results show compelling evidence that, at least in distinct tumour types, a common *DPYD* polymorphism strongly contributes to the occurrence of fluoropyrimidine-related drug adverse events. Carriers of this variant could benefit from individual dose adjustment of the fluoropyrimidine drug or alternate therapies."

table continues

		capecitabine-based chemotherapy. Chemotherapy was discontinued in 2 of these. The association seems stronger with combination therapy than with monotherapy. Variant IVS10-15T>C: Association with grade III and IV toxicity: OR = 3.38 [95% CI = 1.71-8.78] for 39 patients with toxicity. The association was significant in patients with breast and gastro-oesophageal cancer (n=46 and n=146), but was non-significant in colon cancer patients (n=58). Variant *2A (IVS14+1G>A): Low allele frequency in these groups (0.03 in patients with severe toxicity; 0 in healthy people and patients without severe toxicity) (NS difference). 16 other variants identified: No significant association with severe toxicity.	
ref. 19 – FU, mono Capitain O et al. The influence of fluorouracil outcome parameters on tolerance and efficacy in patients with advanced colorectal cancer. Pharmacogenomics J 2008;8:256-67.	Level of evidence score: 3 (gene act. 1 + gene act. 1,5):CTC-AE 4(2)[#]	76 French patients with advanced colon cancer received weekly or two-weekly FU plus folinic acid (initial FU dose 1200 and 2500 mg/m^2 respectively; by continuous infusion, two-weekly regimen partially using a bolus (400 mg/m^2); dose adjustments based on a target AUC of 25 mg.h/L; dose reduction of 10% in the event of significant grade II toxicity, discontinuation and dose decrease of 25% in the event of grade III toxicity and discontinuation of therapy in the event of grade IV toxicity), screening for *2A (IVS14+1G>A), c.2846A>T, *13 (1679 T>G) and 464T>A and for DPD-deficient patients and also for 19 other variants. - 11.8% of the patients (n=9) displayed abnormally low clearance of FU associated with abnormal dihydrouracil/uracil plasma ratio prior to therapy. An SNP was found in 3 of these (2x c.2846A>T, 1x *2A). - Despite pharmacological dose adjustments, the incidence of grade III and IV toxicity was higher in the group with reduced DPD activity (n=9) than in the group with normal DPD activity (33.3% versus 7.5%; S by 347%; OR = 6.20 [95% CI = 1.18-32.56]). - The incidence of grade III and IV toxicity was higher in the group with SNPs (n=3) than in the group without SNPs (66.7% versus 8.2%; S by 711%). - The authors indicated that the increased toxicity in DPD-deficient patients may have been prevented by reduced initial doses followed by pharmacokinetic dose adjustments.	Authors' conclusion: "Toxicity was linked to low UH2/U ratio, c.2846 A>T, IVS14+1 G>A for DPD."
ref. 20 – FU Sulzyc-Bielicka V et al.	Level of evidence score: 3	252 Polish colon cancer patients received FU chemotherapy and screening for *2A (IVS14+1G>A). - 1 patient was heterozygous. This patient was 1 of the 4 patients with grade III-IV neutropenia.	Authors' conclusion: "We conclude that IVS14 + 1G > A *DPYD* (*DPYD**2A) variant occurs in the Polish

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5-Fluorouracil toxicity-attributable IVS14 + 1G > A mutation of the dihydropyrimidine dehydrogenase gene in Polish colorectal cancer patients. Pharmacol Rep 2008;60:238-42.	gene act. 1:CTC-AE 4(2)[#]		population and is responsible for a significant proportion of life-threatening toxicity of 5-FU."
ref. 21 – FU, mono Schwab M et al. Role of genetic and nongenetic factors for fluorouracil treatment-related severe toxicity: a prospective clinical trial by the German 5-FU Toxicity Study Group. J Clin Oncol 2008;26:2131-8.	Level of evidence score: 3 gene act. 1: CTC-AE 4	683 German patients (670x *1/*1, 13x *1/*2A), of whom 110 with grade III/IV toxicity; FU monotherapy with folinic acid or levamisole; screening for *2A (IVS14+1G>A) and also sequencing of exons and exon/intron transitions in 28 patients with grade IV toxicity, grade III toxicity or grade 0-II toxicity. *1/*2A versus *1/*1: Increased risk of grade III/IV toxicity: OR = 4.67 [95% CI = 1.54-14.2]. Significantly increased risk of grade III/IV leukopenia and mucositis (OR = 10.19 [95% CI = 3.0-35.1] and OR = 5.8 [95% CI = 1.71-19.4] respectively), but not of grade III/IV diarrhoea. Significantly increased risk of grade III/IV toxicity in men (OR = 41.8 [95% CI = 9.2-190]), but not in women. The sensitivity of *2A genotyping for overall toxicity was 5.5% [95% CI = 0.02-0.11] with a positive predictive value of 0.46 [95% CI = 0.19-0.75]. Sequencing of 3x 28 patients with different toxicity classes: 12 additional SNPs, including 4 new ones. 5 variants (623G>A, *4 (1601G>A), *6 (2194G>A), c.2846 A>T and 2585G>C) further investigated in ≥ 250 patients. 2585G>C was found in 1 patient with grade IV mucositis, but not in other patients (NS). The percentage of patients with toxicity was increased for c.2846A>T (60% versus 16.1% in the overall population) (NS). All other variants did not show a significant association with toxicity.	Authors' conclusion: "*DPYD*, TYMS, and MTHFR play a limited role for FU related toxicity but a pronounced *DPYD* gene/sex-interaction increases prediction rate for male patients."

table continues

		Inclusion of the additional variants only led to a marginal improvement in the prediction of overall toxicity. The method of administration is an independent risk factor: the risk of grade III/IV toxicity was greater for the bolus Mayo regimen than for the high-dose infusion (OR=2.44 [95% CI 1.52-3.91]).	
ref. 22 – FU, comb Mercier C et al. Prospective phenotypic screening for DPD deficiency prior to 5-FU administration: decrease in toxicity, not in efficacy. J Clin Oncol 2008;26(May 20 suppl):abstr 14556. (meeting abstract)	Level of evidence score: 3	59 French patients with inoperable head and neck cancer; determination of DPD activity (dihydrouracil/uracil ratio) prior to FU combination therapy or radio-chemotherapy; mild DPD deficiency (dihydrouracil/uracil ratio < 0.5): FU dose was 80% of the standard dose, severe DPD deficiency (ratio < 0.33): FU dose was 50% of the standard dose, complete DPD deficiency: no FU. - 25% of the patients had mild and 22% severe DPD deficiency. - 12% of the patients with DPD deficiency and dose reduction showed severe toxicity. The incidence of severe toxicity was twofold lower in the overall group compared to the regimen without dose reduction. - There were no toxicity-induced fatalities. - The effectiveness was similar to the regimen without dose reduction (percentages of responders 64% and 81% for first-line chemotherapy and radio-chemotherapy and 50% and 38% for treatment for relapsed cancer).	Authors' conclusion: "5-FU dose tailoring based upon DPD status evaluation led to 2 fold decrease in occurrence of severe toxicities without impairing efficacy."
ref. 23 – FU, comb Jatoi A et al. Paclitaxel, carboplatin, 5-fluorouracil, and radiation for locally advanced esophageal cancer: phase II results of preliminary pharmacologic and molecular efforts to mitigate toxicity and predict outcomes: North Central Cancer	Level of evidence score: 3 gene act. 1: Clinical Relevance Score: AA	50 American patients with locally advanced oesophageal cancer (11x *1/*1, 1x *1/*2A, 16x *1/*5, 3x *1/*6, 13x *1/*9A, 4x *9A/*9A, 1x *5/*5) participating in a phase II study received FU 225 mg/m^2 per day by continuous infusion in combination with carboplatin, paclitaxel and radiotherapy; FU was temporarily discontinued in the event of FU-related grade III-IV toxicity, after which the dose was decreased by 20%; patients received median 81% and 66% of the standard FU dose during 1 and 2 cycles respectively; screening for *2A (IVS14+1G>A), *5 (1627A>G), *6 (2194G>A) and *9A (85T>C). - Almost all patients (94%) had at least 1 incident of grade III-IV toxicity, including 3 fatalities. - No significant associations of the polymorphisms with pathological complete response, time to progression/relapse of cancer, overall survival or grade III/IV toxicity. NB: *5, *6 and *9A do not have reduced DPD activity.	Authors' conclusion: "Genotyping for polymorphisms of dihydropyrimidine dehydrogenase, cytochrome P3A4, and glutathione-S-transferase did not predict tumour response or serious adverse events."

table continues

Treatment Group (N0044). Am J Clin Oncol 2007;30:507-13.			
ref. 24 – FU, comb Magné N et al. Dihydropyrimidine dehydrogenase activity and the IVS14+1G>A mutation in patients developing 5FU-related toxicity. Br J Clin Pharmacol 2007;64:237-40.	Level of evidence score: 3 gene act. 1:CTC-AE 4(2)#	131 French patients with poor tolerance to FU combination or monotherapy (grade II neurotoxicity or grade III-IV toxicity), including 9 fatalities, and 185 unselected patients; screening for DPD activity in peripheral mononuclear blood cells and for *2A (IVS14+1G>A). - 81% of the toxicity occurred during the 1st cycle of FU chemotherapy. - Inverse association between DPD activity and toxicity score (sum of the different toxicity grades per patient) (S). - Percentage of patients with clear or severe DPD deficiency was higher in the case group than in the control group (17% versus 2.7% and 6% versus 0% respectively). - Inverse association between lethal toxicity and DPD activity (S). - Inverse association between the severity of the individual types of toxicity (grade II central neurotoxicity; grade IV mucositis, diarrhoea, neutropenia or thrombocytopenia) and DPD activity (all five S). Median DPD activity was 1.6-3.2x lower in patients with severe toxicity. - Only 2 in 93 screened cases (2.2%) had *2A (both *1/*2A). Both had low DPD activity and high toxicity scores during the 1st cycle. Neither died.	Authors' conclusion: "Present data suggest that IVS14+1 mutation screening has limited effectiveness in identifying patients at risk for severe 5FU toxicity."
ref. 25 - FU/CAP, mono Saif MW et al. Dihydropyrimidine dehydrogenase deficiency (GPD) in GI malignancies: experience of 4-years. Pak J Med Sci Q 2007;23:832-9.	Level of evidence score: 2 gene act. 1: CTC-AE 4	23 patients with excessive toxicity on FU (n=8) or capecitabine therapy (n=15), including 16 Caucasians, 3 Afro-Americans and 3 South-Asians; screening for DPD activity in peripheral mononuclear blood cells and by genotyping. - 30% of the patients had DPD deficiency (n=7), including 3 who were treated with FU (500 mg/m^2 per week or 425 mg/m^2 per week) and folinic acid, 2 who were treated with capecitabine 1800 mg/m^2 and 2 who were treated with high-dose bolus FU (1400 mg/m^2) in combination with the uridine prodrug 2',3',5'-tri-O-acetyluridine. The deficiency was confirmed by genotyping in 1 patient: he was *1/*2A. - 28% of the DPD-deficient patients died due to toxicity (n=2), including 1 to capecitabine and 1 to high-dose bolus FU. - Re-challenge with capecitabine of a patient treated with FU/ folinic acid led to grade III hand-foot syndrome.	Authors' conclusion: "Screening patients for DPD deficiency prior to administration of 5-FU or capecitabine using 2-13C uracil breath test could potentially lower risk of toxicity."

table continues

ref. 26 – FU, mono Boisdron-Celle M et al. 5-Fluorouracil-related severe toxicity: a comparison of different methods for the pretherapeutic detection of dihydropyrimidine dehydrogenase deficiency. Cancer Lett 2007;249:271-82.	Level of evidence score: 3 gene act. 1: CTC-AE 4 gene act. 1,5: CTC-AE 4 gene act. 0,5:CTC-AE 5(2)#	252 French patients with advanced colon cancer (163x *1/*1, 6x *1/c.2846A>T, 1x *9A/c.2846A>T, 1x *1/*2A, 1x -1590C/*2A, 1x *2A/c.2846A>T+85C, 1x *1/-1590C, 67x *1/*9A, 1x -1590C/*9A, 10x *9A/*9A) received either FU 400 mg/m^2 bolus + 2500 mg/m^2 by 46-hour infusion every 2 weeks (n=168) or FU 1200 mg/m^2 by 4-hour infusion per week (n=84) (both regimens: plus folinic acid); dose adjustment from the second cycle based on the FU plasma concentration at the end of the previous infusion (C_{ss}); discontinuation of treatment in the event of grade IV toxicity; screening for *2A (IVS14+1G>A), c.2846A>T, *7 (295-298delTCAT), 1156G>T, *9A (85T>C), *9B (2657G>A), *10 (2983G>T), -1590T>C. (*1/*2A + -1590C/*2A) versus *1/*1: Clearance decreased by 80% (S; from 104.7 to 21.22 L/h per m^2) Increase in the percentage of patients with grade III-IV toxicity by 793% (S; from 5.6% to 50.0%). (*1/c.2846A>T + 1x *9A/c.2846A>T) versus *1/*1: Clearance decreased by 40% and 58% for the two-weekly and weekly regimens respectively (both S; from 136.0 to 81.2 L/h per m^2 and from 104.7 to 43.9 L/h per m^2). Increase in the percentage of patients with grade III-IV toxicity by 1175% (S; from 5.6% to 71.4%). *2A/c.2846A>T+85T versus *1/*1: Clearance decreased to almost 0 (NS; by almost 100%). Increase in the percentage of patients with grade III-IV toxicity by 1686% (NS; from 5.6% to 100%). The patient had grade IV multi-organ toxicity and died after 40 days in Intensive Care. (1x *9A + 2x *9A) versus *1/*1: No difference in clearance and incidence of toxicity (NS). 1x -1590C versus *1/*1: No difference in clearance and incidence of toxicity (NS). Analysis of relevant SNPs had a high specificity (98.3%), but a low sensitivity (47.1%) for detecting DPD deficiency.	Authors' conclusion: "Except in cases where alternative treatment is recommended because the 5-FU metabolism is close to zero, IVS14 + 1G>A or 2846A>T heterozygote are not strict contra-indications to 5-FU treatment, provided that the physician is aware of it and that added precautions are taken, such as an initial 5-FU dose reduction and an individual dose adjustment based on a close clinical and pharmacokinetic follow-up." "In the case of a homozygous status for a relevant SNP, with a uracil plasma level higher than 100 lg/L or a UH2/U ratio below 1, then fluoropyrimidine administration must be discussed and an alternative treatment proposed." Clearance versus gene activity 2: gene act.1.5: 55% gene act.1: 20% gene act.0.5: almost 0%
ref. 27 – FU, mono Cho HJ et al. Thymidylate synthase (TYMS) and	Level of evidence score: 3 gene act. 1,5:	21 Korean colon cancer patients with grade III-IV toxicity on FU therapy (500 mg/m^2 by continuous infusion on days 1-5, plus folinic acid) and 100 healthy volunteers; screening by sequencing all exons and flanking introns.	Authors' conclusion: "The findings, from Korean patients with colon cancer, suggest that polymorphisms of the *DPYD* gene are

table continues

dihydropyrimidine dehydrogenase (*DPYD*) polymorphisms in the Korean population for prediction of 5-fluorouracil-associated toxicity. Ther Drug Monit 2007;29:190-6.	Clinical Relevance Score: AA gene act. 1: Clinical Relevance Score: AA	- Very common variants (allele frequency 14-22%) in this Korean group were *5, 1737T>C and 1896T>C. No *2A was found. - The percentage of patients without SNPs was similar to that in healthy volunteers (9.5% versus 10%). - There was no significant correlation between specific genotypes and toxic response. NB: *5 does not have reduced DPD activity.	not associated with an in-creased risk for toxic response to 5-FU."
ref. 28 – CAP, comb Salgado J et al. Polymorphisms in the thymidylate synthase and dihydropyrimidine dehydrogenase genes predict response and toxicity to capecitabine-raltitrexed in colorectal cancer. Oncol Rep 2007;17:325-8.	Level of evidence score: 3 gene act. 1:CTC-AE 4(2)#	58 Spanish patients with advanced colon cancer received capecitabine (1000 mg/m^2 twice daily for 14 days) and raltitrexed every 3 weeks; screening for *2A (IVS14+1G>A). 1 patient was *1/*2A. This patient developed severe toxicity after the first cycle, after which FU was discontinued and more appropriate chemotherapy was started.	Authors' conclusion: "Considering the common use of fluoropyrimidines, genetic screening would be highly recommendable for the presence of the DPD gene mutation (IVS14+1G>A) related to toxicity, prior to 5-FU administration."
ref. 29 – FU, comb Morel A et al. Clinical relevance of different dihydropyrimidine dehydrogenase gene single nucleotide polymorphisms on 5-fluorouracil tolerance.	Level of evidence score: 3 gene act. 0-1,5: E gene act. 1:CTC-AE 5(2)# gene act. 0:CTC-AE 4(2)#	487 French patients (300x *1/*1, 10x *1/c.2846A>T, 8x *1/*2A, 1x -1590C/*2A, 1x *2A/*2A, 6x *1/-1590C, 144x *1/*9A, 15x *9A/*9A, 1x *1/*13) received FU monotherapy (n=168) or one of 4 different FU combination therapies (n=319); dose adjustment from the second cycle based on the FU plasma concentration at the end of the previous infusion (C_{ss}); discontinuation of treatment or continuation with individual dose adjustment in the event of grade III/IV toxicity; screening for 22 relevant SNPs, including 9 in all patients *2A (IVS14+1G>A), c.2846A>T, *7 (295-298delTCAT), 1156G>T, *9A (85T>C), *9B (2657G>A), *10 (2983G>T), -1590T>C and *13 (1679T>G)) in 171 patients with or without toxicity. 5 variants were found in the population.	Authors' conclusion: "Pretreatment detection of three *DPYD* SNPs could help to avoid serious toxic adverse events. This approach is suitable for clinical practice and should be compared or combined with pharmacologic approaches. In the case of dihydropyrimidine

table continues

Mol Cancer Ther 2006;5:2895-904.		(*1/*2A + *2A/*2A + *1/c.2846A>T + *1/*13) versus *1/*1: Clearance decreased by 43% (S; from 132.3 to 74.9 L/h per m^2) Increase in the percentage of patients with grade III-IV toxicity by 838% (S; from 6.6% to 61.9%). One *1/*2A patient died due to toxicity. The *2A/*2A patient developed grade IV diarrhoea, neutropenia and mucositis a few days after initiation of low-dose bolus FU in combination with epirubicin and cyclophosphamide. She was treated in Intensive Care for 15 days. Patients with SNPs: treatment was discontinued in 40% of the patients with severe toxicity and continued with a 25-50% dose reduction and pharmacokinetic follow-up in the other 60%. (*1/*2A + *1/*13) versus *1/*1: Clearance decreased by 54% (NS; from 132.5 to 60.8 L/h per m^2) *1/c.2846A>T versus *1/*1: Clearance decreased by 45% (NS; from 132.5 to 72.3 L/h per m^2) (*1/*9A + *9A/*9A + *1/-1590C) versus *1/*1: No difference in clearance (NS, increased by 3%). No significant difference in the percentage of patients with grade III-IV toxicity (NS). None of the homozygous patients had grade III/IV toxicity. The sensitivity and specificity of the analysis of the 3 most important SNPs for predicting toxicity were 0.31 and 0.98 respectively.	dehydrogenase deficiency, 5-FU administration often can be safely continued with an individual dose adjustment." Clearance versus gene activity 2: gene act.1.5: 55% gene act.1: 46%
ref. 30 – CAP, mono Largillier R et al. Pharmacogenetics of capecitabine in advanced breast cancer patients. Clin Cancer Res 2006;12:5496-502.	Level of evidence score: 3 gene act. 1:CTC-AE 5(2)[#]	105 French patients with advanced breast cancer received capecitabine monotherapy; screening for *2A (IVS14+1G>A). 1 patient was *1/*2A. This patient died due to haematological toxicity after treatment with capecitabine 1820 mg/m^2 per day for 12 days.	Authors' conclusion: "Our case report clearly identifies DPD deficiency as a source of life-threatening toxicity under capecitabine treatment."
ref. 31 – FU, mono Salgueiro N et al.	Level of evidence score: 3	73 Portuguese colon cancer patients (71x *1/*1, 1x *1/*2A, 1x *1/1845T), including 8 with grade III/IV toxicity; various FU regimens; sequencing of exon 14.	Authors' conclusion: "We conclude that mutations in exon 14 of *DPYD* gene are

table continues

Mutations in exon 14 of dihydropyrimidine dehydrogenase and 5-fluorouracil toxicity in Portuguese colorectal cancer patients. Genet Med 2004;6:102-7.	gene act. 1:CTC-AE 4	SNPs in exon 14 (n=2) versus no SNPs in exon 14: Increase in the percentage of patients with grade III-IV toxicity by 1076% (S; from 8.5% to 100%).	responsible for a significant proportion of life-threatening toxicity to 5-FU, and should therefore be excluded before its administration to cancer patients."
ref. 32 – FU Van Kuilenburg AB et al. High prevalence of the IVS14 + 1G>A mutation in the dihydropyrimidine dehydrogenase gene of patients with severe 5-fluorouracil-associated toxicity. Pharmacogenetics 2002;12:555-8.	Level of evidence score: 3 gene act. 1 + gene act. 0): CTC-AE 4	60 Dutch patients with grade III/IV toxicity on FU therapy (43x *1/*1, 16x *1/*2A, 1x *2A/*2A) and 54 controls, including 35 cancer patients; screening for DPD activity in peripheral mononuclear blood cells and for *2A. - 60% of the cases had reduced DPD activity (< 70% of the average activity in controls). - 29% of the cases had 1 or 2 *2A alleles. - Significantly higher *2A allele frequency in the cases than in the general population (S; increase by 1548% from 0.91% to 15%).	Authors' conclusion: "Our study demonstrates that a DPD deficiency is the major determinant of 5FU-associated toxicity. The apparently high prevalence of the IVS14 + 1G>A mutation warrants genetic screening for this mutation in cancer patients before the administration of 5FU."
ref. 33 – FU, mono Raida M et al. Prevalence of a common point mutation in the dihydropyrimidine dehydrogenase (DPD) gene within the 5'-splice donor site of intron 14 in patients with severe 5-	Level of evidence score: 3 gene act. 1:CTC-AE 5(2)[#] gene act. 0:CTC-AE 5(2)[#]	25 German patients (19x *1/*1, 5x *1/*2A, 1x *2A/*2A) with grade III/IV toxicity on FU monotherapy (n=20), FU chemo-radiotherapy (n=2) or FU combination therapy (n=3) and 851 controls, including 800 cancer patients; screening for *2A. - 24% of the cases had 1 or 2 *2A alleles. - Higher *2A allele frequency in the cases than in the controls (NS; increase by 2879% from 0.47% to 14%). - The homozygous patient and two heterozygous patients died due to toxicity.	Authors' conclusion: "Routine screening for the exon 14-skipping mutation and subsequent individual determination of the 5-FU pharmacokinetics of heterozygous patients provides a concept of individualized therapy and allows the avoidance of undesired treatment toxicity."

table continues

fluorouracil (5-FU)-related toxicity compared with controls. Clin Cancer Res 2001;7:2832-9.			
ref. 34 – FU, comb Yamaguchi K et al. Germline mutation of dihydropyrimidine dehydrogenase gene among a Japanese population in relation to toxicity to 5-fluorouracil. Jpn J Cancer Res 2001;92:337-42.	Level of evidence score: 3 (gene act. 2 + gene act. 1,5): Clinical Relevance Score: AA	69 Japanese patients (61x *1/*1, 4x *1/*9A; 1x *1/*5; 1x *1/74G, 1x *1/812delT, 1x *1/1714G); FU combination therapy or monotherapy (FU: either 800 mg/m^2 by 1-hour infusion or 500 mg/m^2 per day on days 1 and 5 by continuous infusion); screening by PCR and sequencing. - The percentage of patients with grade III/IV toxicity was lower among the 8 heterozygous patients than among the *1/*1 patients (NS; decrease by 18% to 0%). NB: *5 and *9A do not have reduced DPD activity.	Authors' conclusion: "Our observations of Japanese patients implied that the heterozygote is not associated with increased toxic response to 5FU."
ref. 35 – FU van Kuilenburg AB et al. Clinical implications of dihydropyrimidine dehydrogenase (DPD) deficiency in patients with severe 5-fluorouracil-associated toxicity: identification of new mutations in the DPD gene. Clin Cancer Res 2000;6:4705-12.	Level of evidence score: 3 (gene act. 1,5 + gene act. 1): CTC-AE 4	37 Dutch patients with grade III/IV toxicity on FU therapy and 22 controls; sequencing of introns and intron-exon transitions. - 59% of the cases had reduced DPD activity (< 70% of the average activity in controls). - Weak but significant correlation between DPD activity and time to toxicity. - Higher prevalence of grade IV neutropenia in patients with reduced DPD activity compared to those with normal DPD activity (S; increased by 323%, from 13% to 55%). No higher prevalence of other types of toxicity. - 79% of 14 patients with reduced DPD activity had 1 or 2 allele variants (3x *1/*1, 4x *1/*2A, 1x *2A/*9A, 1x *2A/*5, 1x *9A/496G, 1x *9A/496G/c.2846A>T, 1x *1/*5, 1x *5/*9A, 1x *6/*6). NB: *5, *6 and *9A do not have reduced DPD activity.	Authors' conclusion: "Our results demonstrated that at least 57% (8 of 14) of the patients with a reduced DPD activity have a molecular basis for their deficient phenotype."

table continues

ref. 36 – FU, cutaneous Johnson MR et al. Life-threatening toxicity in a dihydropyrimidine dehydrogenase-deficient patient after treatment with topical 5-fluorouracil. Clin Cancer Res 1999;5:2006-11.	Level of evidence score: 2 gene act. 0: CTC-AE 3	A 76-year-old white man developed severe stomatitis, severe inflammatory colitis, erythematous rash, neutropenia $0.6x10^9$/L and thrombocytopenia $57x10^9$/L one week after initiation of 5% FU cream twice daily on the scalp for the treatment of basal cell cancer. FU was discontinued and the patient made a gradual recovery over 3 weeks. The patient was *2A/*2A and had no detectable DPD enzyme activity in peripheral mononuclear blood cells. Assuming 10% cutaneous absorption, the authors estimate that application of 2 g of 5% FU cream leads to a total absorbed dose of ~20 mg/day (~0.33 mg/kg for this patient). This is much lower than the IV bolus FU dose of 500-550 mg/kg that is generally used for chemotherapy.	Authors' conclusion: "This study represents the first characterization of a DPD deficient patient who developed life-threatening toxicity after exposure to topical 5-FU. Considering the previously reported low cutaneous absorption rate (~10%) of topical 5-FU, we suggest that life-threatening toxicity in the population of patients receiving topical 5-FU will be limited to profoundly DPD-deficient patients (no measurable DPD enzyme activity)."
ref. 37 – FU SPC Fluorouracil PCH 15-10-12.	Level of evidence score: 0 gene act. 0-1,5: CTC-AE 4	Warning: There have been reports of increased 5-FU toxicity in patients with partially functional or non-functional dihydropyrimidine dehydrogenase (DPD). If appropriate, DPD enzyme activity should be determined prior to treatment with 5-fluoropyrimidines.	
ref. 38 – FU SPC Efudix (fluorouracil) crème 07-09-16.	Level of evidence score: 0 gene act. 0-1,5: CTC-AE 4	Warning: Individuals with a defective dihydropyrimidine dehydrogenase (DPD) enzyme may be susceptible to severe systemic toxicity on use of standard doses of Efudix due to an increased systemic 5-FU concentration. Evaluation of DPD activity may be considered in patients with confirmed or suspected systemic toxicity. Due to the relationship between DPD deficiency and systemic toxicity, individuals known to have DPD enzyme deficiency should be intensively monitored for systemic toxicity during Efudix treatment. Adverse events: Frequency not known: haematological conditions, such as pancytopenia, neutropenia, thrombocytopenia, leukocytosis; haemorrhagic diarrhoea, diarrhoea, vomiting, stomach pain, stomatitis, rash, nasal mucositis.* * Haematological conditions, stomatitis, rash, nasal mucositis (associated with systemic toxicity to medicinal products).	
ref. 39 - CAP	Level of evidence score: 0	Contraindications: Patients with known complete absence of dihydropyrimidine dehydrogenase (DPD) activity.	

table continues

SPC Xeloda (capecitabine) 26-07-16.	gene act. 0: CTC-AE 5 gene act. 0.5-1.5: CTC-AE 4	Warning: Rarely, unexpected, severe toxicity (e.g. stomatitis, diarrhoea, mucosal inflammation, neutropenia and neurotoxicity) associated with 5-FU has been attributed to a deficiency of DPD activity. Patients with low or absent DPD activity, an enzyme involved in 5-FU degradation, are at increased risk for severe, life-threatening, or fatal adverse reactions caused by 5-FU. Although DPD deficiency cannot be precisely defined, it is known that patients with certain homozygous or certain compound heterozygous mutations in the *DPYD* gene locus, which can cause complete or near complete absence of DPD enzymatic activity (as determined from laboratory assays), have the highest risk of life-threatening or fatal toxicity and should not be treated with Xeloda. No dose has been proven safe for patients with complete absence of DPD activity. For patients with partial DPD deficiency (such as those with heterozygous mutations in the *DPYD* gene) and where the benefits of Xeloda are considered to outweigh the risks (taking into account the suitability of an alternative non-fluoropyrimidine chemotherapeutic regimen), these patients must be treated with extreme caution and frequent monitoring with dose adjustment according to toxicity. There is insufficient data to recommend a specific dose in patients with partial DPD activity as measured by specific test. In patients with unrecognised DPD deficiency treated with capecitabine, life-threatening toxicities manifesting as acute overdose may occur. In the event of grade 2-4 acute toxicity, treatment must be discontinued immediately.
ref. 40 – FU SPC Fluorouracil 29-07-16 (USA) and other[a]	Level of evidence score: 0 gene act. 0: CTC-AE 5 gene act. 0.5-1.5: CTC-AE 5	Warning: Based on post-marketing reports, patients with certain homozygous or certain compound heterozygous mutations in the DPD gene that result in complete or near complete absence of DPD activity are at increased risk for acute early-onset of toxicity and severe, life-threatening, or fatal adverse reactions caused by 5-FU (e.g., mucositis, diarrhoea, neutropenia, and neurotoxicity). Patients with partial DPD activity may also have increased risk of severe, life-threatening, or fatal adverse reactions caused by 5-FU. Withhold or permanently discontinue 5-FU based on clinical assessment of the onset, duration and severity of the observed toxicities in patients with evidence of acute early-onset or unusually severe toxicity, which may indicate near complete or total absence of DPD activity. No 5-FU dose has been proven safe for patients with complete absence of DPD activity. There is insufficient data to recommend a specific dose in

table continues

		patients with partial DPD activity as measured by any specific test.
ref. 41 – FU SPC Carac (fluorouracil) cream 16-12-03 (USA).	Level of evidence score: 0 gene act. 0: CTC-AE 4	Contraindications: Carac should not be used in patients with dihydropyrimidine dehydrogenase (DPD) deficiency. DPD deficiency may lead to 5-FU entering the anabolic route, resulting in cytotoxic activity and possible toxicity. Warning: Patients should discontinue treatment with Carac if symptoms of DPD deficiency develop. Rare, unexpected systemic toxicity (e.g. stomatitis, diarrhoea, neutropenia and neurotoxicity) associated with parenteral administration of 5-FU has been attributed to DPD deficiency. A case of life-threatening systemic toxicity has been reported following topical use of 5% 5-FU by a patient with fully non-functional DPD. Symptoms included severe abdominal pain, haemorrhagic diarrhoea, vomiting, fever and chills. Physical examination showed stomatitis, erythematous rash, neutropenia, thrombocytopenia, inflammation of the oesophagus, stomach and small intestine. Although this patient had used 5% 5-FU cream, it is not known whether patients with severe DPD deficiency develop systemic toxicity in response to lower concentrations of topically administered 5-FU.

[#] For studies that did not show significant differences for intermediate metabolizers (IM) or poor metabolizers (PM) due to very low numbers of IM or PM in the study (<4), the effect for IM or PM was scored as if this concerned a case. This was indicated by placing the case code (2) behind the score.
[a] SPC Xeloda (capecitabine) 14-12-16 (USA).
Abbreviations: 5-FU: 5-fluorouracil; 95% CI: 95% confidence interval; CAP: capecitabine; Cl: clearance; comb: combination therapy (≥ 2 oncolytic drugs), C_{ss}: steady-state plasma concentration; DPD: dihydropyrimidine dehydrogenase; gene act.: gene activity score; gene activity score 2: two fully functional alleles (extensive metaboliser); gene activity score 1.5: one fully functional and one partially functional allele; gene activity score 1: one fully functional and one non-functional allele or two partially functional alleles; gene activity score 0.5: one non-functional and one partially functional allele; gene activity score 0: two non-functional alleles; mono: monotherapy (one oncolytic drug); NS: non-significant; RR: relative risk; S: significant; SNP: single nucleotide polymorphism.

Supplementary Table 2. Literature review of *DPYD*/[tegafur with DPD inhibitor] interactions to support the therapeutic dose guidelines to optimize dose

Reference	Code	Effect	Comments
ref. 1 Cubero DI et al. Tegafur-uracil is a safe alternative for the treatment of colorectal cancer in patients with partial dihydropyrimidine dehydrogenase deficiency: a proof of principle. Ther Adv Med Oncol 2012;4:167-72. PubMed PMID: 22754590.	Level of evidence score: 2 gene act. 1: AA	Four patients with colorectal cancer developed grade 3-4 toxicity after the first cycle of chemotherapy with 5-FU (intravenous bolus of 425 mg/m^2 on days 1 and 5, in combination with folinic acid). They were found to be *1/*2A. After recovery, treatment with tegafur-uracil in combination with folinic acid was initiated. A full dose (100%) was tegafur 100 mg/m^2 three times daily for 21 days followed by a week-long rest period. Doses were rounded down to multiples of 100 mg tegafur. Doses were guided by adverse events. The first patient received 60% in the first cycle, 80% in the second cycle, 100% in the third cycle and 90% in the fourth and fifth cycles of the full dose of tegafur without development of grade 3-4 toxicity. This patient had developed grade 4 mucositis, diarrhoea and myelotoxicity on 5-FU. The following 3 patients received 90% of the full dose of tegafur during 5 cycles without development of grade 3-4 toxicity in any of the cycles. Of the three patients, one developed grade 4 diarrhoea and grade 3 mucositis on 5-FU, the second grade 3 diarrhoea and myelotoxicity, and the third grade 3 mucositis, diarrhoea and myelotoxicity. The best response in the first and the last patient, who both had metastatic disease, was achieving stable disease. The second and third patients receiving adjuvant chemotherapy were disease-free two years after the therapy.	Authors' conclusion: "Here, we demonstrate a complete absence of severe toxicity in all patients and cycles analysed. We believe that UFT is a safe alternative for the treatment of patients with partial DPD deficiency."
ref. 2 Deenen MJ et al. Standard-dose tegafur combined with uracil is not safe treatment after severe toxicity from 5-fluoro-uracil or capecitabine. Ann Intern Med 2010;153:767-8. PubMed PMID: 21135311.	Level of evidence score: 2 gene act. 1: E gene act. 1,5: E	- One patient developed severe abdominal cramps, grade 4 diarrhoea, grade 4 neutropenia, dehydration and severe mucositis 10 days after initiation of capecitabine 1000 mg/m^2 BSA twice daily (in combination with oxaliplatin and bevacizumab). She recovered after discontinuation of capecitabine and 25 days at the hospital. A few months later she received tegafur-uracil 300 mg/m^2 per day in combination with folinic acid. After 10 days, she developed severe diarrhoea, mucositis, fever, dehydration and grade 4 neutropenia. She recovered after 25 days at the hospital. The patient was *1/*2A. - Three other patients requiring hospitalisation due to severe toxicity on 5-FU or capecitabine therapy also developed severe toxicity following treatment with standard-dose tegafur-uracil. The patients were *1/*2A, *1/c.2846A>T and *1/c.1236G>A respectively. The DPD activity was approximately 50% in the latter two patients. This confirms that	Authors' conclusion: "The standard dose of UFT is not safe after severe toxicity to 5-FU or capecitabine in DPD-deficient patients."

table continues

		they were heterozygous and did not have a second unknown non-functional allele. The authors stated that tegafur-uracil is probably not safe in patients with partial DPD deficiency due to the greater effect of the DPD inhibitor uracil in these patients. They referred to an article that showed that uracil increases the half-life of fluorouracil to a greater extent in DPD-deficient patients, which leads to an increased risk of toxicity. The authors also stated that the tegafur dose in tegafur-gimeracil-oteracil is 3x as low as in tegafur-uracil, while the DPD inhibitor is 200x more potent. However, 5-FU is still metabolised by DPD after administration of tegafur-gimeracil-oteracil. This means that DPD also remains essential for detoxification of 5-FU in this instance.
ref. 3 SPC Teysuno (tegafur/gimeracil/ oteracil) 05-04-17.	Level of evidence score: 0 gene act. 0: CTC-AE 4 gene act. 0,5-1,5: E	Contraindications: Known dihydropyrimidine dehydrogenase (DPD) deficiency. History of severe and unexpected reactions to fluoropyrimidine therapy. Pharmacodynamics: Mean 5-FU maximum plasma concentration (C_{max}) and area under the concentration-time curve (AUC) values were approximately 3-fold higher after Teysuno administration than after administration of tegafur alone, despite a 16-fold lower Teysuno dose (50 mg of tegafur) compared to tegafur alone (800 mg), and are attributed to inhibition of DPD by gimeracil. Maximum plasma uracil concentration was observed at 4 hours, with a return to baseline levels within approximately 48 hours after dosing, indicating the reversibility of DPD inhibition by gimeracil. In man, the apparent terminal elimination half-life (T1/2) of 5-FU observed after administration of Teysuno (containing tegafur, a 5-FU prodrug) was longer (approximately 1.6-1.9 hours) than that previously reported after intravenous administration of 5-FU (10 to 20 minutes). Following a single dose of Teysuno, T1/2 values ranged from 6.7 to 11.3 hours for tegafur, from 3.1 to 4.1 hours for gimeracil and from 1.8 to 9.5 hours for oteracil. Interactions: Sorivudine or its chemically related analogues such as brivudine irreversibly inhibit DPD, resulting in a significant increase in 5-FU exposure. This may lead to increased clinically significant fluoropyrimidine-related toxicities with potentially fatal outcomes.

Abbreviations: 5-FU: 5-fluorouracil; DPD: dihydropyrimidine dehydrogenase; gene act.: gene activity score; gene activity score 2: two fully functional alleles (extensive metaboliser); gene activity score 1.5: one fully functional and one partially functional allele; gene activity score 1: one fully functional and one non-functional allele or two partially functional alleles; gene activity score 0.5: one non-functional and one partially functional allele; gene activity score 0: two non-functional alleles.

Supplementary Table 3. Relationship between genotype result and predicted phenotype in patients carrying no variants or one or more variants leading to decreased DPD enzyme activity

Patients carrying no or one variant(s)		
Genotype result	**Genotype (given as functionality of both alleles)**	**Predicted Phenotype**
No aberrant variant (*1/*1)	Full functionality/ full functionality	Gene activity score 2 (100% of normal DPD enzyme activity)
Heterozygous for variant with reduced functionality (*1/c.2846A>T or *1/c.1236G>A)	Fully functionality/ reduced functionality	Gene activity score 1.5 (75% of normal DPD enzyme activity)
Heterozygous for variant with inactive functionality (*1/*2A or *1/*13)	Full functionality/ inactive functionality	Gene activity score 1 (50% of normal DPD enzyme activity)
Homozygous for variant with reduced functionality (c.2846A>T/c.2846A>T or c.1236G>A/c.1236G>A)	Reduced functionality/ reduced functionality	Gene activity score 1 (50% of normal DPD enzyme activity)
Homozygous for variant with inactive functionality (*2A/*2A or*13/*13)	Inactive functionality/ inactive functionality	Gene activity score 0 (0% of normal DPD enzyme activity)
Patients carrying two variants		
Genotype result	**Possible predicted phenotype**	**Reasoning**
Heterozygous for two different variants with reduced functionality (c.2846A>T/c.1236G>A or *1/c.2846A>T+c.1236G>A)	Gene activity score 1 to 1.5 (50% to 75% of normal DPD enzyme activity), phenotyping is required to quantify DPD enzyme activity	When two variants are located on different alleles the predicted gene activity score is 1. When two variants are located on the same allele the predicted gene activity score is dependent on the effect that the two variants have on each other. This effect is unknown. If one of the two variants has no additional effect on the functionality, then the activity of the allele is equal to that without the second variant, thus 0.5, and the gene activity score is 1.5. When the two variants act synergistic and the allele becomes fully inactive, then the activity ofthe allele is 0 and the gene activity score is 1. Since the c.2846A>T and c.1236G>A variants result in reduced DPD enzyme activity through different biological mechanisms (Asp949Val amino acid substitution and an mRNA splicing-defect, respectively), it is probable that they are independent of each other regarding their effect on the allele's functionality. This would result in an allele activity of 0.25 (each variant resulting in half of the allele functionality) and thus a gene activity score of 1.25. Unfortunately there is no recommendation available for gene activity score 1.25.

table continues

		However, other factors than genetic variants can also affect the DPD enzyme activity. For this reason, one should resort to the recommendation for the gene activity score of 1 when the measured DPD enzyme activity is approximately equal to 50% of normal DPD enzyme activity and to the recommendation for the gene activity score of 1.5 when the measured DPD enzyme activity is approximately equal to 75% of normal DPD enzyme activity. When the measured DPD enzyme activity is between 50% and 75% (e.g. 63%) one should resort to the recommendation for gene activity score 1. In this case, one should record a gene activity score of 1.25 in the patients' medical record.
Heterozygous for variants with reduced functionality or inactive functionality (*2A/c.2846A>Tc.2846A>T or *1/*2A+c.2846A>T; *13/c.2846A>Tc.2846A>T or *1/*13+c.2846A>T; *2A/c.1236G>A or *1/*2A+c.1236G>A; *13/c.1236G>A or *1/*13+c.1236G>A)	Gene activity score 0.5 or 1 (25% or 50% of normal DPD enzyme activity), phenotyping is required to quantify DPD enzyme activity	When two variants are located on different alleles the gene activity score is 0.5 (one allele with reduced functionality and one allele with inactive functionality). When two variants are located on the same allele, the gene activity score is 1 (one allele with full functionality and one allele with inactive functionality).
Heterozygous for two different variants with inactive functionality (*2A/*13 or *1/*2A+*13)	Gene activity score 0 or 1 (0% or 50% of normal DPD enzyme activity), phenotyping is required to quantify DPD enzyme activity	When two variants are located on different alleles the gene activity score is 0 (two alleles with inactive functionality). When two variants are located on the same allele the gene activity score is 1 (one allele with full functionality and one allele with inactive functionality).
Homozygous for one variant with reduced functionality and heterozygous for the other variant with reduced functionality	Gene activity score 0.5 to 1 (25% to 50% of normal DPD enzyme activity), phenotyping is required to quantify DPD enzyme activity	One of the alleles has an activity of 0.5. The activity of the other allele is unknown, but lies between 0 and 0.5 (see reasoning for heterozygous for two different alleles with reduced functionality). One should resort to the recommendation for gene activity score 0.5 when the measured
(c.2846A>T/c.2846A>T+ c.1236G>A or c.1236G>A/c.2846A>T+ c.1236G>A)		DPD enzyme activity is approximately 25% of normal DPD enzyme activity and to the recommendation of gene activity score 1 when the DPD enzyme activity is 50% of normal DPD enzyme activity. When the measured DPD enzyme activity is between 25% and 50% (e.g. 38%) one should resort to the recommendation for gene activity score 0.5. In this case, one should record a gene activity score of 0.75 in the patients' medical record.

table continues

Homozygous for a variant with reduced functionality and heterozygous for a variant with inactive functionality (c.2846A>Tc.2846A>T/*2A+ c.2846A>Tc.2846A>T or c.2846A>Tc.2846A>T/*13+ c.2846A>Tc.2846A>T or c.1236G>A/*2A+c.1236G>A or c.1236G>A/*13+ c.1236G>A)	Gene activity score 0.5	One of the alleles has an activity of 0.5, the activity of the other allele is 0. Therefore the gene activity score is 0.5.
Heterozygous for a variant with reduced functionality and homozygous for a variant with inactive functionality 2A/*2A+c.2846A>Tc.2846A>T or *2A/*2A+c.1236G>A or *13/*13+ c.2846A>T/c.2846A>T or *13/*13+c.1236G>A)	Gene activity score 0	Both alleles have an activity of 0. Therefore the gene activity score is 0.
Homozygous for a variant with inactive functionality and heterozygous for the other variant with inactive functionality (*2A/*2A+*13 or *13/*2A+*13)	Gene activity score 0	Both alleles have an activity of 0. Therefore the gene activity score is 0.
Homozygous for two different variants with reduced functionality (c.2846A>T+c.1236G>A/ c.2846A>T+c.1236G>A)	Gene activity score 0 to 1 (0% to 50% of normal DPD enzyme activity), phenotyping is required to quantify DPD enzyme activity	The activity of both alleles is unknown, but lies between 0 and 0.5 (see reasoning for heterozygous for two different reduced functionality alleles). One should resort to the recommendation for gene activity score 0 when the measured DPD enzyme activity is approximately 0% of normal DPD enzyme activity and the recommendation of gene activity score 1 when the DPD enzyme activity is 50% of normal DPD enzyme activity. When the measured DPD enzyme activity is between 0% and 50% (e.g. 25%) one should resort to the recommendation for gene activity score 0.5.
Homozygous for a variant with reduced functionality and a variant with inactive functionality +c.2846A>T/*2A+c.2846A>T or *13+c.2846A>T/*13+ c.2846A>T or *2A+c.1236G>A/*2A+ c.1236G>A or *13+c.1236G>A/*13+ c.1236G>A)	Gene activity score 0	Both alleles have an activity of 0. Therefore the gene activity score is 0.

table continues

Homozygous for two different variants with inactive functionality (*2A+*13/*2A+ *13)	Gene activity score 0	Both alleles have an activity of 0. Therefore the gene activity score is 0.
Patients carrying three or more variants		
Genotype result	**Reasoning for finding the possible predicted phenotype**	
Three or more variants	Since patients carrying three or more different variants are rare, only a general explanation of how to predict the phenotype is given. If one does encounter a patient carrying three or more variants, one must determine how these variants can be located among two alleles and determine if this leads to different predicted phenotypes. Since there are only two validated variants which result in a reduced functionality, an allele with three different variants will always have a variant with an inactive functionality and therefore the allele will have an activity of 0. The predicted allele activities for alleles with 0, 1 or 2 variants are indicated in the tables above. If all possible distributions of the variants across the alleles lead to the same gene activity score of the genotype (i.e. the sum of allele activities), then one can conclude this as the patient's gene activity score. If different distributions lead to genotypes with different gene activity scores, phenotyping is required to quantify DPD enzyme activity.	

Abbreviation: DPD: dihydropyrimidine dehydrogenase.

Supplementary Table 4. Genotype to predicted phenotype translation to be programmed into laboratory information system

Genotype	rs number variants	Nucleotide at position	Dose recommendation according to gene activity score
DPYD: WILDTYPE/WILDTYPE	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:G T:T G:G A:A	GENE ACTIVITY SCORE 2
DPYD: WILDTYPE/*2A	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:A *2A T:T G:G A:A	GENE ACTIVITY SCORE 1
DPYD: WILDTYPE/*13	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:G T:G *13 G:G A:A	GENE ACTIVITY SCORE 1
DPYD: WILDTYPE/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:G T:T G:G A:T c.2846A>T	GENE ACTIVITY SCORE 1,5
DPYD: WILDTYPE/c.1236G>A	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:G T:T G:A c.1236G>A A:A	GENE ACTIVITY SCORE 1,5
DPYD: *2A/*2A	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	A:A *2A T:T G:G A:A	GENE ACTIVITY SCORE 0
DPYD: *13/*13	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:G G:G *13 G:G A:A	GENE ACTIVITY SCORE 0
DPYD: c.2846A>T/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:G T:T G:G T:T c.2846A>T	GENE ACTIVITY SCORE 1
DPYD: c.1236G>A/c.1236G>A	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:G T:T A:A c.1236G>A A:A	GENE ACTIVITY SCORE 1
DPYD: WILDTYPE/*2A WILDTYPE/*13	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:A*2A T:G *13 G:G A:A	Unable to predict the gene activity score. Phenotyping should distinguish if both variants are present on separate alleles (GENE ACTIVITY SCORE 0) or on the same allele (GENE ACTIVITY SCORE 1).
DPYD: WILDTYPE/*2A WILDTYPE/c.1236G>A	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:A *2A T:T G:A c.1236G>A A:A	Unable to predict the gene activity score. Phenotyping should distinguish if both variants are present on separate alleles (GENE ACTIVITY SCORE 0.5) or on the same allele (GENE ACTIVITY SCORE 1).

table continues

DPYD: WILDTYPE/*2A WILDTYPE/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:A *2A T:T G:G A:T c.2846A>T	Unable to predict the gene activity score. Phenotyping should distinguish if both variants are present on separate alleles (GENE ACTIVITY SCORE 0.5) or on the same allele (GENE ACTIVITY SCORE 1).
DPYD: WILDTYPE/*13 WILDTYPE/c.1236G>A	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:G T:G*13 G:A c.1236G>A A:A	Unable to predict the gene activity score. Phenotyping should distinguish if both variants are present on separate alleles (GENE ACTIVITY SCORE 0.5) or on the same allele (GENE ACTIVITY SCORE 1).
DPYD: WILDTYPE/*13 WILDTYPE/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:G T:G*13 G:G A:T c.2846A>T	Unable to predict the gene activity score. Phenotyping should distinguish if both variants are present on separate alleles (GENE ACTIVITY SCORE 0.5) or on the same allele (GENE ACTIVITY SCORE 1).
DPYD: WILDTYPE/c.1236G>A WILDTYPE/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:G T:T G:A c.1236G>A A:T c.2846A>T	Unable to predict the gene activity score. Phenotyping should distinguish if both variants are present on separate alleles (GENE ACTIVITY SCORE 1) or on the same allele (GENE ACTIVITY SCORE 1 to 1.5). When both variants are located on the same allele, it is not known whether the variants have an independent or synergistic effect or whether the second variant does not have an additional effect.
DPYD: c.1236G>A/c.1236G>A c.2846A>T/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:G T:T A:A c.1236G>A T:T c.2846A>T	Unable to predict the gene activity score (GENE ACTIVITY SCORE 0 to 1). Both variants are located on the same allele, but it is not known whether the variants have an independent or synergistic effect or whether the second variant does not have an additional effect.
DPYD: WILDTYPE/c.1236G>A c.2846A>T/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:G T:T G:A c.1236G>A T:T c.2846A>T	Unable to predict the gene activity score (GENE ACTIVITY SCORE 0.5 to 1). There is one allele with one variant and one allele with two variants, but it is not known whether the two variants on the same allele have an independent or synergistic effect or whether the second variant does not have an additional effect.
DPYD: c.1236G>A/c.1236G>A WILDTYPE/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:G T:T A:A c.1236G>A A:T c.2846A>T	Unable to predict the gene activity score (GENE ACTIVITY SCORE 0.5 to 1). There is one allele with one variant and one allele with two variants, but it is not known whether the two variants on the same allele have an independent or synergistic effect or whether the second variant does not have an additional effect.
DPYD: *13/*13 c.1236G>A/c.1236G>A	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:G G:G *13 A:A c.1236G>A A:A	GENE ACTIVITY SCORE 0

table continues

DPYD: *13/*13 WILDTYPE/c.1236G>A	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:G G:G *13 G:A c.1236G>A A:A	GENE ACTIVITY SCORE 0
DPYD: *13/*13 c.2846A>T/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:G G:G *13 G:G T:T c.2846A>T	GENE ACTIVITY SCORE 0
DPYD: *13/*13 WILDTYPE/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:G G:G *13 G:G A:T c.2846A>T	GENE ACTIVITY SCORE 0
DPYD: *13/*13 *2A/*2A	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	A:A*2A G:G *13 G:G A:A	GENE ACTIVITY SCORE 0
DPYD: *13/*13 WILDTYPE/*2A	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:A *2A G:G *13 G:G A:A	GENE ACTIVITY SCORE 0
DPYD: *2A/*2A c.1236G>A/c.1236G>A	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	A:A*2A T:T A:A c.1236G>A A:A	GENE ACTIVITY SCORE 0
DPYD: *2A/*2A WILDTYPE/c.1236G>A	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	A:A*2A T:T G:A c.1236G>A A:A	GENE ACTIVITY SCORE 0
DPYD: *2A/*2A c.2846A>T/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	A:A*2A T:T G:G T:T c.2846A>T	GENE ACTIVITY SCORE 0
DPYD: *2A/*2A WILDTYPE/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	A:A*2A T:T G:G A:T c.2846A>T	GENE ACTIVITY SCORE 0
DPYD: *2A/*2A WILDTYPE/*13	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	A:A*2A T:G*13 G:G A:A	GENE ACTIVITY SCORE 0
DPYD: c.1236G>A/c.1236G>A WILDTYPE/*2A	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:A*2A T:T A:A c.1236G>A A:A	GENE ACTIVITY SCORE 0,5
DPYD: c.1236G>A/c.1236G>A WILDTYPE/*13	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:G T:G*13 A:A c.1236G>A A:A	GENE ACTIVITY SCORE 0,5
DPYD: c.2846A>T/c.2846A>T WILDTYPE/*2A	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:A *2A T:T G:G T:T c.2846A>T	GENE ACTIVITY SCORE 0,5

table continues

DPYD: c.2846A>T/c.2846A>T WILDTYPE/*13	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:G T:G *13 G:G T:T c.2846A>T	GENE ACTIVITY SCORE 0,5
DPYD: *13/*13 c.1236G>A/c.1236G>A c.2846A>T/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:G G:G *13 A:A c.1236G>A T:T c.2846A>T	GENE ACTIVITY SCORE 0
DPYD: *13/*13 wildtype/c.1236G>A c.2846A>T/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:G G:G *13 G:A c.1236G>A T:T c.2846A>T	GENE ACTIVITY SCORE 0
DPYD: *13/*13 c.1236G>A/c.1236G>A wildtype/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:G G:G*13 A:A c.1236G>A A:T c.2846A>T	GENE ACTIVITY SCORE 0
DPYD: *13/*13 wildtype/c.1236G>A wildtype/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:G G:G*13 G:A c.1236G>A A:T c.2846A>T	GENE ACTIVITY SCORE 0
DPYD: wildtype/*13 c.1236G>A/c.1236G>A c.2846A>T/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:G T:G *13 A:A c.1236G>A T:T c.2846A>T	Unable to predict the gene activity score (GENE ACTIVITY SCORE 0 to 0.5). The activity of the allele with two variants (c.1236G>A and c.2846A>T) is not known, because it is not known whether the variants have an independent or synergistic effect or whether the second variant does not have an additional effect.
DPYD: wildtype/*13 wildtype/c.1236G>A c.2846A>T/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:G T:G *13 G:A c.1236G>A T:T c.2846A>T	Unable to predict the gene activity score. Phenotyping should distinguish if *13 and c.1236G>A are present on separate alleles (GENE ACTIVITY SCORE 0 to 0.5) or on the same allele (GENE ACTIVITY SCORE 0.5). When both variants are located on separate alleles, the activity of the allele with the two variants c.1236G>A and c.2846A>T is not known, because it is not known whether the variants have an independent or synergistic effect or whether the second variant does not have an additional effect.
DPYD: wildtype/*13 c.1236G>A/c.1236G>A wildtype/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:G T:G *13 A:A c.1236G>A A:T c.2846A>T	Unable to predict the gene activity score. Phenotyping should distinguish if *13 and c.2846A>T are present on separate alleles (GENE ACTIVITY SCORE 0 to 0.5) or on the same allele (GENE ACTIVITY SCORE 0.5). When both variants are located on separate alleles, the activity of the allele with the two variants c.1236G>A and c.2846A>T is not known, because it is not known whether the variants have an independent or synergistic effect or whether the second variant does not have an additional effect.

table continues

DPYD: wildtype/*13 wildtype/c.1236G>A wildtype/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:G T:G*13 G:A c.1236G>A A:T c.2846A>T	Unable to predict the gene activity score (GENE ACTIVITY SCORE 0 to 1). Phenotyping should distinguish which variants are present on the same allele. The activity of an allele with the two variants c.1236G>A and c.2846A>T is not known, because it is not known whether the variants have an independent or synergistic effect or whether the second variant does not have an additional effect.
DPYD: *2A/*2A c.1236G>A/c.1236G>A c.2846A>T/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	A:A *2A T:T A:A c.1236G>A T:T c.2846A>T	GENE ACTIVITY SCORE 0
DPYD: *2A/*2A wildtype/c.1236G>A c.2846A>T/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	A:A *2A T:T G:A c.1236G>A T:T c.2846A>T	GENE ACTIVITY SCORE 0
DPYD: *2A/*2A c.1236G>A/c.1236G>A wildtype/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	A:A *2A T:T A:A c.1236G>A A:T c.2846A>T	GENE ACTIVITY SCORE 0
DPYD: *2A/*2A wildtype/c.1236G>A wildtype/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	A:A *2A T:T G:A c.1236G>A A:T c.2846A>T	GENE ACTIVITY SCORE 0
DPYD: *2A/*2A *13/*13 c.1236G>A/c.1236G>A	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	A:A *2A G:G *13 A:A c.1236G>A A:A	GENE ACTIVITY SCORE 0
DPYD: *2A/*2A *13/*13 wildtype/c.1236G>A	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	A:A *2A G:G *13 G:A c.1236G>A A:A	GENE ACTIVITY SCORE 0
DPYD: *2A/*2A *13/*13 c.2846A>T/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	A:A*2A G:G*13 G:G T:T c.2846A>T	GENE ACTIVITY SCORE 0
DPYD: *2A/*2A *13/*13 wildtype/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	A:A*2A G:G*13 G:G A:T c.2846A>T	GENE ACTIVITY SCORE 0
DPYD: *2A/*2A wildtype/*13 c.1236G>A/c.1236G>A	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	A:A*2A T:G*13 A:A c.1236G>A A:A	GENE ACTIVITY SCORE 0
DPYD: *2A/*2A wildtype/*13 wildtype/c.1236G>A	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	A:A *2A T:G *13 G:A c.1236G>A A:A	GENE ACTIVITY SCORE 0
DPYD: *2A/*2A wildtype/*13 c.2846A>T/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	A:A *2A T:G *13 G:G T:T c.2846A>T	GENE ACTIVITY SCORE 0

table continues

DPYD: *2A/*2A wildtype/*13 wildtype/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	A:A *2A T:G *13 G:G A:T c.2846A>T	GENE ACTIVITY SCORE 0
DPYD: wildtype/*2A c.1236G>A/c.1236G>A c.2846A>T/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:A *2A T:T A:A c.1236G>A T:T c.2846A>T	Unable to predict the gene activity score (GENE ACTIVITY SCORE 0 to 0.5). The activity of the allele with two variants (c.1236G>A and c.2846A>T) is not known, because it is not known whether the variants have an independent or synergistic effect or whether the second variant does not have an additional effect.
DPYD: wildtype/*2A wildtype/c.1236G>A c.2846A>T/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:A *2A T:T G:A c.1236G>A T:T c.2846A>T	Unable to predict the gene activity score. Phenotyping should distinguish if *2A and c.1236G>A are present on separate alleles (GENE ACTIVITY SCORE 0 to 0.5) or on the same allele (GENE ACTIVITY SCORE 0.5). When both variants are located on separate alleles, the activity of the allele with the two variants c.1236G>A and c.2846A>T is not known, because it is not known whether the variants have an independent or synergistic effect or whether the second variant does not have an additional effect.
DPYD: wildtype/*2A c.1236G>A/c.1236G>A wildtype/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:A *2A T:T A:A c.1236G>A A:T c.2846A>T	Unable to predict the gene activity score. Phenotyping should distinguish if *2A and c.2846A>T are present on separate alleles (GENE ACTIVITY SCORE 0 to 0.5) or on the same allele (GENE ACTIVITY SCORE 0.5). When both variants are located on separate alleles, the activity of the allele with the two variants c.1236G>A and c.2846A>T is not known, because it is not known whether the variants have an independent or synergistic effect or whether the second variant does not have an additional effect.
DPYD: wildtype/*2A wildtype/c.1236G>A wildtype/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:A *2A T:T G:A c.1236G>A A:T c.2846A>T	Unable to predict the gene activity score (GENE ACTIVITY SCORE 0 to 1). Phenotyping should distinguish which variants are present on the same allele. The activity of an allele with the two variants c.1236G>A and c.2846A>T is not known, because it is not known whether the variants have an independent or synergistic effect or whether the second variant does not have an additional effect.
DPYD: wildtype/*2A *13/*13 c.1236G>A/c.1236G>A	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:A *2A G:G *13 A:A A:A c.1236G>A	GENE ACTIVITY SCORE 0
DPYD: wildtype/*2A *13/*13 wildtype/c.1236G>A	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:A *2A G:G *13 G:A c.1236G>A A:A	GENE ACTIVITY SCORE 0

table continues

DPYD: wildtype/*2A *13/*13 c.2846A>T/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:A *2A G:G *13 G:G T:T c.2846A>T	GENE ACTIVITY SCORE 0
DPYD: wildtype/*2A *13/*13 wildtype/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:A *2A G:G *13 G:G A:T c.2846A>T	GENE ACTIVITY SCORE 0
DPYD: wildtype/*2A wildtype/*13 c.1236G>A/c.1236G>A	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:A *2A T:G *13 A:A c.1236G>A A:A	Unable to predict the gene activity score. Phenotyping should distinguish if *2A and *13 are present on separate alleles (GENE ACTIVITY SCORE 0) or on the same allele (GENE ACTIVITY SCORE 0.5).
DPYD: wildtype/*2A wildtype/*13 wildtype/c.1236G>A	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:A *2A T:G *13 G:A c.1236G>A A:A	Unable to predict the gene activity score (GENE ACTIVITY SCORE 0 to 1). Phenotyping should distinguish which variants are present on the same allele.
DPYD: wildtype/*2A wildtype/*13 c.2846A>T/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:A *2A T:G *13 G:G T:T c.2846A>T	Unable to predict the gene activity score. Phenotyping should distinguish if *2A and *13 are present on separate alleles (GENE ACTIVITY SCORE 0) or on the same allele (GENE ACTIVITY SCORE 0.5).
DPYD: wildtype/*2A wildtype/*13 wildtype/c.2846A>T	*DPYD*_rs3918290 *DPYD*_rs55886062 *DPYD*_rs56038477 *DPYD*_rs67376798	G:A *2A T:G *13 G:G A:T c.2846A>T	Unable to predict the gene activity score (GENE ACTIVITY SCORE 0 to 1). Phenotyping should distinguish which variants are present on the same allele.

[#]NOTE: In patients with two different gene variants, the gene activity score is dependent on location of the variants on the alleles. The variants can either be located on the same allele (resulting in one affected allele with reduced or absent DPD activity and one fully functional allele) or located on different alleles (resulting in two affected alleles).

Supplementary Table 5. Dutch Pharmacogenetics Working Group (DPWG) Guideline for *DPYD* and 5-FU/capecitabine: the therapeutic recommendation and its rationale, and the kinetic and clinical consequences for each aberrant gene activity score

Predicted phenotype: Gene activity score 0 **Ref.** [1-13]	
Therapeutic recommendation	SYSTEMIC ROUTE OF ADMINISTRATION: **Choose an alternative.** Tegafur is not an alternative, as this is also metabolised by DPD. **If an alternative is not available: determine the residual DPD activity in mononuclear cells from peripheral blood and adjust the initial dose accordingly.** A patient with 0.5% of normal DPD activity tolerated 0.8% of the standard dose (150 mg capecitabine every five days). A patient with undetectable DPD activity tolerated 0.43% of the standard dose (150 mg capecitabine every 5 days with every third dose skipped) The average Caucasian DPD activity is 9.9 nmol/hour per mg protein. Adjust the initial dose based on toxicity and efficacy. NOTE: If a patient carries two different genetic variations that lead to a non-functional DPD enzyme (e.g. *2A and *13), this recommendation only applies if the variations are on different alleles. If both variations are on the same allele, the patient is assigned a gene activity score of 1 and the recommendation for that gene activity score should be followed. These two situations can only be distinguished by determining the enzyme activity (phenotyping). CUTANEOUS ROUTE OF ADMINISTRATION: **Choose an alternative** NOTE: If a patient has two different genetic variations that lead to a non-functional DPD enzyme (e.g. *2A and *13), this recommendation only applies if the variations are on a different allele. If both variations are on the same allele, this patient is assigned a gene activity score of 1, for which no increased risk of severe, potentially fatal toxicity has been found with cutaneous use. These two situations can only be distinguished by determining the enzyme activity (phenotyping).
Rationale of the therapeutic recommendation	There are not enough data available to be able to make a substantiated recommendation on dose adjustments for patients assigned gene activity score 0. The recommendation for *1/*2A is a dose reduction by 50%. This would be equivalent to a dose reduction by 100% for *2A/*2A and therefore a dose reduction to 0%. This is equivalent to severe toxicity found in one patient with genotype *2A/*2A when using 5-FU cream on the scalp. Because of the indications that the tolerated dose is close to zero and the scarce data on tolerated doses in patients assigned a gene activity score of 0 (see below), an alternative is advised. The calculated dose reduction based on two patients is a reduction to 0.81% of the normal dose (0.72-0.89%; median 0.81%). However, this is based on too few patients to be used for a substantiated dose recommendation. In addition, in one of these patients, having undetectable DPD activity, the dose had to be reduced from 0.65% to 0.43% of the normal dose during treatment. However, there is a fairly good correlation between the residual DPD enzyme activity in peripheral blood mononuclear cells and the tolerated dose (Meulendijks 2016, Deenen 2016, Henricks 2017 JCO Precis Oncol and Henricks 2017 Int J Cancer). Therefore, if an alternative is not possible, adjusting the dose according to the residual DPD enzyme activity in peripheral blood mononuclear cells is advised. This strategy has been shown to be feasible in two patients with genotype *2A/*2A. A patient with 0.5% of the normal DPD activity tolerated 0.8% of the normal dose (150 mg capecitabine every five days) (Henricks 2017 Int J Cancer). A patient with undetectable DPD activity, tolerated 0.43% of the normal dose (150 mg capecitabine every five days with every third dose skipped) (Henricks 2017 JCO Precis Oncol).

table continues

Kinetic consequence	For two patients with genotype *2A/*2A the dose-corrected AUC of 5-FU increased by a factor 113 and 138 respectively after the first systemic capecitabine dose. Extrapolation of the decrease in clearance by 50% identified for *1/*2A would suggest a clearance of 0% for *2A/*2A (gene activity score 0). This is equivalent to severe toxicity found in one patient with *2A/*2A after using 5-FU cream on the scalp and the two previously described patients using very low tolerated systemic doses (0.8% and 0.43% of the standard dose).
Clinical consequence	SYSTEMIC ROUTE OF ADMINISTRATION: All patients assigned a gene activity score of 0 with known toxicity (n=2, both *2A/*2A), had grade III/IV toxicity and 50% died due to toxicity. Moreover, a patient with *2A/*2A developed severe toxicity after treatment with cutaneous 5-FU cream. CUTANEOUS ROUTE OF ADMINISTRATION: A patient with *2A/*2A developed severe toxicity after treatment with cutaneous 5-FU cream. All patients using systemic 5-FU assigned a gene activity score of 0 with known toxicity (n=2, both *2A/*2A), had grade III/IV toxicity and 50% died due to toxicity.
Predicted phenotype: Gene activity score 0.5 **Ref.** [3-5,8-12,14,15]	
Therapeutic recommendation	**Start with 25% of the standard dose or choose an alternative.** Adjustment of the initial dose should be guided by toxicity and effectiveness. Tegafur is not an alternative, as this is also metabolised by DPD. NOTE: This recommendation only applies if the two genetic variations are on a different allele. If both variations are on the same allele, this patient has gene activity score 1 and the recommendation for that gene activity score should be followed. These two situations can only be distinguished by determining the enzyme activity (phenotyping).
Rationale of the therapeutic recommendation	Clearance has only been determined for one patient assigned a gene activity score of 0.5 (Boisdron-Celle, 2007). The clearance found for this patient with genotype *2A/c.2846A>T was almost zero. Extrapolation of the required dose reduction by 50% for *1/*2A and the required dose reduction by 25% for *1/c.2846A>T and *1/c.1236G>A would, however, lead to a required dose reduction by 75% for *2A/c.2846A>T. The dose reductions for *1/*2A, *1/2486T and *1/c.1236G>A are based on more than one patient. Moreover, the Boisdron-Celle article found a much lower clearance for one patient with genotype *1/*2A than the weighted average for this genotype (reduction by 80% instead of by 50%). For this reason, the recommendation given is based on extrapolation and therefore constitutes a dose reduction to 25% of the normal dose. Instead of dose adjustment, physicians may also choose an alternative.
Kinetic consequence	Clearance decreased by almost 100% in one patient assigned a gene activity score of 0.5 (*2A/c.2846A>T). Extrapolation of the dose reductions identified for *1/*2A, *1/c.2846A>T and *1/c.1236G>A would, however, lead to a dose reduction by 75%.
Clinical consequence	Clinical consequences are only known for three patients (all genotype *2A/c.2846A>T). The first patient developed grade III/IV toxicity and died due to toxicity. The second patient developed grade V toxicity and tolerated only one cycle of FOLFOX plus cetuximab. The third patient received half of the standard dose, but despite this the fluoropyrimidine therapy was stopped after the first cycle due to side effects (≤ grade 3).
Predicted phenotype: Gene activity score 1 **Ref.** [1,3-6,8-12,14-39]	
Therapeutic recommendation	**Start with 50% of the standard dose or choose an alternative.** Adjustment of the initial dose should be guided by toxicity and effectiveness. Tegafur is not an alternative, as this is also metabolised by *DPD.*

table continues

	NOTE 1: The dose reduction described here is well substantiated for *1/*2A and c.1236G>A/c.1236G>A. The dose reduction for patients with c.2846A>T (c.2846A>T/c.2846A>T or c.1236G>A/c.2846A>T) is based on, among other factors, the dose reductions identified for *1/c.2846A>T. NOTE 2: If a patient has two different genetic variations that result in a partially functional DPD enzyme (e.g. c.2846A>T and c.1236G>A), this recommendation applies if the variations are on a different allele. If both variations are on the same allele, the gene activity score is between 1 and 1.5, depending on whether and how the two gene variations influence each other and on other factors that influence the DPD activity. Whether a gene activity score of 1 or 1.5 needs to be assigned in the case of two different genetic variations can only be determined by measuring the enzyme activity (phenotyping).
Rationale of the therapeutic recommendation	For 25 patients with genotype *1/*2A, one with genotype *1/*13, one with genotype c.2846A>T/c.2846A>T and one with genotype c.1236G>A/c.2846A>T, the weighted average of the dose adjustments calculated based on 5-FU clearance or AUC was a reduction to 45% (18-49%, median 33%). Because the relatively low median was caused by the low values found in the two smallest studies (n = 1 and n = 2 respectively), it was decided to base the dose recommendation on the weighted mean. The weighted mean of 45% was translated to 50% to be more achievable in clinical practice. This is similar to the dose reduction to 56% and 60% of the standard dose found by Deenen 2011 and Meulendijks 2016 when investigating patients with respectively *1/*2A and c.1236G>A/c.1236G>A in whom toxicity-guided dose adjustments were made. It is also similar to the mean tolerated dose of 55% found by Henricks 2017 JCO Precis Oncol for 2x c.1236G>A/c.1236G>A, 1x c.1236G>A/c.2846A>T and 1x c.2846A>T/c.2846A>T, although in this study a strongvariation between patients (and genotypes) was found. In addition, Deenen 2016 found no difference in toxicities between 18 patients with *1/*2A on an initial dose of maximally 50% of the standard dose and *1/*1-patients on the standard dose. Lunenburg 2016 found no grade ≥ 3 toxicity when treating three patients with *1/*2A with an initial dose of 50% of the standard dose. There are no data on clearance or AUC for c.1236G>A/c.1236G>A and only scarce data on clearance or AUC or on maximum tolerated dose in clinical practice for c.2846A>T/c.2846A>T and c.1236G>A/c.2846A>T. Deenen 2011 found a dose reduction to 74% of the standard dose for patients with *1/c.2846A>T when toxicity-guided dose adjustments were made. Extrapolation of the required dose reduction for *1/c.2846A>T would lead to a required dose reduction to 50% for c.2846A>T/c.2846A>T. This is equivalent to the dose reduction for *1/*2A and c.1236G>A/c.1236G>A, which are also in the gene activity score 1 group. Instead of dose adjustment, physicians may also choose an alternative.
Kinetic consequence	Increase in the AUC of 5-FUby 103% (16x *1/*2A), 127% (1x c.1236G>A/c.2846A>T) or 766% (1x c.2846A>T/c.2846A>T). 52-80% decrease in clearance. 69-109% increase in half-life.
Clinical consequence	7 of the 10 studies and two meta-analyses found an increased risk of grade ≥ 3 toxicity. Increased grade ≥ 3 toxicity: OR = 4.67-24.9; RR = 4.40-9.76. The highest ORs were found for haematological toxicity. There was a 74-793% increase in the percentage of patients with grade ≥ 3 toxicity. Out of 48 patients with genotype *1/*2A in published cohort studies, 73% developed grade ≥ 3 toxicity. The allele frequency of *2A in a group with grade III/IV toxicity was 1548-2879% higher. Toxicity generally occurred in the first cycle. Six patients died due to toxicity, including two that had used capecitabine. No association with grade ≥ 3 toxicity was found for breast cancer patients receiving adjuvant/neoadjuvant therapy with 5-FU, epirubicin and cyclophosphamide in a phase II study that showed 94% grade ≥ 3 toxicity and in a small study of 21 patients with grade ≥ 3 toxicity. 5-FU toxicity is not common in breast cancer patients treated with this combination therapy.

table continues

	A large study found that the *2A allele only increased the risk of grade ≥ 3 toxicity in men (OR = 41.8) and not in women. Other studies did not find any differences between men and women. When the dose was guided by toxicity, the average dose in the sixth cycle was 56% of the standard dose in seven patients with genotype *1/*2A. Dose reduction down to 40% or 50% of the standard dose was not adequate in two *1/*2A patients in another study. There was no difference in grade ≥ 3 toxicity between 18 patients with genotype *1/*2A at ≤ 50% of the standard dose and non-selected patients on the standard dose. In another study, four patients with genotype *1/*2A did not develop grade ≥ 3 toxicity at 50% of the standard dose. One of them had previously developed grade ≥ 3 toxicity during the first cycle at the standard dose. One of them tolerated a dose increase to 60%, the other two did not tolerate a dose increase to 80% and 100% respectively. Of the three patients with genotype c.1236G>A/c.1236G>A, one tolerated a standard dose. A second patient tolerated the treatment after dose reduction to 60% of the standard dose. Another study found a mean tolerated dose of 55% of the standard dose for 2x c.1236G>A/c.1236G>A, 1x c.1236G>A/c.2846A>T and 1x c.2846A>T/c.2846A>T, although in this study a strong variation between patients (and genotypes) was found (17-100% of the standard dose).
Predicted phenotype: Gene activity score 1.5 **Ref.** [3-5,8-12,14-16,20,21,25-27,33,38-41]	
Therapeutic recommendation	**Start with 75% of the standard dose or choose an alternative.** Adjustment of the initial dose should be guided by toxicity and effectiveness. Tegafur is not an alternative, as this is also metabolised by DPD.
Rationale of the therapeutic recommendation	For *1/c.2846A>T, the weighted average of the calculated dose adjustments was a reduction to 55%. However, Deenen 2011 investigated 8 patients with *1/c.2846A>T and found a toxicity-guided dose reduction to 74% of the standard dose. In addition, Lunenburg 2016 found no grade ≥ 3 toxicity when treating five patients with *1/c.1236G>A with an initial dose of 75% of the standard dose. As oncolytic under dosing should be avoided, the dose adjustment determined in clinical practice has been included in the recommendation. Instead of dose adjustment, physicians may also choose an alternative.
Kinetic consequence	40-58% decrease in clearance.
Clinical consequence	Four of the five studies and one meta-analysis found an increased risk of grade ≥ 3 toxicity. Increased grade ≥ 3 toxicity: OR = 4.42-9.35. The percentage of patients with grade ≥ 3 toxicity was 109-1175% higher. One patient (*1/496G) died due to toxicity. No association with grade ≥ 3 toxicity was found in one small study of 21 patients with grade ≥ 3 toxicity. When the dose for eight patients with genotype *1/c.2846A>T was guided by toxicity, the average dose in the sixth cycle was 76% of the standard dose. Five patients with genotype *1/c.1236G>A did not develop grade ≥ 3 toxicity at 75 % of the standard dose. The two patients for who the dose was then increased tolerated the standard dose. One patient with genotype *1/c.1236G>A, who was started at the standard dose, developed grade 3-4 toxicity in the first cycle.

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Supplementary Table 6. Dutch Pharmacogenetics Working Group (DPWG) Guideline for *DPYD* and tegafur with DPD inhibitors: the therapeutic recommendation and its rationale, and the kinetic and clinical consequences for each aberrant gene activity score

Predicted phenotype: Gene activity score 0 **Ref.** [1]	
Therapeutic recommendation	**Choose an alternative.** Do not choose 5-FU or capecitabine, as these are also metabolised by DPD. **If an alternative is not possible: start with a very low dose and adjust the initial dose based on toxicity and efficacy.** A substantiated recommendation for dose reduction cannot be made based on the literature. The recommendation for 5-FU and capecitabine is to determine the residual DPD activity in mononuclear cells from peripheral blood and to adjust the initial dose accordingly. A patient with 0.5% of the normal DPD activity tolerated 0.8% of the standard capecitabine dose (150 mg every 5 days). A patient with undetectable DPD activity tolerated 0.43% of the standard capecitabine dose (150 mg every five days with every third dose skipped) The average Caucasian DPD activity is 9.9 nmol/hour per mg protein. NOTE: If a patient carries two different gene variations that lead to a non-functional DPD enzyme (e.g. *2A and *13), this recommendation only applies if the variations are on different alleles. If both variations are on the same allele, this patient is assigned a gene activity score of 1 and the recommendation for that gene activity score should be followed. These two situations can only be distinguished by determining the enzyme activity (phenotyping).
Rationale of the therapeutic recommendation	There are no data available on the use of tegafur in combination with a DPD inhibitor for patients assigned a gene activity score of 0. The SPCs state that tegafur in combination with a DPD inhibitor is contraindicated in patients with dihydropyrimidine dehydrogenase deficiency, but do not substantiate this. However, two patients using standard doses of tegafur-uracil, who developed severe toxicity, were found to be assigned partially deficient phenotypes (gene activity scores of 1 and 1.5). The toxicity was similar to that found in patients treated with capecitabine or 5-FU, both of which are given without a DPD inhibitor. The DPD inhibitor is 200 times more potent in the tegafur-gimeracil-oteracil combination. However, 5-FU is still metabolised by DPD after administration of this combination and DPD is therefore also involved in 5-FU clearance. For 5-FU and capecitabine, the maximally tolerated dose of 50% of the normal dose for *1/*2A indicates that the maximally tolerated dose for *2A/*2A (gene activity score 0) is close to zero, as do the scarce data on tolerated doses in patients with gene activity score 0. For this reason, an alternative is advised. There is a fairly good correlation between the residual DPD enzyme activity in peripheral blood mononuclear cells and the tolerated 5-FU or capecitabine dose. Therefore, if an alternative is not available, adjusting the dose according to the residual DPD enzyme activity in peripheral blood mononuclear cells is advised. This strategy has been shown to be feasible for capecitabine in two patients with genotype *2A/*2A. A patient with 0.5% of the normal DPD activity tolerated 0.8% of the normal capecitabine dose (150 mg every five days). A patient with undetectable DPD activity, tolerated 0.43% of the normal capecitabine dose (150 mg every five days with every third dose skipped).This is why this strategy is also recommended for tegafur in case an alternative is not possible.

table continues

Kinetic consequence	Studies regarding the kinetic consequences are unavailable.
Clinical consequence	Studies regarding the clinical consequences are unavailable. The SmPC states that this combination is contraindicated in patients with DPD deficiency. This probably refers to gene activity score 0. No safe dose for 5-FU (the metabolite of tegafur) has been found for patients assigned a gene activity score of 0. In addition, four patients with a less deficient DPD activity (assigned a gene activity score of 1 or 1.5) had a comparable toxicity for treatment with tegafur/uracil as found for treatment with 5-FU or capecitabine.
Predicted phenotype: Gene activity score 0.5 **Ref.** [1]	
Therapeutic recommendation	**Choose an alternative or start with a low dose and adjust the initial dose based on toxicity and efficacy.** Do not choose 5-FU or capecitabine, as these are also metabolised by DPD. A substantiated recommendation for dose reduction cannot be made based on the literature. For 5-FU and capecitabine, starting with 25% of the standard dose is recommended. NOTE: This recommendation only applies if the two gene variations are on different alleles. If both variations are on the same allele, this patient is assigned a gene activity score of 1 and the recommendation for that gene activity score should be followed. These two situations can only be distinguished by determining the enzyme activity (phenotyping).
Rationale of the therapeutic recommendation	There are no data available on the use of tegafur in combination with a DPD inhibitor for gene activity score 0.5. The SPCs state that tegafur in combination with a DPD inhibitor is contraindicated in patients with a history of serious and unexpected reactions to fluoropyrimidine therapy, but do not substantiate this. However, two patients using standard doses of tegafur-uracil who developed severe toxicity were found to be assigned partially deficient phenotypes of gene activity scores of 1 and 1.5. The toxicity was similar to that found in patients treated with capecitabine or 5-FU, both of which are given without a DPD inhibitor. The recommendation for 5-FU and capecitabine in patients with gene activity score 0.5 is to reduce the dose to 25% of the standard dose or to choose an alternative. This is why a dose reduction or alternative is also recommended for tegafur.
Kinetic consequence	Studies regarding the kinetic consequences are unavailable.
Clinical consequence	Studies regarding the clinical consequences are unavailable. However, four patients with a less deficient DPD activity (assigned a gene activity score of 1 or 1.5) had a comparable toxicity for treatment with tegafur/uracil as found for treatment with 5-FU or capecitabine. In addition to this, four patients assigned a gene activity score of 1 could be treated with 90 % of the standard tegafur/uracil dose without grade 3-4 toxicity occurring.
Predicted phenotype: Gene activity score 1.0 **Ref.** [1-3]	
Therapeutic recommendation	**Choose an alternative or start with a low dose and adjust the initial dose based on toxicity and efficacy.** Do not choose 5-FU or capecitabine, as these are also metabolised by DPD. A substantiated recommendation for dose reduction cannot be made based on the literature. For 5-FUand capecitabine, starting with 50 % of the standard dose is recommended. NOTE: If a patient has two different gene variations that result in a partially functional DPD enzyme (e.g. c.2846A>T and c.1236G>A), this recommendation

table continues

	only applies if the variations are on different alleles. If both variations are on the same allele, the gene activity score assigned is between 1 and 1.5, depending on whether and how the two gene variations influence each other and on other factors that influence the DPD activity. Whether a gene activity score of 1 or 1.5 needs to be assigned in the case of two different genetic variations can only be determined by measuring the enzyme activity (phenotyping).
Rationale of the therapeutic recommendation	Treatment with tegafur in combination with the DPD inhibitor uracil in two patients with gene activity score 1 led to similar toxicity as found after treatment with 5-FU or capecitabine. However, four patients with an assigned gene activity score of 1 could be treated with 90% of the standard tegafur-uracil dose without grade 3-4 toxicity occurring. Similar to data found for 5-FU and capecitabine, treatment with a reduced dose of tegafur-uracil seems possible for patients who are assigned a gene activity score of 1. This is why a dose reduction or alternative is recommended.
Kinetic consequence	Studies regarding the kinetic consequences are unavailable.
Clinical consequence	In a study, two patients had a comparable toxicity for treatment with tegafur/uracil as found for treatment with 5-FU or capecitabine. In another study, four patients could be treated with 90 % of the standard tegafur/uracil dose without grade 3-4 toxicity occurring. All six patients had the genotype *1/*2A.
Predicted phenotype: Gene activity score 1.5 **Ref.** [1,3]	
Therapeutic recommendation	**Choose an alternative or start with a low dose and adjust the initial dose based on toxicity and efficacy.** Do not choose 5-FU or capecitabine, as these are also metabolised by DPD. A substantiated recommendation for dose reduction cannot be made based on the literature. For 5-FU and capecitabine, starting with 75 % of the normal dose is recommended.
Rationale of the therapeutic recommendation	Treatment with tegafur in combination with the DPD inhibitor uracil in two patients with gene activity score 1.5 led to similar toxicity as found after treatment with 5-FU or capecitabine. However, four patients with the more deficient phenotype (gene activity score 1) could be treated with 90% of the standard tegafur-uracil dose without grade 3-4 toxicity occurring. Similar to data found for 5-fluorouracil and capecitabine, treatment with a reduced dose of tegafur-uracil seems possible for patients with gene activity score 1 or higher. This is why a dose reduction or alternative is recommended.
Kinetic consequence	Studies regarding the kinetic consequences are unavailable.
Clinical consequence	Two patients with gene activity score 1.5 had a comparable toxicity for treatment with tegafur/uracil as found for treatment with 5-FU or capecitabine. Four patients with gene activity score 1 could be treated with 90 % of the standard tegafur/uracil dose without grade 3-4 toxicity occurring.

Abbreviations: Ref.: References; 5-FU: 5-fluorouracil; AUC: Area Under the Curve; DPD: dihydropyrimidine dehydrogenase; OR: Odds Ratio.

References

1. SPC Teysuno (tegafur/gimeracil/oteracil) 05 April 2017.
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Supplementary Table 7. Suggested clinical decision support texts for various health care professionals for 5-FU/capecitabine

DPD gene act. 0: 5-fluorouracil (5-FU)/capecitabine, SYSTEMIC

Pharmacist text / Hospital text / Prescriber text
Genetic variation increases the risk of severe, potentially fatal toxicity. A reduced conversion of 5-fluorouracil/ capecitabine to inactive metabolites means that the standard dose is a more than 100-fold overdose.

Recommendation:
- Choose an alternative
  Tegafur is not an alternative, as this is also metabolised by DPD.
- If an alternative is not possible:
  - Determine the residual DPD activity in mononuclear cells from peripheral blood and adjust the initial dose accordingly.
    A patient with 0.5% of the normal DPD activity tolerated 0.8% of the standard dose (150 mg capecitabine every 5 days). A patient with undetectable DPD activity tolerated 0.43% of the standard dose (150 mg capecitabine every 5 days with every third dose skipped)
    The average Caucasian DPD activity is 9.9 nmol/hour per mg protein.
  - Adjust the initial dose based on toxicity and efficacy.

NOTE: If a patient has two different genetic variations that lead to a non-functional DPD enzyme (e.g. *2A and *13), this recommendation only applies if the variations are on a different allele. If both variations are on the same allele, this patient has gene activity score 1 and the recommendation for that gene activity score should be followed. These two situations can only be distinguished by determining the enzyme activity (phenotyping).

Background information
Mechanism:
5-Fluorouracil and its prodrug capecitabine are mainly converted by dihydropyrimidine dehydrogenase (DPD) to inactive metabolites. Genetic variations result in reduced DPD activity and thereby to reduced conversion of 5-fluorouracil to inactive metabolites. As a result, the intracellular concentration of the active metabolite of 5-fluorouracil can increase, resulting in severe, potentially fatal toxicity.
For more information about the phenotype gene activity score 0: see the general background information about DPD on the KNMP Knowledge Bank or on www.knmp.nl (search for DPD).

Clinical consequences:
All patients with gene activity score 0 with known toxicity (n=2, both *2A/*2A), had grade III/IV toxicity and 50% died due to toxicity. Moreover, a patient with *2A/*2A developed severe toxicity after treatment with cutaneous 5-fluorouracil cream.

Kinetic consequences:
For 2 patients with genotype *2A/*2A the dose-corrected AUC of 5-fluorouracil increased by a factor 113 and 138 respectively after the first systemic capecitabine dose. Extrapolation of the decrease in clearance by 50% identified for *1/*2A would suggest a clearance of 0% for *2A/*2A (gene activity score 0). This is equivalent to severe toxicity found in one patient with *2A/*2A after using 5-fluorouracil cream on the scalp and the two previously described patients using very low tolerated systemic doses (0.8% and 0.43% of the standard dose).

Literature
1. Rosmarin D et al. Genetic markers of toxicity from capecitabine and other fluorouracil-based regimens: investigation in the QUASAR2 study, systematic review, and meta-analysis. J Clin Oncol 2014; 32:1031-9.
2. Deenen MJ et al. Relationship between single nucleotide polymorphisms and haplotypes in *DPYD* and toxicity and efficacy of capecitabine in advanced colorectal cancer. Clin Cancer Res 2011; 17:3455-68.
3. Gross E et al. Strong association of a common dihydropyrimidine dehydrogenase gene polymorphism with fluoropyrimidine-related toxicity in cancer patients. PLoS ONE 2008;3:e4003.
4. Boisdron-Celle M et al. 5-Fluorouracil-related severe toxicity: a comparison of different methods for the pretherapeutic detection of dihydropyrimidine dehydrogenase deficiency. Cancer Lett 2007;249:271-82.

5. Morel A et al. Clinical relevance of different dihydropyrimidine dehydrogenase gene single nucleotide polymorphisms on 5-fluorouracil tolerance. Mol Cancer Ther 2006;5:2895-904.
6. Van Kuilenburg AB et al. High prevalence of the IVS14 + 1G>A mutation in the dihydropyrimidine dehydrogenase gene of patients with severe 5-fluorouracil-associated toxicity. Pharmacogenetics 2002;12:555-8.
7. Raida M et al. Prevalence of a common point mutation in the dihydropyrimidine dehydrogenase (DPD) gene within the 5'-splice donor site of intron 14 in patients with severe 5-fluorouracil (5-FU)- related toxicity compared with controls. Clin Cancer Res 2001;7:2832-9.
8. van Kuilenburg AB et al. Clinical implications of dihydropyrimidine dehydrogenase (DPD) deficiency in patients with severe 5-fluorouracil-associated toxicity: identification of new mutations in the DPD gene. Clin Cancer Res 2000;6:4705-12.
9. Johnson MR et al. Life-threatening toxicity in a dihydropyrimidine dehydrogenase-deficient patient after treatment with topical 5-fluorouracil. Clin Cancer Res 1999;5:2006-11.
10. SPC Carac cream (VS), Efudix crème, Fluorouracil P and Xeloda.

DPD gene act. 0: 5-fluorouracil (5-FU) CUTANEOUS

Pharmacist text/ Hospital text / Prescriber text
Genetic variation increases the risk of severe, potentially fatal toxicity. A reduced conversion of 5-fluorouracil/ capecitabine to inactive metabolites means that the normal dose is an overdose.

Recommendation:
- Choose an alternative
  NOTE: If a patient has two different genetic variations that lead to a non-functional DPD enzyme (e.g. *2A and *13), this recommendation only applies if the variations are on a different allele. If both variations are on the same allele, this patient has gene activity score 1, for which no increased risk of severe, potentially fatal toxicity has been found with cutaneous use. These two situations can only be distinguished by determining the enzyme activity (phenotyping).

Background information
Mechanism:
5-Fluorouracil is mainly converted by dihydropyrimidine dehydrogenase (DPD) to inactive metabolites. Genetic variations result in reduced DPD activity and thereby to reduced conversion of 5-fluorouracil to inactive metabolites. As a result, the intracellular concentration of the active metabolite of 5-fluorouracil can increase, resulting in severe, potentially fatal toxicity.
For more information about the phenotype gene activity score 0: see the general background information about DPD on the KNMP Knowledge Bank or on www.knmp.nl (search for DPD).

Clinical consequences:
A patient with *2A/*2A developed severe toxicity after treatment with cutaneous 5-fluorouracil cream. All patients using systemic 5-fluorouracil with gene activity score 0 with known toxicity (n=2, both *2A/*2A), had grade III/IV toxicity and 50% died due to toxicity.

Kinetic consequences:
For 2 patients with genotype *2A/*2A the dose-corrected AUC of 5-fluorouracil increased by a factor 113 and 138 respectively after the first systemic capecitabine dose.
Extrapolation of the decrease in clearance by 50% identified for *1/*2A would suggest a clearance of 0% for *2A/*2A (gene activity score 0). This is equivalent to severe toxicity found in one patient with *2A/*2A after using 5-fluorouracil cream on the scalp and the two previously described patients using very low tolerated systemic doses (0.8% and 0.43% of the standard dose).

Literature
1. Rosmarin D et al. Genetic markers of toxicity from capecitabine and other fluorouracil-based regimens: investigation in the QUASAR2 study, systematic review, and meta-analysis. J Clin Oncol 2014; 32:1031-9.
2. Gross E et al. Strong association of a common dihydropyrimidine dehydrogenase gene polymorphism with fluoropyrimidine-related toxicity in cancer patients. PLoS ONE 2008;3:e4003.
3. Boisdron-Celle M et al. 5-Fluorouracil-related severe toxicity: a comparison of different methods for the pretherapeutic detection of dihydropyrimidine dehydrogenase deficiency. Cancer Lett 2007;249:271-82.
4. Morel A et al. Clinical relevance of different dihydropyrimidine dehydrogenase gene single nucleotide polymorphisms on 5-fluorouracil tolerance. Mol Cancer Ther 2006;5:2895-904.
5. Van Kuilenburg AB et al. High prevalence of the IVS14 + 1G>A mutation in the dihydropyrimidine dehydrogenase gene of patients with severe 5-fluorouracil-associated toxicity. Pharmacogenetics 2002;12:555-8.
6. Raida M et al. Prevalence of a common point mutation in the dihydropyrimidine dehydrogenase (DPD) gene within the 5'-splice donor site of intron 14 in patients with severe 5-fluorouracil (5-FU)- related toxicity compared with controls. Clin Cancer Res 2001;7:2832-9.
7. van Kuilenburg AB et al. Clinical implications of dihydropyrimidine dehydrogenase (DPD) deficiency in patients with severe 5-fluorouracil-associated toxicity: identification of new mutations in the DPD gene. Clin Cancer Res 2000;6:4705-12.
8. Johnson MR et al. Life-threatening toxicity in a dihydropyrimidine dehydrogenase-deficient patient after treatment with topical 5-fluorouracil. Clin Cancer Res 1999;5:2006-11.
9. SPC Carac cream (VS) en Efudix crème.

DPD gene act. 0.5: 5-fluorouracil (5-FU)/capecitabine

Pharmacist text / Hospital text / Prescriber text
Genetic variation increases the risk of severe, potentially fatal toxicity. A reduced conversion of 5-fluorouracil/ capecitabine to inactive metabolites means that the normal dose is an overdose.

Recommendation:
- Start with 25% of the standard dose or choose an alternative.
  Adjustment of the initial dose should be guided by toxicity and effectiveness.
  Tegafur is not an alternative, as this is also metabolised by DPD.
  NOTE: This recommendation only applies if the two genetic variations are on a different allele. If both variations are on the same allele, this patient has gene activity score 1 and the recommendation for that gene activity score should be followed. These two situations can only be distinguished by determining the enzyme activity (phenotyping).

Background information
Mechanism:
5-Fluorouracil and its prodrug capecitabine are mainly converted by dihydropyrimidine dehydrogenase (DPD) to inactive metabolites. Genetic variations result in reduced DPD activity and thereby to reduced conversion of 5-fluorouracil to inactive metabolites. As a result, the intracellular concentration of the active metabolite of 5-fluorouracil can increase, resulting in severe, potentially fatal toxicity.
For more information about the phenotype gene activity score 0.5: see the general background information about DPD on the KNMP Knowledge Bank or on www.knmp.nl (search for DPD).

Clinical consequences:
Clinical consequences are only known for 3 patients (all genotype *2A/2846T). The first patient developed grade III/IV toxicity and died due to toxicity. The second patient developed grade V toxicity and tolerated only one cycle of FOLFOX plus cetuximab. The third patient received half the standard dose, but despite this the fluoropyrimidine therapy was stopped after the first cycle due to side effects (≤ grade 3).

Kinetic consequences:
Clearance decreased by almost 100% in one patient with gene activity score 0.5 (*2A/2846T). Extrapolation of the dose reductions identified for *1/*2A, *1/2846T and *1/1236A would, however, lead to a dose reduction by 75%.

Literature
1. Deenen MJ et al. Relationship between single nucleotide polymorphisms and haplotypes in *DPYD* and toxicity and efficacy of capecitabine in advanced colorectal cancer. Clin Cancer Res 2011; 17:3455-68.
2. Boisdron-Celle M et al. 5-Fluorouracil-related severe toxicity: a comparison of different methods for the pretherapeutic detection of dihydropyrimidine dehydrogenase deficiency. Cancer Lett 2007;249:271-82.
3. Morel A et al. Clinical relevance of different dihydropyrimidine dehydrogenase gene single nucleotide polymorphisms on 5-fluorouracil tolerance. Mol Cancer Ther 2006;5:2895-904.
4. SPC Efudix crème and Fluorouracil PCH.

DPD gene act. 1: 5-fluorouracil (5-FU)/capecitabine

Pharmacist text / Hospital text / Prescriber text
Genetic variation increases the risk of severe, potentially fatal toxicity. A reduced conversion of 5-fluorouracil/capecitabine to inactive metabolites means that the normal dose is an overdose.

Recommendation:
- Start with 50% of the standard dose or choose an alternative.
  Adjustment of the initial dose should be guided by toxicity and effectiveness.
  Tegafur is not an alternative, as this is also metabolised by DPD.
  NB1: The dose reduction described here is well substantiated for *1/*2A and 1236A/1236A. The dose reduction for patients with 2846T (2846T/2846T or 1236A/2846T) is based on, among other factors, the dose reductions identified for *1/2846T.
  NB2: If a patient has two different genetic variations that result in a partially functional DPD enzyme (e.g. 2846T and 1236A), this recommendation applies if the variations are on a different allele. If both variations are on the same allele, the gene activity score is between 1 and 1.5, depending on whether and how the two gene variations influence each other and on other factors that influence the DPD activity. Whether a gene activity score of 1 or 1.5 needs to be assigned in the case of two different genetic variations can only be determined by measuring the enzyme activity (phenotyping).

Background information
Mechanism:
5-Fluorouracil and its prodrug capecitabine are mainly converted by dihydropyrimidine dehydrogenase (DPD) to inactive metabolites. Genetic variations result in reduced DPD activity and thereby to reduced conversion of 5-fluorouracil to inactive metabolites. As a result, the intracellular concentration of the active metabolite of 5-fluorouracil can increase, resulting in severe, potentially fatal toxicity.
For more information about the phenotype gene activity score 1: see the general background information about DPD on the KNMP Knowledge Bank or on www.knmp.nl (search for DPD).

Clinical consequences:
7 of the 10 studies and two meta-analyses found an increased risk of grade ≥ 3 toxicity. Increased grade ≥ 3 toxicity: OR = 4.67-24.9; RR = 4.40-9.76. The highest ORs were found for haematological toxicity. There was a 74-793% increase in the percentage of patients with grade ≥ 3 toxicity. Out of 48 patients with genotype *1/*2A in published cohort studies, 73% developed grade ≥ 3 toxicity. The allele frequency of *2A in a group with grade III/IV toxicity was 1548-2879% higher. Toxicity generally occurred in the first cycle. Six patients died due to toxicity, including two that had used capecitabine.
No association with grade ≥ 3 toxicity was found for breast cancer patients receiving adjuvant/neoadjuvant therapy with 5-fluorouracil, epirubicin and cyclophosphamide in a phase II study that showed 94% grade ≥ 3 toxicity and in a small study of 21 patients with grade ≥ 3 toxicity. 5-Fluorouracil toxicity is not common in breast cancer patients treated with this combination therapy.
A large study found that the *2A allele only increased the risk of grade ≥ 3 toxicity in men (OR = 41.8) and not in women. Other studies did not find any differences between men and women.
When the dose was guided by toxicity, the average dose in the sixth cycle was 56% of the standard dose in 7 *1/*2A. Dose reduction down to 40% or 50% of the standard dose was not adequate in two *1/*2A patients in another study. There was no difference in grade ≥ 3 toxicity between 18 *1/*2A at ≤ 50% of the standard

dose and non-selected patients on the standard dose. In another study, 4 *1/*2A did not develop grade ≥ 3 toxicity at 50% of the standard dose. One of them had previously developed grade ≥ 3 toxicity during the first cycle at the standard dose. One of them tolerated a dose increase to 60%, the other two did not tolerate a dose increase to 80% and 100% respectively. Of the 3 patients with genotype 1236A/1236A, one tolerated a standard dose. A second patient tolerated the treatment after dose reduction to 60% of the standard dose. Another study found a mean tolerated dose of 55% of the standard dose for 2x 1236A/1236A, 1x 1236A/2846T and 1x 2846T/2846T, although in this study a strong variation between patients (and genotypes) was found (17-100% of the standard dose).

Kinetic consequences:
Increase in the AUC of 5-fluorouracil by 103% (16x *1/*2A), 127% (1x 1236A/2846T) or 766% (1x 2846T/2846T).

52-80% decrease in clearance.
69-109% increase in half-life.

Literature

1. Rosmarin D et al. Genetic markers of toxicity from capecitabine and other fluorouracil-based regimens: investigation in the QUASAR2 study, systematic review, and meta-analysis. J Clin Oncol 2014; 32:1031-9.
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24. SPCs Efudix crème and Fluorouracil PCH.

DPD gene act. 1.5: 5-fluorouracil (5-FU)/capecitabine

Pharmacist text / Hospital text / Prescriber text
Genetic variation increases the risk of severe, potentially fatal toxicity. A reduced conversion of 5-fluorouracil/ capecitabine to inactive metabolites means that the normal dose is an overdose.

Recommendation:

- Start with 75% of the standard dose or choose an alternative.
  Adjustment of the initial dose should be guided by toxicity and effectiveness.
  Tegafur is not an alternative, as this is also metabolised by DPD.

Background information
Mechanism:
5-Fluorouracil and its prodrug capecitabine are mainly converted by dihydropyrimidine dehydrogenase (DPD) to inactive metabolites. Genetic variations result in reduced DPD activity and thereby to reduced conversion of 5-fluorouracil to inactive metabolites. As a result, the intracellular concentration of the active metabolite of 5-fluorouracil can increase, resulting in severe, potentially fatal toxicity.
For more information about the phenotype gene activity score 1.5: see the general background information about DPD on the KNMP Knowledge Bank or on www.knmp.nl (search for DPD).

Clinical consequences:
4 of the 5 studies and one meta-analysis found an increased risk of grade ≥ 3 toxicity. Increased grade ≥ 3 toxicity: OR = 4.42-9.35. The percentage of patients with grade ≥ 3 toxicity was 109-1175% higher. One patient (*1/496G) died due to toxicity.
No association with grade ≥ 3 toxicity was found in one small study of 21 patients with grade ≥ 3 toxicity.
When the dose for 8 *1/2846T was guided by toxicity, the average dose in the sixth cycle was 76% of the standard dose. 5 patients with genotype *1/1236A did not develop grade ≥ 3 toxicity at 75 % of the standard dose. The two patients for who the dose was then increased tolerated the standard dose. One patient with genotype *1/1236A, who was started at the standard dose, developed grade 3-4 toxicity in the first cycle.

Kinetic consequences:
40-58% decrease in clearance.

Literature
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12. SPCs Efudix crème and Fluorouracil PCH.

Supplementary Table 8. Suggested clinical decision support texts for health care professionals for tegafur with DPD inhibitors

DPD gene act. 0: tegafur
Pharmacist text / Hospital text / Prescriber text

Genetic variation increases the risk of severe, possibly fatal toxicity. A reduced conversion of tegafur to inactive metabolites means that the normal dose is an overdose.

Recommendation:
- Choose an alternative
  Do not choose 5-fluorouracil or capecitabine, as these are also metabolised by DPD.
- If an alternative is not possible: start with a very low dose and adjust the initial dose based on toxicity and efficacy.
  A substantiated recommendation for dose reduction cannot be made based on the literature. The recommendation for 5-fluorouracil and capecitabine is to determine the residual DPD activity in mononuclear cells from peripheral blood and to adjust the initial dose accordingly. A patient with 0.5% of the normal DPD activity tolerated 0.8% of the standard capecitabine dose (150 mg every 5 days).
  A patient with undetectable DPD activity tolerated 0.43% of the standard capecitabine dose (150 mg every 5 days with every third dose skipped)
  The average Caucasian DPD activity is 9.9 nmol/hour per mg protein.

NOTE: If a patient has two different gene variations that lead to a non-functional DPD enzyme (e.g. *2A and *13), this recommendation only applies if the variations are on a different allele. If both variations are on the same allele, this patient has gene activity score 1 and the recommendation for that gene activity score should be followed. These two situations can only be distinguished by determining the enzyme activity (phenotyping).

Background information

Mechanism:
Tegafur is mainly converted by CYP2A6 to 5-fluorouracil. 5-Fluorouracil is mainly (> 80 %) converted by dihydropyrimidine dehydrogenase (DPD) to inactive metabolites. Genetic variations result in reduced DPD activity and thereby to reduced conversion of 5-fluorouracil to inactive metabolites. As a result, the intracellular concentration of the active metabolite of 5-fluorouracil can increase, resulting in severe, potentially fatal toxicity. Tegafur is used in combination with the DPD inhibitor gimeracil (molar ratio 1:0.4) and was used in combination with the DPD inhibitor uracil (molar ratio 1:4). Both DPD inhibitors exhibit competitive inhibition of DPD. This is why efficacy is achieved at lower concentrations of the metabolites formed by DPD, which seem to contribute to the toxicity. Inhibition by DPD inhibitors is reversible and reduces over time.
For more information about the phenotype gene activity score 0: see the general background information about DPD on the KNMP Knowledge Bank or on www.knmp.nl (search for “DPD”).

Clinical consequences:
There are no studies into the clinical consequences of tegafur in combination with a DPD inhibitor for gene activity score 0. The SmPC states that this combination is contra-indicated in patients with DPD deficiency. This probably refers to gene activity score 0. No safe dose has been found for gene activity score 0 for 5-fluorouracil (the metabolite of tegafur). In addition to this, four patients with a less strongly reduced DPD activity (gene activity score 1 or 1.5) had a comparable toxicity for treatment with tegafur/uracil as found for treatment with 5-fluorouracil or capecitabine.

Kinetic consequences:
There are no studies into the kinetic consequences.

Literature
1. Deenen MJ et al. Standard-dose tegafur combined with uracil is not safe treatment after severe toxicity from 5-fluorouracil or capecitabine. Ann Intern Med 2010;153:767-8.
2. SPC Teysuno.

DPD gene act. 0.5: tegafur

Pharmacist text / Hospital text / Prescriber text
Genetic variation increases the risk of severe, possibly fatal toxicity. A reduced conversion of tegafur to inactive metabolites means that the normal dose is an overdose.

Recommendation:
- Choose an alternative or start with a low dose and adjust the initial dose based on toxicity and efficacy
  5-fluorouracil and capecitabine are not alternatives, as these are also metabolised by DPD.
  It is not possible to offer substantiated advice for dose reduction based on the literature. For 5-fluorouracil and capecitabine, starting with 25% of the standard dose is recommended.
  NOTE: This recommendation only applies if the two gene variations are on a different allele. If both variations are on the same allele, this patient has gene activity score 1 and the recommendation for that gene activity score should be followed. These two situations can only be distinguished by determining the enzyme activity (phenotyping).

Background information

Mechanism:
Tegafur is mainly converted by CYP2A6 to 5-fluorouracil. 5-Fluorouracil is mainly (> 80 %) converted by dihydropyrimidine dehydrogenase (DPD) to inactive metabolites. Genetic variations result in reduced DPD activity and thereby to reduced conversion of 5-fluorouracil to inactive metabolites. As a result, the intracellular concentration of the active metabolite of 5-fluorouracil can increase, resulting in severe, potentially fatal toxicity. Tegafur is used in combination with the DPD inhibitor gimeracil (molar ratio 1:0.4) and was used in combination with the DPD inhibitor uracil (molar ratio 1:4). Both DPD inhibitors exhibit competitive inhibition of DPD. This is why efficacy is achieved at lower concentrations of the metabolites formed by DPD, which seem to contribute to the toxicity. Inhibition by DPD inhibitors is reversible and reduces over time.
For more information about the phenotype gene activity score 0.5: see the general background information about DPD on the KNMP Knowledge Bank or on www.knmp.nl (search for “DPD”).

Clinical consequences:
There are no studies into the clinical consequences of tegafur in combination with a DPD inhibitor for gene activity score 0.5. However, four patients with a less strongly reduced DPD activity (gene activity score 1 or 1.5) had a comparable toxicity for treatment with tegafur/uracil as found for treatment with 5-fluorouracil or capecitabine. In addition to this, four patients with gene activity score 1 could be treated with 90 % of the standard tegafur/uracil dose without grade 3-4 toxicity occurring.

Kinetic consequences:
There are no studies into the kinetic consequences.

Literature
1. Cubero DI et al. Tegafur-uracil is a safe alternative for the treatment of colorectal cancer in patients with partial dihydropyrimidine dehydrogenase deficiency: a proof of principle. Ther Adv Med Oncol 2012;4:167-72.
2. Deenen MJ et al. Standard-dose tegafur combined with uracil is not safe treatment after severe toxicity from 5-fluorouracil or capecitabine. Ann Intern Med 2010;153:767-8.
3. SPC Teysuno.

DPD gene act. 1.0: tegafur

Pharmacist text / Hospital text / Prescriber text
Genetic variation increases the risk of severe, possibly fatal toxicity. A reduced conversion of tegafur into inactive metabolites means that the normal dose is an overdose.

Recommendation:
- Choose an alternative or start with a low dose and adjust the initial dose based on toxicity and efficacy
  5-Fluorouracil and capecitabine are not alternatives, as these are also metabolised by DPD.
  It is not possible to offer substantiated advice for dose reduction based on the literature. For 5-fluorouracil and capecitabine, starting with 50 % of the standard dose is recommended.
  NOTE: If a patient has two different gene variations that result in a partially functional DPD enzyme (e.g. 2846T and 1236A), this recommendation only applies if the variations are on a different allele. If both variations are on the same allele, the gene activity score is between 1 and 1.5, depending on whether and how the two gene variations influence each other and on other factors that influence the DPD activity. Whether a gene activity score of 1 or 1.5 needs to be assigned in the case of two different genetic variations can only be determined by measuring the enzyme activity (phenotyping).

Background information

Mechanism:
Tegafur is mainly converted by CYP2A6 to 5-fluorouracil. 5-Fluorouracil is mainly (> 80 %) converted by dihydropyrimidine dehydrogenase (DPD) to inactive metabolites. Genetic variations result in reduced DPD activity and thereby to reduced conversion of 5-fluorouracil to inactive metabolites. As a result, the intracellular concentration of the active metabolite of 5-fluorouracil can increase, resulting in severe, potentially fatal toxicity. Tegafur is used in combination with the DPD inhibitor gimeracil (molar ratio 1:0.4) and was used in combination with the DPD inhibitor uracil (molar ratio 1:4). Both DPD inhibitors exhibit competitive inhibition of DPD. This is why efficacy is achieved at lower concentrations of the metabolites formed by DPD, which seem to contribute to the toxicity. Inhibition by DPD inhibitors is reversible and reduces over time.
For more information about the phenotype gene activity score 1: see the general background information about DPD on the KNMP Knowledge Bank or on www.knmp.nl (search for “DPD”).

Clinical consequences:
In a study, two patients had a comparable toxicity for treatment with tegafur/uracil as found for treatment with 5-fluorouracil or capecitabine. In another study, four patients could be treated with 90 % of the standard tegafur/uracil dose without grade 3-4 toxicity occurring. All six patients had the genotype *1/*2A.

Kinetic consequences:
There are no studies into the kinetic consequences.

Literature
1. Cubero DI et al. Tegafur-uracil is a safe alternative for the treatment of colorectal cancer in patients with partial dihydropyrimidine dehydrogenase deficiency: a proof of principle. Ther Adv Med Oncol 2012;4:167-72.
2. Deenen MJ et al. Standard-dose tegafur combined with uracil is not safe treatment after severe toxicity from 5-fluorouracil or capecitabine. Ann Intern Med 2010;153:767-8.
3. SPC Teysuno.

DPD gene act. 1.5: tegafur

Pharmacist text / Hospital text / Prescriber text
Genetic variation increases the risk of severe, possibly fatal toxicity. A reduced conversion of tegafur into inactive metabolites means that the normal dose is an overdose.

Recommendation:
- Choose an alternative or start with a low dose and adjust the initial dose based on toxicity and efficacy
5-Fluorouracil and capecitabine are not alternatives, as these are also metabolised by DPD.
It is not possible to offer substantiated advice for dose reduction based on the literature. For 5-fluorouracil and capecitabine, starting with 75 % of the normal dose is recommended.

Background information

Mechanism:
Tegafur is mainly converted by CYP2A6 to 5-fluorouracil. 5-Fluorouracil is mainly (> 80 %) converted by dihydropyrimidine dehydrogenase (DPD) to inactive metabolites. Genetic variations result in reduced DPD activity and thereby to reduced conversion of 5-fluorouracil to inactive metabolites. As a result, the intracellular concentration of the active metabolite of 5-fluorouracil can increase, resulting in severe, potentially fatal toxicity. Tegafur is used in combination with the DPD inhibitor gimeracil (molar ratio 1:0.4) and was used in combination with the DPD inhibitor uracil (molar ratio 1:4). Both DPD inhibitors exhibit competitive inhibition of DPD. This is why efficacy is achieved at lower concentrations of the metabolites formed by DPD, which seem to contribute to the toxicity. Inhibition by DPD inhibitors is reversible and reduces over time.
For more information about the phenotype gene activity score 1.5: see the general background information about DPD on the KNMP Knowledge Bank or on www.knmp.nl (search for “DPD”).

Clinical consequences:
Two patients with gene activity score 1.5 had a comparable toxicity for treatment with tegafur/uracil as found for treatment with 5-fluorouracil or capecitabine. Four patients with gene activity score 1 could be treated with 90 % of the standard tegafur/uracil dose without grade 3-4 toxicity occurring.

Kinetic consequences:
There are no studies into the kinetic consequences.

Literature
1. Cubero DI et al. Tegafur-uracil is a safe alternative for the treatment of colorectal cancer in patients with partial dihydropyrimidine dehydrogenase deficiency: a proof of principle. Ther Adv Med Oncol 2012;4:167-72.
2. Deenen MJ et al. Standard-dose tegafur combined with uracil is not safe treatment after severe toxicity from 5-fluorouracil or capecitabine. Ann Intern Med 2010;153:767-8.
3. SPC Teysuno.

Supplementary Table 9. The clinical implication score of *DPYD*-fluoropyrimidines is "essential", based on the criteria and corresponding scores given by the DPWG

Clinical Implication Score Criteria	Possible score	Given score
Clinical effect associated with gene/drug interaction		
3 (D) ≤ CTCAE Grade ≤4 (E)	+	
4 (E) < CTCAE Grade ≤5 (F)	++	++[a]
Increased efficacy	+	
Level of evidence supporting the associated clinical effect		
One study with level of evidence score 3	+	
At least two studies with level of evidence score 3	++	
Three or more studies with level of evidence score 3	+++	+++[b]
Effectiveness of the intervention		
Number needed to genotype (NNG)		
100 < NNG ≤ 1000	+	
10 < NNG ≤ 100	++	++[c]
NNG ≤ 10	+++	
PGx information in the drug-label		
Recommendation to genotype	+	
At least one genotype/phenotype mentioned as a contraindication	+	+[d]
Total Score	9+	8+
Corresponding Clinical Implication Score[e]		**Essential**

[a] Patients assigned to be DPD deficient but have received normal doses of fluoropyrimidines been associated with CTCAE grade 5 toxicity;

[b] Eight studies of sufficient quality have shown an association with CTCAE grade 5 toxicity (references in Supplementary Table 1: 10, 15, 16, 18, 26, 29, 30 and 33);

[c] The NNG was calculated using the "Calculations of the number of adverse events prevented with an effective pre-emptive genotyping program".[1] The pooled odds ratios and relative risks for*2A, 1236A, 2846T and *13 was 5.2, extracted from meta-analyses Meulendijks et al., Terrazzino et al., and Rosmarin et al.[2-4] The calculated NNG was 53.9;

[d] In the European Union, DPD deficiency is mentioned in the current version of the summary of product characteristics (SPC) of capecitabine in the sections Contraindications and Special Warnings and Precautions for Use.[5] Similar information on DPD deficiency is provided in the United States by the Food and Drug Administration (FDA) for capecitabine.[6] Comparable reports are made in SPCs of 5-FU;[7,8]

[e] essential, beneficial, potentially beneficial or not required.

Abbreviations: DPD: dihydropyrimidine dehydrogenase; CTCAE: Common terminology criteria for adverse events.

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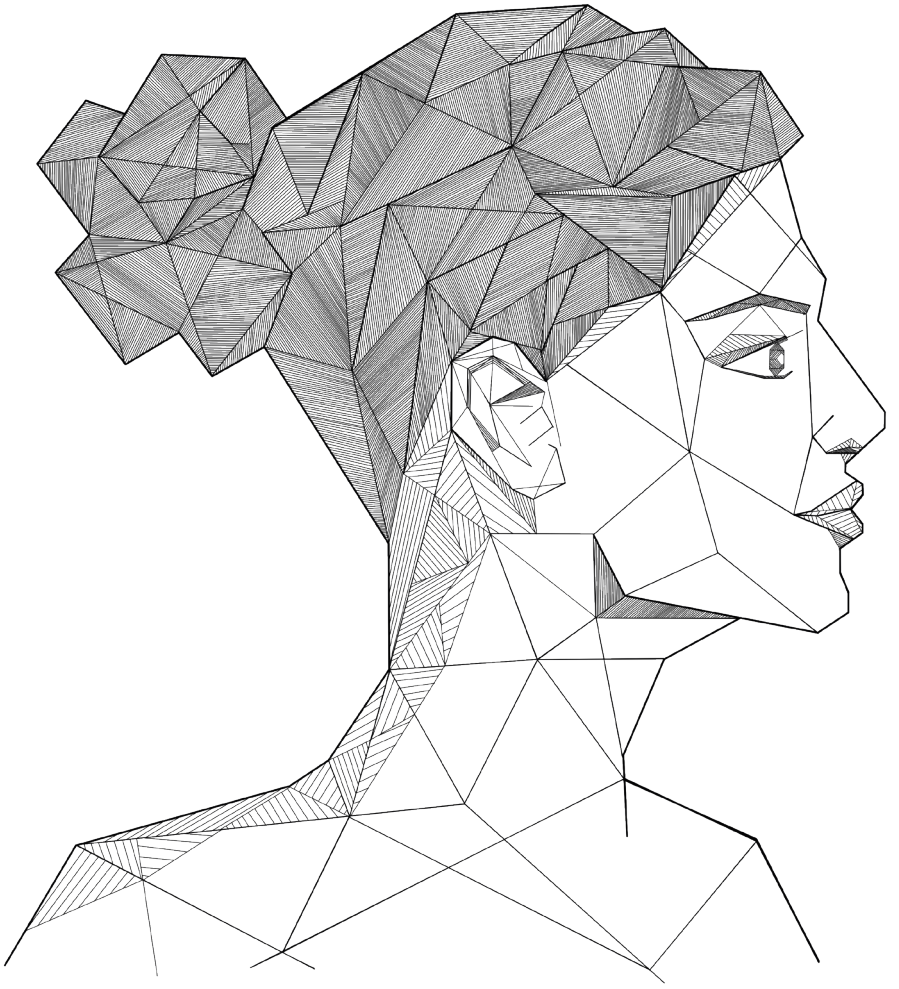

CHAPTER 5

DPYD genotype-guided dose individualisation of fluoropyrimidine therapy in patients with cancer: a prospective safety analysis

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Linda M. Henricks*, Carin A.T.C. Lunenburg*, Femke M. de Man*, Didier Meulendijks, Geert W.J. Frederix, Emma Kienhuis, Geert-Jan Creemers, Arnold Baars, Vincent O. Dezentjé, Alexander L.T. Imholz, Frank J.F. Jeurissen, Johanna E.A. Portielje, Rob L.H. Jansen, Paul Hamberg, Albert J. ten Tije, Helga J. Droogendijk, Miriam Koopman, Peter Nieboer, Marlène H.W. van de Poel, Caroline M.P.W. Mandigers, Hilde Rosing, Jos H. Beijnen, Erik van Werkhoven, André B.P. van Kuilenburg, Ron H.N. van Schaik, Ron H.J. Mathijssen, Jesse J. Swen, Hans Gelderblom, Annemieke Cats, Henk-Jan Guchelaar, Jan H.M. Schellens

**Contributed equally*

Abstract

Fluoropyrimidine treatment can result in severe toxicity in up to 30% of patients and is often the result of reduced activity of the key metabolic enzyme dihydropyrimidine dehydrogenase (DPD), mostly caused by genetic *DPYD* variants. In a prospective clinical trial, we investigated whether upfront screening for four *DPYD* variants and *DPYD*-guided dose individualization can reduce fluoropyrimidine-induced toxicity.

Prospective genotyping of *DPYD**2A, c.2846A>T, c.1679T>G, and c.1236G>A was performed in adult cancer patients for which fluoropyrimidine-based chemotherapy was considered in their best interest. All patients about to start with a fluoropyrimidine regimen (capecitabine or 5-fluorouracil as single agent or in combination with other chemotherapeutic agents and/or radiotherapy) could be included in the study. Heterozygous *DPYD* variant allele carriers received an initial dose reduction of 25% (c.2846A>T, c.1236G>A) or 50% (*DPYD**2A, c.1679T>G), *DPYD* wild-type patients were treated according to standard of care. The primary endpoint of the study was the incidence of severe (CTC-AE grade≥3) overall fluoropyrimidine-related toxicity. This toxicity incidence was compared between *DPYD* variant allele carriers and *DPYD* wild-type patients in the study in an intention-to-treat analysis, and relative risks for severe toxicity were compared between the current study and a historical cohort of *DPYD* variant allele carriers treated with full dose fluoropyrimidine-based therapy (derived from a previously published meta-analysis). This trial is registered under clinicaltrials.gov identifier NCT02324452 and is completed.

In total, 1,103 evaluable patients were enrolled, of whom 85 *DPYD* variant carriers (7.7%). Overall grade≥3 toxicity was higher in *DPYD* variant carriers than in wild-type patients (39% vs 23%, p=0.0013). The relative risk (RR) for grade≥3 toxicity was 1.31 (95% confidence interval [95%CI]:0.63–2.73) for genotype-guided dosing vs 2.87(95%CI:2.14–3.86) in the historical cohort for *DPYD**2A, no toxicity vs 4.30(95%CI:2.10–8.80) in c.1679T>G, 2.00(95%CI:1.19–3.34) vs 3.11(95%CI:2.25–4.28) for c.2846A>T, and 1.69(95%CI:1.18–2.42) vs 1.72(95%CI: 1.22–2.42) for c.1236G>A.

Upfront *DPYD* genotyping was feasible in routine clinical practice, and improved patient safety of fluoropyrimidine treatment. For *DPYD**2A and c.1679T>G carriers, a 50% initial dose reduction seems adequate. For c.1236G>A and c.2846A>T carriers, a larger dose reduction of 50% (instead of 25%) needs to be investigated. As fluoropyrimidines are among the most commonly used anticancer agents, the findings of this study are of high clinical importance, as they endorse implementing *DPYD* genotype-guided dosing as the new standard of care.

Acknowledgments

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