27 March 2020
EMA/274404/2020
Pharmacovigilance Risk Assessment Committee (PRAC)

Assessment report

Referral under Article 31 of Directive 2001/83/EC resulting from pharmacovigilance data

Fluorouracil and fluorouracil related substances (capecitabine, tegafur and flucytosine) containing medicinal products

INN/active substances: capecitabine, fluorouracil, tegafur, flucytosine

Procedure numbers:

EMEA/H/A-31/1481
Xeloda EMEA/H/A-31/1481/C/000316/0085
Teysuno EMEA/H/A-31/1481/C/001242/0040
Capecitabine Accord EMEA/H/A-31/1481/C/002386/0032
Capecitabine medac EMEA/H/A-31/1481/C/002568/0021
Capecitabine Teva EMEA/H/A-31/1481/C/002362/0031
Ecansya EMEA/H/A-31/1481/C/002605/0023

Note:

Assessment report as adopted by the PRAC and considered by the CHMP with all information of a commercially confidential nature deleted.

Table of contents

1. Information on the procedure

Dihydropyrimidine dehydrogenase (DPD) is the rate limiting enzyme of the catabolism of 5-fluorouracil (5-FU) and has therefore a pivotal role in 5-fluorouracil (and related substances) elimination patterns. Treatment of patients with DPD deficiency with 5-fluorouracil or 5-fluorouracil related substances can therefore result in severe and fatal toxicity.

DPD deficiency is a known risk for the use of systemic fluoropyrimidines and is reflected in their product information. While DPD deficiency testing by genotyping method is recommended for 5-flurouracil and capecitabine in oncological indications, no pre-treatment screening is mandated in the product information.

In 2014, the French National Cancer Institute (INCa) funded and launched a 3-year hospital clinical research program (PHRC) FUSAFE (2015-2017), coordinated by the French Group for Clinical Onco-Pharmacology (GPCO-Unicancer) and the French Network for pharmacogenetics (RNPGx). The objective of FUSAFE was to elaborate collegial national recommendations to allow a secured prescription of fluoropyrimidines, based on upfront detection of DPD deficiency.

In December 2018, INCa published detailed recommendations on the most appropriate methods to screen DPD deficiency in view of the current national clinical practices in oncology: (1) to screen DPD deficiency before initiating any chemotherapy containing 5-FU or capecitabine; (2) to perform DPD phenotyping by measuring plasma uracil (U) concentrations (possibly associated with dihydrouracil/U ratio), and *DPYD* genotyping (variants *2A, *13, p.D949V, HapB3); (3) to reduce the initial FU dose (first cycle) according to DPD status, if needed, and further, to consider increasing the dose at subsequent cycles according to treatment tolerance.

Based on these recommendations, the French medicines agency (ANSM) considered that the product information of systemic fluorouracil and its prodrugs (capecitabine and tegafur) do not reflect the current evidence on the different screening tests to detect DPD deficiency and on 13 March 2019, France triggered a referral under Article 31 of Directive 2001/83/EC resulting from pharmacovigilance data, requesting the PRAC to assess the need to take action at EU level regarding the detection of DPD deficient patients (especially through genotyping and/or phenotyping) in patients treated with systemic fluorouracil and fluorouracil related substances (capecitabine and tegafur) and issue a recommendation on whether the relevant marketing authorisations should be maintained, varied, suspended or revoked.

As the risk of systemic exposure of 5-fluorouracil after administration of topical formulation or after metabolism of flucytosine cannot be completely excluded, the PRAC further agreed during its March 2019 plenary meeting to extend the scope of this referral procedure to include these products in the review.

2. Scientific discussion

2.1. Introduction

5-fluorouracil (5-FU) is a pyrimidine analogue which competitively inhibits the enzyme thymidylate synthase (TS), thereby creating a thymine deficiency and resulting in inhibition of deoxyribonucleic acid (DNA) synthesis and cytotoxicity. It also inhibits, to a lesser extent, the formation of ribonucleic acid (RNA). These effects are most marked in rapidly growing cells and may lead to cell death.

Dihydropyrimidine dehydrogenase (DPD catabolizes more than 80% of an administered dose of 5-fluorouracil to the inactive metabolite 5,6-dihydrofluorouracil, the first step of the catabolic cascade. Remarkably, DPD enzyme activity is subject to a wide variability, resulting in a possible range of

enzymatic deficiencies that span from partial to complete loss of enzyme activity. DPD deficiency is partly linked to genetic polymorphisms in its gene DPYD but may also have other causes. Prevalence of partial and complete DPD deficiencies in the entire population vary between different sources and has been estimated with approximately 3%–9% and 0.01%–0.5%, respectively in the Caucasian population. There is only very limited data on prevalence of partial and complete DPD deficiency in other ethnicities, although it has been suggested that Asian and African populations are at greater risk of DPD deficiency.

Due to the pivotal role of DPD activity in 5-fluorouracil elimination patterns, the variability in DPD activity may have dramatic impact on clinical outcome of patients. Thus, treatment of patients with DPD deficiency with fluoropyrimidines can result in severe toxicity, which impacts the benefit-risk balance of fluoropyrimidines in this subpopulation.

Systemic fluoropyrimidines are widely used in oncology as the backbone of a large percentage of current chemotherapy regimens across a broad spectrum of cancers. They consist in a group of anticancer drugs including 5-fluorouracil and its prodrugs capecitabine and tegafur, with different presentations:

- Parenteral 5-fluorouracil: a component of the standard therapy for a variety of malignancies, including colorectal, pancreatic, gastric, breast and head and neck cancers. Parenteral 5-fluorouracil (intravenous use) can be administered as bolus, infusion or continuous infusion for up to several days. In general, 5-fluorouracil is administered in combination other agents, modulating the metabolism of 5-fluorouracil. The most frequent used 5-fluorouracil modulator is the calcium salt of folinic acid, also called leucovorin (LV). LV is an intracellular source of reduced folates, which stabilize the ternary complex that they form with TS and 5-FdUMP, and thus increase and prolong the inhibition of TS and so enhance the efficacy of the drug. At the present time, the combination 5-fluorouracil/LV is considered the standard chemotherapy for colon cancer. The dose of 5-fluorouracil and the treatment schedule depend on the chosen treatment regimen, the indication, the general status and previous treatment of the patient. Treatment regimens vary in the combination of 5-fluorouracil with other cytotoxic agents or dose of concomitantly used folinic acid.

- Tegafur: an oral prodrug of 5-fluorouracil and one of the active substances of the combined product Teysuno, in which tegafur is combined with 2 modulators of 5-fluorouracil metabolism, gimeracil and oteracil. The combination of tegafur/gimeracil/oteracil is also better known as S-1. The main clinical study supporting the application for Teysuno was a randomized controlled phase III study (FLAGS) comparing S-1 plus cisplatin with 5-fluorouracil plus cisplatin. In this study, median overall survival times of 8.6 months and 7.9 months for S-1 plus cisplatin and 5-fluorouracil plus cisplatin, respectively, were observed (hazard ratio, 0.92; 95% confidence interval, 0.80 –1.05). Furthermore, in some EU-countries tegafur is available in capsules of 400 mg (without 5-fluorouracil metabolism modulators). It is approved for treatment of rectal, colon and breast cancer, as well as gastric cancer and some types of brain tumors in selected patients in whom the disease is considered surgically or by other means incurable.

- Capecitabine: an oral prodrug of 5-fluorouracil currently authorised for the treatment of colorectal, gastric and breast cancers. For the indications of colorectal and advanced gastric cancer, a meta-analysis of six randomized controlled clinical trials supports capecitabine replacing 5-fluorouracil in mono- and combination treatment (Cassidy J *et al.*, 2011). For the indication of locally advanced or metastatic breast cancer, data from a multicentre, randomised, controlled phase III clinical trial support the use of capecitabine in combination with docetaxel for treatment of patients with locally advanced or metastatic breast cancer after failure of cytotoxic chemotherapy, including an anthracycline. Furthermore, different prospective, randomized controlled phase II/III clinical trials

support the use of capecitabine as monotherapy or in combination with biologic and novel agents after failure of taxanes and anthracycline containing chemotherapy, and for whom anthracycline therapy is not indicated.

Other medicines also contain 5-fluorouracil or prodrugs of 5-fluorouracil:

- Topical 5-fluorouracil is currently marketed in 2 different formulations of 0.5 and 5%, respectively. The 0.5% solution contains 10% salicylic acid and is indicated for the topical treatment of slightly palpable and/or moderately thick hyperkeratotic actinic keratosis (grade I/II) in immunocompetent adult patients, as well as to treat warts (*Verrucae vulgares, Verrucae planae, Verrucae plantares, Verrucae digitatae et filliformes*). Two phase III trials showed superiority of the 0.5% 5-fluorouracil/10% salicylic acid solution to placebo and to diclofenac gel in patients with actinic keratosis (AK) grade I to II (Stockfleth E *et al.*, 2011; Stockfleth E *et al.*, 2017).

- The 5% cream formula is indicated for the topical treatment of superficial pre-malignant and malignant skin lesions; keratoses including senile, actinic and arsenical forms, keratoacanthoma, Bowen's disease and superficial basal-cell carcinoma.

- Flucytosine (5-FC), another prodrug of 5-fluorouracil, is specifically indicated for severe systemic fungal infections with susceptible pathogens, as an alternative or when switching from parenteral use, particularly: candidiasis, cryptococcosis, chromoblastomycosis and certain forms of aspergillosis. 5-FC must be used in combination with other antifungal agents, in order to avoid as much as possible the selection of resistant mutations, especially in the treatment of candidiasis and cryptococcosis. Combination with amphotericin B is often synergistic: in some cases, it allows a dose reduction and reduces the risk of the emergence of secondary resistance to flucytosine. The benefit-risk of 5-FC as an antifungal agent has been evaluated in numerous clinical trials and post-marketing studies, and therefore can be considered well established.

In 2018, in the EEA, about 600,000 patients have been treated with fluoropyrimidines in oncological indications and about 1,500,000 patients have been treated with topical 5-fluorouracil products. The reported exposure for flucytosine has been 6,054 patients in 2018.

2.2. Clinical aspects

2.2.1. Pharmacokinetics and pharmacogenetics

2.2.1.1. Pharmacokinetics of 5-fluorouracil

2.2.1.1.1. Fluorouracil (parenteral administration)

- **Distribution**

When administered intravenously, 5-fluorouracil rapidly disappears from the circulating blood with a half-life of 8 to 20 minutes. It diffuses rapidly in all tumour and fast-growing tissues (marrow and intestinal mucosa). Six to eight times higher concentrations are observed in these tissues four hours after injection compared with normal growth tissues. It also enters the extracellular spaces (cerebrospinal fluid, ascites, pleural effusion).

It enters the cells using the same transport facilitated as uracil, in particular the nucleobase carriers of SLC29A2 (solute carrier family) and SLC22A7.

- **Elimination**

Following bolus intravenous injection, 5 – 20 % of the parent drug is excreted unchanged in the urine within six hours. The remaining percentage of the administered dose is metabolised, primarily in the liver. Different metabolism patterns of 5-fluorouracil are summarized in figure 1 below (from Launay M, *et al.*, 2017).
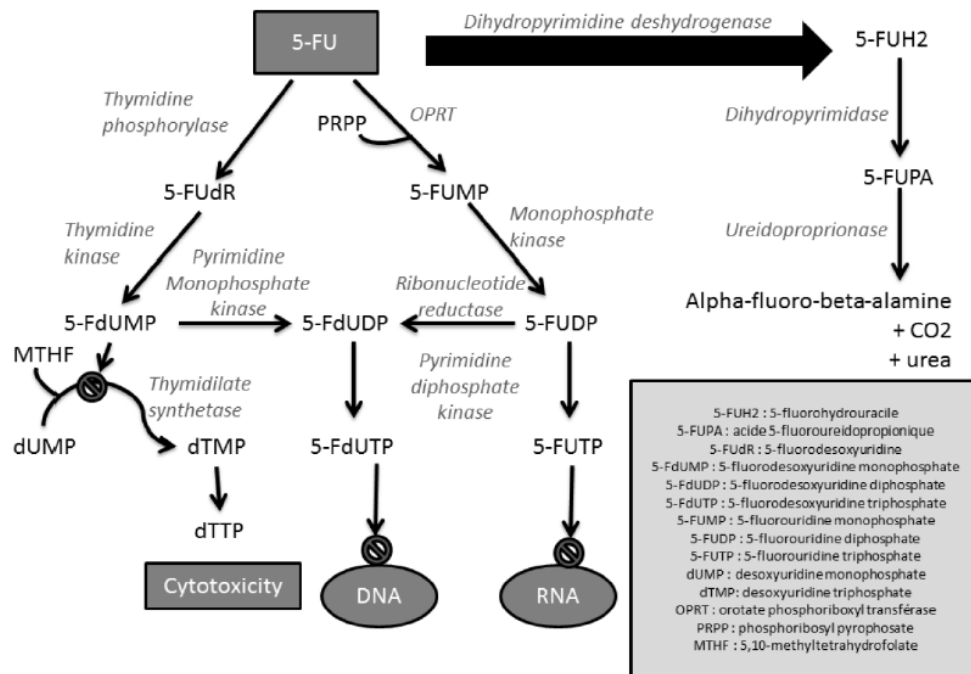

Fig. (1). Metabolism and anabolism of 5-FluoroUracil.

Figure 1. Metabolism and anabolism of 5-fluorouracil (from Launay M, *et al.*, 2017).

It is estimated that between 80 and 85% of the fraction of the dose undergoing metabolism is catabolized by DPD, while 15 to 20% of the initial dose is involved in anabolism.

DPD is found in many tissues, but mostly in the liver. This enzyme, encoded by the DPYD gene, is responsible for the catabolism of 5-fluorouracil to dihydro-5-fluorouracil (FUH2). The elimination of 5-fluorouracil and the main metabolite (5-fluorouracilH2) follows non-linear kinetics, with a saturation of elimination after therapeutic doses. In fact, a log-linear decrease in concentrations and an increase in the half-life with the dose have been observed, suggesting that the non-linearity of the elimination is due to a potential role of self-inhibition.

2.2.1.1.2. Topical fluorouracil

Absorption

Inconsistent results are found in the scientific literature about the absorption of 5-fluorouracil from the different available formulations (dosed 0.5-5% 5-fluorouracil). These conflicting results could reflect the actual differences in the systemic absorption or bioavailability across preparations, possibly as a result of the different vehicles (e.g., the Microsponge copolymers) used in the respective formulations. It has been reported that when fluorouracil is administered topically to intact skin, ~10% of the dose appears to undergo systemic absorption. A study in patients with actinic keratosis (AK) who received 1g doses of radio-labelled 5% fluorouracil twice daily found that 6% of the fluorouracil dose was absorbed systemically. However, a study comparing the absorption of topical fluorouracil in healthy and diseased skin found that absorption may be up to 75 times greater in diseased than in healthy skin.

Levy *et al.* (2001) compared the flux and percutaneous absorption of the 0.5% fluorouracil formulation evaluated with those of 5% fluorouracil. The flux of the 5% formulation was 20 to 40 times higher than that of the 0.5% formulation.

As regards the 0.5% formulations, the systemic bioavailability is very low (0.1%). Systemic toxicities have not been reported and appear unlikely.

Disposition

The distribution and elimination of 5-fluorouracil after topical administration will follow the same pathways described for parenteral administration.

2.2.1.2. *Pharmacokinetics of capecitabine*

Absorption

After oral administration, capecitabine is rapidly and extensively absorbed, followed by extensive conversion to its metabolites, 5'-DFCR and 5'-DFUR. Administration with food decreases the rate of capecitabine absorption, but only results in a minor effect on the AUC of 5'-DFUR, and on the AUC of the subsequent metabolite 5-fluorouracil. At the dose of 1250 mg/m^2 on day 14 with administration after food intake, the peak plasma concentrations (C_{max} in µg/ml) for capecitabine, 5'-DFCR, 5'-DFUR, 5-fluorouracil and FBAL were 4.67, 3.05, 12.1, 0.95 and 5.46 respectively. The time to peak plasma concentrations (T_{max} in hours) were 1.50, 2.00, 2.00, 2.00 and 3.34. The AUC0-∞ values in μg·h/ml were 7.75, 7.24, 24.6, 2.03 and 36.3.

Distribution

In vitro human plasma studies have determined that capecitabine, 5'-DFCR, 5'-DFUR and 5-fluorouracil are 54%, 10%, 62% and 10% protein bound, mainly to albumin.

Elimination

Capecitabine is first metabolised by hepatic carboxylesterase to 5'-DFCR, which is then converted to 5'-DFUR by cytidine deaminase, principally located in the liver and tumour tissues. Further catalytic activation of 5'-DFUR then occurs by thymidine phosphorylase (ThyPase). The enzymes involved in the catalytic activation are found in tumour tissues but also in normal tissues, albeit usually at lower levels. The sequential enzymatic biotransformation of capecitabine to 5-fluorouracil leads to higher concentrations within tumour tissues. In the case of colorectal tumours, 5-fluorouracil generation appears to be in large part localised in tumour stromal cells. Following oral administration of capecitabine to patients with colorectal cancer, the ratio of 5-fluorouracil concentration in colorectal tumours to adjacent tissues was 3.2 (ranged from 0.9 to 8.0). The ratio of 5-fluorouracil concentration in tumour to plasma was 21.4 (ranged from 3.9 to 59.9, n=8) whereas the ratio in healthy tissues to plasma was 8.9 (ranged from 3.0 to 25.8, n=8). Thymidine phosphorylase activity was measured and found to be 4 times greater in primary colorectal tumour than in adjacent normal tissue. According to immuno-histochemical studies, thymidine phosphorylase appears to be in large part localised in tumour stromal cells. 5-fluorouracil is further catabolised as described above.

The elimination half-life (in hours) of capecitabine, 5'-DFCR, 5'-DFUR, 5-fluorouracil and FBAL were 0.85, 1.11, 0.66, 0.76 and 3.23 respectively.

2.2.1.3. Pharmacokinetics of tegafur

Tegafur is a prodrug of 5-fluorouracil. Following oral administration, tegafur is converted into fluorouracil mainly through cytochrome P450 (CYP) 2A6 in the liver. However, to what extent the bioactivation of tegafur by CYP2A6 accounts for the formation of 5-fluorouracil *in vivo* remains unclear.

2.2.1.4. Pharmacokinetics of flucytosine (5-FC)

The pharmacokinetics of 5-FC have been investigated and reviewed extensively. 5-FC absorption is very rapid and almost complete: 76-89% absorption takes place after oral administration as compared to the intravenous route of administration. However, food, antacids, and renal insufficiency can delay absorption. In the absence of delay in absorption, peak levels are obtained in serum and other body fluids within 1 to 2 hours. 5-FC has a good penetration into body tissues due to its low molecular weight, its high-water solubility, and its low binding to serum proteins. Penetration of 5-FC is excellent into most body sites, including cerebrospinal, vitreous, and peritoneal fluids, and into inflamed joints. 5-FC is principally eliminated by the kidneys and the plasma clearance of the drug is closely related to the creatinine clearance. 5-FC is only minimally metabolized in the liver. Renal elimination involves filtration at the glomeruli, but no tubular reabsorption or secretion takes place. The half-life of 5-FC is approximately 3 to 4 hours in patients with normal renal function but can be extended up to 85 hours in patients with severe renal insufficiency. Renal insufficiency alters 5-FC pharmacokinetics since it results in a slower rate of absorption, a prolongation of the serum half-life, and a decreased clearance.

The apparent volume of distribution of 5-FC approaches that off total body water and is not altered by renal failure. Liver disease in laboratory animals does not significantly alter the pharmacokinetics off intravenously administered 5-FC, but in man the available information is very limited.

The antimycotic activity of 5-FC depends on its deamination to fluorouracil (5-fluorouracil). 5-FC is taken up by mycotic cells by means of the enzyme cytosine permease and converted into 5-fluorouracil by the specific enzyme cytosine deaminase (Vermes *et al.*, 2000). 5-fluorouracil in its turn is converted to 5-fluorodeoxyuridylic acid monophosphate, a non-competitive inhibitor of thymidylate synthetase, by which the RNA and DNA synthesis of the mycotic cells is inhibited.
It has initially been postulated that since the necessary enzyme cytosine deaminase is absent or only weakly active in mammalian cells, the conversion of 5-FC to 5-fluorouracil can hardly take place in mammalian cells and hence toxicity should be minimal. However, investigators have shown that certain bacteria residing in the human gastrointestinal tract are able to deaminate 5-FC to 5-fluorouracil, which could play a significant role in the toxicity of 5-FC in humans. Moreover, a correlation was found in humans between the gut flora status and the amount of 5-fluorouracil metabolites in urine.

2.2.1.5. Genetic and epigenetic aspects

DPD enzyme inactivates approximately 80-85% of 5-fluorouracil in the body. Deficiency in the enzymatic activity of DPD is associated with a reduced degradation of fluorouracil, an increased exposure to its (active/cytotoxic) metabolites and consequently an increased risk of adverse drug reactions.

DPD deficiency is most often the result of genetic variations in *DPYD*, the gene encoding the DPD enzyme. *DPYD* is a highly polymorphic gene with more than 160 genetic variants described to date (Palmirotta *et al.*, 2018).

The most known deleterious *DPYD* variants associated with loss of function of the DPD enzyme are DPYD*2A and DPYD*13, whereas other DPYD variants have been associated with partially reduced DPD activity, such as c.2846A>T or HapB3.

Table 1. Main DPYD variants screened for and frequencies estimated/calculated in populations of Caucasian origin (from INCa, HAS report "DPD deficiency screening with a view to preventing some severe toxicities occurring with treatments including fluoropyrimidines', 2018).

DPYD variants	Frequency in population (bibliographic sources)	Proportion of carriers		Number of carriers / 100,000 patients	
		heterozygous	homozygous	heterozygous	homozygous
DPYD*2A (IVS14+1G>A, c.1905G>A)	0.8% (11, 13, 24, 29)	1.5%	0.01%	1,500	10
DPYD*13 (c.1679T>G / p.I560S)	0.1% (8, 11, 23, 24, 29)	0.2%	0.0001%	200	0.1
c.2846A>T (p.D949V)	0.6% (11, 23, 24, 28, 29)	1%	0.004%	1,000	4
HapB3[15]	2.4% (24, 29)	4.6%	0.06%	4,600	60

These variants can be found in homozygous or heterozygous state in carriers and are transmitted across generations in a recessive autosomal way (see Figure 3 below).

According to other sources, in European population, HapB3 with c.1129–5923C>G is the most common decreased function DPYD variant with carrier frequencies of 4.7%, followed by c.190511G>A (carrier frequency: 1.6%) and c.2846A>T (carrier frequency: 0.7%). Considering all four variants combined, around 7% of Europeans carry at least one decreased function DPYD variant. In individuals with African ancestry, the decreased function variant c.557A>G (rs115232898, p.Y186C) is relatively common (3–5% carrier frequency). Most other DPYD variants of phenotypic consequence are very rare and were not observed even in large cohort studies (Amstutz *et al.*, 2017; Lunenburg *et al.*, 2015).
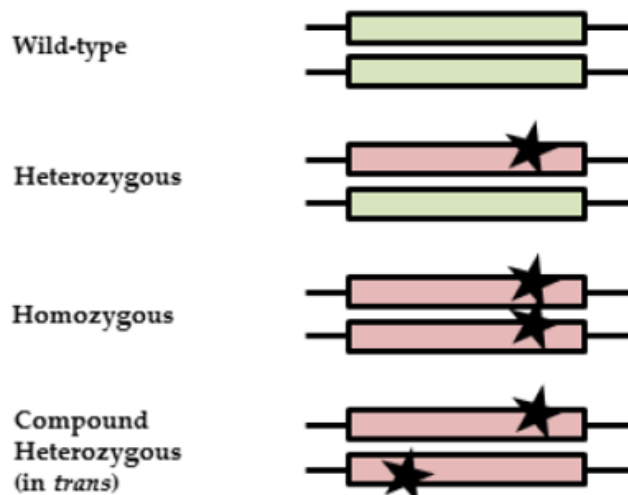
Figure 2. Illustration of zygosity and clinical interpretations (from Lunenburg *et al.*, 2015)

Black stars represent variants; boxes represent alleles. A wild-type patient carries no variants, resulting in normal-activity alleles (green). A heterozygous patient carries one variant, resulting in one reduced or inactive allele (red) and one active allele (green). A partly reduced enzyme activity is expected, since there is still one active allele left. For homozygous patients, both variants result in a reduced or inactive allele (red). Depending on the effect of the variants on the protein, a reduced or absent enzyme activity is expected. Compound heterozygous patients can carry variants on different alleles (in trans) or on one allele (in cis), resulting in differences in enzyme function, either like that of a heterozygous patient or a homozygous patient.

Depending on the type of the variant, the extent of the enzyme activity would change. The complete or partial defect in activity as well as heterozygous/homozygous state are taken into account to assign the activity.

Epigenetic regulations

In addition to genetic mutations, the importance of genetic and epigenetic regulations of the DPYD gene may be critical, although not yet fully elucidated. Strong correlations have been reported between DPD activity and mRNA levels, suggesting that transcriptional regulation should be an important mechanism leading to marked variation in DPD activity. SP1 and SP3 proteins have been identified as transcription activators of the DPYD gene. These proteins could thus be used as a marker for DPD expression. Conflicting data related to the association between methylation of the DPYD promoter region with severe toxicity to 5- FU have also been reported. Thus, the exact mechanisms associating methylation with DPD down-regulation is still unclear and still under investigation.

Other alterations could lead to a reduced enzyme production, like microRNA-related mechanisms such as MIR27A.

Deficiency of other enzymes in the metabolic pathway of fluoropyrimidines

In addition to DPYD variants, variants in other genes have been associated with an impaired metabolism of fluoropyrimidines. Variants in genes such as *MTHFR* and *TS*, encoding MTHFR and TYMS enzymes, have been reported.

2.2.1.6. *Mechanism of action and pharmacodynamic aspects*

5-fluorouracil is a pyrimidine analogue which competitively inhibits the enzyme thymidylate synthase (TS), thereby creating a thymine deficiency and resulting in inhibition of deoxyribonucleic acid (DNA) synthesis and cytotoxicity. It also inhibits, to a lesser extent, the formation of ribonucleic acid (RNA). These effects are most marked in rapidly growing cells and may lead to cell death.

Only a small fraction of 5-fluorouracil (1%–5%) is converted intracellularly into the cytotoxic metabolites fluorodeoxyuridine monophosphate (FdUMP), fluorodeoxyuridine triphosphate (FdUTP), and fluorouridine triphosphate (FUTP). These metabolites have several effects including the inhibition of thymidylate synthase by FdUMP, incorporation of FUTP into RNA and incorporation of FdUTP into DNA.

The anabolic pathways are presented on the left part of the Figure 3 below.
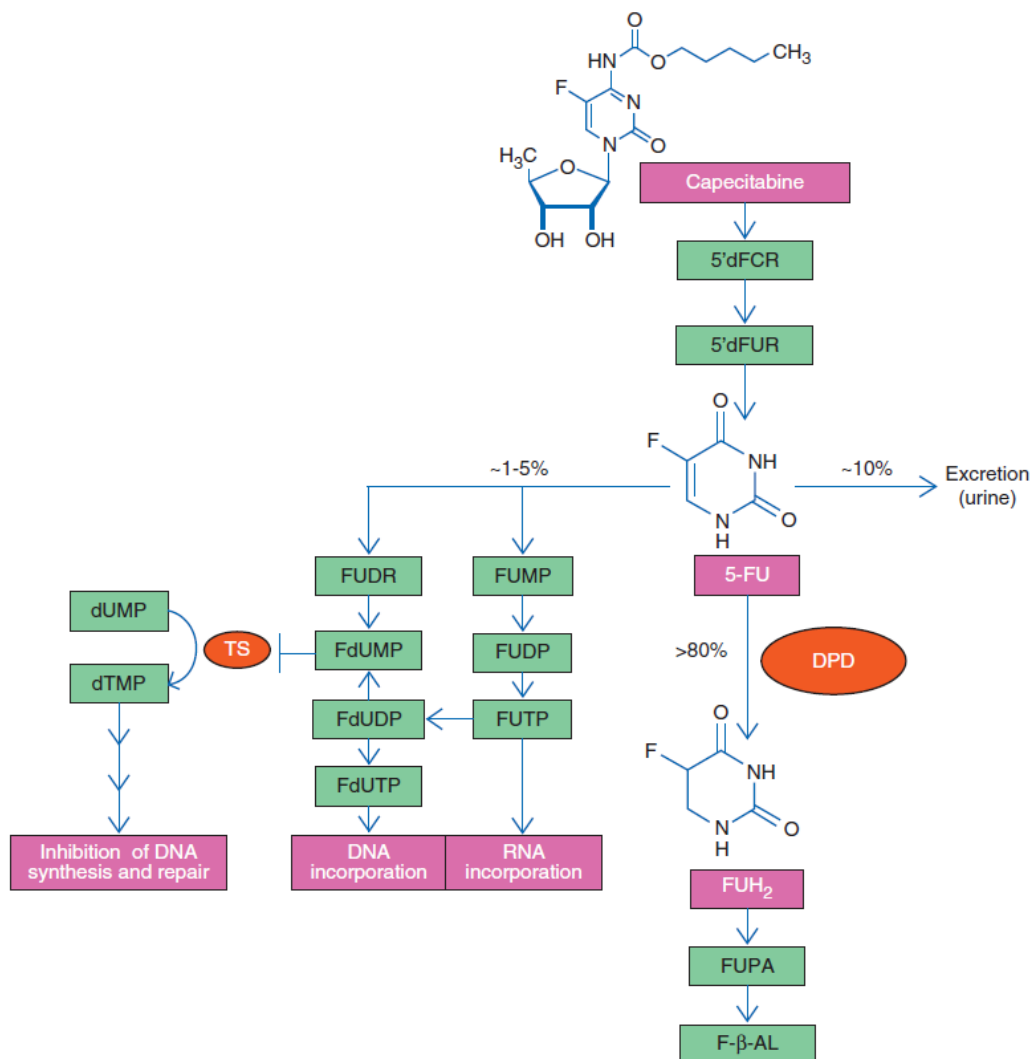
Figure 3. Metabolic pathway of fluoropyrimidines (from Meulendijks D. *et al.*, 2016)

Metabolic pathway of fluoropyrimidines. 50dFCR, 50-deoxy-5-fluorocytidine; 50dFUR, 50-deoxy-5-fluorouridine; 5-fluorouracil, 5-fluorouracil; DPD, dihydropyrimidine dehydrogenase; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; FdUDP, fluorodeoxyuridine diphosphate; FdUMP, fluorodeoxyuridine monophosphate; FdUTP, fluorodeoxyuridine triphosphate; FUDP, fluorouridine diphosphate; FUDR, fluorodeoxyuridine; FUH2, 5,6-dihydro-5-fluorouracil; FUMP, fluorouridine monophosphate; FUPA, fluoro-b-ureidopropionate; FUTP, fluorouridine triphosphate; F-b-AL, fluoro-b-alanine; TS, thymidylate synthase.

After oral administration, the prodrug capecitabine is stepwise converted into 5-fluorouracil. Only a small fraction of 5-fluorouracil (1%–5%) is converted intracellularly into the cytotoxic metabolites fluorodeoxyuridine monophosphate (FdUMP), fluorodeoxyuridine triphosphate (FdUTP), and fluorouridine triphosphate (FUTP; Figure 3). There is evidence that the metabolism of 5-fluorouracil in the anabolic pathway blocks the methylation reaction of deoxyuridylic acid to thymidylic acid, thereby interfering with the synthesis of deoxyribonucleic acid (DNA). The incorporation of 5-fluorouracil also

leads to inhibition of RNA and protein synthesis. Since DNA and RNA are essential for cell division and growth, the effect of 5-fluorouracil may be to create a thymidine deficiency that provokes unbalanced growth and death of a cell. The effects of DNA and RNA deprivation are most marked on those cells which proliferate more rapidly and metabolise 5-fluorouracil at a more rapid rate.

Capecitabine is activated via several enzymatic steps. The enzyme involved in the final conversion to 5-fluorouracil, thymidine phosphorylase (ThyPase), is found in tumour tissues, but also in normal tissues, albeit usually at lower levels. In human cancer xenograft models capecitabine demonstrated a synergistic effect in combination with docetaxel, which may be related to the upregulation of thymidine phosphorylase by docetaxel.

As regards catabolic pathways presented on the right side of the Figure 3, the DPD enzyme converts about 80% of the administered dose of 5-fluorouracil into the inactive metabolite 5,6-dihydro-5-fluorouracil (FUH2), which makes DPD the rate-controlling enzyme for inactivation of 5-fluorouracil. The amount of 5-fluorouracil available for conversion into cytotoxic metabolites is therefore primarily determined by systemic DPD activity. The DPD enzyme is mainly expressed in the liver, the main site of 5-fluorouracil metabolism (Henricks *et al.*, 2017).

2.2.2. 5-fluorouracil related toxicities and DPD deficiency

As part of this review, the MAHs were requested to provide information on the clinical consequences of partial and full DPD deficiency with the use of their product(s). MAHs performed search either in their own safety database or in the scientific literature.

5-fluorouracil has an effect not only on cancer cells, but also on healthy cells, leading to dose-dependent toxicities. The toxicity of 5-fluorouracil has been reported to be focused on rapidly dividing cell lines (particularly in bone marrow cells and intestinal epithelial cells) leading to a wide range of adverse effects.

The safety profile of 5-fluorouracil and related fluoropyrimidines has been extensively studied. The overall described safety profile is consistent across all authorised indications.

However, since the drug is often administered in combination therapy, it is sometimes difficult to determine with certainty the contribution of 5-FU to the adverse drug reaction. Depending on the regimen, administration of 5-fluorouracil usually leads to severe toxicities in one third of the patients, and up to 3% of deaths have been reported in frail patients such as the elderly. Ethnicity (e.g. Afro-American) and gender (e.g. women) could be additional risk factors. For the purpose of this review, severe fluoropyrimidine-associated toxicities are classified as toxicities graded $\geq$ 3 as per Common Terminology Criteria for Adverse Events (CTCAE).

The safety profile of fluoropyrimidines is mainly characterised by haematological (myelosuppression, infection, bleeding) and gastrointestinal toxicity (diarrhoea, abdominal pain, nausea, stomatitis), which are also the most frequent dose limiting toxicities (Xeloda SmPC, Leichman *et al.*, 1995). However, there are differences in the safety profile of each substance. Skin toxicity (hand-foot skin reaction (HFS), palmar-plantar erythrodysesthesia) is a common dose limiting toxicity for capecitabine, which is significantly more often observed compared to 5-fluorouracil. However, neutropenia and stomatitis are more pronounced for 5-fluorouracil compared to capecitabine (Petrelli *et al.*, 2012). The combination product of tegafur with gimeracil and oteracil potassium exhibit significantly less gastrointestinal toxicity.

Apart from the type of fluoropyrimidine (e.g. 5-FU +/-LV vs. capecitabine) administered, incidence rate, type and grading of toxicities is further dependent on the method of administration (5-FU bolus vs. 5-FU continuous infusion), the combination with other cytotoxic treatments (such as oxaliplatin,

irinotecan) and characteristics of the patients included in the different studies (age, renal function, performance status). Consequently, incidences of severe toxicities vary significantly in the published literature (see below table, modified from INCa-HAS, 2018).

Assessed toxicities	Bibliographic sources	Incidences of toxicities
Overall toxicities, grade ≥ 3	Gamelin, 2007	20% - 25%
	Cancer Research UK, 2017; Hamzic, 2018	25%
	Centre Antoine Lacassagne, 2014; Launay, 2016; Lunenburg, 2016; Meulendijks, 2015	10% - 30%
	Terazzino *et al.*, 2013,	6% - 57%
	Launay *et al.*, 2017	25% - 50%
	PHRC FUSAFE (section 1)	24%
	PHRC FUSAFE (section 2)	43%
Severe toxicities, grade ≥ 4	Centre Antoine Lacassagne, 2014; Boisdron-Celle, 2017	3% - 5%
	PHRC FUSAFE (section 1)	6.7%
	PHRC FUSAFE (section 2)	2%
Lethal toxicities, grade 5	Gamelin, 2007; Launay, 2016; Lunenburg, 2016; Meulendijks, 2015; Boisdron-Celle, 2007; Deenen, 2016; Henricks, 2017	0.1% - 1%
	PHRC FUSAFE (section 1)	0.05%
	PHRC FUSAFE (section 2)	0.2%

Table 2. Estimated incidences of severe toxicities with fluoropyrimidine treatments

Fatal cases attributed to toxicity have been reported, with different types of adverse events being observed and leading to fatal outcomes, in some cases after a progressive increase in severity of adverse events.

Role of DPD deficiency in the 5-fluorouracil related toxicity

DPD is encoded by the gene DPYD, which consists of 23 exons spanning 950 kb on chromosome 1p22. More than 160 single nucleotide polymorphisms have been described, some resulting in altered or near-complete loss of enzyme activity. In a study including 2594 patients, three DPYD variants (DPYD*2A, D949V and I560S) were associated to increased 5-fluorouracil toxicity. Statistically significant associations were found between two DPYD variants (i.e., DPYD*2A and D949V) and increased incidence of grade 3 or greater 5FU-induced adverse events (AEs) in patients treated with adjuvant 5-fluorouracil- based combination chemotherapy. A statistically significant association could not be demonstrated with I560S because of its low frequency. In a retrospective study encompassing 568 patients with colorectal cancer, Deenen and colleagues (2016) demonstrated that DPYD*2A and D949V were also significantly associated with capecitabine-induced grade 3 to 4 toxicities and toxicity-driven dose reductions. The most well-studied variant is a splice-site mutation, IVS14+1G>A, also known as DPYD*2A, located on exon 14-flanking region and causing skipping of 165 base pairs involved in more than 50% of the cases in Western populations. Association between severe toxicity and DPD variants DPYD*2A, D949V, I560S and HapB3 has been studied and totally confirmed by several meta-analyses. However, HapB3 was not significantly associated with grade-3 AEs in a study including 1953 stage III colon cancer patients.

Nevertheless, it is noted that the number of patients bearing a mutated allele is still far below the number of patients developing severe toxicities, thus raising questions about the sensitivity of genotyping DPYD to detect the majority of patients at risk. In addition, only approximatively 50-80% of heterozygous carriers of a low-activity allele develop severe fluoropyrimidine related toxicity

according to several studies. This may indicate allelic regulation of DPYD or compensation by another DPYD variant on the second allele, thus suggesting that specificity could be improved too.

DPD activity follows a circadian rhythm and a Normal or Gaussian distribution. Various studies have been undertaken to identify clinical and paraclinical covariates associated with 5-fluorouracil PK variability: the influences of age, gender, ethnicity, dose and route of administration have been suggested. A wide inter-individual variability has been observed in pharmacokinetics, and DPD deficiency accounts for the most significant changes in PK parameters.

By partially or totally impairing the degradation of 5-fluorouracil, DPD deficiency is thus responsible for an important part of cases of life-threatening toxicities upon 5-fluorouracil intake in patients to whom the drug has been administered at standard dosages.

EudraVigilance analysis and literature review by EMA

In addition to the data provided by the MAHs, the PRAC has considered a detailed analysis of cases of toxicities as reported in EudraVigilance database for the dihydropyrimidine dehydrogenase deficiency related toxicities to fluorouracil and related substances containing medicinal products.

The primary objectives of the EudraVigilance analysis were:

- to identify and describe case reports to fluorouracil and related substances and;

- to identify and characterise case reports to these products where dihydropyrimidine dehydrogenase deficiency (DPD) was also reported.

A secondary objective of the EudraVigilance analysis was to estimate the number of case reports of fluorouracil and fluorouracil related substances that might have been due to DPD related toxicity but for which the DP genotype was not reported.

Whereas the manifestations of toxicity due to DPD are extensive, the terms within the following standardised MedDRA queries (SQM) were used as being indicative of DPD related toxicity to create a group of Dihydropyrimidine dehydrogenase deficiency toxicity spectrum: Gastrointestinal perforation, ulcer, haemorrhage, obstruction non-specific findings/procedures; Gastrointestinal ulceration; Gastrointestinal perforation; Gastrointestinal haemorrhage ; Non-infectious diarrhoea; Agranulocytosis; Haematopoietic thrombocytopenia; Haemorrhages.

Noticeably, most, if not all, of these adverse reactions may be caused either by the underlying malignancy or its treatment.

A literature review was also performed by the EMA with the aim to identify published papers on DPD screening since the last review conducted by the INCa, i.e. from May 2018 to March 2019. Thus, a similar methodology to the one applied by the INCa was used.

Based on the case definitions DPD deficiency, DPD toxicity spectrum and No DPD toxicity' group were defined as follows:
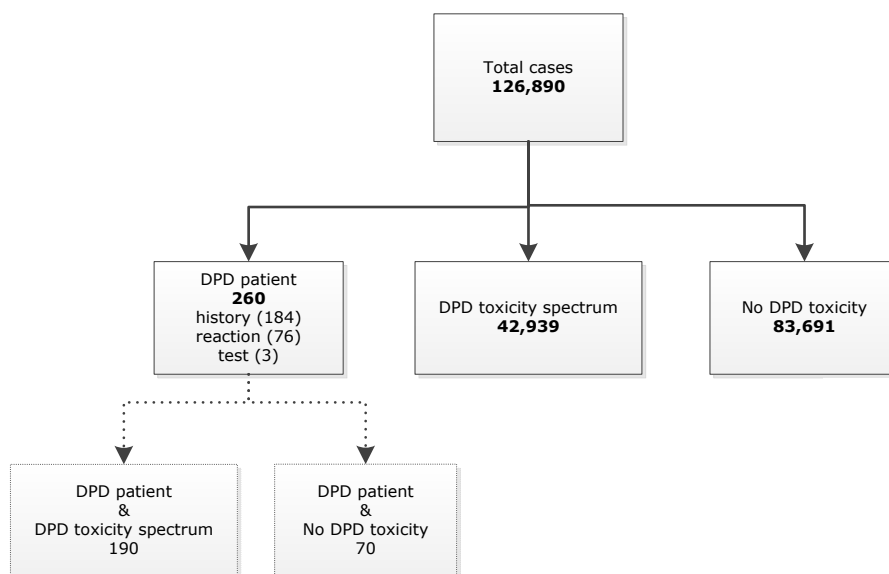
Figure 4. Flowchart defining DPD toxicity spectrum and No DPD toxicity' group

Amongst the DPD patients, diarrhoea and neutropenias are the most frequent reactions.

When considering outcome at case level, the fatality rate is 19.2% in DPD patients, which is twice as high as the fatality rate within the DPD toxicity spectrum (9.4%) and the 'no DPD toxicity' group (11.8%).
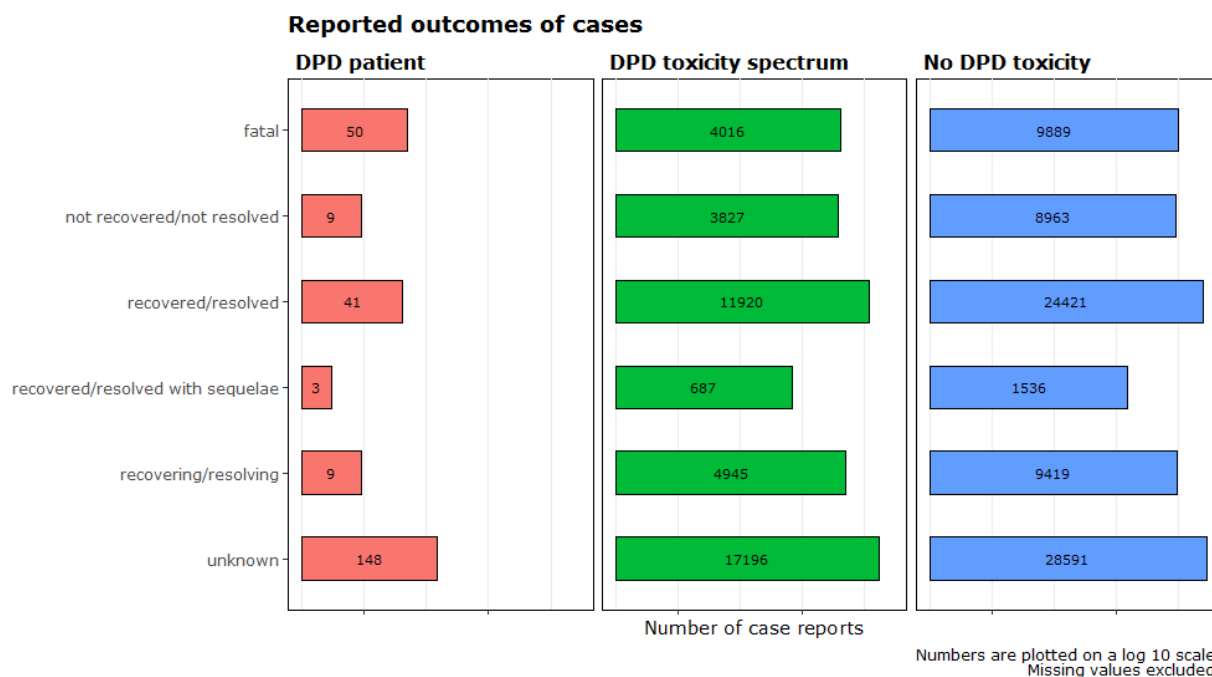
Figure 5. Reported outcomes of cases

There has been a steady increase in the number of DPD patient cases since 2014, with a small increase over the past two years, possibly attributable to increasing awareness.

Median time-to-onset is consistent across substances, with the exception of flucytosine for which it is shorter. Time to onset is fairly consistent across the most frequently reported indications.

More than half of the DPD confirmed patients had a fatal or life-threatening reaction, a proportion that was much lower (23.6%) within the DPD spectrum group. This may be due to the fact that toxicity is higher in DPD patients and/or that patients experiencing severe reactions are more likely to be tested for DPD deficiency.
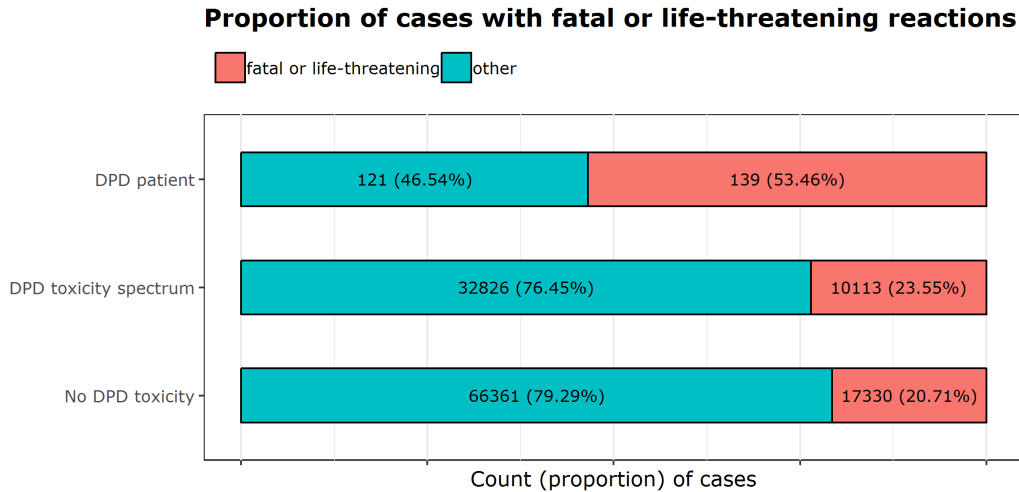
Proportion of cases with fatal or life-threatening reactions

Figure 6. Proportion of cases with fatal or life-threatening reactions

The proportion of cases with immediate (1 - 2 days), short (3 – 21 days) and long (> 21 days) time-to-onset for DPD patients and those with DPD toxicity spectrum reactions were compared. Seventy-three percent (73%) of fatal or life-threatening reactions occurred early in confirmed DPD patients, while a slight majority (53%) of fatal or life-threatening reactions within the DPD spectrum group had a late onset (53%). Again, this may be due to the fact that toxicity occurs earlier in DPD patients and/or that patients experiencing early reactions are more likely to be tested for DPD deficiency.

Proportions of cases with early or late onset for fatal or life-threatening reactions
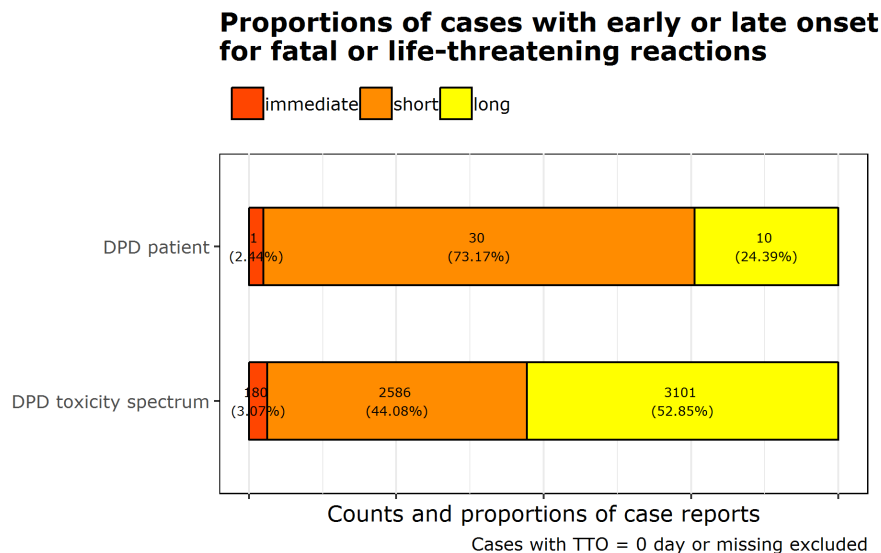
Figure 7. proportion of cases with early or late onset for fatal or life-threatening reactions

A similar pattern was observed for capecitabine and fluorouracil, although a slight majority (51.9%) of DPD spectrum patients experienced early reactions with capecitabine. There were no DPD patients with time-to-onset information for flucytosine and tegafur.

To estimate the count of DPD cases, machine learning methods were applied. A cautious interpretation of the results suggests that 7% to 12% of all case reports to fluorouracil related substances have an adverse reaction profile similar to that reported in dihydropyrimidine dehydrogenase deficient patients. This frequency is within the range of prevalence of DPD and the variable importance follows the expected profile of the reaction and gender imbalances.

Overall, the findings were in line with the known safety profile of 5-fluorouracil, the role of DPD and the current status of DPD detection.

2.2.3. Screening methods for detection of DPD deficiency

In this review, the MAHs were asked to provide up-to-date reviews of the clinical data investigating the gene encoding DPD (DPYD) genotyping and identification of DPYD variants known to be associated with decreased DPD activity and the reliability of the translation of genotyping into the phenotype of each clinically relevant variant, of the clinical data investigating DPD phenotyping for detection of patients at increased risk for toxicities, as well as of the clinical data investigating the advantages of DPD combined methods (genotyping + phenotyping) for detection of patients at increased risk for toxicities.

2.2.3.1. Genotyping approaches

In 2017, Meulendijks and colleagues published a systematic review on 7365 subjects from 8 studies. DPYD c.1679T>G, DPYD*2A, c.1236G>A/HapB3 and c.2846A>T were significantly associated with severe fluoropyrimidine-associated toxicities, whereas c.1601G>A was not found to be a risk factor. For c.1679T>G, only eleven patients with this mutation had been described and the risk of global severe toxicity was four times increased with this variant. The decrease in DPD activity is expected to result in a 50-100% increase in tissue exposure to fluorouracil, thus leading to recommending a dose reduction of 50% in patients with c.1679T>G. For c.1236G>A/HapB3, the authors observed a half reduction in DPD activity compared with DPYD*2A, and thus recommended a dose reduction of 25% in heterozygous patients. This later recommendation was not supported by later findings: a 50% dose reduction is now also recommended for heterozygous patients. Although the risks of severe gastrointestinal and haematological toxicities were increased in c.1679T>G and c.1236G>A/HapB3 carriers, the risk of hand foot syndrome was not, suggesting a weaker association between DPYD variants and occurrence of hand-foot syndrome occurring after several cycles of fluoropyrimidine treatments. This illustrated how DPYD genetic polymorphism is more associated with early severe toxicities, and less with cumulative ones.

As stated in the French INCa-HAS report, the only completely deficient patients who can potentially be identified using this genotyping are homozygous carriers of the DPYD*2A, or DPYD*13 variants. However, as the frequency of these variants is extremely low in populations of Caucasian origin and practically null in other populations, it can only explain a limited number of cases of complete deficiency.

In addition, in the event of the identification of two different variants in the same patient, referral to genotyping alone will not indicate whether these variants are carried on the same allele or not and, for this reason, a supplementary DPD screening method such as TDM would appear to be necessary. As such, DPYD genotyping, which is currently based on screening for four variants (*2A, *13, HapB3 and c.2846A>T) is unable to cover the identification of all completely DPD deficient patients.

Partial DPD deficiency screening using genotyping, based on combined screening for DPYD*2A, DPYD*13 and c.2846A>T variants, exhibits a low sensitivity and, based on the data available, a mediocre positive predictive value. In other words, this approach is only suitable for identifying a very small proportion of patients presenting with a DPD deficiency and may also give rise to false-positive diagnoses and to the administration of a less effective treatment.

Nonetheless, it is to be noted that over 160 variants within the DPYD gene have been described to date. In the light of some in vitro and/or ex-vivo data, the existence of other functional variants is very likely in particular in non-Caucasians.

2.2.3.2. Phenotyping approaches

To overcome some of the limitations of the genotyping method, phenotyping methods to measure DPD activity has been investigated. Phenotyping has a lower level of evidence because most clinical reports published thus far are monocentric or retrospective studies. Phenotyping requires strict and constraining pre-analytical treatment of samples that can be hardly automated. In addition, currently available results show better (negative) predictive performance (sensitivity) for phenotyping methods as compared to genotyping. However, specificity and sensitivity of phenotyping DPD as a mean to detect toxic patients at risk for severe toxicity remains to be further evaluated. Several phenotyping methods are described in this section.

Endogenous plasma uracil (U)

One of the methods used to determine the DPD status phenotype is the monitoring of the endogenous uracil in plasma used as surrogate parameter. Standard liquid–liquid or solid-liquid extractions using a simple and time-effective High-Performance Liquid Chromatography with ultraviolet detector (HPLC-UV) method is used. Mass spec analysis can alternatively be used.

Plasma uracil seems to be currently the most clinically useful of the phenotypic methods available. However, determining appropriate initial dose reduction in patients with suspected DPD deficiency identified by plasma uracil levels (U) is hampered by the lack of data on dose reductions guided by blood uracil levels. In addition, there is a lack of consensus regarding cut-off uracil levels defining complete or partial DPD activity associated with clinical toxicities.

Etienne-Grimaldi *et al.* (2017) investigated the risk of severe toxicity in 243 breast cancer patients receiving capecitabine. DPD phenotype was assessed by pre-treatment plasma uracil and dihydrouracil measurements. They found that compared to genotyping alone, combining genotyping (7 variants) and phenotyping (U > 16 ng/L) did not substantially increase sensitivity and impaired positive predictive value and relative risk. Based on these results the authors recommended extending the set of deleterious DPYD variants that are screened to increase the accurate prediction of grade 3-4 and grade 4 toxicity related to capecitabine. However, the study did find that patients with U >14 ng/L were significantly prone to develop grade 4 toxicity (Fisher Exact test p = 0.047) but significance was not reached for grade 3-4 toxicity. Elevated U > 16 ng/L was significantly associated with a RR of 20.6 to develop grade 4 toxicity.

Meulendijks *et al.* (2017) investigated the predictive value of serum uracil and dihydrouracil levels and found that high pre-treatment uracil concentration (U) was strongly predictive of fluoropyrimidine toxicity and was superior to the UH2/U ratio. The study included 550 patients treated with DPYD*2A genotype-guided fluoropyrimidine dosing for whom pre-treatment serum samples were available. The serum U and UH2 concentrations in the pre-treatment samples were measured. The association between U or the UH2/U ratio and toxicity was assessed. Pre-treatment U was found to correlate better than UH2/U with DPD activity in PBMC. Pre-treatment U was found to be strongly predictive of global severe toxicity (grade > 3) (p = 0.004), fatal-treatment related toxicity (p = 0.007) but not

haematological toxicity (p = 0.383). Patients with U >16 ng/L and those with U > 13.9-16 ng/L were at significantly increased risk of global severe toxicity compared to patients with low pre-treatment U (< 13 ng/L). They concluded that pre-treatment uracil is a promising phenotypic marker of toxicity used alone or with DPYD genotype testing. Recommendations for dose adjustment were not provided in this study.

In their responses to the PRAC questions, the MAHs consistently indicated that measuring endogenous uracil in plasma is feasible and appears currently the most promising method for detection of DPD deficiency. However, they also stated that threshold levels for partial deficiency and related optimal dose adjustment still deserves additional research/exploration.

In order to better characterise the cut-off levels for partial deficiency more research is encouraged.

Dihydrouracil (UH2) to uracil (U) (UH2/U) ratio in plasma, saliva or urine

Because U is metabolized into UH2 by DPD, monitoring (UH2/U) ratio and comparing the value of a given patient with standard values recorded in a reference population (ideally, patients with no severe toxicities after being treated with 5-fluorouracil), should allow the detection of patients at risk of impaired DPD activity and therefore drug-related side effects. Calculation of such a ratio permits the determination of DPD status as a continuous variable.

Early studies investigated the UH2/U ratio in urine, but this methodology no longer appears to be used.

The plasma UH2/U ratio has been proposed as a simple and rapid pre-emptive phenotypic test which overcomes the limitation of genotypic profiling.

Studies investigating the correlation between UH2/U and toxicity are inconclusive. Boisdron-Celle *et al.* (2007), reported that UH2/U ratio and early treatment toxicity correlated significantly (p < 0.001), while Etienne-Grimaldi *et al.*, 2017, observed no significant differences in the UH2/U ratios of patients developing grade 3 and 4 toxicities during the first two cycles of treatment. Gamelin *et al.*, 1999, found a significant correlation between UH2/U ratio and optimal 5-fluorouracil dosage in a study performing TDM (p<0.001). Van Kuilenberg *et al.* (2016) investigated the correlation of different phenotyping methods with DPD activity in healthy controls and observed no correlation between the DPD activity and the UH2/U ratio.

Only one study reports on sensitivity (82.4%), specificity (78.4%) and PPV (30-49%) of the UH2/U ratio with regard to the onset of severe fluoropyrimidine-associated toxicities (Boisdron-Celle *et al.,* 2007). However, apart from the low PPV, the lack of details on the methodology and the patient sample sizes are major limitations.

One of the major limitations of UH2/U is the robustness of the used method. Interference problems of dihydrouracil have been reported, when the most common method HPLC-UV is used. This leads to different results between laboratories.

In addition, there is no scientific consensus on a cut-off level to define DPD deficiency. The cut-off levels used in literature as well as in laboratories to denote patients with increased risk for toxicity differ significantly between UH2/U <1.8 proposed by Gamelin *et al.* (1999) and UH2/U <6 proposed by Boisdron-Celle *et al.* (2007). Complete DPD deficiency was defined by Launay *et al.* (2016; 2017), with UH2/U < 0.5 or UH2 not detected at all and by Boisdron-Celle *et al.* (2017), with UH2/U<1. While the assumption that patients with no detectable UH2 might have a complete DPD deficiency is reasonable, no rationale for the cut-off level of <0.5 or <1.0 were given and no supporting data was provided. There is even evidence that the proposed cut-off level of UH2/U <1 may be too high to define complete deficiency as Launay *et al.* (2016), successfully treated a patient with a ratio between 0.5 and 1 with a reduced fluoropyrimidine dose.

In summary use of UH2/U ratio for pre-emptive DPD testing has some limitations due to a lack of strong correlation between the test result and severe toxicity as well as robustness of the method and clearly defined cut-off.

DPD enzymatic activity in peripheral blood mononuclear cells (PBMC)

Because of its ubiquitous localisation, evaluating DPD activity in easily available surrogate tissues (such as lymphocytes or fibroblasts) can be used as a marker for the actual liver function, despite mixed correlations between activities.

Routine determination of DPD enzymatic activity in peripheral blood mononuclear cells is limited by time-consuming, labour-intensive methods, and the involvement of radioactive elements. Moreover, the methods are not readily available in most outpatient treatment facilities.

The MAHs consistently indicated that testing of DPD activity in PBMC is not suitable for use in routine clinical practice.

Other phenotyping methods

Other phenotyping methods were the ^{13}C-uracil breath test or uracil loading dose. Data regarding these methods is currently very limited to enable giving any recommendation.

2.2.3.3. Combined methods

Only limited data regarding the predictive performances of various combined phenotyping and genotyping approaches are currently available.

In a study by Boisdron-Celle M *et al.* (2017) it was shown that the combination of genotyping and phenotyping was superior to genotyping or phenotyping alone for DPD deficiency prediction in terms of sensitivity and specificity, which was crucial for reliably detecting DPD deficiency and suitable for routine clinical application. The authors provided a two-step combined approach, judged suitable to clinical practice by themselves, as a screening test to identify DPD deficiency. However, this phenotyping approach seems to be much too complicated to execute in regular medical facilities. Furthermore, combined approaches are heterogeneous depending on the studies, since they combine screening for a variable number of DPYD variants with one or more phenotyping methods.

In conclusion, even though some publications show added value of combination, the final results will strongly depend on not only the methods used for genotyping and phenotyping which are very variable but also on the decision criteria and algorithms used for interpretation of the combining results (e.g. method A AND method B vs method A AND/OR method B vs quantitative algorithm used by Boisdron-Celle; 2017).

2.2.4. Pharmacokinetic (PK)-guided Dosing and therapeutic drug monitoring (TDM)

Therapeutic drug monitoring (TDM) can be a valuable tool for monitoring dose escalation in patients treated with a reduced starting dose as result of genotype- or phenotype-guided dosing. Target levels for 5-fluorouracil AUC have been reported to be in the range of 20-30 mgh/mL by different research groups (Beumer *et al.*, 2019; Gamelin *et al.*, 2008; Kaldate *et al.*, 2012; Wilhelm *et al.*, 2016).

In 2016, Lee *et al.* published a meta-analysis where PubMed and abstracts from the American Society of Clinical Oncology were searched up through September 2015 for clinical data relating to 5-fluorouracil TDM. Conclusion by the authors was that dose adjustment of 5-fluorouracil is feasible, and

PK-based dosing can significantly improve clinical outcomes by reducing toxicities and improving efficacy and safety.

2.2.5. Guidelines

Several guidelines are currently addressing upfront DPD screening for patients treated with fluoropyrimidines.

In the ESMO consensus guidelines for the management of patients with metastatic colorectal cancer (Van Cutsem E. *et al.*, 2016). DPD testing before 5-fluorouracil administration remains an option but is not routinely recommended. The guideline states that there is no recommended standardised assessment technique, although several methods are available. None of the current strategies are adequate to mandate routine DPD testing before starting fluoropyrimidine-based therapy.

In addition, the ESMO Clinical Practice Guidelines: Diarrhoea in Adult Cancer Patients (Bossi P. *et al.*, 2018) mentions partial or complete DPD deficiency caused by non-functional DPYD variants as risk for toxicity including diarrhoea. No screening is recommended.

Other guidelines however, such as the ones issued by the Pharmacology and Molecular Mechanisms Group of the European Organisation for Research and Treatment of Cancer (PAMM-EORTC) strongly support the prospective evaluation of DPD activity in patients scheduled for fluoropyrimidines despite its limitations (Ciccolini J. *et al.*, 2018).

Some national guidelines provide further guidance regarding DPD deficiency screening (Loriot MA *et al.*, 2018), thresholds of blood uracil levels deemed indicative of complete or partial DPD deficiency (INCa, 2018) or recommend guided dosing of fluoropyrimidines for patients with reduced DPD activity (Amstutz U. *et al.*, 2017, Lemaitre F. *et al.*, 2018).

3. Expert consultation

3.1. Scientific advisory group on oncology

Upon request from the PRAC, a scientific advisory group (SAG) in oncology meeting was convened on 11 October 2019.

The SAG was asked to advise on the most suitable method(s) to prevent severe DPD-associated fluoropyrimidine toxicity taking into account the strengths and limitations of (1) genotyping, (2) phenotyping and (3) combined approaches, as well as to discuss the level of evidence for the different methods. In particular, the SAG was asked:

- what would be the most effective evidence-based intervention to be recommended for patients identified with partial DPD deficiency, taking into account test performance (in terms of sensitivity, specificity and positive/negative predictive value), and effectiveness of testing in allowing effective treatment with reduced risk of toxicity.

- whether there are particular groups of patients at high risk of fluoropyrimidine toxicity for whom DPD testing should be particularly recommended;

- advice on the dosing adjustment to recommend in case of partial DPD deficiency based on patient characteristics as well as on DPD activity. More specifically, to discuss the proposed cut offs for for uraciliemia (U≤ 16 ng/ml; U>150 ng/ml);

- whether the measures would also applicable for patients treated with flucytosine or Teysuno (tegafur/oteracil/gimeracil).

The SAG views on these questions are described below.

SAG advice on most suitable method(s) to prevent severe DPD-associated fluoropyrimidine toxicity

Currently, the level of the available evidence does not allow making conclusive recommendations on the most effective method and intervention for patients identified with partial DPD deficiency since no directly comparative data are available to assess the clinical utility of different approaches. Such randomised prospective studies would be extremely challenging to conduct and, in any case would only able to address a fraction of the many optimisation questions that are still open. Nevertheless, a number of sensible approaches are proposed in more or less widely implemented and formally developed clinical guidelines, including reduced-dose treatment initiation and subsequent escalation based on clinical considerations. In this respect, the wording of the SmPC section 4.4 Warnings of Xeloda as proposed by the holder of the marketing authorisation is informative and adequately provide information for different methods (including genotyping, phenotyping methods or combination of these methods) and practices. Similar wording would be appropriate for the SmPC of i.v. 5-fluorouracil products.

The prevalence of around 3-8% in Caucasians for partial DPD deficiency is high enough so that generally testing can be recommended according to relevant guidelines and availability of testing facilities and considering alternative risk minimisation measure like increased monitoring and reduced starting dose.

Testing could also be generally recommended in patients with family history (first degree) of severe toxicity related to fluoropyrimidine chemotherapy.

Existing dosing recommendations for genotyping as stated in, e.g., the Dutch Pharmacy or CPIC Guidelines, are still open for debate, especially with respect to the c.1236G>A/HapB3 and c.2846A>T variants. Physicians should refer to the relevant guidelines for up-to-date recommendations.

As concerns phenotyping, even though elevated serum uracil concentration is significantly linked to early fluoropyrimidine-related severe toxicity (the greater the serum uracil concentration, the higher the toxicity risk), (Meulendijks, Henricks *et al.* 2017) validation of a dose reduction guide based on serum uracil concentration is not available (the 15-16 ng/ml cut-off identified in 3 independent studies was not prospectively validated) (Boisdron-Celle, Remaud *et al.* 2007, Etienne-Grimaldi, Boyer *et al.* 2017, Meulendijks, Henricks *et al.* 2017). The upper cut-off (uracilemia ≥150 ng/ml) proposed by the French Cancer Institute corresponds approximately to the 99.9th percentile (obtained on 38,862 patients) and was considered as a result of complete DPD deficiency, thus contra-indicating fluoropyrimidine chemotherapy.

Part of the problem with setting thresholds based on serum uracil concentration, at least for some of the experts, was analytical concerns with the different assays, availability of the different assays, and inexperience of different centres in the proper handling of specimens (although the French experience has shown that adequate standardisation of the assays is possible on a wider scale). Thus, the clinical utility of the proposed cut-offs for serum uracil concentration for dosing adjustment to recommend in case of partial DPD deficiency cannot be considered established. However, the approach is promising and should be investigated further.

As to whether measures would also be applicable for patients treated with flucytosine or tegafur, SAG's views was that flucytosine is used in a very different clinical context of severe fungal infection where

waiting even a few days for test results would be unacceptable. Also, the population and mutations might be very different, and the impact is difficult to predict. There is a need to characterise mutations in non-Caucasian populations.

For tegafur/oteracil/gimeracil there is no clear information about the role of partial DPD deficiency and it is difficult to speculate about appropriate measures.

For topical 5-FU, in principle if the skin barrier is damaged this could result in sufficient systemic exposure resulting in severe toxicity. Thus, in patients with complete DPD deficiency there could be concerns if the skin is not intact.

SAG advice on the feasibility to implement upfront DPD deficiency screening in routine clinical practice

The SAG was asked to discuss the feasibility to implement upfront DPD deficiency screening (genotyping or phenotyping or combined approach) in routine clinical practice, taking into account the current availability of facilities testing for DPD deficiency and the timelines for implementation and validation of genotyping and phenotyping methods and the expected turnaround time for each method; and whether there are patients for whom a delay of treatment until results are available is not acceptable.

The SAG advised that phenotyping is currently not widely available although the French experience shows that it can be implemented effectively on a large scale, with 26,800 patients screened for serum uracil concentration over the past 6 months (April to September 2019), accounting for approximately 82% of French patients currently receiving 5-FU or capecitabine. Genotyping is progressively more widely available throughout EU. Either methods have pros and cons that were debated by different experts with no consensus, like the reliability and handling issues above mentioned for phenotyping, the need for timely results that according to clinicians should be a maximum of 7-10 days. Timelines to get results varied from 6 days on average for serum uracil concentration (French experience, delays being as short as 2-3 days in large private or public laboratories) to 3-5 days for phenotyping and genotyping, respectively, at least in larger facilities with central laboratories.

SAG advice on the clinical utility and feasibility of therapeutic drug monitoring (TDM) in routine clinical practice

The SAG, finally, was asked to discuss the clinical utility and feasibility of therapeutic drug monitoring (TDM) in routine clinical practice (in particular, to monitor the impact of dose titrations in patients treated with a reduced starting dose after detection of partial DPD deficiency taking into account the current evidence on different treatment regimens and active substances).

The experts concluded that there are no systematic approaches reported in the literature to study the relationship between plasma levels of capecitabine or its metabolites, including 5-fluorouracil, to clinical safety or efficacy. Systemic exposure to capecitabine and capecitabine metabolites in plasma also appear to be poorly predictive of safety and efficacy.

They agreed that in general, 5-fluorouracil TDM in case of dose reduction or reduced first dose is considered potentially useful based mostly on reasoning and indirect evidence. 5-fluorouracil TDM is believed to improve clinical outcomes by improving efficacy of 5-fluorouracil-based combination regimens and reducing toxicities. Recent recommendations from the International Association of TDM and Clinical Toxicology strongly recommended 5-fluorouracil TDM for optimal management of colorectal and head and neck cancer patients receiving common 5-fluorouracil dosing regimens (Beumer, Chu *et al.* 2019). These recommendations advise 5-fluorouracil TDM in patients with partial

DPD deficiency in order to more safely and quickly adjust 5-fluorouracil dosing. Some approaches that are mainly looking at clinical effects rather than TDM are also used in practice as drug monitoring is not always easy to carry out also depending on the regimens and timing of sampling. Furthermore, the SAG highlighted that availability of the TDM service is probably restricted to specialist centres.

Published guidelines on antimycotic drug treatment recommend routine TDM of plasma flucytosine (as treatment is usually combined with amphotericin B, which is nephrotoxic). There is an established target range for plasma flucytosine to minimise the risk of bone marrow toxicity. However, the same considerations about availability of the TDM service apply.

3.2. *Pharmacogenomic working party (PgWP)*

At the request of the PRAC, the pharmacogenomic working party (PgWP) was also asked to discuss the strengths and limitations of available methods for screening of DPD deficiency based on (1) genotyping, (2) phenotyping and (3) combined approaches, as well as the level of evidence of the different methods.

Concerning new data on *DPYD* variants associated with severe toxicity, the PGWP was asked to comment on:

a) the need to update the current wording in the SmPCs for capecitabine and 5-fluorouracil containing products describing *DPYD* variants associated with severe toxicity. In particular, new data on variant HapB3 and differences in the expression of *DPYD* variants between different ethnicities should be taken into account.

b) the most recent data on genotype- or phenotype-guided dosing and whether the evidence on effectiveness of genotype-guided dosing, in particular reduction of toxicity and efficacy of treatment, is considered sufficient for a recommendation in the SmPC.

The views of the PgWP are summarised below.

PgWP's comments on strengths and limitations of available methods for screening of DPD deficiency

Comparing genotyping and phenotyping for *DYPD* and DPD, respectively, experience shows that genotyping for *DYPD* variants is easy to implement in clinical and laboratory practice, whereas in general, phenotyping in relation to DPD includes more logistical issues: e.g., phenotyping requires samples to be sent on dry ice and arrive at the laboratory within 24 hours of following withdrawal. Phenotyping also poses a stronger logistic burden on the patient, e.g., due to the fact that the patient needs to be present at a specific time and place for drawing the blood sample. For genotyping, blood, but also cheek swabs or saliva samples can be used, making it a non-invasive procedure. These types of samples can also be sent at room temperature, and do not require arrival in the laboratory within 24 hours.

With respect to the outcome of the assays, genotyping results are dichotomous variables: normal metabolizer (NM), intermediate metaboliser (IM) or poor metaboliser (PM), which corresponds to scientifically confirmed and specific dosing schedules for fluoropyrimidines in the literature (like 100%, 75%, 50%). Phenotyping for DPD activity will give an outcome on a continuous scale, for which then cut-off values need to be decided upon and then translated into dichotomous dosing advices. At present, knowledge on the cut-offs to be applied in DPD phenotyping assays is limited, resulting in the judging by the PGWP that scientific evidence for fluoropyrimidines dosing based on phenotyping is less mature than that for genotyping.

Genotyping for *DYPD* genetic variants will give measured, consistent outcomes (e.g., mutation yes/no), whereas phenotyping assays are prone to variation within and between laboratories. This makes the outcomes for phenotyping less clear, and fluoropyrimidine dose advices may thus differ between laboratories depending on the type of assay ran and local conditions. A drawback of genotyping is that specific but potentially relevant unknown *DYPD* variants will be missed, whereas phenotyping will capture these by measuring DPD enzymatic activity or a surrogate for this. Further, the occurrence of specific variants may differ between ethnicities, resulting in the situation that the detection rate of functionally altered alleles by genotyping may be less optimal in certain (non-Caucasian) populations, a problem not seen with phenotyping. However, as clearly stated in the EMA guideline for 'Good pharmacogenomics practice', any genotyping approach should include all known functionally relevant genetic variations for the interrogated gene, regardless their frequency or ethnic prevalence.

With respect to the available assays, PgWP noted that there is currently more evidence on decrease in toxicity when using genotype as the dosing guidance for fluoropyrimidine use as compared to phenotyping. For both genotyping and phenotyping, limited data is available indicating no detrimental effect of genotype- or phenotype-based dose adjustment on efficacy.

PgWP's comment the description of DPYD variants in the SmPC

At this stage, four *DPYP* variants that may be tested via genotyping approaches are mentioned in the 5-fluorouracil and capecitabine SmPCs, i.e. *DPYD*2A (c.1905+1G>A), c.1679T>G, c. 2846A>T and c.1236G>A/HapB3* variants. Further, the frequency of these variants in the Caucasian population is indicated, as is that knowledge on frequency in other ethnic populations is limited. Finally, it is stated that a dose reduction in patients with the aforementioned *DPYD* variants may be considered to avoid serious toxicity, without detailing on the actual dose modification to be applied. Of note, it is indicated that genotyped patients who test negative for these variants may still present a risk for severe toxicity, due to the possible presence of unrecognised (rare) *DYPD* variants.

With respect to ethnicity, at the present time, the four *DPYD* variants mentioned above are generally considered virtually absent in populations of African(-American) (Offer SM *et al., 2014*) or Asian (Hishinuma E *et al., 2018*) origin. Other, currently less characterised, but functionally relevant *DPYD* variants may be present in these populations. This may also be indicated by the fact that regional differences in tolerability to fluoropyrimidines has been reported (Haller DG *et al.*,2008). Therefore, at present, it is acknowledged that genotyping is the most reliable/robust method in Caucasians, but at this stage, due to the much lower frequency of expression in other ethnic populations, upfront genotyping of these four variants in such ethnic populations bears less evidence of usefulness. Currently, this is indicated in the SmPC with the text "*No data are currently available on important variants in e.g. African or Asian populations*". The current advice from the PGWP is to add that the four variants indicated are considered not to be as relevant for screening of patients from Asian or African background as it is for Caucasian patients.

With respect to the c.1236G>A/HapB3 variant, varying and sometimes contradictory data on the potential association between the expression of this variant and toxicity to fluoropyrimidines have appeared. A number of individual studies did not find evidence for a relationship with grade ≥3 or higher toxicity (Meulendijks D *et al.* 2017; Etienne-Grimaldi *et al.,* 2017; MeulendijksD *et al.,* 2017; Lee *et al.* 2016). In a recent publication by Henricks *et al.*, however, it was noted that the risk of severe toxicity in heterozygous *HapB3* patients was still increased compared to a historic cohort of wild-type patients (i.e. not expressing one of the four *DPYD* variants) after applying a 25% dose reduction (Meulendijks D *et al.* 2017). Further, grade ≥4 toxicity was still observed at the same level as in wild-type patients. Importantly, the authors indicate that a large variation in exposure was observed in this *c.1236G>A/HapB3* patient population, potentially indicating that some patients may

require a larger dose reduction than others. Unfortunately, no data on exposure were provided for the *c.1236G>A/HapB3* group showing grade ≥4 toxicity in this study (Henricks LM *et al.*, 2018). Of note, these individual studies appear less powered than the meta-analysis that was published in support of the potential correlation between the *c.1236G>A/HapB3* variant and severe toxicity (Meulendijks *et al.*, 2015).

Overall, it is acknowledged by the PGWP that the current SmPC advice for the *c.1236G>A/HapB3* variant appears under discussion in the scientific literature. However, at this stage it, would be advised to take a cautionary approach and to retain the c.1336G>A/HapB3 variant in the SmPC as a variant that should be screened for. In this light, it is also considered that the logistic changes with either in- or exclusion of the c.1336G>A/HapB3 variant in the genotyping screen are considered minimal. The actual dose modifications for the various *DPYD* variants are currently not indicated in the SmPC, a situation that appears reasonable considering the current uncertainty and data generation on the actual dose adjustment to be implemented. For that reason, it would be appropriate to add a statement in the SmPC to consult the most recent relevant Guidelines on this matter.

Further, current advice from the PGWP is to add to the SmPC more explicitly that the four variants indicated are considered not to be as relevant for screening patients from Asian or African background as they are for patients with Caucasian background, due to the very low frequency of expression of these variants in these populations. However, in order to be consistent with the guideline on 'Good pharmacogenomics practice', it is recommended to mention that the afore-mentioned guideline advises to test for all known functionally relevant genetic variations for the interrogated gene, regardless of their frequency or ethnic prevalence.

PgWP's comment on recommendations for genotype- or phenotype-guided dosing

The current warning/advice in SmPC section 4.4, to test for *DPYD*2A*, *c.1679T>G*, *c.2846A>T* and *c.1236G>A/HapB3* variants is based on information available up to 2016. At that point in time, the PGWP, although acknowledging that there may still be patients not identified by the proposed genotyping method prior to treatment which may develop serious adverse effects, it was at that time also acknowledged that for a relevant proportion of Caucasian patients (4.8 – 8.5 %) serious adverse effects appear to be avoidable, by using a simple and relatively cheap genotyping method. According to the PGWPs opinion at that time, it was ethically challenging to withhold such a precautionary measure while waiting for other subgroups to be identified and included in the testing procedure.

In 2017, no data were available on the potential effect of a reduced starting dose on efficacy of the treatment. Although normally, the dose advice in case of a phase I or phase II genetic variant is based on the PK of the product, the lack of actual data on efficacy was considered a major issue in case of *DYPD*, partly in due to the high variable 5-fluorouracil pharmacokinetics. Since then, more information on efficacy has been published, applying either pre-emptive phenotyping (Launay *et al.* 2016; 2017) or genotyping (Henricks *et al.,* 2019). In the study by Henricks *et al.* (2019) only the starting dose was reduced, whereas subsequent doses were based on response and toxicity. In the study of Launay *et al.* (2017) the reduced dose appeared to be maintained throughout treatment. In either case, despite the relatively low numbers of patients in these studies, efficacy in standard treated patients and pre-screened patients appears comparable. Although at present only the above-mentioned relatively small studies were conducted on efficacy, and no large, prospectively randomized controlled clinical trials investigating non-inferiority have been conducted, the data so far do not point at reduced efficacy in patients with a lower starting dose based on upfront genotyping or phenotyping for *DPYD* or DPD, respectively, in line with what would be expected based on PK in these subgroups.

A point of note is that genotyping may lead to a false classification with respect to complete loss of DPD function. It is likely that other unknown functional *DPYD* variants exist, apart from those which are identified by genotyping for the four variants currently indicated in the SmPC. It is acknowledged that the currently indicated genotyping method is not capable of identifying all functional *DPYD* variants associated with complete deficiency. It should however be realised that the actual number of such misclassification are expectedly very small, since these will be dependent on so-called rare variants. In line with this expectation and to the PGWPs knowledge, at present no cases of such complete-loss-of-function misclassification following genotyping for the four variants has been reported. Although the chance of unnoticed DPD poor metabolisers following genotyping appears small, this occurrence of false negatives cannot be excluded. In that respect, the use of phenotyping appears from a theoretic point of view, better able to detect such poor metabolisers. However, this advantage of phenotyping over genotyping has not yet been proven in the clinical setting.

At present the data supporting the use of upfront genotyping are more robust than that in support of upfront phenotyping for DPD activity. To the PGWPs opinion, the clinical value of genotyping in the Caucasian population has been demonstrated to a reasonable extent and is relevant for a significant proportion of the Caucasian patients. However, the current dosing recommendation in case of the DPD variants as stated in e.g., the CPIC Guidelines, are still open for debate, especially with respect to the *c.1236G>A/HapB3* and *c.2846A>T* variants. Therefore, at present it would not yet be advisable to include the actual recommended dose reductions per variant in the SmPC, but instead to refer the physician to the relevant (e.g., CPIC and others) guidelines for further/latest information.

With respect to phenotyping, in theory, upfront phenotyping may overcome some limitations of genotyping, especially in situations where non-Caucasian patients are involved, for which the currently advised variants do not bear significance, and for situations where a poor metaboliser phenotype is present. At present however, the clinical value of upfront phenotyping has not been demonstrated to the extent as for upfront genotyping. For example, the most suitable type of phenotyping assay is not clear at this stage. Furthermore, the robustness of the phenotyping assay (e.g., it is unclear if the outcome of the assay is independent of the laboratory where the assay is conducted,) is still under debate. Finally, at present, the phenotype threshold for DPD poor metabolisers is not firmly established. Only when this threshold is known and robust beyond reasonable doubt, this method can be advised for detection of poor metabolisers as well.

Overall, the PGWP considered the clinical utility of upfront genotyping sufficiently established to maintain indicating this possibility in the SmPC section 4.4. Reduced toxicity following genotype-based dosing has been reported in multiple studies, whereas the data so far (albeit limited) do not point to reduced efficacy in patients with a lower starting dose based on upfront genotyping for *DYPD*. At this moment, however, the level of dose-reduction is not considered well enough established to be included in the SmPC section 4.2 Posology, as a dose advice. Instead, it is advised to introduce a reference to relevant guidelines in section 4.4 Warnings and Precautions of use, to be informed on the latest dose advices.

Of note, DPD poor metabolisers that are caused by an unknown *DPYD* genetic variant will not be revealed by genotyping performed according to the guideline on 'Good pharmacogenomic practice' (e.g. interrogating all known functionally relevant variants for the interrogated gene, regardless its frequency or ethnic prevalence). In that respect, the PGWP considers that the possibility of phenotyping for DPD activity may also be included in the SmPC, with the notion that, although the upfront phenotyping procedure is clearly less firmly established than the upfront genotyping procedure, a phenotyping assay potentially offers the possibility to detect reduced DPD activity in other populations than Caucasians or may be able to detect very rare, but potentially relevant variants in Caucasian patients.

4. Stakeholders' input

PRAC received several submissions from third parties in the context of this procedure. These included testimonies from family members of patients with complete DPD deficiency having received 5-fluorouracil or capecitabine; as well as submissions from academia, highlighting current research on DPD testing prior to initiation of treatment with these products. These submissions were all considered by PRAC in the context of this review.

5. Discussion and benefit-risk balance

5-fluorouracil (5-FU) is a pyrimidine analogue which competitively inhibits the enzyme thymidylate synthase (TS), thereby creating a thymine deficiency and resulting in inhibition of deoxyribonucleic acid (DNA) synthesis and cytotoxicity. It also inhibits, to a lesser extent, the formation of ribonucleic acid (RNA). These effects are most marked in rapidly growing cells and may lead to cell death. Depending on the regimen, administration of standard 5-fluorouracil usually leads to severe toxicities in one third of the patients, and up to 3% of treatment-related deaths have been reported in frail patients such as the elderly. Ethnicity (e.g. Afro-American) and gender (e.g. women) could be additional risk factors.

Dihydropyrimidine dehydrogenase (DPD) is the main metabolising enzyme of 5-fluorouracil (80-85% of catabolic clearance). Its activity is subject to a wide variability, resulting in a possible range of enzymatic deficiencies that span from partial to complete loss of enzyme activity. DPD deficiency is partly linked to genetic polymorphisms in its gene DPYD but may also have other causes. Prevalence of partial and complete DPD deficiency in the entire population varies between different sources and has been estimated with approximately 3%–9% and 0.01%–0.5%, respectively.

Due to the pivotal role of DPD activity in 5-fluorouracil elimination patterns, the variability in DPD activity may have dramatic impact on clinical outcome of patients. Thus, treatment of patients with DPD deficiency with fluoropyrimidines can result in severe toxicity, which impacts the benefit-risk balance of fluoropyrimidines in this subpopulation.

As part of this review, the MAHs were requested to provide information on the clinical consequences of partial and full DPD deficiency with the use of their product(s). Based on the data available, and despite the safety datasets carrying limitations in relation to lack or incomplete diagnostic of DPD activity (only few patients were tested), unavailability of related doses at individual levels, reporting bias and retrospective analysis, it was noted that there were high(er) rates of serious and fatal cases in DPD deficient patients as compared to either control (whole population) or non-deficient patients; patients with low or absent DPD activity were at increased risk for severe, life-threatening, or fatal adverse reactions; different variations of DPD deficiency were showed to be associated with specific AEs; time to onset was compatible with the effects of a DPD deficiency (within the first treatment cycle); and a reduction of fluoropyrimidines dose in variant allele carriers resulted in decrease in drug related severe toxicity.

Overall, it can be concluded that by partially or totally impairing the catabolism of 5-fluorouracil, DPD deficiency is responsible for an important part of cases of life-threatening toxicities upon 5-fluorouracil intake in patients to whom the drug has been administered at standard dosages. These severe and life-threatening side effects include stomatitis, diarrhoea, mucosal inflammation, neutropenia and neurotoxicity.

Parenteral 5-flurouracil and its prodrugs capecitabine and tegafur are systemic fluoropyrimidines widely used in oncology as the backbone of a large percentage of current chemotherapy regimens across a broad spectrum of cancers. The increased risk of toxicity of parenteral 5-flurouracil,

capecitabine and tegafur in patients with complete DPD deficiency is known and the benefit-risk of these products is considered unfavourable in this patient population. As outcome of this review, PRAC confirmed that a contra-indication in patients with known complete DPD deficiency should be included in the product information of all 5-fluorouracil (i.v.), capecitabine and tegafur containing products, as well as information on the risks for life-threatening or fatal toxicity in this patient population.

For these products, the clinical situation in case of partial loss of DPD activity is less clear. Partial DPD deficiency is also associated with an increased risk for severe toxicity, but in the absence of suitable alternative treatment, patients may be treated with caution. A reduced starting dose should be considered to limit this toxicity. DPD deficiency should be considered as a parameter to be taken into account in conjunction with other routine measures for dose reduction. Initial dose reduction may impact the efficacy of treatment. In the absence of serious toxicity, subsequent doses may be increased with careful monitoring.

Unlike fluoropyrimidine exposure in cancer, systemic availability of 5-fluorouracil is usually very low after topical application. In the 5% fluorouracil formulation treated patients, with measurable plasma concentrations of 5-fluorouracil and sufficient data points for calculation of pharmacokinetic parameters, the AUC ranged from 14.507 to 37.518 ng-h/ml, which is 100-1,000 times below recommended AUC for fluoropyrimidine-based therapy in cancer. Hence, the benefit-risk balance of topical 5-fluorouracil formulations in all authorised indications remains unchanged in patients with complete or partial DPD deficiency. PRAC however considers that a warning should be introduced in the product information of topical formulations of 5-fluorouracil containing products to inform on the potential increased risk of toxicity in patients with DPD deficiency in case of systemic absorption of 5-fluorouracil (depending on the strength of 5-fluorouracil in the product), together with a guidance to stop treatment in case of suspected systemic toxicity for the higher strengths (topical 5-fluorouracil 5%).

Fluorouracil is a metabolite of flucytosine (5-FC). DPD is a key enzyme involved in the metabolism and elimination of fluorouracil and although only a small amount of flucytosine is metabolised into fluorouracil, the risk of fluorouracil-induced severe toxicities due to DPD deficiency cannot be completely ruled out. On this basis, PRAC considers that flucytosine should not be used in patients with known complete DPD deficiency.

To evaluate methods to identify patients with partial or complete DPD deficiency prior to treatment and mitigate the risk of severe or life-threating toxicities, and their feasibilities, the PRAC has considered data submitted during the referral by the marketing authorisation holders of the products concerned in relation to the risk of toxicity associated with dihydropyrimidine dehydrogenase (DPD) deficiency and to the different screening methods currently available to identify patients with DPD deficiency, as well as an analysis of EudraVigilance data and third parties' interventions. The PRAC also took into account the outcome of a consultation with the oncology scientific advisory group and the pharmacogenomic working party.

Identification of completely and partially DPD deficient patients can guide the decision as to who should not be treated with fluoropyrimidines and who should be treated with a reduced dose, due to their increased risk for severe or life-threatening toxicities. Genotyping and phenotyping are to date considered to be the best available methods for identification of DPD deficient patients but both methods have some limitations.

Pre-treatment testing for rare mutations of the DPYD gene can identify patients with DPD deficiency. The four DPYD variants c.1905+1G>A [also known as DPYD*2A], c.1679T>G [DPYD*13], c.2846A>T and c.1236G>A/HapB3 can cause complete absence or reduction of DPD enzymatic activity. Other rare variants may also be associated with an increased risk of severe or life-threatening toxicity. Patients

with certain heterozygous DPYD variants (including c.1905+1G>A, c.1679T>G, c.2846A>T and c.1236G>A/HapB3 variants) have increased risk of severe toxicity when treated with fluoropyrimidines. Certain homozygous and compound heterozygous mutations in the DPYD gene locus (e.g. combinations of the four variants with at least one allele of c.1905+1G>A or c.1679T>G) are known to cause complete or near complete absence of DPD enzymatic activity.

The frequency of the heterozygous c.1905+1G>A 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. Data on the frequency of the four DPYD variants in other populations than Caucasian is limited. At the present, the four DPYD variants (c.1905+1G>A, c.1679T>G, c.2846A>T and c.1236G>A/HapB3) are considered virtually absent in populations of African (-American) or Asian origin.

Overall, genotyping DPYD provides rapid and univocal information, using techniques that can be automated and made widely available. Sorting patients on their genotype is usually considered to have an acceptable specificity but is hampered by poor sensitivity, due to the presence of other variants not investigated in routine and/or due to the potential non-genetic origin of DPD deficiency. However, low sensitivity (5.3%) and negative predictive value (68.0%) were also observed.

To overcome some limitations of genotyping, phenotyping methods for measuring DPD activity have been developed. Less evidence is available on phenotyping methods as most clinical reports published thus far are monocentric or retrospective studies. Phenotyping requires strict and constraining pre-analytical treatment of samples that can be hardly automated. Currently available results show better (negative) predictive performance (sensitivity) for phenotyping methods as compared to genotyping, but specificity and sensitivity of phenotyping DPD as a mean to detect patients at risk for severe toxicity remains to be further evaluated. Measurement of endogenous plasma uracil levels (U) as a surrogate of the DPD enzyme activity, has been identified as the most clinically useful phenotyping method. There are, however, uncertainties on uracil cut-off levels defining complete and partial DPD deficiency, as these have not been validated prospectively. In addition, solid data on both safety and efficacy of adaptive dosing following a test results of DPD phenotyping are lacking.

Based on the data currently available, PRAC is of the view that a blood uracil level $\geq$ 16 ng/ml and < 150 ng/ml should be considered indicative of partial DPD deficiency and associated with an increased risk for fluoropyrimidine toxicity. A blood uracil level $\geq$ 150 ng/ml should be considered indicative of complete DPD deficiency and associated with a risk for life-threatening or fatal fluoropyrimidine toxicity. In order to better characterize the cut-off levels for DPD deficiency and related optimal dose adjustment, more research is still needed.

Two expert groups have been consulted on these methods in the context of this referral procedure. According to the SAG on oncology, the level of the available evidence does not allow to make conclusive recommendations on the most effective method and intervention for patients identified with partial DPD deficiency since no directly comparative data are available to assess the clinical utility of different approaches. The PgWP outlined that genotyping for DYPD variants is easy to implement in clinical and laboratory practice. The PgWP further reported that genotyping for DYPD genetic variants will give measured, consistent outcomes (e.g., mutation yes/no), whereas phenotyping assays are prone to variation within and between laboratories. PgWP however highlighted that a drawback of genotyping is that specific but potentially relevant unknown DYPD variants will be missed, whereas phenotyping will capture these by measuring DPD enzymatic activity or a surrogate for this. Further, the occurrence of specific variants may differ between ethnicities, resulting in the situation that the detection rate of functionally altered alleles by genotyping may be less optimal in certain (non-Caucasian) populations, a problem not seen with phenotyping.

Overall, due to the limitations of the data available as described above, the inadequacies with routine clinical practice, which would require simple, rapid and inexpensive detection methods with good sensitivity and specificity, technical limitations, and the limited availability of testing facilities, no approach for detection of DPD status in patients stands yet as a standard. However, pre-treatment testing for DPD deficiency is considered a valuable tool to identify patients at increased risk for severe toxicity and is therefore recommended. Clinical availability and implementation is currently more advanced for genotyping than for phenotyping but it is acknowledged that both types of methods have pros and cons. In the absence of data comparing both methods, PRAC proposes that both methods are included in the product information of 5-fluorouracil (i.v.), capecitabine and tegafur containing medicinal products, as possible approaches to identify DPD deficient patients.

For patients requiring treatment with flucytosine though, pre-treatment DPD testing may not be compatible with the need for immediate treatment required for systemic yeast and fungal infections and is therefore not required.

Increasing efforts have been placed on optimising 5-fluorouracil dosing with the main goals of increasing antitumor efficacy while reducing drug-associated toxicity. There is growing evidence to show that 5-fluorouracil dosing based on plasma 5-fluorouracil drug level is feasible and that 5-fluorouracil therapeutic drug monitoring can improve clinical outcomes. Therapeutic drug monitoring is a useful tool for characterizing the actual exposure to 5-fluorouracil drug in an individual patient during chemotherapy given that 5-fluorouracil is characterised by a strong exposure-toxicity relationship and a narrow therapeutic window. The area under the curve (AUC) of 5-fluorouracil concentrations is considered the most relevant PK parameter associated to 5-fluorouracil related efficacy and toxicity and it is generally considered that an AUC range of 20-30 mg*h/L is required for successful therapy in patients treated with 5-fluorouracil i.v.. TDM may constitute a valuable complementary method to the upfront DPD deficiency detection methods such as phenotyping or genotyping and overcome the limited knowledge on safety and efficacy of a reduced dose. Combining upfront phenotyping or genotyping with TDM can improve the benefit-risk balance of 5-fluorouracil-based therapy in patients with DPD deficiency and information about TDM is recommended to be included in the SmPC of 5-fluorouracil (i.v.) containing products. As 5-Fluorouracil concentrations in plasma after administration of capecitabine or tegafur are an indirect reflection of the true exposure of tissues to 5-fluorouracil, and as systemic exposure to capecitabine or tegafur, and metabolites in plasma appears to be poorly predictive of safety and efficacy, TDM for capecitabine and tegafur is not recommended.

The optimal treatment of patients with partial DPD deficiency as well as the best testing methodology to identify patients at increased risk for severe toxicity remains uncertain and should be further explored. MAHs and other relevant stakeholders, including academia, are encouraged to perform further research focussing on current gaps and uncertainties in knowledge, including but not exclusive to, the optimal test method to identify patients at risk of severe DPD-associated toxicity, the optimal dose for patients tested positive for partial DPD deficiency, clinical outcome in terms of efficacy (OS, PFS) and safety (frequency of ≥ grade 3 toxicity) in patients with partial DPD deficiency, the robustness of the proposed upper (>150 ng/ml) and lower (≤16 ng/ml) cut-off values for uracilemia to discriminate patients with normal DPD activity, partial DPD deficiency and complete DPD deficiency, and the implementation of the recommendation to screen patients for DPD deficiency and to use TDM in the different EU MS.

6. Risk management

6.1. Risk minimisation activities

6.1.1. Amendments to the product information

For 5-fluorouracil, capecitabine, tegafur and flucytosine containing products, the PRAC considered that routine risk minimisation measures in the form of updates to the product information would be necessary in order to minimise the risk of increased toxicity in patients with DPD deficiency.

PRAC considered that use of 5-fluorouracil (i.v.), capecitabine, tegafur and flucytosine containing products should be contraindicated in patients with known complete DPD deficiency. In addition, further warnings and precautions of use relating to the increased risk of toxicity in patients with DPD deficiency were also included in the product information of 5-fluorouracil (all formulations), capecitabine, tegafur and flucytosine containing products.

A recommendation to test for DPD deficiency prior to initiation of treatment as well as information on the currently most suitable testing methodologies to identify patients with DPD deficiencies was added in the product information of 5-fluorouracil (i.v.), capecitabine, tegafur. In addition, information about therapeutic drug monitoring in patients receiving continuous 5-fluorouracil infusions was added in the product information of 5-fluorouracil (i.v.).

The package leaflets were amended accordingly.

6.1.2. Direct Healthcare Professional Communications /Communication plan

The PRAC adopted two DHPCs to inform healthcare professionals of the outcome of the review for 5-fluorouracil, capecitabine and tegafur containing products as well as for flucytosine containing products. The PRAC also agreed on a communication plan for each DHPC.

7. Grounds for recommendation

Whereas,

- The PRAC considered the procedure under Article 31 of Directive 2001/83/EC resulting from pharmacovigilance data for medicinal products containing 5-fluorouracil and related substances.

- PRAC considered the totality of the data submitted during this review in relation to the risk of toxicity associated with dihydropyrimidine dehydrogenase (DPD) deficiency and to the different screening methods currently available to identify patients with DPD deficiency. These data included the responses submitted by the marketing authorisation holders in writing, an analysis of EudraVigilance data by EMA, third parties' interventions, as well as the outcome of consultation with the Oncology scientific advisory group and the EMA pharmacogenomic working party.

- PRAC confirmed the current knowledge that the use of 5-fluorouracil for systemic use and related substances in patients with DPD deficiency is associated with an increased risk of toxicity.

- The PRAC concluded that the benefit-risk balance of 5-fluorouracil (i.v.) and related substances capecitabine, tegafur and flucytosine is negative in patients with complete DPD deficiency and

confirmed that these medicinal products should be contra-indicated in patients with known complete DPD deficiency. PRAC also concluded that patients with partial DPD deficiency should be treated with an adjusted starting dose.

- To minimise the risk of increased toxicity, PRAC recommended that DPD deficiency testing is conducted before initiation of treatment. PRAC considered genotyping and phenotyping by evaluation of blood uracil levels tests as being currently the most suitable methods to identify patients with DPD deficiency. Although both methods have limitations, PRAC agreed that the product information of 5-fluorouracil (i.v.), capecitabine and tegafur containing products should provide information on these two testing methodologies together with a guidance to consider applicable clinical guidelines.

- For patients requiring treatment with flucytosine, PRAC considered that pre-treatment DPD testing would not be compatible with the need for immediate treatment required for systemic yeast and fungal infections and therefore agreed that pre-treatment testing for DPD deficiency is not required.

- Taking into account the low systemic availability of 5-fluorouracil after topical application, PRAC concluded that the benefit-risk balance of topical 5-fluorouracil formulations remains unchanged in all authorised indications but that information on the risk of toxicity in patients with DPD deficiency in case of systemic exposure should be introduced in the product information.

- PRAC also agreed on direct healthcare professional communications (DHPC), together with the timelines for their distribution.

In view of the above, the Committee considers that the benefit-risk balance of 5-fluorouracil and related substances capecitabine, tegafur and flucytosine containing products remains favourable subject to the agreed amendments to the product information.

The Committee, as a consequence, recommends the variation to the terms of the marketing authorisations for medicinal products containing 5-fluorouracil or related substances capecitabine, flucytosine and tegafur.

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Xeloda Summary of Product Characteristics (SmPC)