DPD: flucytosine 7208/7209/7210/7211

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), AUC = area under the concentration-time curve, CTCAE = common terminology criteria for adverse events, 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, SmPC = Summary of Product Characteristics

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:
Cells of sensitive fungi are able to take up flucytosine and deaminate it to 5-fluorouracil by means of a specific cytosine deaminase. The incorporation of 5-fluorouracil in the ribonucleic acids of the fungi has a quantitative correlation with its fungistatic action. After longer contact, flucytosine also has fungicide activity. Flucytosine is active against certain fungi species both *in vitro* and *in vivo*. This indicates that the fungi can incorporate 5-fluorouracil in the ribonucleic acids and are not dependent on the host for converting 5-fluorouracil into the active metabolites. So, the effectiveness of flucytosine is only dependent on the plasma concentration of flucytosine and independent of the plasma concentration of 5-fluorouracil in the patient.
Flucytosine is almost exclusively excreted unchanged in the urine. A small part is deaminated (probably by gut bacteria) to 5-fluorouracil, that can result in adverse events. The AUC ratio 5-fluorouracil/flucytosine is 4%. This indicates that the plasma concentration of 5-fluorouracil is low (1-4 mg/L since the therapeutic range of flucytosine is 25-100 mg/L). This also indicates that the dosing range of flucytosine for adults of 25-50 mg/kg 4 times a day would correspond to dosing 4-8 mg/kg 5-fluorouracil per day. This is lower than the maximum of 1000 mg/day for 5-fluorouracil monotherapy for colorectal carcinoma. This is confirmed by the low incidence of haematological adverse events of flucytosine (frequency unknown according to the SmPC, so not or only incidentally observed in the registration trials).
5-Fluorouracil is mainly (> 80%) converted by dihydropyrimidine dehydrogenase (DPD) to inactive metabolites. Lower metabolic activity of DPD in the patient leads to increased intracellular concentrations of fluorodeoxyuridine monophosphate, the active metabolite of 5-fluorouracil. This increases the risk of toxicity due to 5-fluorouracil such as neutropenia, thrombocytopenia and diarrhoea. In the majority of cases, flucytosine adverse events occur in the first two to three weeks of treatment.
There are no studies demonstrating the increased risk of adverse events in patients with (genetically determined) diminished DPD activity. Also the Direct Healthcare Professional Communication only refers to results for 5-fluorouracil and its prodrugs in general, not to results for flucytosine. However, the effect of genetically diminished DPD activity on 5-fluorouracil toxicity has been well established (see the risk analysis for DPD: fluorouracil/capecitabine). This is why it was decided that this concerns a gene-drug interaction and that therapy adjustment or enhanced alertness for adverse events is required (yes/yes-interactions).
You can find an overview of the deduced clinical and kinetic effects per phenotype group in the background information text of the gene-drug interactions in the KNMP Kennisbank. You may also have access to this background text via your pharmacy or physician information system. Substantiation for the recommendation for the different phenotype groups is provided below.
Justification of the recommendation for the different phenotype groups
AS 0: The Direct Healthcare Professional Communication and the SmPC of flucytosine indicate that flucytosine is contraindicated in patients with full DPD deficiency, i.e. gene activity score 0. This is reasonable, because these patients have been shown to tolerate only very small amounts of 5-fluorouracil (< 1% of the normal 5-fluorouracil dose) (see the risk analysis for DPD: fluorouracil/capecitabine and the background information

text for DPD AS 0: fluorouracil/capecitabine,systemic). This is why the recommendation to avoid flucytosine is adopted by the KNMP Pharmacogenetics Working Group.

FENO, AS 1, and AS 1.5: The Direct Healthcare Professional Communication and the SmPC of flucytosine indicate that the risk of (serious) drug toxicity is increased in patients with partial DPD deficiency, i.e. 'fenotyping', gene activity score 1, and gene activity score 1.5, but do not mention precautions or measures to be taken. The increased risk of serious 5-fluorouracil toxicity is in line with what has been found for 5-fluorouracil treatment (see the risk analysis for DPD: fluorouracil/capecitabine). However, dose reductions as recommended for 5-fluorouracil treatment in these patients are no option for flucytosine treatment. Because the effectiveness of flucytosine is dependent on its plasma concentration, dose reductions would diminish effectiveness. Avoiding flucytosine is not a good recommendation either. In contrast to 5-fluorouracil treatment, the risk for serious 5-fluorouracil toxicity in the general patient population is low for flucytosine treatment due to low 5-fluorouracil plasma concentrations. In addition, patients with phenotypes 'fenotyping', gene activity score 1 and gene activity score 1.5 do tolerate low doses of 5-fluorouracil (approximately 25-75% of the normal 5-fluorouracil dose) (see the risk analysis for DPD: fluorouracil/capecitabine and the therapeutic recommendations and background information texts for DPD FENO: fluorouracil/capecitabine, DPD AS 1: fluorouracil/capecitabine, and DPD AS 1.5: fluorouracil/capecitabine). So, many patients with one of these DPD phenotypes might tolerate flucytosine without problems. For these reasons, only additional alertness for adverse events is recommended in these patients.

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

The KNMP Pharmacogenetics Working Group considers genotyping before starting flucytosine to be beneficial for drug safety. It is advised to consider genotyping these patients before (or directly after) drug therapy has been initiated to guide drug selection.

The clinical implication of the gene-drug interaction scores 3 out of the maximum of 10 points (with pre-emptive genotyping considered to be beneficial for scores ranging from 3 to 5 points) (see also the clinical implication score tables at the end of this risk analysis):

The Direct Healthcare Professional Communication states that the risk of life-threatening drug toxicity (code E = CTCAE grade 4) is increased for patients with full DPD deficiency (gene activity score 0) and the risk for serious drug toxicity (code D = CTCAE grade 3) for patients with partial DPD deficiency ('fenotyping', gene activity score 1, and gene activity score 1.5). This results in 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).

There are no published studies showing a higher risk of adverse events in patients with genetically diminished DPD activity. This results in 0 out of the maximum of 3 points for the second criterion of the clinical implication score, the level of evidence supporting an associated clinical effect grade ≥ 3 (only points for at least one publication with level of evidence score ≥ 3).

The risk for serious 5-fluorouracil toxicity, like haematological adverse events, is low in the patient population treated with flucytosine (frequency unknown according to the SmPC, so not or only incidentally observed in the registration trials). So, this frequency is likely to be lower than 1 in 1000. This indicates that the number needed to genotype to prevent such an adverse event is likely to be more than 1000. This results in 0 of the maximum of 3 points for the third criterion of the clinical implication score, the number needed to genotype (NNG) to prevent one clinical effect grade ≥ 3 (only points for NNG ≤ 1000).

The Summary of Product Characteristics (SmPC) of flucytosine mentions known full DPD-deficiency (gene activity score 0) to be a contraindication for flucytosine treatment. This results in the maximum 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 contraindication in the corresponding section in the SmPC).

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 publication.

Source	Code	Effect	Comments
ref. 1 Mylan Healthcare LLC Important risk information: Adapted recommendations for the use of flucytosine (Ancotil, solution for infusion 10 mg/ml, RVG 08533) in patients	0 AS 0: E AS 1-1.5 + FENO: D	In consultation with the European and Dutch Medicines Agencies (EMA and CBG), and the Dutch Inspection Healthcare and Youth (IGJ), Mylan Healthcare LCC informs you of a contraindication concerning the product Ancotil (flucytosine): • There is a new contraindication for patients with a known full dihydropyrimidine dehydrogenase (DPD)-deficiency who are treated with flucytosine because of the risk of life-threatening toxicity. • Patient with a partial DPD-deficiency also have an increased risk of serious toxicity. • Consider determining the DPD-activity in case of confirmed or suspected drug toxicity.	

with dihydropyrimidine dehydrogenase (DPD) deficiency. Direct Healthcare Professional Communication (Orange Hand Letter) 05-06-20. **ref. 1, continuation**		• Consider ending the treatment with flucytosine in case of drug toxicity. • However, testing for DPD-deficiency before start of treatment is not required to avoid delay of antimycotic treatment. Supplementary information: Flucytosine is a 5-fluorouracil (5-FU) prodrug. Relevant systemic exposure to 5-FU has been observed in patients treated with flucytosine. A reduced DPD-enzyme function result in an increased risk of serious or life-threatening toxicity (stomatitis, mucositis, diarrhoea, neutropenia of neurotoxicity) in patients treated with 5-FU or prodrugs of 5-FU. Patients with a DPD-enzyme deficiency have an increased risk of serious drug toxicity, with the level of toxicity correlating with the extent of DPD-deficiency. Patients with a full DPD-deficiency have a higher risk to develop life-threatening or fatal toxicity. If it is known that patients have a full DPD-deficiency, treatment with flucytosine is contraindicated.	
ref. 2 SmPC Ancotil (flucytosine) 17-12-20.	0 AS 0-1.5 + FENO: D	Contra-indication: known complete deficiency of dihydropyrimidine dehydrogenase (DPD). Warning: Deficiency of the enzyme dihydropyrimidine dehydrogenase (DPD): 5-fluorouracil is a metabolite of flucytosine. DPD is an important enzyme involved in metabolism and elimination of 5-fluorouracil. Therefore, the risk of severe drug toxicity is increased when Ancotil is used in patients with a deficiency of dihydropyrimidine dehydrogenase (DPD). In case of confirmed or suspected drug toxicity, determining the DPD activity can be considered. When drug toxicity is suspected, ending the treatment with Ancotil should be considered. Adverse events: Frequency unknown: anaemia, leukopenia, neutropenia, granulocytopenia and thrombocytopenia (dependent on possibly enhanced serum concentrations), and agranulocytosis, eosinophilia, aplastic and haemolytic anaemia, bone marrow toxicity (irreversible) associated with pancytopenia and bone marrow suppression in immune-compromised patients with fatal outcome. In the majority of cases, adverse events occur in the first two to three weeks of treatment.	

Risk group	AS 1-1.5 and FENO with DPD inhibitors (antiviral nucleoside drugs, such as ganciclovir, valganciclovir, brivudine, sorivudine, and their analogues) or with impaired kidney function

Comments:
-

Date of literature search: 23 October 2021.

	Phenotype	**Code**	**Gene-drug interaction**	**Action**	**Date**
KNMP Pharmacogenetics Working Group decision	AS 0	0E	Yes	Yes	15 November 2021
	FENO	0D	Yes	Yes	
	AS 1	0D	Yes	Yes	
	AS 1.5	0D	Yes	Yes	

Mechanism:
Cells of sensitive fungi are able to take up flucytosine and deaminate it to 5-fluorouracil by means of a specific cytosine deaminase. The incorporation of 5-fluorouracil in the ribonucleic acids of the fungi has a quantitative correlation with its fungistatic action. After longer contact, flucytosine also has fungicide activity. Flucytosine is active against certain fungi species both *in vitro* and *in vivo*. This indicates that the fungi can incorporate 5-fluorouracil in the ribonucleic acids and are not dependent on the host for converting 5-fluorouracil into the active metabolites. So, the effectiveness of flucytosine is only dependent on the plasma concentration of flucytosine and independent of the plasma concentration of 5-fluorouracil in the patient.
Flucytosine is almost exclusively excreted unchanged in the urine. A small part is deaminated (probably by gut bacteria) to 5-fluorouracil, that can result in adverse events. The AUC ratio 5-fluorouracil/flucytosine is 4%. This indicates that the plasma concentration of 5-fluorouracil is low (1-4 mg/L since the therapeutic range of flucytosine is 25-100 mg/L). This also indicates that the dosing range of flucytosine for adults of 25-50 mg/kg 4 times a day would correspond to dosing 4-8 mg/kg 5-fluorouracil per day. This is lower than the maximum of 1000 mg/day for 5-fluorouracil monotherapy for colorectal carcinoma. This is confirmed by the low incidence of haematological adverse events of flucytosine (frequency unknown according to the SmPC, so not or only incidentally observed in the registration trials).
5-Fluorouracil is mainly (> 80%) converted by dihydropyrimidine dehydrogenase (DPD) to inactive metabolites. Lower metabolic activity of DPD in the patient leads to increased intracellular concentrations of fluorodeoxyuridine monophosphate, the active metabolite of 5-fluorouracil. This increases the risk of toxicity due to 5-fluorouracil such as neutropenia, thrombocytopenia and diarrhoea. In the majority of cases, flucytosine adverse events occur in the first two to three weeks of treatment.

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 consider genotyping 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**
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+	3+
Corresponding Clinical Implication Score:		Beneficial