DPD: 5-fluorouracil/capecitabine 2551/2552/4893/4894/6192

95% CI = 95% confidence interval, AS = gene activity score, AS 0 = gene activity score 0 = two non-functional alleles (*2A/*2A, *2A/*13 or *13/*13) or more general two gene variants leading to non-functional alleles (*2A-homozygosity, *13-homozygosity, or both *2A and *13), AS 1 = gene activity score 1 = one fully functional and one non-functional allele (*1/*2A or *1/*13), AS 1.5 = gene activity score 1.5 = one fully functional and one partially functional allele (*1/1236A or *1/2846T), AS 2 = gene activity score 2 = two fully functional alleles (normal metaboliser; *1/*1), CAP = capecitabine, Cl = clearance, comb = combination therapy (≥ 2 oncolytic drugs), C_{ss} = steady-state plasma concentration, DPD = dihydropyrimidine dehydrogenase, FENO = fenotyping = two partially functional alleles (1236A/1236A, 1236A/2846T or 2846T/2846T) or one non-functional and one partially functional allele (*2A/1236A, *2A/2846T, *13/1236A or *13/2846T) or more general two gene variants leading to partially functional alleles (1236A-homozygosity, 2486T-homozygosity, or both 1236A and 2846T) or a gene variant leading to a non-functional allele and a gene variant leading to a partially functional allele (*2A plus 1236A, *2A plus 2846T, *13 plus 1236A or *13 plus 2846T), FU = fluorouracil, mono = monotherapy (1 oncolytic drug), NS = non-significant, OR = odds ratio, OR_{adj} = adjusted odds ratio, RR = relative risk, RR_{adj} = adjusted relative risk, S = significant, SmPC = Summary of Product Characteristics, SNP = single nucleotide polymorphism

Disclaimer: The Pharmacogenetics Working Group of the KNMP 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.

Brief summary and justification of choices:
5-Fluorouracil is mainly (> 80%) converted by dihydropyrimidine dehydrogenase (DPD) to inactive metabolites. Lower metabolic activity of DPD leads to increased intracellular concentrations of fluorodeoxyuridine monophosphate, the active metabolite of 5-fluorouracil and its prodrug capecitabine. This increases the risk of toxicity due to overdosing such as neutropenia, thrombopenia and diarrhoea.
18 of 20 studies and all 3 meta-analyses found an increased risk of grade ≥ 3 toxicity (either global or at least one specific kind of toxicity) for patients with gene variants resulting in reduced DPD activity (gene activity score (AS) 0 to 1.5 and fenotyping (FENO)). This increased risk was shown separately for AS 1 and 1.5, but AS 0 and FENO, were only investigated combined with other genotypes. However, both the stronger deficiency of these latter genotype groups and the development of severe toxicity in cases, confirm that the risk is also increased for AS 0 and FENO. In one study investigating response, there was no effect of gene variants. Because of the increased toxicity risk, the working group concludes that gene-drug interactions are present and that they necessitate therapy adjustment (yes/yes-interactions).
The recommended therapy adjustment per genotype group and the justification for these adjustments are indicated below.
Recommended therapy adjustments
Dose adjustments have been calculated on the basis of 5-fluorouracil clearance or AUC, also for patients receiving capecitabine. In addition, titrated doses and residual DPD enzyme activities are taken into account. Data on 5-fluorouracil clearance or AUC are only available for *1/*2A, *1/2846T and *2A/2846T. There are data from one patient with *1/*13 who developed severe toxicity on 5-fluorouracil, from one patient with 2846T/2846T and from one patient with 1236A/2846T.

AS 1.5: For *1/2846T, the weighted average of the calculated dose adjustments was a reduction to 55%. However, Deenen 2011 investigated 8 patients with *1/2846T and found a toxicity-guided dose reduction to 76% of the standard dose. In addition, Lunenburg 2016 found no grade ≥ 3 toxicity when treating five patients with *1/1236A with an initial dose of 75% of the standard dose. Deenen 2011 did not find significantly more dose decreases for *1/1236A + 1236A/1236A compared to *1/*1 when treating 568 patients with chemotherapy with capecitabine 1000 mg/m^2 twice daily. Kleinjan 2019 found the tolerated dose for 6 patients with *1/1236A to be 78% of the normal dose. The single patients with *1/2846T in Lunenburg 2018 and Kleinjan 2019 tolerated 60% and 85% of the normal dose, respectively. In a study with 51 patients with *1/1236A and 17 patients with *1/2846T, Henricks 2018 Lancet Oncol found the titrated dose to be 74% of

the normal dose for *1/1236A and 64% for *1/2846T. The mean and median DPD enzyme activity in this study were respectively 80% (standard deviation 30%) and 74% of that for *1/*1 for *1/1236A, and respectively 66% (standard deviation 20%) and 67% for *1/2846T. In this study, the (planned) starting dose was 75% of the normal dose, but bad tolerance was induced in 6% of patients with *1/1236A by increasing the dose afterwards and in 12% of patients with *1/2846T, either by not applying a reduced starting dose or by increasing the dose after tolerance for the reduced starting dose. In this study, the percentage of patients with overall grade ≥ 3 toxicity was still higher for *1/236A and for *1/2846T on (planned) reduced starting dose than for *1/*1 on the normal starting dose. To avoid this additional toxicity in patients with *1/1236A and *1/2846T, the KNMP Pharmacogenetics Working Group recommends starting with a lower than the calculated dose (i.e. 50% instead of 75% or 65% of the normal dose) and subsequently adjust the dose based on toxicity and efficacy.
Instead of dose adjustment, physicians may also choose an alternative.

AS 1: For 25x *1/*2A and 1x *1/*13, the weighted average of the dose adjustments calculated based on 5-fluorouracil clearance or AUC was a reduction to 47% (20-49%, median 46%) (Deenen 2016, Bois-dron-Celle 2007 and Morel 2006). The weighted mean of 47% was translated to 50% to be more achievable in clinical practice. Several studies on tolerated doses, residual enzyme activities and a 50% starting dose for AS 1 support this dose recommendation. Titrated or tolerated doses were 56% of the normal dose in Deenen 2011 (n = 5), 61% in Van Kuilenburg 2012 (n = 9), < 50% in Magnani 2013 (n = 3), 48% in Deenen 2016 (n = 18), 53% in Lunenburg 2016 (n = 4), 57% in Henricks 2018 Lancet Oncol (n = 17) and 54% in Kleinjan 2019 (n = 4). DPD enzyme activities were 64% of that for *1/*1 in Deenen 2016 (n = 15) and 54% in Henricks 2018 Lancet Oncol (n = 9; standard deviation for *1/*2A of 8% (n = 8); median 56% (n = 8)). In addition, Henricks 2018 Int J Cancer found no differences in toxicity and efficacy between 40 *1/*2A-patients (37 for efficacy) on a starting dose of approximately 50% of the normal dose and *1/*1-patients on the normal dose. Henricks 2018 Lancet Oncol found no differences in toxicity between 17 patients with AS 1 on a starting dose of 50% of the normal dose and *1/*1-patients on the normal dose, despite bad tolerance being induced by a subsequent dose increase in 12% of the patients with AS 1. Deenen 2016 found no difference in toxicities between 18 patients with *1/*2A on an initial dose of maximally 50% of the normal dose and *1/*1-patients on the normal dose. Lunenburg 2016 found no grade ≥ 3 toxicity when treating three patients with *1/*2A with an initial dose of 50% of the normal dose.
Based on these data, the KNMP Pharmacogenetics Working Group recommends to start with 50% of the normal dose. If appropriate, the dose can subsequently be adjusted based on toxicity and efficacy.
Instead of dose adjustment, physicians may also choose an alternative.

FENO: Clearance has only been determined for one patient with genotype *2A/2846T (Boisdron-Celle 2007). The clearance found for this patient was almost zero. For 1x 2846T/2846T and 1x 1236A/2846T, the average of the dose adjustment calculated based on 5-fluorouracil AUC was a reduction to 19% (12% for 2846T/2846T and 44% for 1236A/2846T) (Henricks 2017). Data on titrated or tolerated doses and on residual DPD enzyme activity showed a similar high range in values, both within as between the different genotypes. For 5 patients with 1236A/1236A, the tolerated dose varied from 40% to 100% of the normal dose (Meulendijks 2016 and Henricks 2017). For one patient with 2846T/2846T, the tolerated dose was 17% of the normal dose (Henricks 2017). For one patient with both 1236A and 2846T, the tolerated dose was 51% of the normal dose (Henricks 2017). For 4 patients with 1236A/1236A, the DPD enzyme activity varied from 41% to 79% of the activity in patients without a gene variant (Meulendijks 2016 and Henricks 2017). For 2 patients with 2846T/2846T, the DPD enzyme activity varied from 10% to 29% of the normal value (Henricks 2017). For one patient with both 1236A and 2846T, the DPD enzyme activity was 45% of the normal value (Henricks 2017). Lunenburg 2018 Genes (Basel) found 2 patients with gene activity 0.25-0.5 with null allele to tole-rate 50% of the normal dose. These patients had respectively 60% and 72% of the normal DPD enzyme activity. However, 4 patients with this phenotype who had previously developed sever toxicity on full dose fluoropyrimidines had DPD enzyme activities ranging from 1% to 38% of the normal value. The low DPD enzyme activities in these patients might be due to neutropenia, because DPD enzyme activity is measured in mononuclear cells. So in these 6 patients, the DPD activity was 33% of that measured in *1/*1-patients, but 66% if measured prior to toxicity.
Because of the high interpatient variability, the scarcity of data for calculating a dose recommendation, and the low prevalence of patients with two gene variants (0.35% of the 1138 genotyped patients in Henricks 2018 Lancet Oncol), the KNMP Pharmacogenetics Working Group recommends to fully personalise therapy in these patients, i.e. measure DPD enzyme activity and adjust the fluoropyrimidine dose accordingly.
Instead of dose personalisation, physicians may also choose an alternative.

AS 0: There are not enough data to be able to make a substantiated recommendation on dose adjustments for gene activity 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 agrees with the severe toxicity found in one patient with genotype *2A/*2A when using 5-fluorouracil 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 with gene activity 0 (see below), an alternative is recommended.
The calculated dose reduction based on 2 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 and Henricks 2018 Int J Cancer, Henricks 2018 Lancet Oncol and Lunenburg 2018 Genes (Basel)). Therefore, if an alternative is not possible, adjusting the dose according to the residual DPD enzyme activity in peripheral blood mononuclear cells is recommended. This strategy has been shown to be feasible in two patients with gene activity 0. A patient with 0.5% of the normal DPD activity tolerated 0.8% of the normal dose (150 mg capecitabine every 5 days) (Henricks 2018 Int J Cancer). A patient with undetectable DPD activity, tolerated 0.43% of the normal dose (150 mg capecitabine every 5 days with every third dose skipped) (Henricks 2017).

You can find a detailed overview of the observed kinetic and clinical effects in the background information text of the gene-drug interactions on the KNMP Kennisbank. You might also have access to this background information text via your pharmacy or physician electronic decision support system.

Recommendation concerning pre-emptive genotyping, including justification of choices:

Capecitabine and systemic fluorouracil

The Dutch Pharmacogenetics Working Group considers genotyping before starting systemic fluorouracil or capecitabine to be essential for drug safety. Genotyping must be performed before drug therapy has been initiated to guide drug and dose selection.

The clinical implication of the gene-drug interaction scores 8 out of the maximum of 10 points (with pre-emptive genotyping considered to be essential for scores ranging from 6 to 10 points):

The risk of serious life-threatening toxicity is increased for patients with a genotype resulting in diminished DPD enzyme activity (gene activity scores 0-1.5 and fenotyping). This toxicity can be fatal (grade 5). This results in the maximum score of 2 points for the first criterion of the clinical implication score, the clinical effect associated with the gene-drug interaction (2 points for CTCAE grade 5).

The increased risk for serious life-threatening toxicity (code E corresponding to grade 4) has been shown in 18 studies and 3 meta-analyses. This results in the maximum score of 3 points for the second criterion of the clinical implication score, the level of evidence supporting the associated clinical effect grade ≥ 3 (3 points for three or more publications with level of evidence score ≥ 3).

The number needed to genotype was deduced from two articles on a large Dutch study to be 163. Deenen 2016 found 18 *1/*2A in 1631 patients. Based on historical data, 73% of *1/*2A and 23% of non-genotyped patients (predominantly *1/*1) would have developed grade ≥ 3 toxicity on full dose fluoropyrimidine. This corresponds to 9 additional patients with toxicity. Meulendijks 2017 found 19 carriers of 2846T, 3 carriers of *13 and 58 carriers of 1236A in 1606 of the 1615 *2A-negative patients. The positive predictive value for grade ≥ 3 toxicity in the first cycle was 13% for all 3 gene variants combined. The negative predictive value was 88%, indicating that 12% of patients without a gene variant developed grade ≥ 3 toxicity in the first cycle. This corresponds to 1 additional patient with toxicity (1% of the 80 gene variant carriers). Thus the number needed to genotype to prevent a patient developing grade ≥ 3 toxicity would be 163 (1631/10). There are good indications that dose reduction can indeed abolish the increased toxicity risk in these patients, so that the deduced number to genotype indeed reflects the number of patients to genotype to prevent one case of grade ≥ 3 toxicity. Deenen 2016 showed that treatment of 18 *1/*2A with 50% of the normal initial dose resulted in a comparable toxicity as treatment of *1/*1 with the normal initial dose. The calculated number to genotype of 163 results in 1 out of the maximum of 3 points for the third criterion of the clinical implication score, the number needed to genotype (NNG) in the Dutch population to prevent one clinical effect grade ≥ 3 (1 point for 100 ≤ NNG ≤ 1000).

The Summary of Product Characteristics (SmPC) of capecitabine indicates that capecitabine is contra-indicated in patients with complete DPD deficiency (corresponding to gene activity score 0). This results in the maximum number of 2 points for the fourth and last criterion of the clinical implication score, the pharmacogenetics information in the SmPC (2 points for at least one genotype/phenotype mentioned as a contra-indication in the corresponding section).

In addition to the clinical implication score indicating pre-emptive genotyping to be essential, three cost analyses suggest that the costs for genotyping of all patients are less than the costs of treating the additional patients with toxicity if genotyping is not performed (Henricks 2019, Cortejoso 2016 and Deenen 2016). Thus, pre-emptive genotyping might not only be essential, but also cost-saving.

Cutaneous fluorouracil

The Dutch Pharmacogenetics Working Group considers genotyping before starting cutaneous fluorouracil to be potentially beneficial for drug safety. Genotyping can be considered on an individual patient basis. If, however, the genotype is available, the Dutch Pharmacogenetics Working Group recommends adhering to the gene-drug guideline.

The clinical implication of the gene-drug interaction scores 2 out of the maximum of 10 points (with pre-emptive genotyping considered to be potentially beneficial for scores ranging from 0 to 2 points):

The literature reports only one case of a patient with gene activity score 0 (*2A/*2A) developing toxicity code D, corresponding to grade 3, when treated with fluorouracil cream. This results in a score of 1 out of the maximum of 2 points for the first criterion of the clinical implication score, the clinical effect associated with the gene-drug interaction (1 point for CTCAE grade 3 or 4). In addition, it results in a score of 0 of the maximum of 3 points for the second crite-

rion of the clinical implication score, the level of evidence supporting the associated clinical effect grade ≥ 3 (only points for at least one publication with level of evidence score ≥ 3).
In the Netherlands, the frequency of *2A is 0.6% and the frequency of *13 0.1%. This indicates, that even if all compound heterozygotes for these variants have the variants on different alleles, the prevalence of gene activity score 0 is only 1 out of 20.408. Thus, more than 20.000 patients should be genotyped to find 1 patient with an increased risk. In addition, it is not known how large the risk is for this patient, because only one case has been reported, Thus, it can only been concluded that the number needed to genotype to prevent one clinical effect grade ≥ 3 is more than 20.400. This results in 0 out of the maximum of 3 points for the third criterion of the clinical implication score, the number needed to genotype (NNG) in the Dutch population to prevent one clinical effect grade ≥ 3 (no points for NNG > 1000).
The Dutch Summary of Product Characteristics (SmPC) of fluorouracil cream contains a warning for a possible risk of severe systemic toxicity in patients with a defective DPD enzyme. However, it does not contain a recommendation to genotype, nor does it mention a genotype/phenotype as a contra-indication. This results in 1 out of the maximum of 2 points for the fourth and last criterion of the clinical implication score, the pharmacogenetics information in the SmPC (1 point for at least one genotype/phenotype being mentioned in the SmPC in the absence of a recommendation to genotype and/or a genotype/phenotype being mentioned as a contra-indication in the corresponding section).

The table below follows the KNMP nomenclature for the gene variants. The nomenclature for the gene variants used in the table below may therefore differ from the nomenclature used by the authors in the article.

Source	Code	Effect	Comments
ref. 1 – CAP, mono/comb Kleinjan JP et al. Tolerance-based capecitabine dose escalation after DPYD genotype-guided dosing in heterozygote DPYD variant carriers: a single-center observational study. Anticancer Drugs 2019 Jan 8 [Epub ahead of print]. PubMed PMID: 30628914.	3	11 patients, heterozygous for a gene variant, received capecitabine treatment with reduced doses (75% of the normal dose for gene activity 1.5 and 50% of the normal dose for gene activity 1), that were subsequently adjusted on basis of tolerance according to a prespecified protocol. Only patients receiving capecitabine mono- or combination therapy without radiation and completing at least one treatment cycle were considered. Capecitabine doses according to protocol (normal doses) were 1000-1250 mg/m^2 twice daily. Therapy was evaluated after 1-2 cycles. Clinically relevant capecitabine-induced toxicities resulted in a dose reduction according to standard practice, whereas no or minimal toxicities allowed for a 15% dose escalation. In the remaining cases, the dose was maintained because of acceptable toxicities. The capecitabine dose could be increased repeatedly on the basis of tolerability, but could never exceed the conventional dose for the intended treatment. As is standard practice, the patients' clinical condition was also taken into account. Doses were only increased if the patient had a good clinical condition and if oncologist and patient agreed on increasing the dose. The dose was increased in 6 patients and subsequently reduced again in 2 patients. Of the 6 patients with a dose increase 1 (17%) experienced a grade 3 toxicity. 2 of the 11 patients (18%) developed a grade 3 adverse event on the reduced starting dose. For one patient with grade 3 diarrhoea the dose was reduced because of this, for the other patient with grade 3 neutropenia, the dose was not reduced. The 3 patients maintained on the reduced starting dose because low-grade toxicities and a moderate clinical condition did not allow for a dose increase, developed disease progression. Of the 5 patients who tolerated a dose increase, 2 developed disease progression and a 3rd patient had his colon resected. A median dose increase of 8.5% (range 4-31%) was achieved. Diarrhoea and haematological toxicities grade ≥ 3 and hand-foot syndrome grade ≥ 2 were considered severe. Hospitalisations were defined as capecitabine treatment related or possibly treatment related hospitalisations. Cycles were noted only if at least 50% of the capecitabine days were completed. Results were compared with 174 patients without gene variant treated with normal dose capecitabine.	Authors' conclusion: 'Tolerance-based capecitabine dose escalation did not lead to more toxicity in DPYD variant carriers compared with wild-type patients.'

<table>
<tr>
<td>ref. 1, continuation</td>
<td>AS 1-1.5 on genotype guided dose: AA</td>
<td>
Genotyping:
- 7x gene activity score 1.5 (6x *1/1236A, 1x *1/2846T)
- 4x gene activity score 1 (*1/*2A)

Results:
<table>
<tr><td colspan="3">Result for carriers of a gene variant on reduced dose compared to non-carriers on the normal dose:</td></tr>
<tr><td>outcome</td><td></td><td>value for non-carriers</td></tr>
<tr><td>% of patients with severe toxicity</td><td>NS
The % of patients with severe toxicity was numerically lower for carriers of a gene variant on reduced dose (27.3%).</td><td>37.9%</td></tr>
<tr><td>% of patients with diarrhoea grade ≥ 3</td><td>NS</td><td>19.5%</td></tr>
<tr><td>% of patients with diarrhoea resulting in hospitalisation</td><td>NS</td><td>11.5%</td></tr>
<tr><td>% of patients with hand-foot syndrome grade ≥ 2</td><td>NS</td><td>20.1%</td></tr>
<tr><td>% of patients with haematological toxicity grade ≥ 3</td><td>NS</td><td>3.4%</td></tr>
<tr><td>total prevalence of severe toxicities</td><td>NS</td><td>43.1%</td></tr>
<tr><td>hospitalisations</td><td>NS</td><td>11.5%</td></tr>
<tr><td>median days of hospitalisation</td><td>NS</td><td>8 days</td></tr>
<tr><td>cycle of first severe toxicity</td><td>NS</td><td>2</td></tr>
</table>
- Of the 6 patients with genotype *1/1236A:
  - 2 tolerated a dose increase in cycle 3 (to 83% or 98% of the normal dose). The patient with the highest increased dose developed disease progression in cycle 3. The other patient only moderately tolerated the dose increase.
  - 1 received an initial dose increase (to 85% of the normal dose in cycle 2 and 93% in cycle 3), but due to the general condition changing from good to poor, the dose increase was reversed (decrease to 85% of the normal dose in cycle 4 and to 75% in cycle 6).
  - 2 were maintained on the starting dose, 1 due to a moderate general condition combined with the absence of toxicity and 1 due to grade 3 neutropenia in cycle 2 being accepted. The first patient developed disease progression (in cycle 2).
  - 1 received a dose reduction to 66% in cycle 2 after grade 3 diarrhoea occurred in cycle 1. In this patient the colon was resected.
- The mean dose in the last cycle was 78% of the normal dose for these 6 patients.

- The only patient with genotype *1/2846T tolerated a dose increase in cycle 3 to 85% of the normal dose. She developed disease progression in cycle 9.

- Of the 4 patients with gene activity score 1 (*1/*2A):
  - 1 tolerated three dose increases (to 57% of the normal dose in cycle 2, 63% in cycle 3 and 73% in cycle 4).
</td>
<td>Tolerated dose compared to AS 2:
AS 1.5: 79%
AS 1: 54%</td>
</tr>
</table>

ref. 1, continuation		The patient received a colon resection in cycle 6. - 1 developed grade 3 diarrhoea after dose increase to 59% of the normal dose in cycle 2. - 2 were maintained on the starting dose due to a moderate general condition (and either no or grade 1 toxicity). Both developed disease progression (in respectively cycle 3 and 6). - The mean dose in the last cycle was 54% of the normal dose for these 4 patients. NOTE: Because 42% of patients were not genotyped for 1236G>A, approximately 4 patients in the group without gene variant, treated with the normal dose, were actually *1/1236A. However, reducing the number of patients experiencing toxicity in the group without a gene variant with 4, still results in a numerically higher percentage of patients in the group without gene variant experiencing toxicity than in the group with gene variant (36% versus 24%). NOTE: All patients were genotyped for *2A and 2846A>T. 58% of patients was also genotyped for *13 and 1236G>A. *13 was not found in these patients.	
ref. 2 – CAP, mono/comb Lunenburg CATC et al. Diagnostic and therapeutic strategies for fluoropyrimidine treatment of patients carrying multiple DPYD variants. Genes (Basel) 2018;9:E585. PubMed PMID: 30487465.	2 FENO: F FENO on 50% of the normal dose: AA	6 patients with multiple gene variants were identified either by additional retrospective genotyping after development of toxicity grade ≥ 3 from capecitabine-containing therapy in the study of Deenen 2016 (n = 4) or prior to treatment in routine clinical care (n = 2). One of these patients (with *2A and 2846T) was already described in Lunenburg 2016, but was included because the data on DPD activity are new for these patient. A 7th patient (1236A/2846T), already described in Henricks 2017, was not included in this summary. DPD enzyme activity in peripheral blood mononuclear cells was determined either retrospectively after the occurrence of toxicity grade ≥ 3 (n = 4) or during treatment (n = 2). Genotyping: - 6x 'fenotyping' (3x *2A/1236A or *1/*2A+1236A, 3x *2A/2846T or *1/*2A+2846T) Results: - The 6 patients had 1%, 9%, 16%, 38%, 60% or 72% of the normal DPD activity (9%, 16% and 38% for *2A/1236A or *1/*2A+1236A; 1%, 60% and 72% for *2A/2846T or *1/*2A+2846T). Of the 2 patients with a DPD activity higher than 50% of normal, the patient with 72% of the normal DPD activity was verified to be *1/*2A+2846T (gene variants on the same allele), but the patient with 60% of the normal DPD activity was verified to be *2A/2846T (gene variants on separate alleles). - the 4 patients with the lowest DPD activity had developed toxicity grade ≥ 3 (1x grade 3, 2x grade 4, 1x grade 5) on the normal dose. Three of them were admitted to hospital for 7-14 days. The authors indicate that low DPD enzyme activities might be due to neutropenia, because DPD activity is measured in mononuclear cells. - the 2 patients with the highest DPD activity tolerated 50% of the normal starting dose (toxicity grade 0 for the patient with 72% of the normal DPD activity (starting dose based on the detection of the *2A allele prior to treatment in Deenen 2016) and grade 1-2 for the patient with 60% of the normal DPD activity (starting dose based on the genotype and the previous tolerance for adjuvant therapy	Authors' conclusion: 'In patients carrying multiple DPYD variants, we recommend that a DPD phenotyping assay be carried out to determine a safe starting dose.' Tolerated dose compared to AS 2: FENO: 50% DPD activity compared to AS 2: FENO: 33% (66% if measured prior to toxicity)

<table>
<tr>
<td>ref. 2, continuation</td>
<td></td>
<td>with 5-fluorouracil 600 mg/m^2 as described earlier in Lunenburg 2016)). A dose increase was not attempted for these patients. The palliative therapy for the patient with 60% of the normal DPD activity was discontinued due to the side effects.

NOTE: Genotyping was for *2A, *13, 1236G>A and 2846A>T.</td>
<td></td>
</tr>
<tr>
<td>ref. 3 – CAP/FU, mono/comb
Henricks LM et al. Effectiveness and safety of reduced-dose fluoropyrimidine therapy in patients carrying the DPYD*2A variant: a matched pair analysis.
Int J Cancer 2018 Nov 28 [Epub ahead of print].
PubMed PMID: 30485432.</td>
<td>3

AS 1 on genotype guided dose: AA</td>
<td>40 patients with genotype *1/*2A and treated with an approximately 50% reduced fluoropyrimidine dose were compared to patients without *2A and to *1/*2A treated with full dose. To compare efficacy, 37 *1/*2A patients treated with reduced dose were matched to patients without *2A, *13, 1236A, and 2846T, treated with full dose. Only 13 matched pairs could be evaluated for disease control. To compare safety, *1/*2A patients treated with a reduced dose were compared with 1606 patients without *2A treated with full dose from Deenen 2016 and with 86 historical controls (*2A-carriers treated with full dose; including the historical controls in Deenen 2016).
16 of the 40 *1/*2A patients were from the study of Deenen 2016. The mean dose during the first cycle of these patients was 52% of the normal dose and during the entire treatment duration 53%. It was allowed to titrate the dose upwards during treatment after two cycles based on tolerance, as decided by the treating physician. In 11 patients, doses were titrated upwards during treatment, in 7 patients doses had to be further reduced after the initial dose reduction of 50%.
Overall survival was defined as the time between initiation of treatment and death by any cause. Progression-free survival was defined as the time between initiation of treatment and first signs of disease progression by either radiology or clinical signs, or death, whichever came first. Objective tumour response was defined according to RECIST 1.1 criteria. Disease control was defined as complete response, partial response or stable disease.
When anticipating a 15% decrease in overall survival (from 45% in the group without *2A to 30% in the *1/*2A-group at the end of the study, with a hazard ratio of no *2A versus *1/*2A of 0.66), 154 pairs (192 events in total) will be needed to reach 80% power, with a 5% significance level. With the 37 pairs of patients in this study, only a difference of at least 33% is detectable with 80% power.

Genotyping:
- 1606x gene activity score 2 (37 for efficacy analysis)
- 40x gene activity score 1 (*1/*2A) (37 for efficacy analysis)

Results:
<table>
<tr><td colspan="3">Result for *1/*2A on approximately 50% of the normal dose compared to patients without *2A on the normal dose:</td></tr>
<tr><td>outcome</td><td></td><td>value for patients without *2A</td></tr>
<tr><td>median overall survival</td><td>NS</td><td>24 months</td></tr>
<tr><td>median progression-free survival</td><td>NS</td><td>10 months</td></tr>
<tr><td>% of patients with controlled disease</td><td>NS</td><td>48%</td></tr>
<tr><td>overall toxicity grade ≥ 3</td><td>NS</td><td>23%</td></tr>
<tr><td>hand-foot syndrome grade 3</td><td>NS</td><td>5%</td></tr>
<tr><td>haematological toxicity grade ≥ 3</td><td>NS</td><td>10%</td></tr>
<tr><td>gastrointestinal toxicity</td><td>NS</td><td>9%</td></tr>
</table>
</td>
<td>Authors' conclusion: 'Our study is the first to show that DPYD *2A genotype-guided dosing appears to have no negative effect on effectiveness of fluoropyrimidine-based chemotherapy, while resulting in significantly improved patient safety.'</td>
</tr>
</table>

<table>
<tr>
<td>ref. 3, continuation</td>
<td>genotype guided versus not-genotype guided therapy for AS 1: AA#</td>
<td>
<table>
<tr><td>grade ≥ 3</td><td></td><td></td></tr>
<tr><td>treatment interruptions</td><td>NS</td><td>19%</td></tr>
<tr><td>treatment discontinuation due to toxicity</td><td>NS</td><td>16%</td></tr>
<tr><td>treatment-related hospitalisation</td><td>NS</td><td>11%</td></tr>
<tr><td>treatment-related death</td><td>NS</td><td>0%</td></tr>
</table>
<table>
<tr><td colspan="3">Result for *1/*2A on approximately 50% of the normal dose compared to *2A-carriers on the normal dose:</td></tr>
<tr><td>outcome</td><td></td><td>value for *2A-carriers on the normal dose</td></tr>
<tr><td>overall toxicity grade ≥ 3</td><td>RR = 0.23 (95% CI: 0.12-0.45) (S)</td><td>77%</td></tr>
<tr><td>haematological toxicity grade ≥ 3</td><td>x 0.18 (S)</td><td>56%</td></tr>
<tr><td>gastrointestinal toxicity grade ≥ 3</td><td>x 0.26 (S)</td><td>38%</td></tr>
<tr><td>treatment-related death</td><td>trend for a decrease (p = 0.096) (NS)</td><td>8%</td></tr>
</table>
NOTE: Genotyping was for *2A. For the 37 patients without *2A included in the efficacy analysis, the presence of 1236A, 2846T and *13 was excluded.
</td>
<td></td>
</tr>
<tr>
<td>ref. 4 – CAP/FU, mono
Lunenburg CATC et al.
Standard fluoropyrimidine dosages in chemoradiation therapy result in an increased risk of severe toxicity in DPYD variant allele carriers.
Eur J Cancer 2018;104:210-8.
PubMed PMID: 30361102.</td>
<td>3</td>
<td>828 patients were treated with chemoradiation with a fluoropyrimidine. 22 patients with DPYD variant alleles treated with a reduced fluoropyrimidine dose and 34 patients with DPYD variant alleles treated with the normal dose were compared to 771 patients without variant alleles treated with the normal dose. In addition, a *1/*2A case started on reduced dose followed by a strong dose increase was described. Starting doses in 22 patients with DPYD variant alleles were reduced to 50% or 75% of the normal starting dose according to Dutch Pharmacogenetics Working Group 2015 and Clinical Pharmacogenetics Implementation Consortium 2017 guidelines (i.e. 50% of the normal dose for *1/*2A, 60% for *1/2846T and 50-75% for *1/1236A). 495 of the 828 patients were from the study of Deenen 2016. In addition, 240 patients from the Leiden University Medical Center and 93 from the Italian Cancer Institute were included in the analysis. Differences in toxicities were observed between the locations. In chemoradiotherapy the fluoropyrimidine dosing is different (for example capecitabine 825 mg/m^2 twice daily continuously instead of 1250 mg/m^2 twice daily for 2 weeks followed by 1 week rest for (colo)rectal cancer).
Of the gastrointestinal toxicity, diarrhoea and mucositis were scored for all of the included patients, but nausea and vomiting were only scored in the Leiden plus Italian groups and the Leiden group respectively.

Genotyping:
Reduced dose group:
- 12x gene act. 1.5 (11x *1/1236A, 1x *1/2846T)
- 10x gene act. 1 (*1/*2A)

Normal dose group:
- 771x gene act. 2 (*1/*1)
- 20x gene act. 1.5 (20x *1/1236A, 9x *1/2846T)
- 3x gene act. 1 (2x *1/*2A, 1x *1/*13)
- 2x ‘feno’ (1236A/1236A)</td>
<td>Authors’ conclusion: ‘DPYD variant allele carriers who received dose reductions (n = 22) showed a comparable frequency of severe gastrointestinal toxicity compared with wild-type patients, but more (not statistically significant) severe haematological toxicity. Hospitalisations for all DPYD variant allele carriers were comparable, independent of dose adjustments; however, the mean duration of hospitalisation was significantly shorter in the dose reduction group.’</td>
</tr>
</table>

ref. 4, continuation

AS 1-1.5 on genotype guided dose: AA

Results:

Result for gene variant carriers on reduced dose compared to gene activity 2 on the normal dose:

outcome		value for gene act. 2 on normal dose
overall toxicity grade ≥ 3	NS	13.6%
haematological toxicity grade ≥ 3	trend for an increase (p = 0.083) (NS)	2.9%
gastrointestinal toxicity grade ≥ 3	NS	8.0%
dose reductions	NS	4.4%
dose increases	NS	0.5%
treatment interruptions	NS	4.9%
prematurely stopped	NS	9.9%
treatment-related hospitalisation	NS	7.8%
days of hospitalisation	NS	13

AS 1-1.5 + FENO: E

Result for gene variant carriers on normal dose compared to gene activity 2 on normal dose:

outcome		value for gene act. 2 on normal dose
overall toxicity grade ≥ 3	NS	13.6%
haematological toxicity grade ≥ 3	OR_{adj} = 4.19 (95% CI: 1.32-13.25) (S)	2.9%
gastrointestinal toxicity grade ≥ 3	OR_{adj} = 2.58 (95% CI: 1.02-6.53) (S)	8.0%
dose reductions	NS Doses were reduced from 100 to 60-77%.	4.4%
dose increases	NS	0.5%
treatment interruptions	NS	4.9%
prematurely stopped	NS	9.9%
treatment-related hospitalisation	NS	7.8%
days of hospitalisation	NS	13

genotype guided versus not-genotype guided therapy for AS 1-1.5: AA[#]

Result for gene variant carriers on reduced dose compared to gene variant carriers on the normal dose:

outcome		value for carriers on normal dose
treatment-related hospitalisation	NS	17.6%
days of hospitalisation	x 0.17 (S)	23

- One *1/*2A was started on 50% of the normal dose. Despite diarrhoea grade I-II after 2 weeks, the dose was increased to 83% of the normal dose. After 4 weeks, severe toxicity (diarrhoea, vomiting, nausea grade III and dermatitis grade II) occurred, and chemotherapy, and later radiotherapy, was stopped prematurely. The patient was hospitalised for 31 d, of which 3 d at the intensive

<table>
<tr><td>ref. 4, continuation</td><td></td><td>care unit. After hospitalisation, the patient had to recover completely from toxicity for 39 d in a nursing home (rehabilitation).

NOTE: Genotyping was for *2A, *13, 1236G>A and 2846A>T.</td><td></td></tr>
<tr><td>ref. 5 – CAP/FU, mono/comb
Henricks LM et al. DPYD genotype-guided dose individualisation of fluoropyrimidine therapy in patients with cancer: a prospective safety analysis. Lancet Oncol 2018;19:1459-67. PubMed PMID: 30348537

and personal communication (titrated dose and median DPD activity)</td><td>3

AS 1.5: F (2)#

AS 1.5 on 75% of the normal starting dose: D

AS 1 on 50% of the normal starting dose: AA</td><td>1103 patients with either no or a single gene variant were treated with genotype-guided fluoropyrimidine-based chemotherapy for a median of 71 days. The median number of treatment cycles was 3. Patient without a gene variant received the normal starting dose. *1/*2A and *1/*13 received 50% of the normal starting dose. *1/1236A and *1/2846T received 75% of the normal starting dose. Dose increase was allowed after the first two cycles provided that treatment was well tolerated, and the decision was left to the discretion of the treating physician. For one *1/*2846T no dose reduction was applied. The patient died after two cycles on full dose. Doses were increased more than 4 times as often in patients with a gene variant (13% of patients) than in patients without a gene variant (3% of patients). The dose increase was not well tolerated in 6 of the 11 patients with a gene variant (2 of the 4 *1/*2A, 3 of the 4 *1/1236A, 1 of the 2 *1/2846T and 0 of the 1 *1/*13), resulting in the *1/2846T discontinuing therapy because of toxicity and a dose reduction in the other patients. So, in 8% of the 85 patients with a gene variant, intolerance/toxicity was caused by either a dose increase or the starting dose not being reduced.
Toxicities scored as possibly, probably, or definitely related to fluoropyrimidine treatment were considered treatment-related. DPD enzyme activity in peripheral blood mononuclear cells was determined in pre-treatment samples (in 56 patients with a gene variant and 82 patients without).
Relative toxicity risks for patients with a gene variant and patients without a gene variant were compared to the relative risks in a historical control of patients receiving not-genotype-guided therapy (i.e. the normal starting dose for all patients) (the meta-analysis of Meulendijks 2015).
Sample size calculations revealed a required sample size of 11 variant carriers.

Genotyping:
- 1018x gene activity score 2 (*1/*1)
- 68x gene activity score 1.5 (51x *1/1236A, 17x *1/2846T)
- 17x gene activity score 1 (16x *1/*2A, 1x *1/*13)

Results:
<table>
<tr><td colspan="4">Result for gene variant carriers on reduced dose compared to patients without gene variant on the normal dose:</td></tr>
<tr><td colspan="2"></td><td></td><td>value for patients without gene variant</td></tr>
<tr><td rowspan="5">overall grade ≥ 3 toxicity</td><td>all carriers</td><td>x 1.7 (S)</td><td rowspan="5">23%</td></tr>
<tr><td>*1/1236A</td><td>RR = 1.69 (95% CI: 1.18-2.42) (S)</td></tr>
<tr><td>*1/2846T</td><td>RR = 2.00 (95% CI: 1.19-3.34) (S)</td></tr>
<tr><td>*1/*2A</td><td>NS</td></tr>
<tr><td>*1/*13</td><td>-</td></tr>
<tr><td colspan="2">grade ≥ 3 gastrointestinal toxicity</td><td>x 2.5 (S)</td><td>8%</td></tr>
<tr><td colspan="2">grade ≥ 3 haematological toxicity</td><td>x 2.5 (S)</td><td>6%</td></tr>
</table></td><td>Authors' conclusion: 'Prospective DPYD genotyping was feasible in routine clinical practice, and DPYD genotype-based dose reductions improved patient safety of fluoropyrimidine treatment. For DPYD*2A and c.1679T>G carriers, a 50% initial dose reduction was adequate. For c.1236G>A and c.2846A>T carriers, a larger dose reduction of 50% (instead of 25%) requires investigation.'</td></tr>
</table>

ref. 5, continuation		grade 3 hand-foot syndrome	NS	4%	
		grade ≥ 3 cardiac toxicity	NS	1%	
		grade ≥ 3 other treatment-related toxicity	NS	8%	
		overall grade ≥ 4 toxicity	NS	3%	
		grade ≥ 4 gastrointestinal toxicity	NS	1%	
		grade ≥ 4 haematological toxicity	NS	1%	
		grade ≥ 4 cardiac toxicity	NS	<1%	
		grade ≥ 4 other treatment-related toxicity	NS	1%	
		fluoropyrimidine-related hospital admission	NS	14%	
		stop of fluoropyrimidines because of adverse events	NS	17%	
		fluoropyrimidine-related death	NS	<1%	

Results per variant genotype:

	*1/ 1236A	*1/ 2846T	*1/*2A	*1/*13
% of normal dose in first cycle	74	73	51	50
% of normal dose whole treatment	74	72	53	54
titrated dose	74	64	57	63
DPD-enzyme activity (% of normal)	80	66	55	40
median DPD-enzyme activity (% of normal)	74	67	56	-

Titrated dose compared to AS 2:
AS 1.5: 72%
AS 1: 57%

DPD activity compared to AS 2:
AS 1.5: 77%
AS 1: 53%

Relative risk (95% CI) for overall grade ≥ 3 toxicity for carriers on reduced dose compared to carriers on the normal dose:

	reduced dose	normal dose
1236G>A	1.69 (1.18-2.42) (S)	1.72 (1.22-2.42) (S)
2846A>T	2.00 (1.19-3.34) (S)	3.11 (2.25-4.28) (S)
*2A	1.31 (0.63-2.73) (NS)	2.87 (2.14-3.86) (S)
*13	-	4.30 (2.10-8.80) (S)

NOTE: The authors did not investigate whether the overall grade ≥ 3 toxicity in carriers on reduced dose after correction for the toxicity caused by dose increase and exclusion of the patient that never received a dose reduction was still significantly higher than in patients without gene variant on full dose. This correction reduces the overall grade ≥ 3 toxicity in all carriers from 39% to 31%, in *1/1236A from 39% to 33% and in *1/2846T from 47% to 38%. (For *1/*2A, this correction reduces the overall grade ≥ 3 toxicity from 31% to 19%, while the value for *1/*13 remains 0%.)

NOTE: The authors indicated that although the mean DPD enzyme activity for *1/1236A was reduced by around 20%, the large variation in this activity suggests that a proportion of patients needs a larger dose reduction, while other patients

<table>
<tr><td>ref. 5, continuation</td><td></td><td>might tolerate a full dose. However, the absence of a correlation between DPD enzyme activity and the occurrence of severe fluoropyrimidine-related toxicity for each of the genotypes, also for *1/236A (and for *1/2846T), argues against an important role of DPD activity variation in the occurrence of toxicity.

NOTE: In the discussion, the authors mentioned twice that the applied genotype-guided dose reduction is unlikely to result in underdosing/undertreatment. However, they did not address this issue in their recommendation of a more cautious initial dose reduction of 50% for *1/236A and *1/2846T, followed by close monitoring and individual dose titration. Because the titrated dose in these patients is respectively 74% and 64% of the normal dose and corresponding percentages for the remaining mean and median DPD activity were found, a starting dose of 50% would be underdosing for the majority of these patients.

NOTE: Genotyping was for *2A, *13, 1236G>A and 2846A>T.</td><td></td></tr>
<tr><td>ref. 6 – CAP/FU, comb
Madi A et al. Pharmacogenetic analyses of 2183 patients with advanced colorectal cancer; potential role for common dihydropyrimidine dehydrogenase variants in toxicity to chemotherapy. Eur J Cancer 2018;102:31-9. PubMed PMID: 30114658.</td><td>3</td><td>2116 patients with advanced colorectal cancer were treated with fluoropyrimidine-oxaliplatin chemotherapy with or without cetuximab for 24 weeks. 62% of patients received capecitabine plus oxaliplatin and 38% received infusional 5-fluorouracil plus oxaliplatin. 37% of patients also received cetuximab.
After 12 weeks, therapies also differed in being either continuous or intermittent.
Any 12-week toxicity was defined as a dose reduction or delay in chemotherapy in the first 12 weeks of treatment due to any toxicity except peripheral neuropathy. Peripheral neuropathy is an oxaliplatin-associated toxicity.
Associations were tested with a codominant model (i.e. homozygous variant versus heterozygous versus homozygous wild type). Odds ratios were calculated using the best model that fitted the data (dominant (i.e. homozygous+heterozygous variant versus homozygous wild type), recessive (i.e. homozygous variant versus heterozygous+homozygous wild type), or additive (i.e. homozygous variant versus heterozygous versus homozygous wild type)) and were adjusted for cetuximab use and type of fluoropyrimidine.
The power was > 85% to detect odds ratios of 1.3 for variants with frequencies > 20% and to detect odds ratios of 1.6 for variants with frequencies > 5%. This corresponds to respectively a 7% difference in response or toxicity (45% responded and 35% had toxicity) and an 11% difference in response.

Genotyping:
2846A>T (Asp949Val):
- 2086x *1/*1
- 30x *1/2846T
*2A (2105 patients genotyped):
- 2082x *1/*1
- 23x *1/*2A

Results:
<table>
<tr><td colspan="4">Results for gene variant carriers compared to patients without the gene variant:</td></tr>
<tr><td></td><td>2846A>T</td><td>*2A</td><td>value for patients without gene variant</td></tr>
<tr><td>dose reduction or the-</td><td>OR = 2.2 (95% CI: 1.1-4.5)</td><td>NS</td><td>36%</td></tr>
</table>
</td><td>Authors' conclusion: 'Our data suggest that both common and rare DPYD variants may be associated with toxicity to fluoropyrimidine-based chemotherapy. …. No common variant associations remained significant after Bonferroni correction.'</td></tr>
</table>

ref. 6, continuation		rapy delay in the first 12 weeks due to any toxicity except peripheral neuropathy	(S, but NS after correction for the 8 tested gene variants)			
		neutropenia grade ≥ 2	OR = 3.2 (95% CI: 1.2-8.2) (S, but NS after correction for the 8 tested gene variants)	NS	13%	
		lethargy grade ≥ 2	NS	OR = 5.3 (95% CI: 1.9-14.9) (S, also after correction for the 8 tested gene variants)	34%	
		nausea and vomiting grade ≥ 2	OR = 3.4 (95% CI: 1.5-7.3) (S, also after correction for the 8 tested gene variants)	NS	20%	
	AS 1.5: D	diarrhoea grade ≥ 2	OR = 4.6 (95% CI: 2.1-10.1) (S, also after correction for the 8 tested gene variants)	OR = 4.4 (95% CI: 1.7-11.0) (S, also after correction for the 8 tested gene variants)	25%	
		stomatitis grade ≥ 2	NS	OR = 4.6 (95% CI: 1.7-12.6) (S, also after correction for the 8 tested gene variants)	10%	
		hand-foot syndrome grade ≥ 2	NS	OR = 3.8 (95% CI: 1.2-11.8) (S, but NS after correction for the 8 tested gene variants)	9%	
	AS 1: E	infection with neutropenia grade ≥ 3	OR = 5.5 (95% CI: 1.3-24.2) (S, but NS after correction for the 8 tested gene variants)	OR = 19 (95% CI: 5.0-73.8) (S, also after correction for the 8 tested gene variants)	3%	
		NOTE: Only data on the gene variants proven to be associated with toxicity and reduced DPD enzyme activity were included in the table above. For the common gene variants *9A (Cys29Arg) and *6 (Val732Ile), this study found an association with a decrease and an increase in any toxicity in the first 12 weeks respectively. However, significance disappeared after Bonferroni correction for multiple testing. For *9A, no associations with specific toxicities were found. For *6, this study found an association with neutropenia grade ≥ 2, but significance disappeared after correction for the testing of 8 variants of the DPD encoding gene.				

ref. 6, continuation		The study found an association of *4 (Ser534Asn) with any toxicity in the first 12 weeks, but not with specific toxicities and significance was lost after correction for the testing of 8 variants of the DPD encoding gene. The study found an association of 775A>G (Lys259Glu) with stomatitis, but significance was both lost after calculation of an odds ratio and after correction for the testing of 8 variants of the DPD encoding gene. The study found no association of 496A>G (Met166Val) and 1627A>G (Ile543Val) with either any or specific toxicity. So, the study confirms the lack of sufficient proof for an association with toxicity for these gene variants. NOTE: Genotyping was for the indicated gene variants.	
ref. 7 – CAP, comb Henricks LM et al. Capecitabine-based treatment of a patient with a novel DPYD genotype and complete dihydropyrimidine dehydrogenase deficiency. Int J Cancer 2018;142:424-30. PubMed PMID: 28929491.	2 AS 0: 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 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 fluorouracil 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, 2846 A>T and 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.	Authors' conclusion: 'This case report demonstrates that a more comprehensive genotyping and phenotyping approach, combined with pharmacokinetically-guided dose administration, enables save fluoropyrimidine-treatment with adequate drug exposure in completely DPD deficient patients.' Dose-corrected AUC versus AS 2: AS 0: 11271%
ref. 8 – 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 - published online 2017 Oct 6.	2 FENO: A	5 patients, being either homozygous for a gene variant or having two different gene variants, received capecitabine or 5-fluorouracil 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 2846T/2846T, who did not receive treatment, because she was disease free after surgery. For 3 patients, the AUC of fluorouracil 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 1236A/1236A - 2x 2846T/2846T - 1x *2A/*2A - 1 carrier of both 1236A and 2846T (verified as 1236A/2846T (variants on separate alleles) by Lunenburg, Genes 2018) Results: - Of the four patients homozygous for a partially functional allele, the two patients with genotype 1236A/1236A 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-fluorouracil dose. The patients did not have severe toxicity on the reduced doses. The two patients with genotype 2846T/2846T had	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.'

<table>
<tr>
<td>ref. 8, continuation</td>
<td>AS 0: A

FENO: A</td>
<td>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 well without occurrence of severe toxicity and surgery was performed after treatment. The dose-corrected AUC of 5-fluorouracil 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-fluorouracil in this patient was 13.812% of that of control patients.

- The carrier of both 1236A and 2846T had 45% of the normal DPD activity, corresponding to a patient with 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-fluorouracil in this patient was 227% of that of control patients.

NOTE: Patients were genotyped for *2A, *13, 2846A>T and 1236G>A.</td>
<td>Dose-corrected AUC versus AS 2:
FENO: 546%
AS 0: 13812%

Tolerated dose compared to AS 2:
FENO: 55%
AS 0: 0.43%

DPD activity compared to AS 2:
FENO: 41%
AS 0: 0%</td>
</tr>
<tr>
<td>ref. 9 – 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.</td>
<td>4</td>
<td>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 2846T
- 3 carriers of *13
- 58 carriers of 1236A

Results:
Result for carriers compared to non-carriers of the gene variant:
<table>
<tr><th>gene variant</th><th>outcome</th><th>OR$_{adj}$ (95% CI)</th></tr>
</table></td>
<td>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.’</td>
</tr>
</table>

<table>
<tr>
<td>ref. 9, continuation</td>
<td>AS 1: E

AS 1-1.5: E

AS 1.5: AA</td>
<td>
<table>
<tr><td>2846T</td><td>global toxicity</td><td>NS, trend for an increase (p = 0.095)</td></tr>
<tr><td></td><td>gastrointestinal toxicity</td><td>NS</td></tr>
<tr><td></td><td>haematological toxicity</td><td>NS, trend for an increase (p = 0.066)</td></tr>
<tr><td></td><td>hospitalisation</td><td>NS</td></tr>
<tr><td>*13</td><td>global toxicity</td><td>NS</td></tr>
<tr><td></td><td>gastrointestinal toxicity</td><td>NS, trend for an increase (p = 0.090)</td></tr>
<tr><td></td><td>haematological toxicity</td><td>24.9 (1.74-354) (S)</td></tr>
<tr><td></td><td>hospitalisation</td><td>NS, trend for an increase (p = 0.094)</td></tr>
<tr><td>2846T and *13</td><td>global toxicity</td><td>3.0 (1.05-8.77) (S)</td></tr>
<tr><td>1236A</td><td>global toxicity</td><td>NS</td></tr>
<tr><td></td><td>gastrointestinal toxicity</td><td>NS</td></tr>
<tr><td></td><td>haematological toxicity</td><td>NS</td></tr>
<tr><td></td><td>hospitalisation</td><td>NS, trend for an increase (p = 0.069)</td></tr>
<tr><td colspan="3">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.</td></tr>
</table>

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.
</td>
<td></td>
</tr>
<tr>
<td>ref. 10 – 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.</td>
<td>2

AS 1: E</td>
<td>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-fluorouracil 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.</td>
<td>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.'</td>
</tr>
<tr>
<td>ref. 11 – CAP, mono/comb
Meulendijks D et al.
Patients homozygous for DPYD c.1129-5923C>G/ haplotype B3 have partial DPD deficiency and require a dose</td>
<td>2</td>
<td>Three patients treated with capecitabine containing chemotherapy were retrospectively determined to have genotype 1236A/1236A. Gene variants *2A, *13 and 2846T were not present in these patients. More than 4 weeks after the last treatment with fluoropyrimidines, DPD enzyme activity in 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</td>
<td>Authors' conclusion: 'The presented functional and clinical data indicate that the c.1129-5923 C>G variant is both functionally and clinically relevant, and support an upfront dose reduction of the fluoropyri-</td>
</tr>
</table>

reduction when treated with fluoropyrimidines. Cancer Chemother Pharmacol 2016;78:875-80. PubMed PMID: 27544765. **ref. 11, continuation**	FENO: C	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 stopcodon. 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 patient with genotype 1236A/1236A, who developed fatal toxicity during the first cycle with full-dose 5-FU plus cisplatin. NOTE: Patients were genotyped for 1129-5923C>G and checked for the presence of 1236G>A and 959-51T>G, which are in complete linkage disequilibrium with 1129-5923C>G in haplotype B3.	midine starting dose in patients carrying c.1129-5923C>G homozygously.' Tolerated dose versus AS 2: FENO: 60% DPD activity versus AS 2: FENO: 48%
ref. 12 – 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.	3	The results of routine prospective genotyping and genotype-guided dosing were retrospectively evaluated in patients receiving capacetabine 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 1236G>A was added (n = 109). Recommended dosing reductions were 50% of the normal dose per *2A- and *13-variant and 25% per 1236A-variant. Recommended dosing reduction per 2846T-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.	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.'

PubMed PMID: 27181275. **ref. 12, continuation**	AS 1.5: E (2)# AS 1: E (2)# FENO on 50% of the normal dose: C (2)#	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 *1/1236A - 5x *1/*2A - 1 carrier of both *2A and 2846T (either *2A/2846T (on separate alleles) or *1/(*2A+2846T) (variants on the same allele)) Results: - 8 patients (5x *1/1236A 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 *1/1236A 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. - 3 patients (1x *1/1236A 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 2846T, 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 *1/1236A) did not start fluoropyrimidine therapy.	
ref. 13 – 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	3	A subset of patients from Lee 2014 was reanalysed: 1953 patients, negative for *2A, *13 and 2846T, and treated with 12 cycles of adjuvant FOLFOX therapy (5-fluorouracil, 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-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 (12.5%), 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	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>
<tr>
<td>Genomics 2016;26:133-7. PubMed PMID: 26658227.

ref. 13, continuation</td>
<td>AS 1.5 + FENO: E</td>
<td>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. Results were adjusted for this, but this indicates that adverse events common to 5-fluorouracil are not the same as 5-fluorouracil-induced adverse events.

Genotyping:
- 1875x *1/*1
- 77x *1/1236A
- 1x 1236A/1236A

Results:
<table>
<tr><td colspan="2">Risk of grade ≥ 3 adverse event for 1236A/1236A versus *1/1236A versus *1/*1:</td></tr>
<tr><td rowspan="2">any adverse event</td><td>NS, trend for an increase (p = 0.082)</td></tr>
<tr><td>OR_{adj} for (*1/1236A + 1236A/1236A) compared to *1/*1 also showed a trend for an increase (NS, p = 0.127).</td></tr>
<tr><td>diarrhoea</td><td>NS</td></tr>
<tr><td>neutropenia</td><td>S for an increase</td></tr>
<tr><td>pain</td><td>NS</td></tr>
<tr><td>fatigue</td><td>NS</td></tr>
<tr><td>nausea/vomiting</td><td>NS</td></tr>
<tr><td>stomatis/mucositis</td><td>NS</td></tr>
<tr><td>dehydration</td><td>NS</td></tr>
<tr><td>leukopenia</td><td>NS</td></tr>
</table>
NOTE: Results were reported for 1129-5923C>G, which was in complete linkage disequilibrium with the also genotyped 1236G>A.</td>
<td></td>
</tr>
<tr>
<td>ref. 14 – 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.</td>
<td>3</td>
<td>1631 patients received genotype-guided therapy with capecitabine (90% of patients) or 5-fluorouracil (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%.
The risk of grade ≥ 3 toxicity was higher in combination therapy than in monotherapy and chemo-radiotherapy regimens.

Genotyping:
- 1613x *1/*1</td>
<td>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.’</td>
</tr>
</table>

ref. 14, continuation

AS 1 on 48% of the normal dose: AA

- 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 2**9**% to 67%) - 2 patients developed toxicity grade 0 (first of the two cycles with 29% and second cycle with 59% of the normal 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; maximum 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 chemoradiotherapy, downstaging of the tumor 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)	73%
	In addition, the observed toxicity was short in duration with reduced dosing and usually long-lasting with full dosing.	
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%
	grade 1-2	NS	54%
haematological toxicity	grade ≥ 3	NS	10%
	grade 1-2	NS	35%
diarrhoea	grade ≥ 3	NS	8%
	grade 1-2	NS	29%

<table>
<tr>
<td>ref. 14, continuation</td>
<td>AS 1: A</td>
<td>
<table>
<tr><td>hand-foot syndrome</td><td>grade ≥ 3</td><td>NS</td><td>5%</td></tr>
<tr><td></td><td>grade 1-2</td><td>NS</td><td>28%</td></tr>
<tr><td colspan="4">The authors indicate that the comparable toxicity burden suggests that *1/*2A is not underexposed when treated with a median dose of 48%.</td></tr>
</table>

<table>
<tr><td colspan="3">Dose-normalised pharmacokinetics and DPD enzyme activity for *1/*2A compared to *1/*1:</td></tr>
<tr><td></td><td></td><td>value for *1/*1</td></tr>
<tr><td>5-fluorouracil AUC normalised to a capecitabine dose of 1250 mg/m^2</td><td>x 2.03 (NS)</td><td>602 ng.h/ml</td></tr>
<tr><td>DPD enzyme activity in peripheral mononuclear blood cells</td><td>x 0.64 (S)</td><td>9.9 nmol/ hr per mg protein</td></tr>
</table>
</td>
<td>AUC versus AS 2:
AS 1: 203%</td>
</tr>
<tr>
<td>ref. 15 – FU/CAP, mono/comb
Meulendijks D et al.
Clinical relevance of DPYD variants c.1679T>G, c.1236G>A/HapB 3, and c.1601G>A as predictors of severe fluoropyrimidine-associated toxicity: a systematic review and meta-analysis of individual patient data.
Lancet Oncol 2015;16:1639-50.
PubMed PMID: 26603945.</td>
<td>4

AS 1: E</td>
<td>Meta-analysis of 8 cohortstudies with in total 7365 patients treated with 5-fluorouracil 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 1236G>A were derived from 6 studies including a total of 4,261 patients and 174 heterozygous carriers and 3 homozygous carriers of 1236A. Data on *2A were derived from 7 studies including a total of 5.737 patients and 60 carriers of *2A. Data on 2846 A>T were derived from all 8 studies including a total of 7,318 patients and 85 carriers of 2846T.
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 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 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:
<table>
<tr><td colspan="3">Risk of grade ≥ 3 toxicity for *1/*13 compared to *1/*1:</td></tr>
<tr><td></td><td>RR_{adj} (95% CI)</td><td>incidence for *1/*1 (% of patients)</td></tr>
<tr><td>any toxicity</td><td>4.40 (2.08-9.30) (S)</td><td>22%</td></tr>
</table>
</td>
<td>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 patients with cancer treated with fluoropyrimidines.'</td>
</tr>
</table>

ref. 15, continuation	AS 1.5 + FENO: E	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 2846T were excluded from the meta-analysis. The association remained significant with p < 0.0167 after exclusion of any study from the meta-analysis, 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 (*1/1236A + 1236A/1236A) 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 subgroup 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 2846T were excluded from the meta-analysis. The association remained significant after exclusion of any study from the meta-analysis.		
		The effect of 1236A on risk of severe toxicity seemed similar in studies with long and short timeframes.		
		The sensitivity of 1236A 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 1236A/1236A 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)

<table>
<tr>
<td>ref. 15, continuation</td>
<td>AS 1.5: E</td>
<td>
<table>
<tr><td>any toxicity</td><td>2.85 (1.75-4.62) (S)</td><td>29%</td></tr>
<tr><td colspan="3">The heterogeneity between the studies was significant and strong.
There was no indication of publication bias.</td></tr>
</table>

<table>
<tr><td colspan="3">Risk of grade ≥ 3 toxicity for 2846T-carriers compared to *1/*1:</td></tr>
<tr><td></td><td>RR_{adj} (95% CI)</td><td>incidence for *1/*1 (% of patients)</td></tr>
<tr><td>any toxicity</td><td>3.02 (2.22-4.10) (S)</td><td>25%</td></tr>
<tr><td colspan="3">The heterogeneity between the studies was significant and strong.
There was no indication of publication bias.</td></tr>
</table>

NOTE: 1236G>A is in complete linkage disequilibrium with 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.
</td>
<td></td>
</tr>
<tr>
<td>ref. 16 – 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.</td>
<td>3</td>
<td>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 outcomes were not. In addition, this indicates that adverse events common to 5-fluorouracil are not the same as 5-fluorouracil-induced adverse events.

Genotyping:
- 2532x *1/*1
- 24x *1/*2A
- 26x *1/2846T
- 1x *2A/2846T
- 1x *1/274C
- 5x *2A-genotyping failed
</td>
<td>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.'</td>
</tr>
</table>

ref. 16, continuation

AS 1 + FENO: E

- 5x 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.

AS 1.5 + FENO: E

Risk of grade ≥ 3 toxicity, premature treatment termination and disease free survival for *2846T-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%

ref. 16, continuation	FENO: F (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 2846T and grade ≥ 3 5-FU toxicity remained significant. The association between 2846T 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/2846T-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. - The gene variants *2A, *13 and 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, 2846G>T and 274G>C) were found in this population from the USA.	
ref. 17 – 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.	4 AS 0-1.5 + FENO: E	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 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, 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 1236G>A. Variant 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 2846A>T Meta-analysis of 6 studies during which Caucasian patients received capecitabine or fluorouracil-based therapy. Of these	Authors' conclusion: "Global capecitabine toxicity (grades 0/1/2 v grades 3/4/5) was associated with the rare, functional DPYD alleles 2846T>A and *2A (combined odds ratio, 5.51)."

ref. 17, continuation		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 fluorouracil infusion, but there was a trend (2 studies, n = 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 fluorouracil 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 2846A>T: - No meta-analysis for capecitabine, fluorouracil infusion and fluorouracil bolus injection (1 study each time) Variant 496G>A: - No meta-analysis for capecitabine and fluorouracil infusion (in both cases only 1 study) - No association with grade III-V toxicity for fluorouracil bolus injection (2 studies, n = 379) (NS) Variant 1236G>A: - No meta-analysis for capecitabine, fluorouracil infusion and fluorouracil bolus injection (1 study each time) Variant 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 fluorouracil infusion and fluorouracil 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. 18 – FU/CAP, mono/comb Terrazzino S et al. DPYD IVS14+1 G>A and 2846 A>T genotyping for the prediction of severe fluoropyrimidine-related toxicity: a meta-analysis. Pharmacogenomics 2013;14:1255-72. PubMed PMID: 23930673.	4 AS 1: E	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 2846 A>T were derived from 7 studies including a total of 2,308 patients and 34 carriers of 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). However, the increase was non-significant in studies including ≥ 40% of patients with toxicity.	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."

ref. 18, continuation	AS 1.5: E	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 fluorouracil-based therapy or fluorouracil 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) 2846A>T versus (no 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 fluorouracil-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 fluorouracil-based therapy. There may have been publication bias. - Increased risk of grade III-V diarrhoea (OR = 6.04; 95% CI: 1.77-20.66) (S) - 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. - 2846A>T had a sensitivity of 4.6% (95% CI: 2.2-9.4) for predicting grade III-V diarrhoea (S)	
ref. 19 – FU/CAP, comb Magnani E et al.	2	3 patients with genotype *1/*2A with gastrointestinal or head and neck tumours received fluorouracil or capecitabine-based therapy (adjuvant or metastatic therapy). A 4th patient with	Authors' conclusion: "Our data suggest that greater dose re-

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. **ref. 19, continuation**	AS 1: E	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 fluorouracil/carboplatin/folinic acid with standard-dose fluorouracil (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.	ductions or alternative therapies are needed for patients with DPD IVS14+1 G>A mutations.”
ref. 20 – FU, comb Vulsteke C et al. Genetic variability in the multidrug 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.	4 AS 1: AA	1012 breast cancer patients received neoadjuvant/adjuvant therapy with fluorouracil, epirubicin and cyclophosphamide. The fluorouracil 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). 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 fluorouracil toxicity is not common among breast cancer patients treated with this combination therapy. The fluorouracil 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.	Authors’ conclusion: “In our study, we did not observe any association with toxicity and IVS14+1 G>A. The absence of 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. 21 – FU, mono/ comb van Kuilenburg AB et al. Evaluation of 5-fluorouracil pharmacokinetics in cancer patients with a c.1905+1	3	Clinical aspects were determined in 20 patients who had been genotyped as *1/*2A beforehand and were treated with fluorouracil. Kinetics were determined in 30 *1/*2A (c.1905+1G>A) and 18 *1/*1, who received a fluorouracil 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 fluorouracil showed grade III-V toxicity, of which 3 showed grade IV neutropenia	Authors’ conclusion: “Profound differences in the elimination of 5FU could be detected between DPD-deficient patients and control patients. Furthermore, treat-

G>A mutation in DPYD by means of a Bayesian limited sampling strategy. Clin Pharmacokinet 2012;51:163-74. PubMed PMID: 22339448. **ref. 21, continuation**	AS 1: F	The severe toxicity occurred in the first cycle each time and 1 patient died. - Among 13 *1/*2A receiving low-dose fluorouracil, 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 fluorouracil 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 underestimate for *1/*2A. The fluorouracil concentration 1 hour after injection was around the detection limit for *1/*1. - The terminal half-life of fluorouracil 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)	ment 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 AS 2: AS 1: 54% AUC_t versus AS 2: AS 1: 132%
ref. 22 – 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.	4 AS 1: F AS 1.5 + FENO: E	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) (*1/1236A + 1236A/1236A) 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 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)	Authors' conclusion: "Of the patients polymorphic for DPYD IVS14+1G>A, 2846 A>T, and 1236G>A, 71% (5 of 7), 63% (5 of 8), and 50% (14 of 28) developed grade 3 to 4 diarrhea, 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 2846A>T variant allele carriers with further dose titration would significantly reduce the total number of severe toxicity events, thereby separate validation is indica-

ref. 22, continuation	AS 1.5: E	*1/2846T versus *1/*1: - 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 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. 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.	ted."
ref. 23 – FU/CAP, mono/comb Kristensen MH et al. Variants in the	3	68 patients with advanced colorectal cancer were given adjuvant or palliative treatment with fluoropyrimidine-based therapy. Therapy consisted of either a fluorouracil 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	Authors' conclusion: "Patients with the genetic variant IVS14+1 G/A or c1896 C/T 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. **ref. 23, continuation**	AS 1.5: E	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.	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 pretreatment detection of genetic variants can help to predict early toxicity experienced by patients receiving 5-FU-based chemotherapy."
ref. 24 – 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.	3 AS 1.5: F AS 1: E	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 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.	Authors' conclusion: "Our results show compelling evidence that, at least in distinct tumor 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."
ref. 25 – FU, mono Capitain O et al. The influence of	3	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, 2-weekly regimen partially using a bolus (400 mg/m^2); dose adjust-	Authors' conclusion: "Toxicity was linked to low UH2/U ratio, 2846 A>T, IVS14+1

fluorouracil outcome parameters on tolerance and efficacy in patients with advanced colorectal cancer. Pharmacogenomics J 2008;8:256-67. **ref. 25, continuation**	AS 1-1.5: E (2)[#]	ments 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), 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 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.	G>A for DPD."
ref. 26 – FU Sulzyc-Bielicka V et al. 5-Fluorouracil toxicity-attributable IVS14 + 1G > A mutation of the dihydropyrimidine dehydrogenase gene in Polish colorectal cancer patients. Pharmacol Rep 2008;60:238-42.	3 AS 1: E (2)[#]	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 population and is responsible for a significant proportion of life-threatening toxicity of 5-FU."
ref. 27 – 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.	3 AS 1: E	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] (S). - 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), 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).	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."

ref. 27, continuation		- The percentage of patients with toxicity was increased for 2846A>T (60% versus 16.1% in the overall population) (NS). - All other variants did not show a significant association with toxicity. - 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. 28 – 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)	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 occurence of severe toxicities without impairing efficacy."
ref. 29 – 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 Treatment Group (N0044). Am J Clin Oncol 2007;30:507-13.	3 AS 2 + AS 1: 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 tumor response or serious adverse events."
ref. 30 – FU, comb Magné N et al. Dihydropyrimidine dehydrogenase activity and the IVS14+1G>A mutation in patients developing 5FU-related toxicity.	3	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	Authors' conclusion: "Present data suggest that IVS14+1 mutation screening has limited effectiveness in identifying patients at risk for severe 5FU toxicity."

Br J Clin Pharmacol 2007;64:237-40. **ref. 30, continuation**	AS 1: E (2)#	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 thrombopenia) 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.	
ref. 31 - 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.	2 AS 1: E	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. - Rechallenge 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."
ref. 32 – 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.	3 AS 1: E AS 1.5: E FENO: F (2)#	252 French patients with advanced colon cancer (163x *1/*1, 6x *1/2846T, 1x *9A/2846T, 1x *1/*2A, 1x -1590C/*2A, 1x *2A/2846T+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), 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%). (*1/2846T + 1x *9A/2846T) 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/2846T+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:	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 alterna-

ref. 32, continuation		- 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.	tive treatment proposed." Clearance versus AS 2: AS 1.5: 55% AS 1: 20% FENO: almost 0%
ref. 33 – FU, mono Cho HJ et al. Thymidylate synthase (TYMS) and dihydropyrimidine dehydrogenase (DPYD) polymorphisms in the Korean population for prediction of 5-fluorouracil-associated toxicity. Ther Drug Monit 2007;29:190-6.	3 AS 1.5: AA AS 1: AA	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. - 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.	Authors' conclusion: "The findings, from Korean patients with colon cancer, suggest that polymorphisms of the DPYD gene are not associated with an increased risk for toxic response to 5-FU."
ref. 34 – 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.	3 AS 1: E (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. 35 – FU, comb 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.	3 AS 0-1.5: E AS 1: F (2)# AS 0: E (2)#	487 French patients (300x *1/*1, 10x *1/2846T, 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), 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. (*1/*2A + *2A/*2A + *1/2846T + *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 cyclophospha-	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 dehydrogenase deficiency, 5-FU administration often can be safely continued with an individual dose adjustment."

ref. 35, continuation		mide. 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) *1/2846T 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.	Clearance versus AS 2: AS 1.5: 55% AS 1: 46%
ref. 36 – CAP, mono Largillier R et al. Pharmacogenetics of capecitabine in advanced breast cancer patients. Clin Cancer Res 2006;12:5496-502.	3 AS 1: F (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. 37 – FU, mono Salgueiro N et al. Mutations in exon 14 of dihydropyrimidine dehydrogenase and 5-fluorouracil toxicity in Portuguese colorectal cancer patients. Genet Med 2004;6:102-7.	3 AS 1: E	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. 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%).	Authors' conclusion: "We conclude that mutations in exon 14 of DPYD gene are responsible for a significant proportion of life-threatening toxicity to 5-Fluorouracil, and should therefore be excluded before its administration to cancer patients."
ref. 38 – 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.	3 AS 1 + AS 0: E	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. 39 – 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-fluorouracil (5-FU)-related toxicity compared with controls. Clin Cancer Res 2001;7:2832-9.	3 AS 1: F (2)# AS 0: F (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."
ref. 40 – FU, comb Yamaguchi K et al. Germline mutation of dihydropyrimidine dehydrogenese gene among a Japanese population in relation to toxicity to 5-fluorouracil. Jpn J Cancer Res 2001;92:337-42.	3 AS 2 + AS 1.5: 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. 41– 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.	3 AS 1-1.5: E	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/2846T, 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."
ref. 42 – FU, cutaneous Johnson MR et al. Life-threatening toxicity in a dihydropyrimidine dehydrogenase-deficient patient after treatment with topical 5-fluorouracil. Clin Cancer Res	2 AS 0: D	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	Authors' conclusion: "This study represents the first characterization of a DPDdeficient patient who developed life-threatening toxicity after exposure to topical 5-FU. Considering the previously reported low cutaneous absorption

1999;5:2006-11. **ref. 42, continuation**		mg/kg that is generally used for chemotherapy.	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. 43 – FU SmPC Fluorouracil PCH 15-10-12.	0 AS 0-1.5 + FENO: E	Warning: There have been reports of increased fluorouracil 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. 44 – FU SmPC Efudix (5-fluorouracil) cream 08-10-18.	0 AS 0-1.5 + FENO: E	Warning: Individuals with a defect in the enzyme dihydropyrimidine dehydrogenase (DPD) may be susceptible to severe systemic toxicity on use of standard doses of Efudix due to an increased systemic fluorouracil 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. 45 - CAP SPC Xeloda (capecitabine) 20-04-18.	0 AS 0: F AS 1-1.5 + FENO: E	Contraindications: Patients with known complete absence of dihydropyrimidine dehydrogenase (DPD) activity. 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 fluorouracil degradation, are at increased risk for severe, life-threatening, or fatal adverse reactions caused by fluorouracil. 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 (e.g. DPYD*2A, c.1679T>G, c.2846A>T and c.1236G>A/HapB3 variants), 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. Patients with certain heterozygous DPYD variants (including DPYD*2A, c.1679T>G, c.2846 A>T and c.1236G>A/HapB3 variants) have been shown to have increased risk of severe toxicity when treated with capecitabine. The frequency of the heterozygous DPYD*2A genotype in the DPYD gene in Caucasian patients is around 1%, 1.1% for c.2846A>T, 2.6-6.3% for c.1236G>A/HapB3 variants and 0.07 to 0.1% for c.1679T>G. Genotyping for these alleles is recommended to identify patients at increased risk for severe toxicity. Data on the frequency of these DPYD variants in other populations than Caucasian is limited. It cannot be excluded	

ref. 45, continuation		that other rare variants may also be associated with an increased risk of severe toxicity. 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 adjusment according to toxicity. A reduction of the starting dose in these patients may be considered to avoid serious toxicity. There is insufficient data to recommend a specific dose in patients with partial DPD activity as measured by specific test. It has been reported that the DPYD*2A, c.1679 T>G variants lead to a greater reduction in enzymatic activity than the other variants with a higher risk of side effects. The consequences of a reduced dose for efficacy are currently uncertain. Therefore, in the absence of serious toxicity the dose could be increased while carefully monitoring the patient. The patients who are tested negative for the above-mentioned alleles may still have a risk of severe adverse events. In patients with unrecognised DPD deficiency treated with capecitabine as well as in those patients who test negative for specific DPYD variations, life-threatening toxicities manifesting as acute overdose may occur. In the event of grade 2-4 acute toxicity, treatment must be discontinued immediately. Permanent discontinuation should be considered based on clinical assessment of the onset, duration and severity of the observed toxicities.	
ref. 46 – FU SPC Fluorouracil 29-07-16 (USA) and other[1]	0 AS 0: F AS 1-1.5 + FENO: F	Warning: Based on postmarketing 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 fluorouracil (e.g., mucositis, diarrhea, neutropenia, and neurotoxicity). Patients with partial DPD activity may also have increased risk of severe, life-threatening, or fatal adverse reactions caused by fluorouracil. Withhold or permanently discontinue fluorouracil 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 fluorouracil dose has been proven safe for patients with complete absence of DPD activity. There is insufficient data to recommend a specific dose in patients with partial DPD activity as measured by any specific test.	
ref. 47 – FU SmPC Carac (5-fluorouracil) cream 16-12-03 (USA).	0 AS 0: E	Contraindications: Carac should not be used in patients with dihydropyrimidine dehydrogenase (DPD) deficiency. DPD deficiency may lead to fluorouracil 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 fluorouracil has been attributed to DPD deficiency. A case of life-threatening systemic toxicity has been reported following topical use of 5% fluorouracil by a patient with fully non-functional DPD. Symptoms included severe abdominal pain, haemorrhagic diarrhoea, vomiting, fever and chills. Physical examination showed stomatitis,	

ref. 47, continuation		erythematous rash, neutropenia, thrombocytopenia, inflammation of the oesophagus, stomach and small intestine. Although this patient had used 5% fluorouracil cream, it is not known whether patients with severe DPD deficiency develop systemic toxicity in response to lower concentrations of topically administered fluorouracil.	

[#] For studies that did not show significant differences for IM or 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.
[1]: SmPC Xeloda (capecitabine) 14-12-16 (USA).

Risk group	AS 1-1.5 and FENO with DPD inhibitors (uracil analogue antiviral drugs)

Comments:

- Cases about systemic fluorouracil or capecitabine therapy were not included in the status report due to the large number of articles available. Kinetic studies from 2009 were only included if the kinetic parameters were given per genotype group. Clinical studies were only included if the patient numbers exceeded 500 (from 2009) or 1000 (from May 2014) 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.
- Existing guidelines:
  - Amstutz U et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing: 2017 update. Clin Pharmacol Ther 2018;103: 210-6. PubMed PMID: 29152729.
    The authors consider *2A, *13, 1236G>A and 2846A>T to be of primary relevance due to their population frequency and established impact on enzyme function and toxicity risk. They indicate that *2A and *13 have the most deleterious impact on DPD activity, whereas 1236G>A and 2846A>T result in moderately reduced DPD activity. In addition, they indicate that 557A>G is a relatively common (3-5% carrier frequency) decreased function variant in individuals with African ancestry. However, in a supplementary table they indicate the evidence for an association of 557A>G with toxicity to be weak and for an association with DPD activity to be moderate. Based on current knowledge, they consider e.g. *5, *6 and *9A to be fully functional. Dose recommendations are only available for *2A, *13, 1236G>A and 2846A>T. CPIC allocates a gene activity score of 0 to *2A and *13 and a gene activity score of 0.5 to 1236G>A and 2846A>T. In calculating the gene activity score of the genotype of a patient with two different decreased/no function variants, they presume these variants to be on different alleles. CPIC distinguishes the phenotypes intermediate metaboliser (including our phenotypes gene activity score 1.5, gene activity score 1, and the genotypes 1236A/1236A, 2846T/2846T and both 1236A and 2846T) and poor metabolisers (including gene activity score 0 and the genotypes *2A plus 1236A, *2A plus 2846T, *13 plus 1236A, and *13 plus 2846T). In the summary below, our phenotype nomenclature is used.
    As evidence linking gene variants with 5-fluorouracil toxicity, CPIC mentions the meta-analysis of Meulendijks 2015. As evidence linking gene variants with DPD activity they mention the publications of Nie 2017 and of Offer 2013 (Nie Q et al. Quantitative contribution of rs75017182 to dihydropyrimidine dehydrogenase mRNA splicing and enzyme activity. Clin Pharmacol Ther 2017 [Epub ahead of print] and Offer SM et al. A DPYD variant (Y186C) in individuals of African ancestry is associated with reduced DPD enzyme activity. Clin Pharmacol Ther 2013;94:158-66). Nie 2017 indicates that the reduction in DPD activity is 50% and 68% in heterozygous carriers of *2A and *13, respectively. In addition, Nie 2017 indicates that the reduction in DPD activity is 30% and 35% in heterozygous carriers of 2846T and 1236A, respectively. In addition, Boisdron-Celle 2007 and Morel 2006 report a reduction in 5-fluorouracil clearance by 40-80% in heterozygous carriers of *2A, 2846T and *13. Offer 2013 indicates a 46% reduction in DPD activity in heterozygous carriers of 557A>G. In addition, CPIC indicates two publications measuring DPD activity after homozygous expression of gene variants *in vitro*.
    The CPIC guideline is based on evidence from 91 included studies. Because we only included the most important clinical and *ex vivo* studies and no *in vitro* studies in our guideline, the number of included studies in our guideline is lower (32 up to March 2017).
    CPIC indicates that the strength of the pharmacogenetic dosing recommendations is based on the known impact of some variants (*2A, *13, 2846T and 1236A) on DPD activity, the demonstrated relationship between DPD activity and 5-fluorouracil clearance, and between 5-fluorouracil exposure and its toxic effects. Patients who are heterozygous for decreased/no function variants demonstrate partial DPD deficien-

cy and should receive reduced starting doses. Based on Deenen 2016, CPIC concludes that a dose reduction of 50% is suitable for heterozygous carriers of no function variants. CPIC classifies this therapeutic recommendation as strong (i.e. “The evidence is high quality and the desirable effects clearly outweigh the undesirable effects”). CPIC indicates that evidence is limited regarding the optimal degree of dose reduction for decreased function variants. Based on Deenen 2011 (average capecitabine dose reduction by 25% in *1/2846T) and Lunenburg 2016 (safe treatment of 5 *1/1236A with a 25% reduced capecitabine starting dose), CPIC concludes that heterozygous carriers of decreased function variants may tolerate higher doses compared to carriers of no function variants. CPIC indicates that in heterozygous carriers of a decreased function variant, the individual circumstances of a given patient should therefore be considered to determine if a more cautious approach (50% starting dose followed by dose titration), or an approach maximising potential effectiveness with a potentially higher toxicity risk (25% dose reduction) is preferable. CPIC classifies this therapeutic recommendation as moderate (i.e. “There is a close or uncertain balance” as to whether the evidence is high quality and the desirable clearly outweigh the undesirable effects).
Because some patients carrying decreased or no function variants tolerate normal doses of 5-fluorouracil, CPIC indicates that to maintain effectiveness, doses should be increased in subsequent cycles in patients experiencing no or clinically tolerable toxicity in the first two chemotherapy cycles or with subtherapeutic plasma concentrations. Similarly, doses should be decreased in patients who do not tolerate the starting dose.
CPIC strongly recommends to avoid use of 5-fluorouracil-containing regimens in patients having two no function alleles or a no function allele and a decreased function allele. However, if no fluoropyrimidine-free regimens are considered a suitable therapeutic option, CPIC indicates that 5-fluorouracil administration at a strongly reduced dose combined with early therapeutic drug monitoring may be considered for patients with both a decreased function and a no function allele. However, CPIC notes that no reports of the successful administration of low-dose 5-fluorouracil in these patients were available at the time of guideline preparation. Assuming additive effects of decreased and no function alleles, it is estimated that a dose reduction of at least 75% would be required (i.e., starting dose < 25% of normal dose). CPIC indicates that in such cases a phenotyping test is advisable to estimate DPD activity and a starting dose. CPIC classifies this therapeutic recommendation as strong (i.e. “The evidence is high quality and the desirable effects clearly outweigh the undesirable effects”).
In an online November 2018 update, CPIC indicates the following:
The current guideline recommends to reduce the dose of fluoropyrimidines by 25-50% (from the full standard dose) in heterozygous carriers of decreased function variants. At the time of the guideline publication, this dose range was recommended due to limited evidence for genotype-guided dosing of decreased function alleles/variants. However, a recent prospective study (Henricks 2018 Lancet Oncol) provides evidence to support a recommendation for a 50% dose reduction in heterozygous carriers of the decreased function variants 2846A>T or 1236G>A. These data suggest that all Intermediate Metabolisers (gene activity score 1.5, gene activity score 1, and the genotypes 1236A/ 1236A, 2846T/2846T and both 1236A and 2846T) should receive a 50% dose reduction. Therefore CPIC revises its recommendation such that all Intermediate Metabolisers should receive a 50% dose reduction from the full standard starting dose, followed by dose titration based on clinical judgement and ideally therapeutic drug monitoring.
In addition, recent case reports from patients who are homozygous for 2846A>T indicate that a dose reduction of more than 50% may be required in some carriers of this genotype. Therefore, in patients with a 2846T/2846T genotype, clinicians should be aware that a > 50% reduction in starting dose might be warranted.
The therapeutic recommendations are indicated below:

Recommended dosing of fluorouracil or capecitabine by DPD phenotype		
Phenotype/ genotypes	Therapeutic recommendation	Classification of recommendation
AS 1.5	Reduce starting dose by 25 to 50% followed by titration of dose based on toxicity[a] or therapeutic drug monitoring (if available). *Online November 2018 update:* Reduce starting dose by 50% followed by titration of dose based on toxicity[a] or therapeutic drug monitoring (if available).	Moderate
AS 1	Reduce starting dose by 50% followed by titration of dose based on toxicity[a] or therapeutic drug monitoring (if available).	Strong
1236A/1236A, 2846T/2846T, or 1236A plus 2846T	Reduce starting dose by 50% followed by titration of dose based on toxicity[a] or therapeutic drug monitoring (if available). *Online November 2018 update:* Reduce starting dose by 50% followed by titration of dose based on toxicity[a] or therapeutic drug monitoring (if available). In patients with a 2846T/2846T genotype, clinicians should be aware that a > 50% reduction in starting dose might be warranted.	Strong

*2A plus 1236A, *2A plus 2846T, *13 plus 1236A, or *13 plus 2846T	Avoid use of 5-fluorouracil or 5-fluorouracil prodrug-based regimens. In the event, based on clinical advice, alternative agents are not considered a suitable therapeutic option, 5-fluorouracil should be administered at a strongly reduced dose[b] with early therapeutic drug monitoring.[c]	Strong
AS 0	Avoid use of 5-fluorouracil or 5-fluorouracil prodrug-based regimens.	Strong

[a]: Increase the dose in patients experiencing no or clinically tolerable toxicity in the first two cycles to maintain efficacy; decrease the dose in patients who do not tolerate the starting dose to minimize toxicities.

[b]: If available, a phenotyping test should be considered to estimate the starting dose. In the absence of phenotyping data, a dose of < 25% of the normal starting dose is estimated assuming additive effects of alleles on 5-FU clearance.

[c]: Therapeutic drug monitoring should be done at the earliest timepoint possible (e.g., minimum timepoint in steady state) in order to immediately discontinue therapy if the drug level is too high.

CPIC also refers to other gene-based dosing recommendations, i.e. the 2011 publication of our dosing recommendations in Clinical Pharmacology and Therapeutics and the warning against use in patients with DPD deficiency in the SmPCs of 5-fluorouracil and capecitabine from the United States of America and Canada.

On 25-2-2019, there was an online November 2018 update of the guideline present on the PharmGKB- and on the CPIC-site, which is included in the summary above.

- Thorn CF et al. PharmGKB summary: fluoropyrimidine pathways. Pharmacogenet Genomics 2011;21:237-42. PubMed PMID: 20601926.

  The PharmGKB mentions four polymorphisms that are associated with toxicity (*2A, 496A>G, *5 and *9A). However, the latter two have not been associated with toxicity in studies and/or with reduced clearance or activity. These are therefore fully functional alleles.

  The PharmGKB writes that *2A has the highest association with fluoropyrimidine toxicity, although the frequency of this variant is less than 1% in Caucasians. However, the *2A allele does not always correlate with reduced DPD activity *in vivo*. A recent study showed that *2A has limited predictive capacity for severe fluorouracil toxicity. This capacity was more pronounced in male patients and with certain treatment protocols.

  The PharmGKB concluded that the effect of DPYD on toxicity is clear. Given the low frequency of the variants, the unclear relationship between genotype and phenotype and the lack of diagnostic tests available before administration, the PharmGKB currently considers the effect not to be clinically relevant. More prospective studies are needed to investigate the influence of gender, treatment protocol, and additional unidentified DPYD variants on DPD-related toxicity.

- A cura del Gruppo di Lavoro di AIOM-SIF. Raccomandazioni per analisi farmacogenetiche.

  This unpublished Italian guideline is consulted for the UPGx project in 2016. For heterozygotes a dose reduction of 50% is recommended. In homozygotes the use of fluorouracil or capecitabine is contra-indicated.

- Van Cutsem E et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. Ann Oncol 2016;27:1386-422.

  According to this guideline, DPYD genotyping before starting fluoropyrimidine treatment is optional.

- Positive and negative predictive values:

  In a study including 683 German patients (Schwab M et al., 2008), the positive predictive power of the *2A allele for grade III/IV toxicity was 46% and the negative predictive power 85%. Of the patients with grade III/IV toxicity among this population, 5.5% had a *2A allele compared to 29% in the Dutch population (Van Kuilenburg AB et al., 2002). This suggests that the *2A allele has more significance in a Dutch population than in a German population.

  In a study with 1606 *2A-negative Dutch patients receiving therapy with capecitabine (90% of patients) or 5-fluorouracil (10% of patients), either as combined chemotherapy (different combinations) or as monotherapy (with or without radiotherapy), the positive predictive value of 2846A>T, *13 and 1236G>A combined for grade ≥ 3 toxicity in the first cycle was 13% and the negative predictive value 88% (Meulendijks 2017).

  In a study with 2594 patients from the USA 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, the positive predictive value of the gene variants *2A, *13 and 2846A>T combined for 5-FU grade ≥ 3 toxicity was 81.8% and negative predictive value 68% (Lee 2014). The low negative predictive value might be attributed to the combination chemotherapy, which may add to the toxicities common to 5-fluorouracil.

  In a meta-analysis of 8 cohort studies with in total 7365 patients, the positive predictive value of *13 in prediction of grade ≥ 3 toxicity was 46% and the positive predictive value of 1236G>A 41% (Meulendijks 2015).

- Cost-effectiveness:

  - Henricks LM et al. A cost analysis of upfront DPYD genotype-guided dose individualisation in fluoropyrimidine-based anticancer therapy. Eur J Cancer 2019;107:60-7. PubMed PMID: 30544060.

    In patients treated with fluoropyrimidines in 17 Dutch hospitals (Henricks 2018 Lancet Oncol), genotype-guided therapy was both cheaper and safer than not-genotype-guided therapy (€ 51 reduced costs per

patient and reduction of grade ≥ 3 toxicity in variant allele carriers). Variation of input data demonstrated that the screening strategy was very likely to be cost saving or worst case cost-neutral. Patients were screened for *2A, *13, 1236G>A and 2846A>T, followed by the use of 50% of the normal dose for *1/*2A and *1/*13 and 75% of the normal dose for *1/1236A and *1/2846T. Patients with more than 1 gene variant (homozygotes or compound heterozygotes) were individually treated and excluded from the study.
The calculation was from a health-care payer perspective. Only direct medical costs were included. The calculated costs of genotype-guided therapy were € 2,599 per patient and the calculated costs of not-genotype-guided therapy € 2,650 per patient. The calculation was based on costs of hospitalisation in a nursing ward of € 636 per day, costs of hospitalisation in an intensive care unit of € 2,015 per day, additional costs for interventions related to toxicity (except hospitalisation) of € 86 for toxicity grade 0-2 and € 234 for toxicity grade ≥ 3, costs of capecitabine tablets of € 144.06 per cycle, costs of 5-fluorouracil and administration at day care of € 335.29 per cycle, cost of a medical doctor visit of € 132 per cycle, a gene variant carrier frequency of 7.71%, and a genetic test price of € 100. The risks of serious adverse events were derived from Henricks 2018 Lancet Oncol and other published studies.
Variation of input data (± 20%) found genotype-guided therapy to have a high likelihood of being cost-saving. It found average cost-savings of € 52 (95% interval range -€ 38 to € 176) per patient. Average gain in safety was 0.89% (95% interval range -0.04% to 1.79%). This gain in safety represents the difference between the proportion of patients treated without severe toxicity (both wild-type patients and gene variant carriers taken together) in the genotype-guided strategy and the non-genotype-guided strategy.
The model was shown to be most sensitive to the frequency of the gene variants, followed by the risk of hospitalisation at the nursing ward for gene variant carriers receiving standard dose, and genotyping costs.

- Cortejoso L et al. Cost-effectiveness of screening for DPYD polymorphisms to prevent neutropenia in cancer patients treated with fluoropyrimidines. Pharmacogenomics 2016;17:979-84. PubMed PMID: 27248859.
The authors conclude that genotyping of *2A, 2846A>T and *13 using real-time PCR, TaqMan probes and a rapid cell lysis to provide PCR-ready DNA is cost effective in all fluoropyrimidine-based treatments. The costs for genotyping were € 6.40 per patient and the mean cost of treating severe neutropenia was € 3,044. Therefore, if severe fluoropyrimidine-induced neutropenia is reduced by genotyping the three DPYD variations in at least 2.21 cases per 1000 treated patients, DPYD genotyping will be cost effective.
The literature differs greatly with regard to the estimation of the percentage of adverse reactions that can be prevented by DPYD genotyping. A meta-analysis of *2A and 2846A>T genotyping results estimated that each variant could identify 5% of patients with grade ≥ 3 adverse reactions to fluoropyrimidines with a specificity of 99%, independently of penetrance (Terrazino 2013). Therefore, a minimum 10% of adverse reactions could be avoided by genotyping both variants, since they are not in linkage disequilibrium. A review comparing capecitabine and 5-FU in monotherapy estimated the incidence of severe neutropenia to range from 2-2.6% (capecitabine) to 19.8-26% (5-FU) (Aguado C et al. Should capecitabine replace 5-fluorouracil in the first-line treatment of metastatic colorectal cancer? World J Gastroenterol 2014;20:6092-101).The frequency of severe neutropenia arising from fluoropyrimidine-based combination therapies has been reported to range between 5% (Skof E et al. Capecitabine plus irinotecan (XELIRI regimen) compared to 5-FU/LV plus irinotecan (FOLFIRI regimen) as neoadjuvant treatment for patients with unresectable liver-only metastases of metastatic colorectal cancer: a randomised prospective Phase II trial. BMC Cancer 2009;9:120) and 44% (Cassidy J et al. XELOX vs FOLFOX-4 as first-line therapy for metastatic colorectal cancer: NO16966 updated results. Br J Cancer 2011;105:58-64). The authors conclude that genotyping is likely to reduce neutropenia in more than 2.21 cases per 1000 treated patients (0.2% of patients).
The mean cost of treating 5-fluorouracil-induced severe neutropenia in 20 Spanish patients was € 3.044 (range € 17.45 to €14,103.25 per treatment). 45% of patients was treated with FOLFOX (5-FU plus oxaliplatin), 35% with FOLFOX plus monoclonal antibody, 15% with XELOX (capecitabine plus oxaliplatin) and 5% with FOLFIRI (5-FU plus irinotecan) plus monoclonal antibody. Neutropenia was grade 3 in 75% of patients and grade 4 (febrile neutropenia) in 25%.
The costs for genotyping 1000 patients in this Spanish centre (1000 genotyping samples per year (DPYD and other), 10 samples per run) were € 6,400 (range based on the number of samples per run and the number of samples analysed per year using the equipment: € 3.5 (2000 genotyping samples per year, 20 per run) to € 43.1 (100 genotyping samples per year, 1 per run)). Therefore, the range of neutropenia to be prevented is 1.15 to 14.16 cases per 1000 treated patients (0.1% to 1.4% of patients) for genotyping to be cost effective.
- 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.
In patients treated with fluoropyrimidines in three Dutch hospitals, *2A-genotype-guided therapy was both cheaper and safer than not-genotype-guided therapy (€ 45 reduced costs and reduction of grade ≥ 3 toxicity in *2A-carriers with 62%). *2A-genotype-guided therapy involved patients with genotype *1/*2A being started on ≤ 50% of the normal dose, followed by dose titration based on tolerance, and patients with genotype *1/*1 being started on the normal dose.
The calculation was from a health care payer perspective. Only direct medical costs were included. The calculated costs of genotype-guided therapy were € 2,772 per patient and the calculated costs of not-genotype-guided therapy € 2,817 per patient. The calculation was based on costs of hospitalisation in a nursing

ward of € 497 per day, costs of hospitalisation in an intensive care unit of € 2,372 per day, additional costs for a medical intervention not requiring hospitalisation of € 50, costs of capecitabine tablets of € 292 per cycle, costs of 5-fluorouracil and administration at day care of € 401 per cycle, cost of a treating physician of € 78 per cycle, and a genetic test price of € 75. The risks of serious adverse events were derived from this study and other published studies.
Variation of input data (± 20%) found *2A-genotype-guided therapy to have a high likelihood of being cost-saving. It found average cost savings of € 44 (range -€ 74 to € 331) per patient.
The model was shown to be most sensitive to the likelihood of toxicity-related hospitalisation of *2A-carriers receiving the standard dose, followed by the *2A-frequency, the genotyping costs and the duration of hospitalisation of *2A-carriers receiving the standard dose.

Date of literature search: 30 January 2019.

	Phenotype	Code	Gene-drug interaction	Action	Date
KNMP Pharmacogenetics Working Group decision	AS 0	4F	Yes	Yes	13 May 2019
	FENO	4F	Yes	Yes	
	AS 1	4F	Yes	Yes	
	AS 1.5	4F	Yes	Yes	

Mechanism:
Fluorouracil is mainly (> 80%) converted by dihydropyrimidine dehydrogenase (DPD) to inactive metabolites. Lower metabolic activity of DPD leads to increased intracellular concentrations of fluorodeoxyuridine monophosphate, the active metabolite of fluorouracil and its prodrug capecitabine. This leads to an increased risk of adverse events such as neutropenia, thrombopenia and hand-foot syndrome.

Clinical Implication Score:

Table 1: Definitions of the available Clinical Implication Scores

Potentially beneficial	PGx testing for this gene-drug pair is potentially beneficial. Genotyping can be considered on an individual patient basis. If, however, the genotype is available, the DPWG recommends adhering to the gene-drug guideline	0-2 +
Beneficial	PGx testing for this gene-drug pair is beneficial. It is advised to genotype the patient before (or directly after) drug therapy has been initiated to guide drug and dose selection	3-5 +
Essential	PGx testing for this gene-drug pair is essential for drug safety or efficacy. Genotyping must be performed before drug therapy has been initiated to guide drug and dose selection	6-10 +

Table 2: Criteria on which the attribution of Clinical Implication Score is based

Clinical Implication Score Criteria	**Possible Score**	**Given Score**	
		systemic	**cutaneous**
Clinical effect associated with gene-drug interaction (drug- or diminished efficacy-induced)	+		+
• CTCAE Grade 3 or 4 (clinical effect score D or E)	++	++	
• CTCAE Grade 5 (clinical effect score F)			
Level of evidence supporting the associated clinical effect grade ≥ 3			
• One study with level of evidence score ≥ 3	+		
• Two studies with level of evidence score ≥ 3	++		
• Three or more studies with level of evidence score ≥ 3	+++	+++	
Number needed to genotype (NNG) in the Dutch population to prevent one clinical effect grade ≥ 3			
• 100 < NNG ≤ 1000	+	+	
• 10 < NNG ≤ 100	++		
• NNG ≤ 10	+++		
PGx information in the Summary of Product Characteristics (SmPC)			
• At least one genotype/phenotype mentioned	+		+
OR			
• Recommendation to genotype	++	++	
OR			
• At least one genotype/phenotype mentioned as a contra-indication in the corresponding section	++		

Total Score:	10+	8+	2+
Corresponding Clinical Implication Score:		Essential	Potentially beneficial