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Pharmacovigilance and Epidemiology Department

# Dihydropyrimidine dehydrogenase deficiency related toxicities to fluorouracil and fluorouracil related substances containing medicinal products

Report of EudraVigilance analysis and literature review

Procedure number: 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

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## 1. Roles and responsibilities

| Role | Responsible |
|---|---|
| Lead investigator | Luis Pinheiro |
| Investigator | Julie Durand |
| Procedure lead | Veronique Le Ber |
| Data extraction | Luis Pinheiro |
| Review | Georgy Genov, Peter Arlett |
| Sign-off | Peter Arlett |

## 2. Milestones

| Milestone | Planned | Actual |
|---|---|---|
| Data analysis plan | 26 March 2019 | 26 March 2019 |
| Internal report | 20 May 2019 | 17 June 2019 |
| Peer-review | NA | 19 June 2019 |
| Implementation of corrections | NA | 19 June 2019 |
| Submission | NA | 19 June 2019 |

## 3. Objectives

The primary objectives of the EudraVigilance analysis were:

- To identify and describe case reports to fluorouracil and fluorouracil 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.

The objective of the literature review was to source any new publications on DPD screening and toxicity published from May 2018 to March 2019.

# 4. Methodology

## *4.1. EudraVigilance analysis*

### 4.1.1. Database

The database used was EudraVigilance. The period of interest was from start of data collection (i.e. 1995) to 15 March 2019.

### 4.1.2. Ontology

The Medical Dictionary for Drug Regulatory Activities (MedDRA) v.21.1 was be used to code the outcomes of interest and extract the data.

### 4.1.3. Exposure

The exposure was defined as use of fluorouracil and fluorouracil related substances, namely capecitabine, fluorouracil, tegafur, flucytosine containing medicinal products.

Dihydropyrimidine dehydrogenase deficiency related toxicity might be masked. That is, reporters might not realise that a patient's genotype contributed to the toxicity. Similarly, it is also possible that reporters might attribute a causal relationship to a different product because they are unaware of the genotype of the patient. Thus, to define exposure, the characterisation of drug role [ICH E2B(R3) G.k.1] included suspect, interacting and concomitant.

### 4.1.4. Case definitions

The toxicity spectrum of DPD related toxicity is extensive. Accumulation of fluorouracil may lead to severe inflammation and ulceration of the gastrointestinal tract, mouth sores, abdominal pain, bleeding, nausea, vomiting, and diarrhoea. Fluoropyrimidine toxicity may also lead to neutropenia and infections. It can also be associated with thrombocytopenia and resulting haemorrhage. Other disorders include hand-foot syndrome, shortness of breath and hair loss.

There is no standardised MedDRA query or published algorithmic diagnostic criteria that can be used to readily classify cases of DPD related toxicity. Only the preferred term "Dihydropyrimidine dehydrogenase deficiency" exists as a MedDRA code under the Congenital, familial and genetic disorders System Organ Class.

This term refers solely to the presence of the mutation and is not fundamentally an adverse drug reaction. Due to its nature, reflecting a genetic mutation, it is, in fact, more likely to be part of the patient history.

#### *4.1.4.1. Dihydropyrimidine dehydrogenase deficiency patient*

The MedDRA preferred term "Dihydropyrimidine dehydrogenase deficiency" was used to identify patients with dihydropyrimidine dehydrogenase deficiency. The term was queried in three fields: the reaction field [ICH E2B(R3) E.i.2.1b], the test field [ICH E2B(R3) F.r.2.2b] and related fields [ICH E2B(R3)

F.r.3.1 to F.r.7] and the medical history field [ICH E2B(R3) D.7.1.r.1b]. Narratives were not queried due to computational power issues.

#### *4.1.4.2. Dihydropyrimidine dehydrogenase deficiency toxicity spectrum*

Whereas the manifestations of toxicity due to DPD are extensive, the terms within the following standardised MedDRA queries will be used as being indicative of DPD related toxicity:

- Gastrointestinal perforation, ulcer, haemorrhage, obstruction non-specific findings/procedures (SMQ),
- Gastrointestinal ulceration (SMQ)
- Gastrointestinal perforation (SMQ)
- Gastrointestinal haemorrhage (SMQ)
- Noninfectious diarrhoea (SMQ)
- Agranulocytosis (SMQ)
- Haematopoietic thrombocytopenia (SMQ)
- Haemorrhages (SMQ)

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

### 4.1.5. Covariates

Age, gender, indication for use, time-to-onset and origin of report were defined as relevant covariates. In EVDAS, certain values are imputed, such as some date variables and the patient onset age. Only the reported values in the variables were used. Imputed variables were recoded to missing.

Time to onset can be calculated at reaction level or at case level. As mentioned above, DPD deficiency can manifest in multiple disorders hence, to ensure comparability between cases, in this analysis it was assumed that all reactions are a spectrum of the same DPD toxicity, hence case level time to onset was used.

Time to onset was calculated by subtracting the earliest start date of any reaction within the DPD toxicity spectrum reported to the start date of the medicinal product. For instance, if the patient started the drug on 15/01/2000, had diarrhoea on 20/01/2000 and neutropenia on 30/01/2000, the time to onset was 5 days.

### 4.1.6. Analytical plan

Descriptive statistics was performed by substance, age, gender, indication for use, origin of reports, reaction and outcome for all case reports. Cases were highlighted according to whether they refer to a DPD patient (see 4.1.4.1. ) or mention any term within the toxicity spectrum (see 4.1.4.2. ).

Where feasible, boxplots of time to onset were plotted and stratified by product and indication for use.

In addition, the proportion of cases with life-threatening or fatal[1] reactions amongst DPD patients and those with DPD toxicity spectrum reactions was compared as were the proportions 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.

To estimate the number of likely DPD related cases, machine learning models were be run deployed (see Annex I). These identify patterns in the terms reported to DPD patients and detect similar patterns in cases were DPD status is unknown.

Features to be used to model DPD related toxicity cases include age, gender and adverse drug reactions. The most relevant variables in each model were profiled and compared to the case definition.

### *4.2. Literature review*

The aim of the literature review was to identify published papers on DPD screening since the last review conducted by the *Institut National du Cancer*[2]. Thus, a methodology similar to that applied by the *Institut National du Cancer* was used.

There are some differences however. The new literature review did not filter on products or on type of study. This means the search was not as restrictive and a higher recall was achieved. The downside is a risk of lower precision however, as the time window is of just one year (except for tegafur and flucytosine), and manual review by two investigators was performed, this seems an acceptable trade-off and ensures all relevant publications on DPD were included.

#### 4.2.1. Databases of references and abstracts

The databases of abstracts and references used were Medline – PubMed and EMBASE. The Cochrane reviews website was also queried.

#### 4.2.2. Period of publications

The period of publications was May 2018 to March 2019, except for tegafur and flucytosine containing products as they were not included in the previous analysis.

#### 4.2.3. Search algorithm

Three distinct search algorithms were used in the different databases of abstracts and references and the Cochrane reviews website.

[1] Fatal code, reaction / case outcome fatal or PT 'death'

[2] DPD deficiency screening with a view to preventing some severe toxicities occurring with treatments including fluoropyrimidines – Haute Autorité de Santé / Institut National du Cancer – December 2018

| Database of abstracts and references | Search algorithm |
|---|---|
| Medline - PubMed | (dihydropyrimidine dehydrogenase OR dpyd OR dihydrouracil dehydrogenase OR dihydropyrimidine dehydrogenase deficiency OR DPYD*2A OR c.2846A>T OR c.1679T>G OR c.1236G>A)<br><br>AND<br><br>((("2018/04/01"[Date - Publication] : "3000"[Date - Publication]))* |
| EMBASE | ('dihydropyrimidine dehydrogenase'/exp OR 'dihydrouracil dehydrogenase'/exp OR 'dihydropyrimidine dehydrogenase deficiency'/exp OR DPYD*2A OR c.2846A OR c.1679T OR c.1236G)<br><br>AND<br><br>[1-4-2018]/sd* |
| Cochrane | "dihydropyrimidine dehydrogenase" with Cochrane Library publication date Between Apr 2018 and Mar 2019*<br><br>AND<br><br>“dihydrouracil dehydrogenase” with Cochrane Library publication date Between Apr 2018 and Mar 2019*<br><br>AND<br><br>“dihydropyrimidine dehydrogenase deficiency” with Cochrane Library publication date Between Apr 2018 and Mar 2019* |

* except for tegafur and flucytosine (from inception)

#### 4.2.4. Study classification and selection

Two investigators performed study classification and selection independently. All combined studies selected by the investigators were included in a summary table. Papers were sourced, where possible.

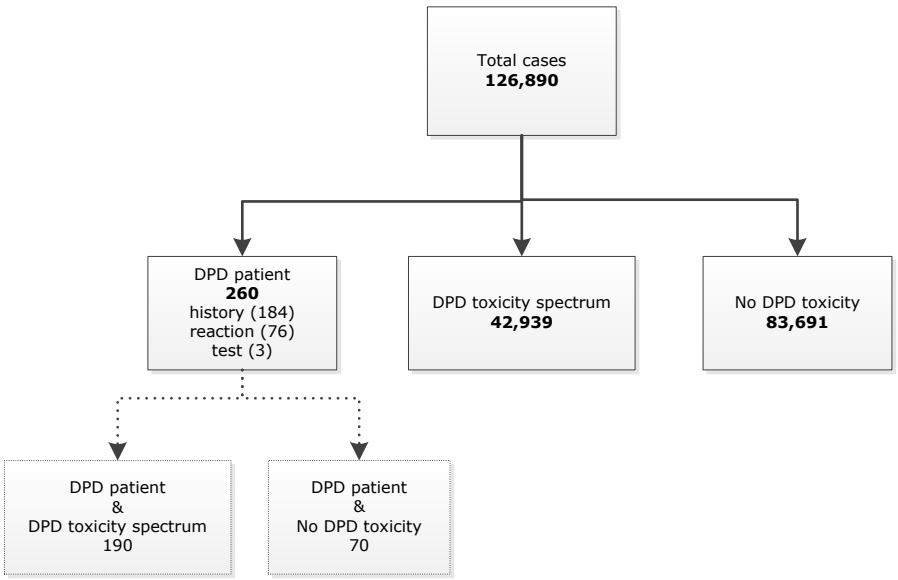
## 5. Results

### *5.1. Descriptive analyses*

There are 126,890 individual case reports in EudraVigilance with capecitabine, fluorouracil, tegafur, or flucytosine containing medicinal products reported as suspect, interacting or concomitant. These cases have not been screened for potential duplicates.

Based on the case definitions (see 4.1.4), DPD deficiency could be ascertained in 260 case reports (‘DPD patient’ group), the majority (70.8%) through the medical history field. A further 42,939 cases report one or more reactions within the DPD toxicity spectrum (‘DPD toxicity spectrum’ group). Approximately two thirds of the case reports neither refer to DPD patients nor report any reactions within the DPD toxicity spectrum as per the case definitions (‘No DPD toxicity’ group). Case reports with both ‘DPD patients’ and

'DPD toxicity spectrum' reactions, as well as cases in DPD patients but without DPD toxicity spectrum reactions are counted only within the 'DPD patient' group, i.e. there is no overlap between the three groups.




Of note, there were 5 confirmed DPD 'normal' patients based on the test fields, 3 of which experienced DPD toxicity spectrum reactions.

### 5.1.1. Age and gender

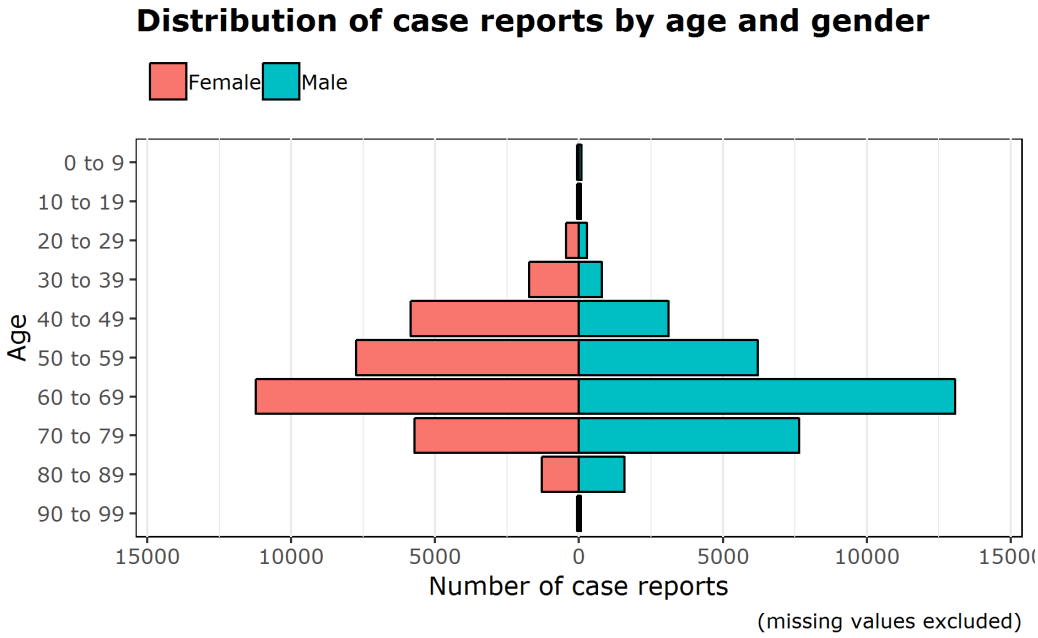
The reports refer more frequently to female patients (48.4%) and patients aged 60 to 69 years (19.9%) (Table 1~~Table 1~~). It should be noted, however, that age is missing from nearly half of the reports.

Forma

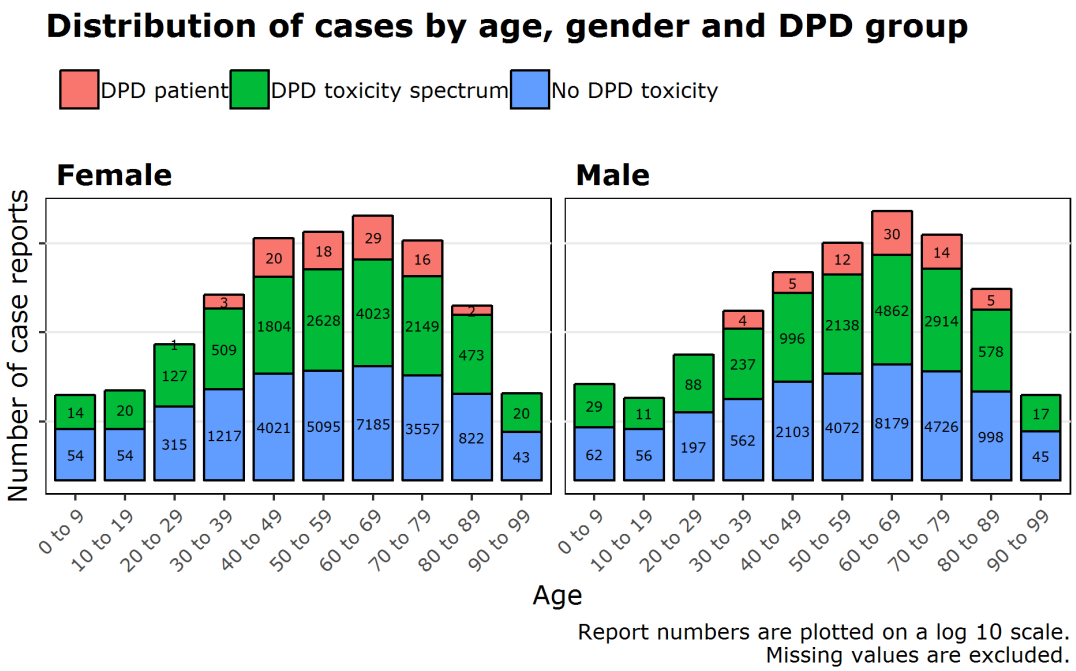
**Table 1**

| Age Group | Female | Male | Not reported | Totals |
|---|---|---|---|---|
| 0 to 9 | 68 (0.1%) | 91 (0.1%) | 35 (0.0%) | 194 (0.2%) |
| 10 to 19 | 74 (0.1%) | 67 (0.1%) | 8 (0.0%) | 149 (0.1%) |
| 20 to 29 | 443 (0.3%) | 285 (0.2%) | 14 (0.0%) | 742 (0.6%) |
| 30 to 39 | 1729 (1.4%) | 803 (0.6%) | 70 (0.1%) | 2602 (2.1%) |
| 40 to 49 | 5845 (4.6%) | 3104 (2.4%) | 258 (0.2%) | 9207 (7.3%) |
| 50 to 59 | 7741 (6.1%) | 6222 (4.9%) | 483 (0.4%) | 14446 (11.4%) |
| 60 to 69 | 11237 (8.9%) | 13071 (10.3%) | 936 (0.7%) | 25244 (19.9%) |
| 70 to 79 | 5722 (4.5%) | 7654 (6.0%) | 567 (0.4%) | 13943 (11.0%) |
| 80 to 89 | 1297 (1.0%) | 1581 (1.2%) | 223 (0.2%) | 3101 (2.4%) |
| 90 to 99 | 63 (0.0%) | 62 (0.0%) | 27 (0.0%) | 152 (0.1%) |
| Other/unknown | 27157 (21.4%) | 22015 (17.3%) | 7938 (6.3%) | 57110 (45.0%) |
| *Totals* | *61376 (48.4%)* | *54955 (43.3%)* | *10559 (8.3%)* | *126890* |

Field C


Distribution of case reports by age and gender
Female
Male
0 to 9
10 to 19
20 to 29
30 to 39
40 to 49
50 to 59
60 to 69
70 to 79
80 to 89
90 to 99
Age
15000
10000
5000
0
5000
10000
Number of case reports
(missing values excluded)

DPD patients are reported in similar proportions in male (0.17%) and female (0.21%) patients, mainly across the 30 to 89 age groups.




### 5.1.2. Substance

The vast majority of reports (92.1%) relate to fluorouracil- or capecitabine containing drugs (Table 2~~Table 2~~).

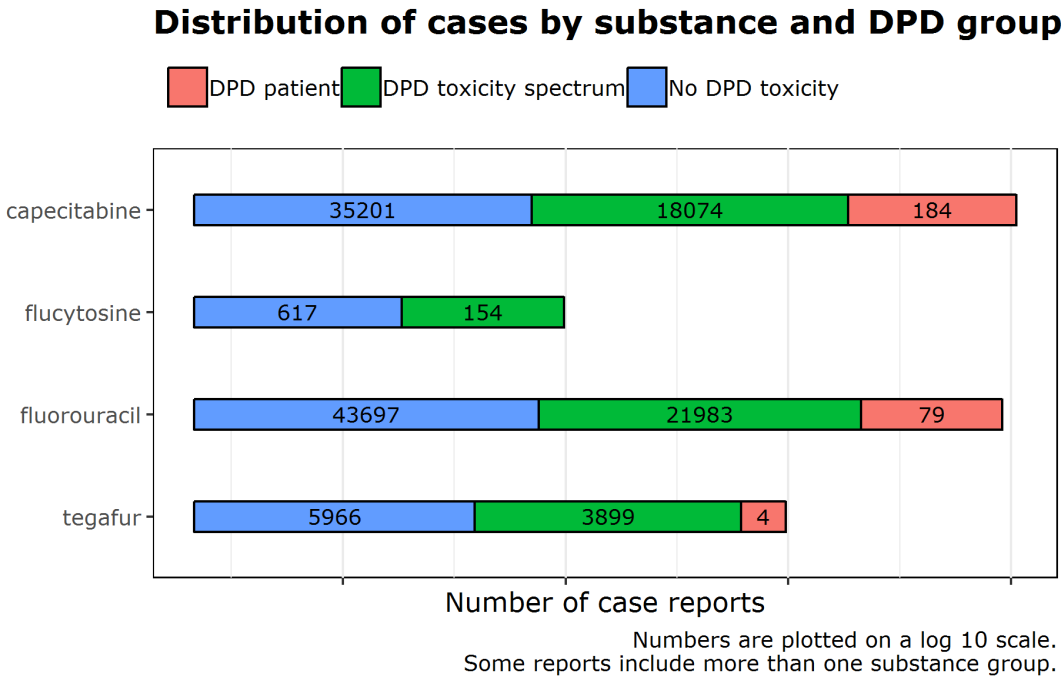
**Table 2**

| Substance group | Reported substances and combinations | Count cases* |
|---|---|---|
| **capecitabine** | capecitabine | 53451 |
| | oxaliplatin, capecitabine | 41 |
| | irinotecan, capecitabine | 6 |
| | capecitabine, ruxolitinib | 1 |
| | ***total*** | ***53459 (42.1%)*** |
| **flucytosine** | flucytosine | 771 |
| | ***total*** | ***771 (0.6%)*** |
| **fluorouracil** | fluorouracil | 65554 |
| | fluorouracil, oxaliplatin, folinic acid | 163 |
| | fluorouracil, salicylic acid | 121 |

| | | |
|---|---|---|
| | fluorouracil, irinotecan, folinic acid | 81 |
| | cyclophosphamide, fluorouracil, methotrexate | 18 |
| | epirubicin, cyclophosphamide, fluorouracil | 11 |
| | fluorouracil, folinic acid | 8 |
| | irinotecan hydrochloride, calcium folinate, fluorouracil, oxaliplatin | 8 |
| | calcium folinate, fluorouracil, oxaliplatin | 6 |
| | cisplatin, fluorouracil | 3 |
| | fluorouracil, tegafur, gimeracil | 3 |
| | fluorouracil, irinotecan, folinic acid, bevacizumab | 2 |
| | fluorouracil, oxaliplatin | 2 |
| | fluorouracil, oxaliplatin, folinic acid, bevacizumab | 2 |
| | doxorubicin, cyclophosphamide, fluorouracil | 1 |
| | doxorubicin, fluorouracil | 1 |
| | fluorouracil, gemcitabine | 1 |
| | heparin, fluorouracil | 1 |
| | irinotecan hydrochloride, calcium folinate, fluorouracil | 1 |
| | tamoxifen citrate, cyclophosphamide, fluorouracil, methotrexate | 1 |
| | ***total*** | ***65759 (51.8%)*** |
| **tegafur** | tegafur, gimeracil, oteracil potassium | 6914 |
| | tegafur, uracil | 2621 |
| | tegafur | 424 |
| | tegafur, gimeracil | 1 |
| | ***total*** | ***9869 (7.8%)*** |

* Reports may include more than one substance group.

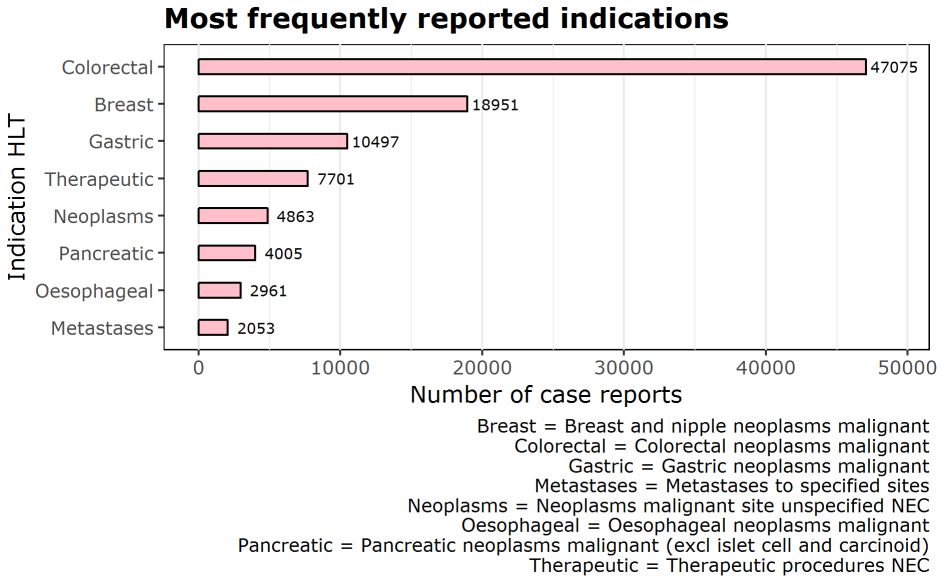
DPD patients more frequently received capecitabine, both in absolute (184) and relative numbers (0.34% of capecitabine reports). No known DPD patient received flucytosine.

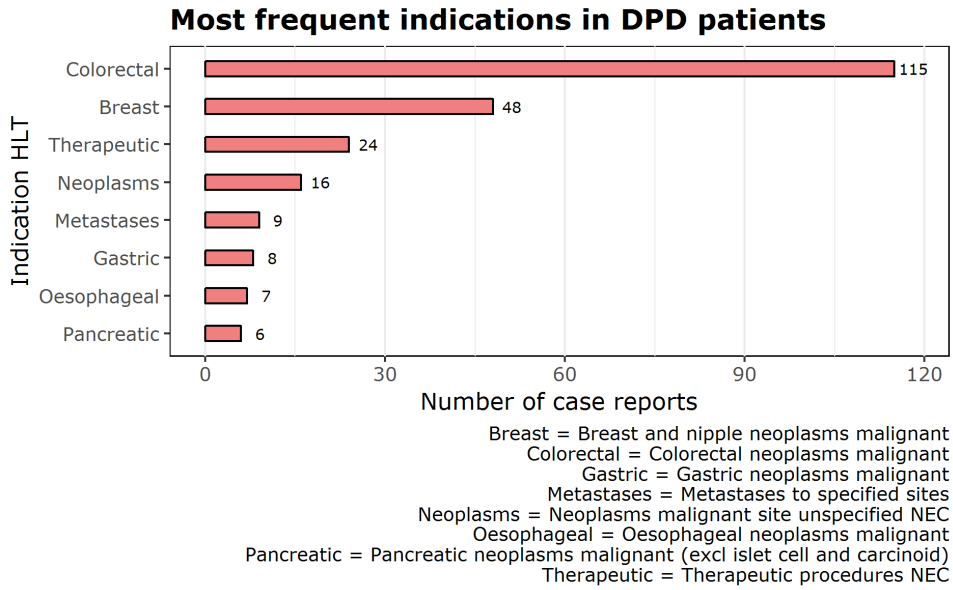


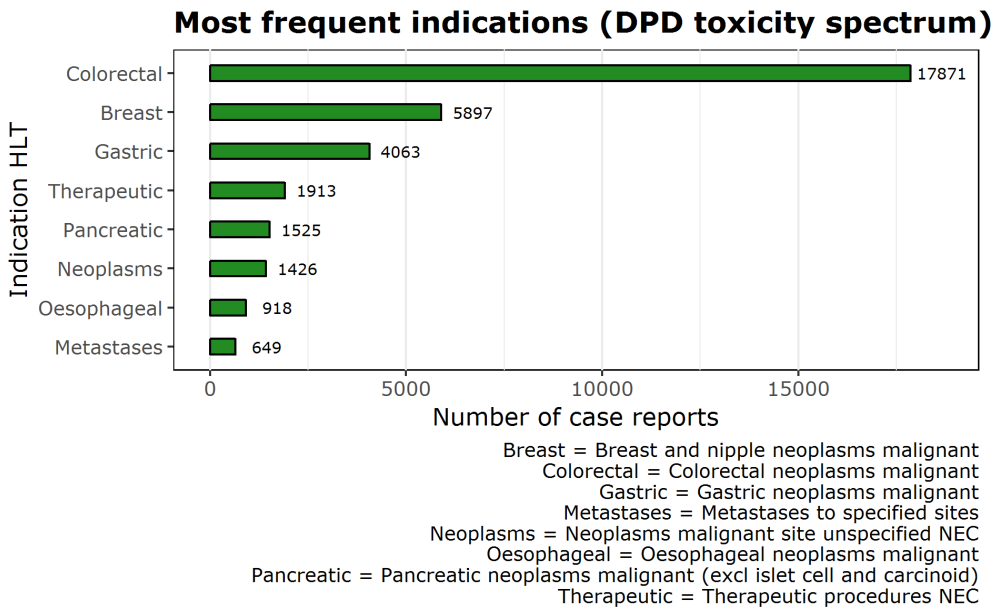

Numbers are plotted on a log 10 scale.
Some reports include more than one substance group.

### 5.1.3. Indication

Colorectal cancer was the most frequently reported indication, followed by breast malignancies. Of note, the indication was missing from 30471 reports (not shown in the graph below). A similar distribution of indications is observed amongst the 'DPD patient' and 'DPD toxicity spectrum' groups.

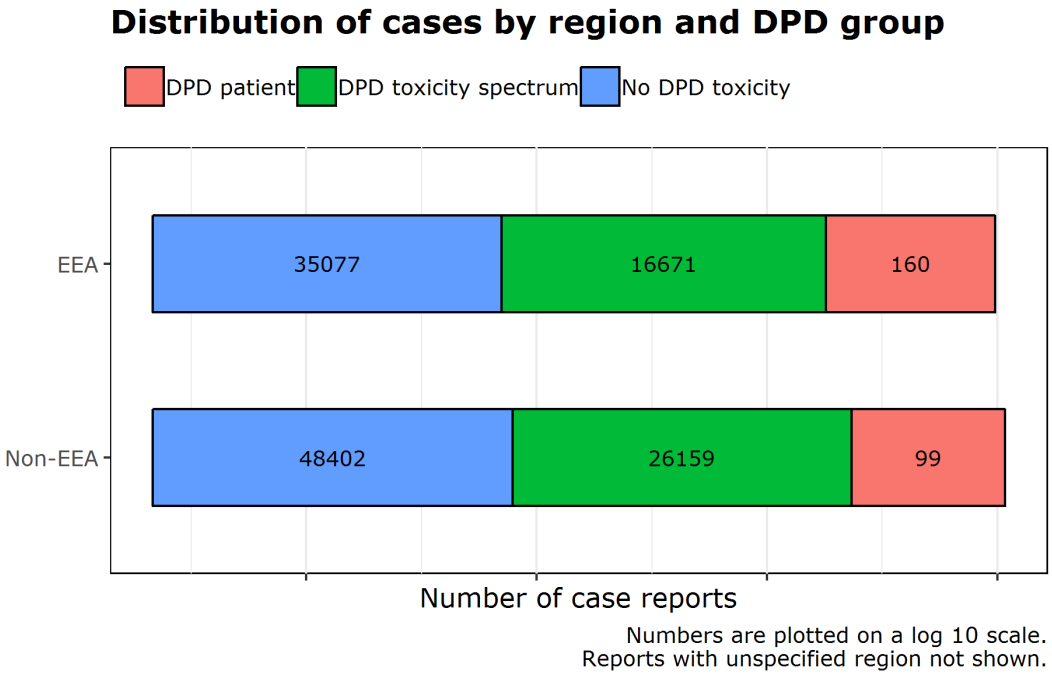


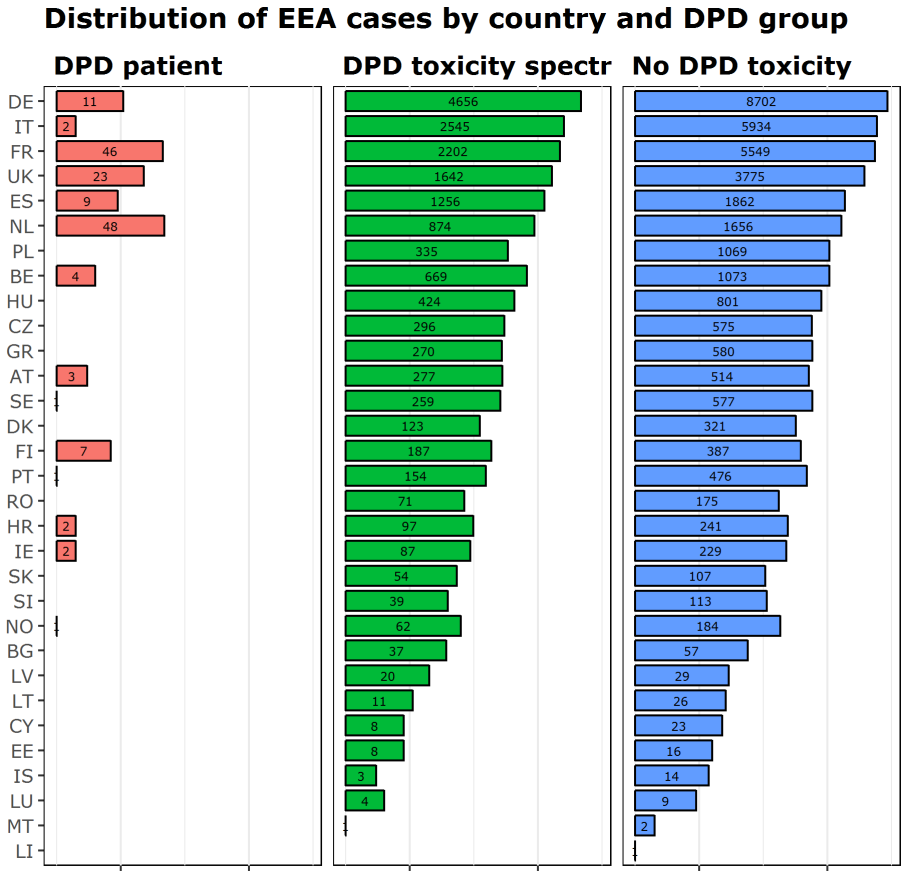



### 5.1.4. Origin

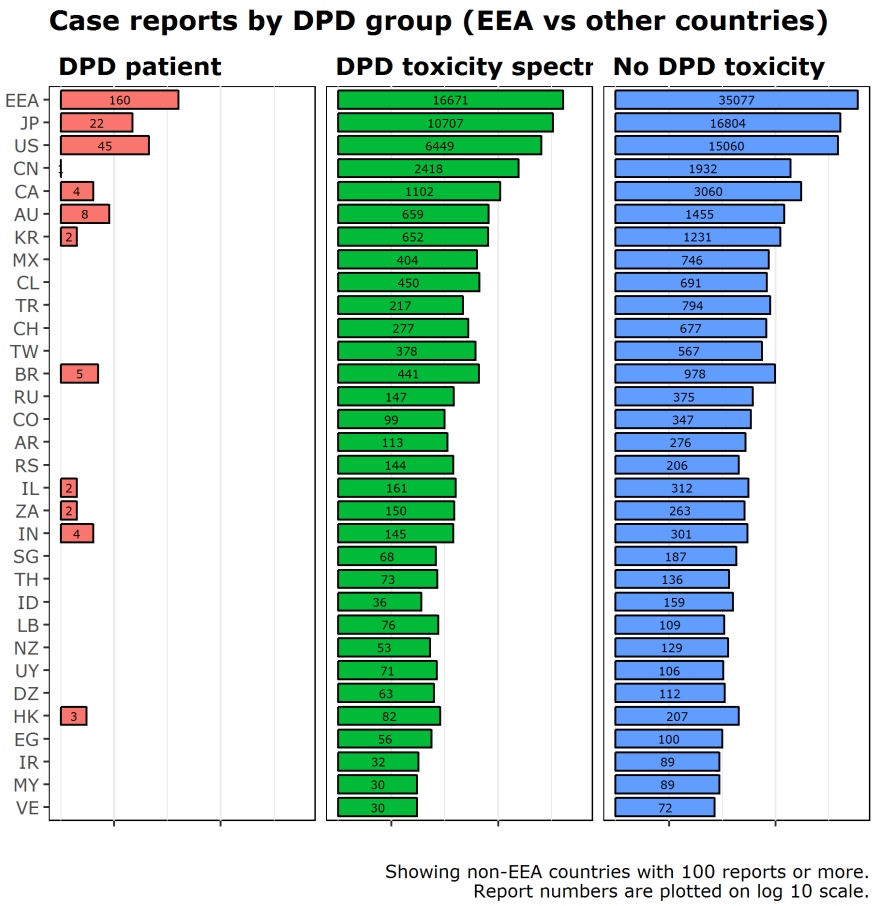
Although the majority (58.8%) of case reports originate from non-EEA countries, there is a higher number of DPD patients amongst EEA reports (+ 61.6%).



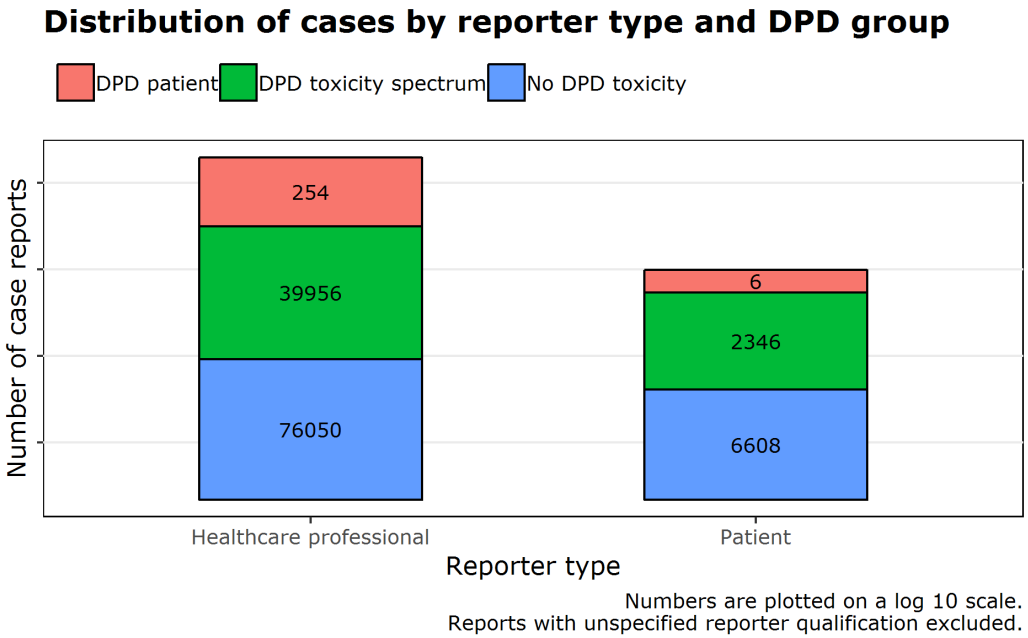
Within the EEA, while Germany accounts for the highest number of reports both overall and within the DPD toxicity spectrum, DPD patients are more frequently seen in the Netherlands (48), France (46) and the UK (23). Outside the EEA, reports most frequently originate from Japan, while DPD patients are more frequently from the US.




Report numbers are plotted on log 10 scale.

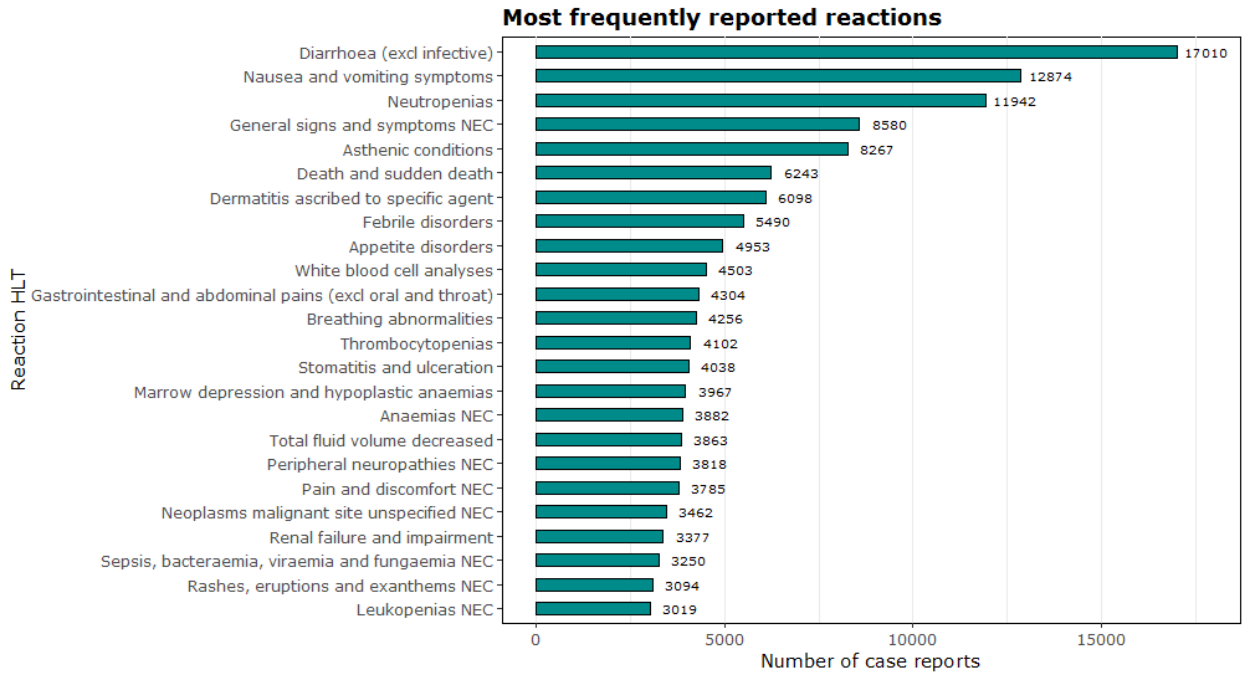



Case reports mostly originate from healthcare professionals (91.6%).

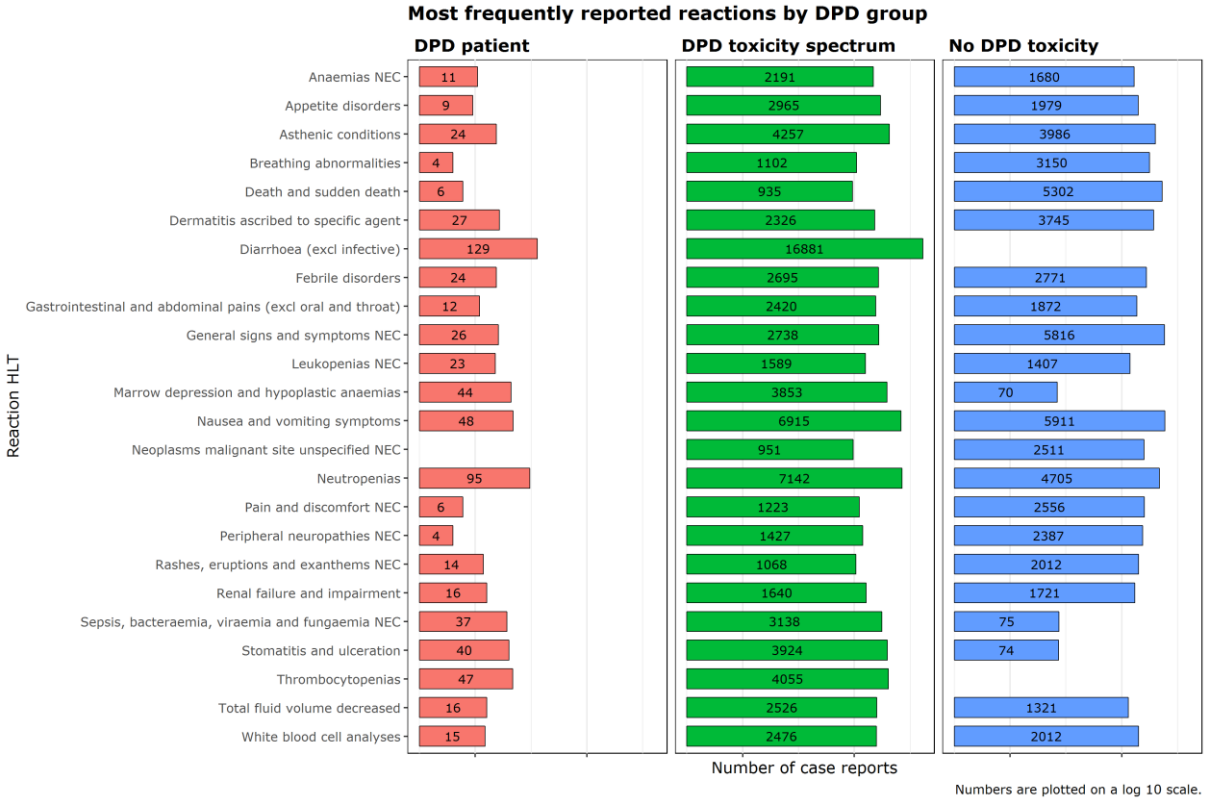


### 5.1.5. Reaction

Gastrointestinal disorders (diarrhoea, nausea and vomiting) are the most frequently reported reactions, followed by neutropenias.

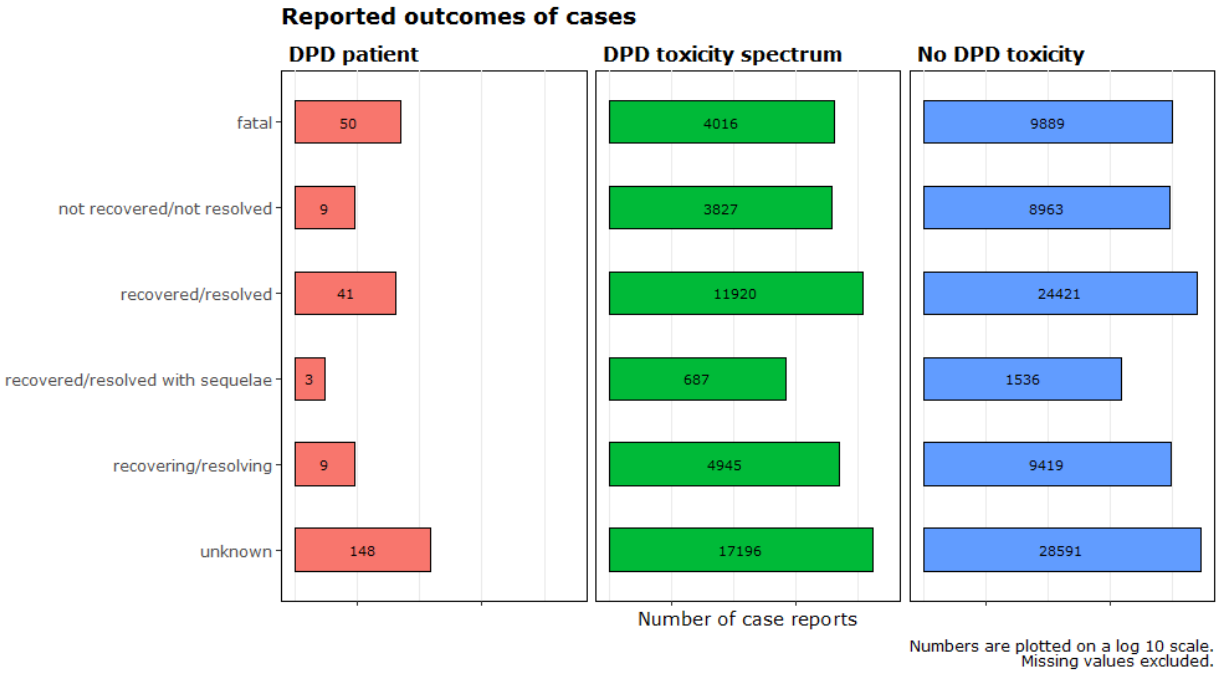


Amongst the DPD patients, diarrhoea and neutropenias are the most frequent reactions. Noticeably due to assumptions in the case definitions, all cases of diarrhoea and thrombocytopenia will appear under DPD patients and DPD toxicity spectrum, even though the underlying malignancy and other treatments might cause these reactions.

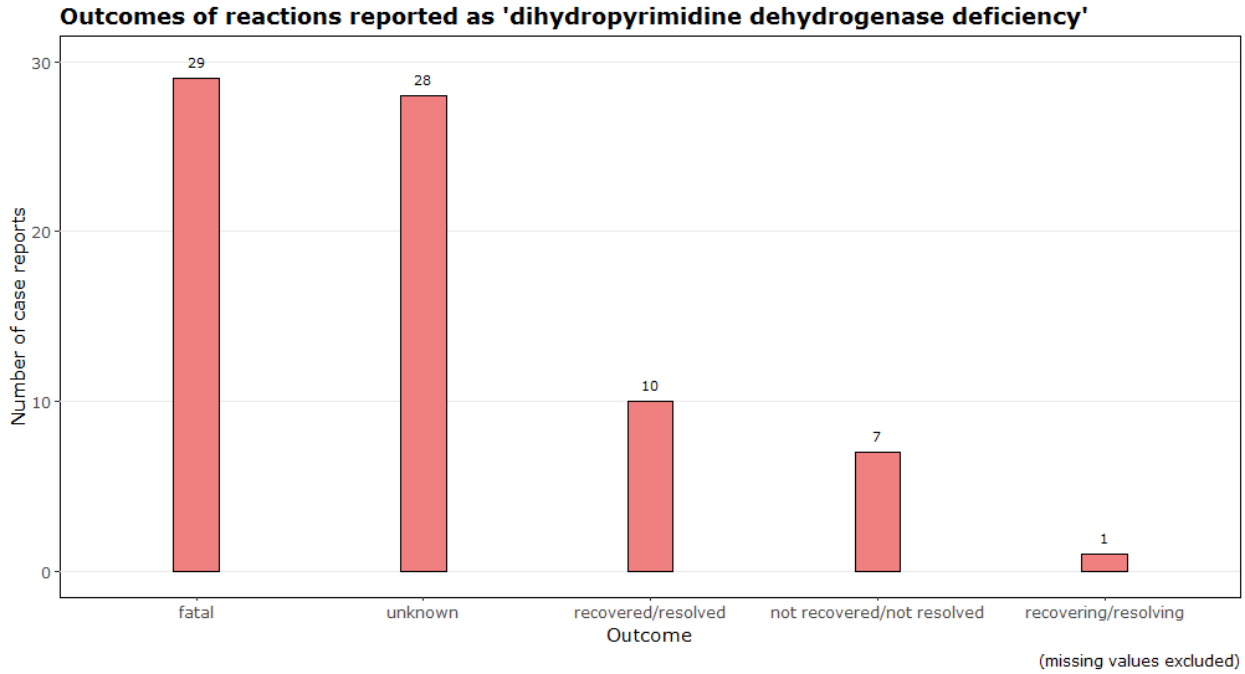


### 5.1.6. Outcome

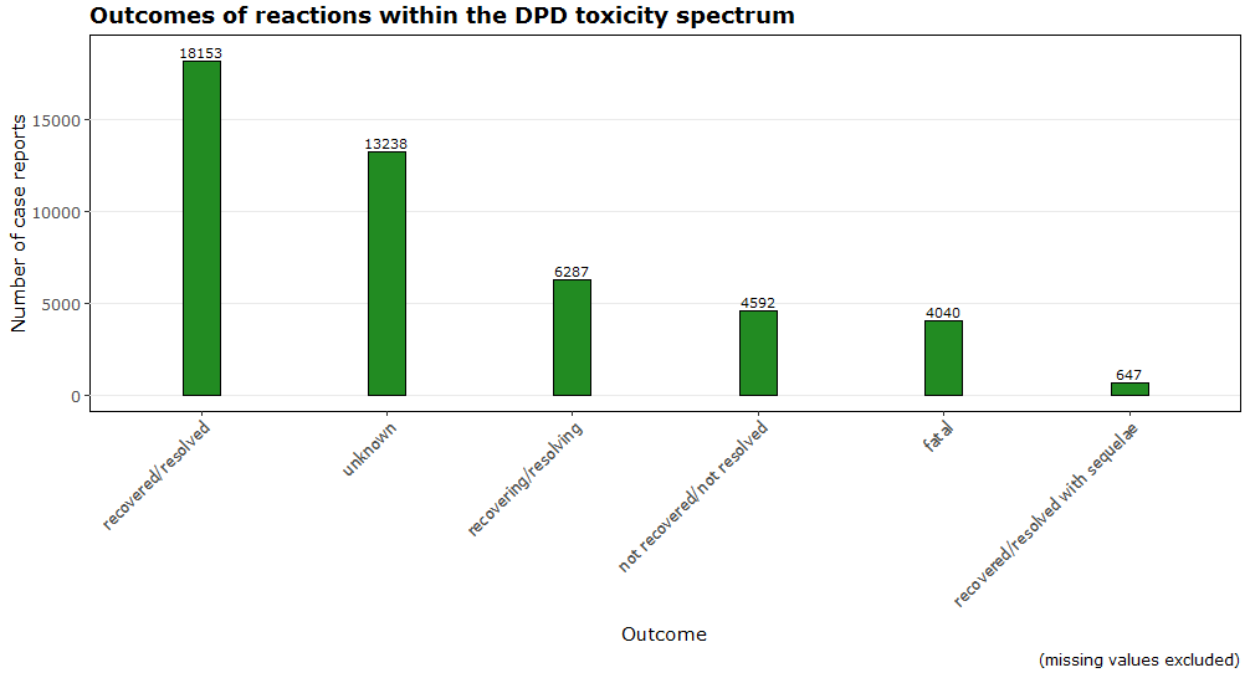
When considering outcome at case level, the fatality rate is 19.2% in DPD patients, which is twice as high as the fatality rate within the DPD toxicity spectrum (9.4%) and the 'no DPD toxicity' group (11.8%).




When looking at the reactions coded as 'dihydropyrimidine dehydrogenase deficiency' (76 cases), the fatality rate is 38.2%.

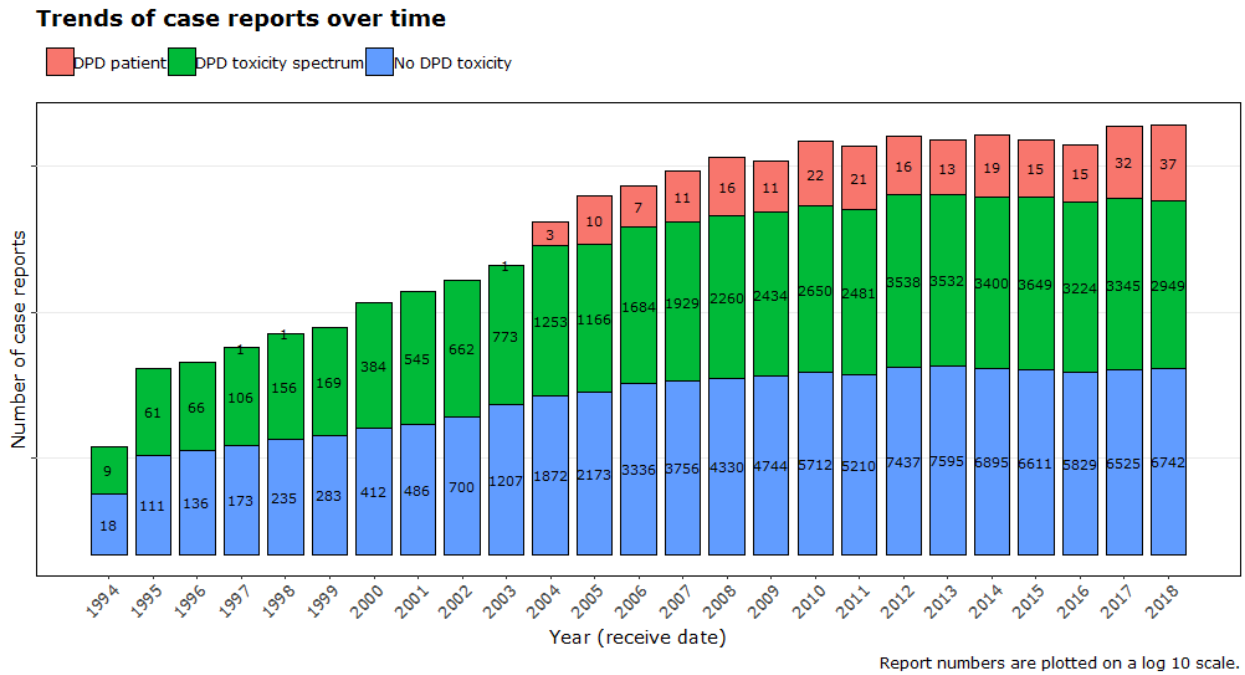


DPD spectrum reactions have a fatality rate of 8.5%.




### 5.1.7. Trend over time

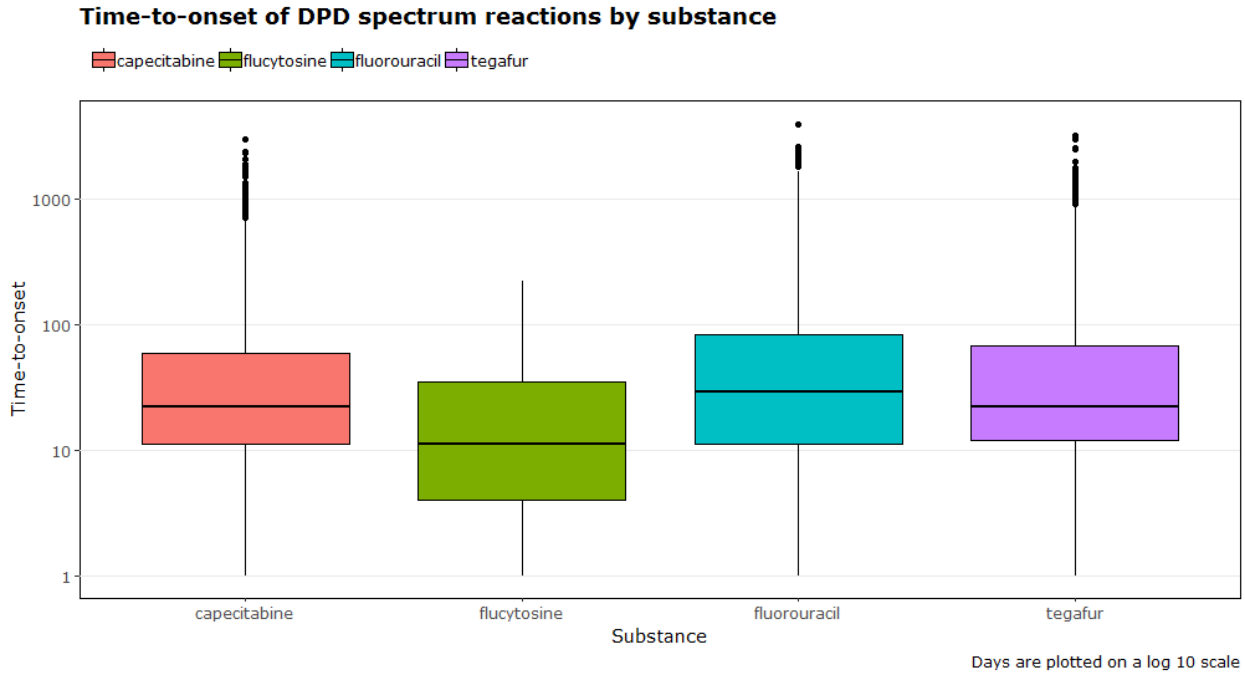
There has been a steady increase in the number of DPD patient cases since 2014, with a small increase over the past two years, possibly attributable to increasing awareness.



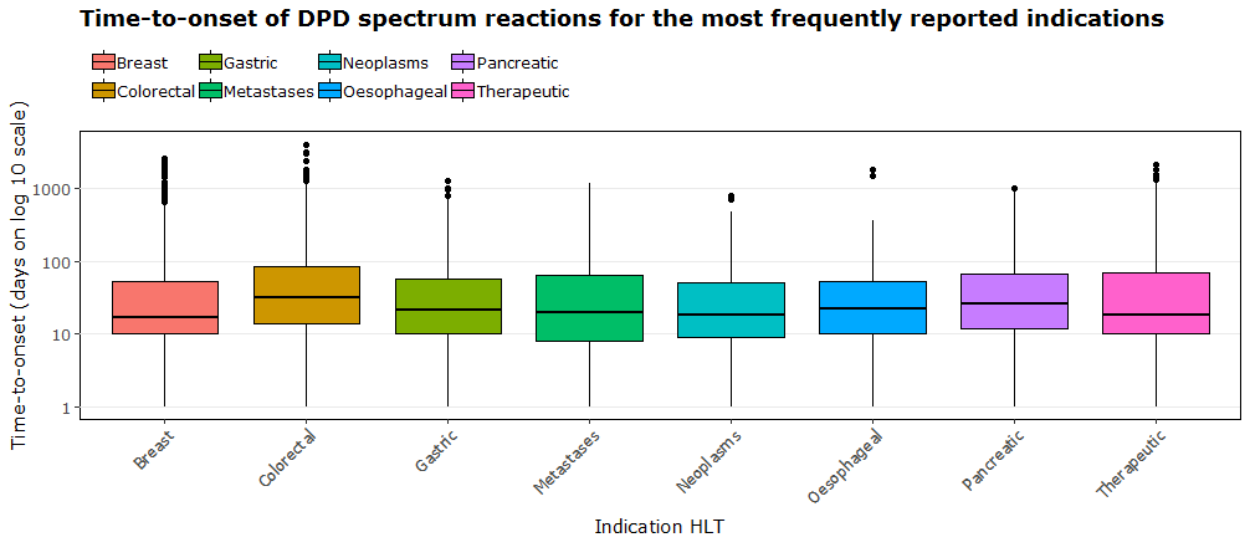
### 5.1.8. Time-to-onset

Time-to-onset calculations are based on 23,161 cases (18% of all cases) / 23,388 observations, after exclusion of 562 cases / 563 observations for which time-to-onset is 0 day.

Median time-to-onset is 26 days overall, 11 days for flucytosine, 22 days for capecitabine and tegafur, and 29 days for fluorouracil.



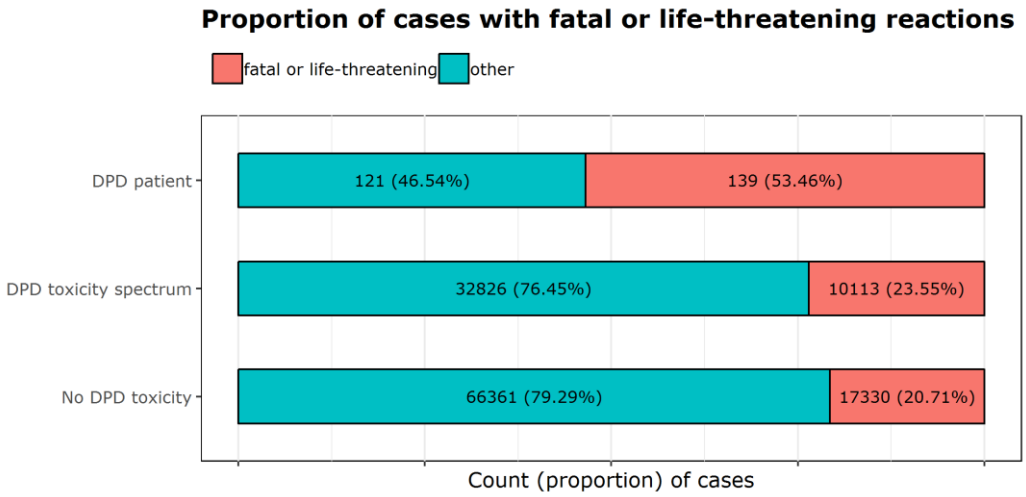

Time to onset is fairly consistent across the most frequently reported indications.



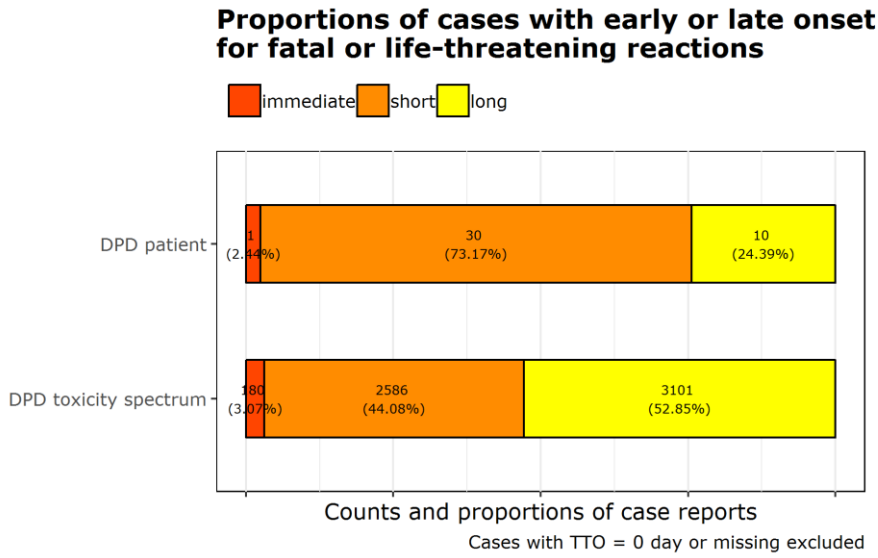

Breast = Breast and nipple neoplasms malignant
Colorectal = Colorectal neoplasms malignant
Gastric = Gastric neoplasms malignant
Metastases = Metastases to specified sites
Neoplasms = Neoplasms malignant site unspecified NEC
Oesophageal = Oesophageal neoplasms malignant
Pancreatic = Pancreatic neoplasms malignant (excl islet cell and carcinoid)
Therapeutic = Therapeutic procedures NEC

### 5.1.9. Additional analyses

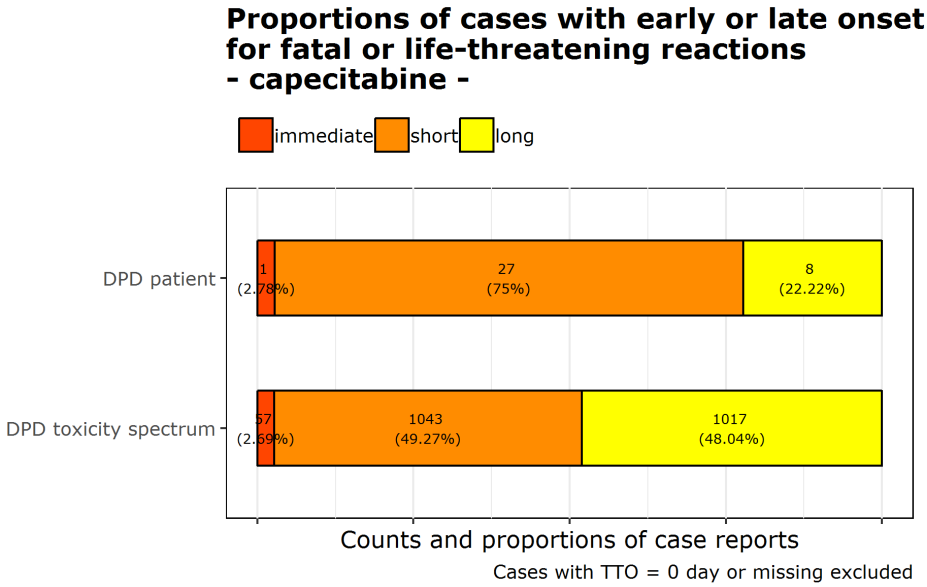
More than half of the DPD confirmed patients had a fatal or life-threatening reaction, a proportion that was much lower (23.6%) within the DPD spectrum group. This may be due to the fact that toxicity is higher in DPD patients and/or that patients experiencing severe reactions are more likely to be tested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

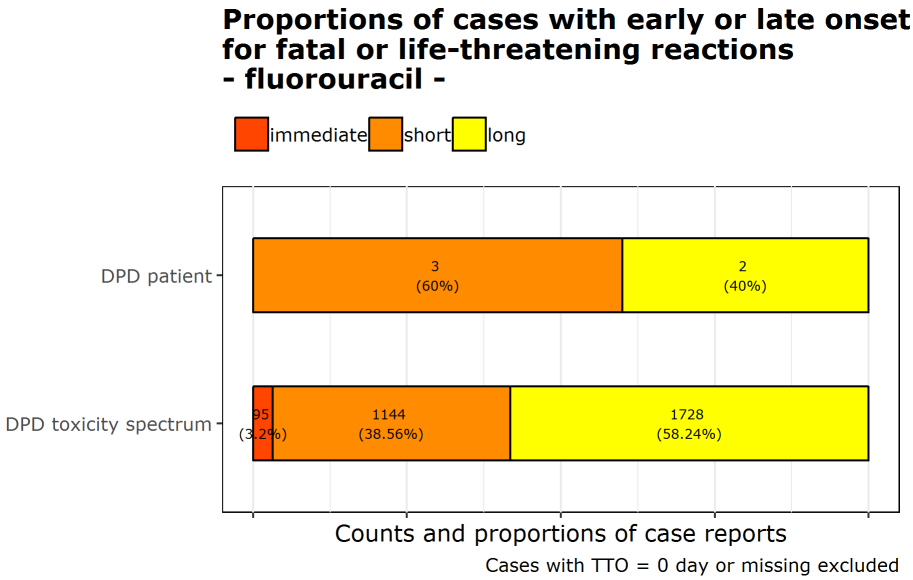



Seventy-~~three~~ six percent (~~73~~76%) of fatal or life-threatening reactions occurred early in confirmed DPD patients, while a slight majority (53%) of fatal or life-threatening reactions within the DPD spectrum group had a late onset ~~(53%)~~. Again, this may be due to the fact that toxicity occurs earlier in DPD patients and/or that patients experiencing early reactions are more likely to be tested for DPD deficiency.




A similar pattern was observed for capecitabine and fluorouracil, although a slight majority (521.9%) of DPD spectrum patients experienced early reactions with capecitabine. There were no DPD patients with time-to-onset information for flucytosine and tegafur.




### *5.2. Estimation of DPD cases using machine learning*

One study suggests that the prevalence of dihydropyrimidine dehydrogenase deficiency in the general population varies according to ethnicity and gender from 1.9% to 12.3%[3].

It is possible that patients with partial dihydropyrimidine dehydrogenase deficiency are unaware of their genotype. Hence, some of the case reports are likely to be of dihydropyrimidine dehydrogenase deficient patients who are unaware of being dihydropyrimidine dehydrogenase deficient.

[3] Mattison LK, Fourie J, Desmond RA, Modak A, Saif MW, Diasio RB: Increased prevalence of dihydropyrimidine dehydrogenase deficiency in African-Americans compared with Caucasians. Clin Cancer Res. 2006;12(18):5491.

To estimate the count of DPD cases, machine learning methods were applied. These methods do not provide information on causality but learn patterns in the adverse drug reactions reported in known DP deficient patients. These patterns were then used to identify similar patterns in other case reports, where the patient is not known to be DP deficient.

The three models that had best performance metrics were two Gradient Boosting Machines (GBM) and a Distributed Random Forest (DRF) as described below.

| Model name | Variable importance (in order of relative importance) | Validation AUC | Estimate of cases |
|---|---|---|---|
| GBM 16 | HLT Mucosal findings abnormal<br>HLT Poisoning and toxicity<br>HLT Diarrhoea (excl infective)<br>Age<br>HLT Thrombocytopenias<br>HLT Marrow depression and hypoplastic anaemias<br>HLT Dermatitis ascribed to specific agent<br>HLT Sepsis, bacteraemia and fungaemia NEC<br>HLT Neutropenias<br>HLT Stomatitis and ulceration | 0.880553 | 15,607 |
| GBM 3 | HLT Mucosal findings abnormal<br>HLT Diarrhoea (excl infective)<br>HLT Poisoning and toxicity<br>Age<br>HLT Thrombocytopenias<br>HLT Neutropenias<br>HLT Marrow depression and hypoplastic anaemias<br>HLT Dermatitis ascribed to specific agent<br>HLT Stomatitis and ulceration<br>Gender | 0.877133 | 9,899 |
| DRF 1 | HLT Diarrhoea (excl infective)<br>HLT Poisoning and toxicity<br>HLT Mucosal findings abnormal<br>HLT Thrombocytopenias<br>HLT Neutropenias<br>HLT Dermatitis ascribed to specific agent<br>Age<br>HLT Stomatitis and ulceration<br>HLT Marrow depression and hypoplastic anaemias<br>HLT Sepsis, bacteraemia and fungaemia NEC | 0.857589 | 8,919 |

The variables that have highest importance are similar across models, even if their relative position varies. Hundreds of HLTs were used as features in the model so it is interesting that the most relevant

HLTs are closely aligned with the spectrum of DPD toxicity. Furthermore, literature describes a gender imbalance in dihydropyrimidine dehydrogenase deficiency, which was also in the top ten relevant variables in the second best performing model.

However, machine learning models learn patterns (or correlations), so it is not adequate to assume causal relationships from these variables. Models identify patterns even in the sample error space – this is also known as overfitting. The feature age, for instance, may solely indicate a higher risk of having a malignant disease or general pharmacokinetic changes and not a specific dihydropyrimidine dehydrogenase relationship.

In fact, considering the simplicity of the features used and complexity of the prediction problem, the performance metrics were probably too good, which suggests some overfitting.

That being said, trying to estimate the number of patients likely to be dihydropyrimidine dehydrogenase deficient but unaware of it is a particularly difficult topic regardless of the methodology chosen. The estimates rely on unspecific surrogates and DPD related toxicity is similar to other drug-related toxicity in malignancy.

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.

### *5.3. Literature review*

Four hundred and eight (408) de-duplicated papers were extracted from PubMed, EMBASE and the Cochrane library. Of these, thirty nine were considered relevant as they were published after April 2018, the data lock point of the Institut National du Cancer's (INCA) report, or were not included in their report, i.e. the papers focus on medicinal products not targeted by INCA (flucytosine, tegafur) or are simply missing.

In addition, twenty-three papers are available online through EMA's subscription or because they are free.

| Title | Authors | Citation | Year | Paper available |
|---|---|---|---|---|
| Dihydropyrimidine dehydrogenase deficiency causes severe adverse effects of capecitabine | Inoue H., Sato Y., Shintani S., Tanabe H., Bamba H., Komai Y., Nakamura T., Imai T., Andou A. | Journal of Japanese Society of Gastroenterology (2018) 115:3 (290-298). Date of Publication: 2018 | 2018 | Yes, in Japanese |
| A Novel DPYD Variant Associated With Severe Toxicity of Fluoropyrimidines: Role of Pre-emptive DPYD Genotype Screening. | Tong CC, Lam CW, Lam KO, Lee VHF, Luk MY. | Front Oncol. 2018 Jul 24;8:279. doi: 10.3389/fonc.2018.00279. eCollection 2018. | 2018 | Yes |
| Association between the pharmacokinetics of capecitabine and the plasma dihydrouracil to uracil ratio in rat: A surrogate biomarker for dihydropyrimidine dehydrogenase activity. | Kobuchi S, Akutagawa M, Ito Y, Sakaeda T. | Biopharm Drug Dispos. 2019 Jan;40(1):44-48. doi: 10.1002/bdd.2168. Epub 2019 Jan 21. | 2019 | Yes |
| Characterization of uracil catabolism variability in healthy volunteers and cancer patients | Schärer D., Kummer D., Nakas C., Fontana S., Jörger M., Amstutz U., | Journal of Laboratory Medicine (2018) 42:4 (eA55-eA56). Date of Publication: 1 Aug 2018 | 2018 | Yes |

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| | Largiadèr C. | | | |
| Clinical implementation of pre-treatment DPYD genotyping in capecitabine-treated metastatic breast cancer patients. | Stavraka C, Pouptsis A, Okonta L, DeSouza K, Charlton P, Kapiris M, Marinaki A, Karapanagiotou E, Papadatos-Pastos D, Mansi J. | Breast Cancer Res Treat. 2019 Feb 12. doi: 10.1007/s10549-019-05144-9. [Epub ahead of print] | 2019 | Yes |
| Clinical value of pharmacogenomic testing in a patient receiving FOLFIRINOX for pancreatic adenocarcinoma | Velez-Velez L.M., Hughes C.L., Kasi P.M. | Front Pharmacol. 2018 Nov 15;9:1309. doi: 10.3389/fphar.2018.01309. eCollection 2018. | 2018 | Yes |
| Cytomegalovirus enterocolitis in a patient with dihydropyrimidine dehydrogenase deficiency after capecitabine treatment: A case report. | Inoue F, Yano T, Nakahara M, Okuda H, Amano H, Yonehara S, Noriyuki T. | Int J Surg Case Rep. 2019;56:55-58. doi: 10.1016/j.ijscr.2019.02.022. Epub 2019 Feb 23. | 2019 | Yes |
| Diagnostic and Therapeutic Strategies for Fluoropyrimidine Treatment of Patients Carrying Multiple DPYD Variants. | Lunenburg CATC, Henricks LM, van Kuilenburg ABP, Mathijssen RHJ, Schellens JHM, Gelderblom H, Guchelaar HJ, Swen JJ. | Genes (Basel). 2018 Nov 28;9(12). pii: E585. doi: 10.3390/genes9120585. | 2018 | Yes |
| Dihydropyrimidine dehydrogenase deficiency as a cause of fatal 5-Fluorouracil toxicity. | Fidai SS, Sharma AE, Johnson DN, Segal JP, Lastra RR. | Autops Case Rep. 2018 Nov 30;8(4):e2018049. doi: 10.4322/acr.2018.049. eCollection 2018 Oct-Dec. | 2018 | Yes |
| Dihydropyrimidine Dehydrogenase Deficiency: Homozygosity for an Extremely Rare Variant in DPYD due to Uniparental Isodisomy of Chromosome 1. | van Kuilenburg ABP, Meijer J, Meinsma R, PÃ©rez-DueÃ±as B, Alders M, Bhuiyan ZA, Artuch R, Hennekam RCM. | JIMD Rep. 2019;45:65-69. doi: 10.1007/8904_2018_138. Epub 2018 Oct 23. | 2018 | Yes |
| Dihydropyrimidine dehydrogenase gene variation and its association with 5-Fluorouracil toxicity in colorectal patients | Salehifar E., Haghighi M.J.A., Negarandeh R., Janbabai G., Safgafi F., Jalali H. | Asia Pacific Journal of Cancer Biology (2018) 3:3 (65-69). Date of Publication: 2018 | 2018 | Yes |
| Dpyd genotype-guided dose individualization of fluoropyrimidine therapy: A prospective safety analysis on four relevant dpyd variants | Swen J.J., Henricks L.M., Lunenburg C.A., De Man F.M., Meulendijks D., Frederix G.W., Kienhuis E., Creemers G.-J., Baars A., Dezentjé V.O., Imholz A.L., Jeurissen F.J., Portielje J.E., Jansen R.L., Hamberg P., Ten Tije A.J., Droogendijk H.J., Koopman M., Nieboer P., Van De Poel M.H., Mandigers C.M., Rosing H., Beijnen J.H., Van Werkhoven E., Van Kuilenburg A.B., Van Schaik R.H., Mathijssen R.H., Gelderblom H., Cats A., Guchelaar H.-J., Schellens J.H. | Clinical Pharmacology and Therapeutics (2019) 105 Supplement 1 (S30). Date of Publication: 1 Mar 2019 | 2019 | Yes |
| DPYD*6 plays an important role in fluoropyrimidine toxicity in addition to DPYD*2A and c.2846A>T: a comprehensive analysis in 1254 patients. | Del Re M, Cinieri S, Michelucci A, Salvadori S, Loupakis F, Schirripa M, Cremolini C, Crucitta S, Barbara C, Di Leo A, Latiano TP, Pietrantonio | Pharmacogenomics J. 2019 Feb 6. doi: 10.1038/s41397-019-0077-1. [Epub ahead of print] | 2019 | Yes |

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| | F, Di Donato S, Simi P, Passardi A, De Braud F, Altavilla G, Zamagni C, Bordonaro R, Butera A, Maiello E, Pinto C, et al. | | | |
| DPYD, TYMS and MTHFR Genes Polymorphism Frequencies in a Series of Turkish Colorectal Cancer Patients. | Amirfallah A, Kocal GC, Unal OU, Ellidokuz H, Oztop I, Basbinar Y. | J Pers Med. 2018 Dec 13;8(4). pii: E45. doi: 10.3390/jpm8040045. | 2018 | Yes |
| Evolution of Dihydropyrimidine Dehydrogenase Diagnostic Testing in a Single Center during an 8-Year Period of Time. | Coenen MJH, Paulussen ADC, Breuer M, Lindhout M, Tserpelis DCJ, Steyls A, Bierau J, van den Bosch BJC. | Curr Ther Res Clin Exp. 2018 Oct 31;90:1-7. doi: 10.1016/j.curtheres.2018.10.001. eCollection 2019. | 2018 | Yes |
| Genotype-guided fluoropyrimidine dosing: ready for implementation | Amstutz U., Largiadèr C.R. | The Lancet Oncology (2018) 19:11 (1421-1422). Date of Publication: 1 Nov 2018 | 2018 | Yes |
| Germline pharmacogenomics of DPYD*9A (c.85T>C) variant in patients with gastrointestinal malignancies treated with fluoropyrimidines. | Khushman M, Patel GK, Hosein PJ, Laurini JA, Cameron D, Clarkson DR, Butler TW, Norden CW, Baliem W, Jones V, Bhadkamkar S, Nelson C, Lee F, Singh AP, Taylor WR. | J Gastrointest Oncol. 2018 Jun;9(3):416-424. doi: 10.21037/jgo.2018.02.03. | 2018 | Yes |
| Rare Dihydropyrimidine Dehydrogenase Variants and Toxicity by Floropyrimidines: A Case Report. | Palmirotta R, Lovero D, Delacour H, Le Roy A, Cremades S, Silvestris F. | Front Oncol. 2019 Mar 11;9:139. doi: 10.3389/fonc.2019.00139. eCollection 2019. | 2019 | Yes |
| Severe adverse events due to dihydropyrimidine dehydrogenase deficiency in a Japanese patient with colon cancer taking capecitabine: a case report | Tsutsui M., Yamamoto S., Yoshikawa Y., Nakanishi R., Takano K., Osumi K., Akatsu T., Yoneyama K., Nakagawa M., Kanai T. | International Cancer Conference Journal (2018) 7:4 (125-129). Date of Publication: 1 Oct 2018 | 2018 | Yes |
| Severe toxicity to capecitabine due to a new variant at a donor splicing site in the dihydropyrimidine dehydrogenase (DPYD) gene. | García-González X, López-Tarruella S, García MI, González-Haba E, Blanco C, Salvador-Martin S, Jerez Y, Thomas F, Jarama M, Sáez MS, Martín M, López-Fernández LA. | Cancer Manag Res. 2018 Oct 11;10:4517-4522. doi: 10.2147/CMAR.S174470. eCollection 2018. | 2018 | Yes |
| SNPs in predicting clinical efficacy and toxicity of chemotherapy: walking through the quicksand. | Palmirotta R, Carella C, Silvestris E, Cives M, Stucci SL, Tucci M, Lovero D, Silvestris F. | Oncotarget. 2018 May 18;9(38):25355-25382. doi: 10.18632/oncotarget.25256. eCollection 2018 May 18. Review. | 2018 | Yes |
| Three different polymorphisms of the DPYD gene associated with severe toxicity following administration of 5-FU: A case report | Mukherji D., Massih S.A., Tfayli A., Kanso M., Faraj W. | Journal of Medical Case Reports (2019) 13:1 Article Number: 76. Date of Publication: 22 Mar 2019 | 2019 | Yes |
| Tolerance-based capecitabine dose escalation after DPYD genotype-guided dosing in heterozygote DPYD variant carriers: a single-center observational study. | Kleinjan JP, Brinkman I, Bakema R, van Zanden JJ, van Rooijen JM. | Anticancer Drugs. 2019 Apr;30(4):410-415. doi: 10.1097/CAD.0000000000000748. | 2019 | Yes |
| A case of colon cancer with DPD deficiency that showed severe myelosuppression by capeox | Watanabe H, Arita S, Takeuchi T, Oshima Y, Koike N. | Gan To Kagaku Ryoho. 2018 Nov;45(11):1661-1664. Review. Japanese. | 2018 | No |

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| adjuvant chemotherapy after colon resection | | | | |
| Alternative chemoradiotherapy to treat locally advanced (LA) anal carcinoma (AC) in patients (PTS) with mutations in thymidylate synthase (TYMS) and dihydropyrimidine dehydrogenase (DPYD) genes: A case series | Saif W.M., Hamal R., Siddiqui N.S., Maloney A., Chen L., Huber K. | Journal of Clinical Oncology (2019) 37 Supplement 4. Date of Publication: 1 Feb 2019 | 2019 | No |
| Determination of endogenous concentrations of uracil and dihydrouracil in dried saliva spots by LC-MS/MS: method development, validation and clinical application. | Antunes MV, Raymundo S, Cezimbra da Silva AC, Muller VV, Vicente Neto OJ, Schwartsmann G, Linden R. | Ther Drug Monit. 2019 Feb 22. doi: 10.1097/FTD.0000000000000615. [Epub ahead of print] | 2019 | No |
| Dihydropyrimidine dehydrogenase gene (DPYD) polymorphism among pts with 5-FU/capecitabine (CAP)-related adverse events (AEs): Experience of 2 decades | Siddiqui N.S., Purvey S., Hamal R., Zhang L., Diasio R.B., Saif W.M. | Journal of Clinical Oncology (2018) 36:15 Supplement 1. Date of Publication: 1 May 2018 | 2018 | No |
| DPYD-Varifier, a computational model to identify 5-FU toxicity-associated DPYD variants | Shrestha S., Zhang C., Jerde C.R., Li H., Offer S.M., Diasio R.B. | Cancer Research (2018) 78:13 Supplement 1. Date of Publication: 1 Jul 2018 | 2018 | No |
| Effectiveness and safety of reduced-dose fluoropyrimidine therapy in patients carrying the DPYD*2A variant: A matched pair analysis. | Henricks LM, van Merendonk LN, Meulendijks D, Deenen MJ, Beijnen JH, de Boer A, Cats A, Schellens JHM. | Int J Cancer. 2019 May 1;144(9):2347-2354. doi: 10.1002/ijc.32022. Epub 2019 Jan 4. | 2019 | No |
| How can we best monitor 5-FU administration to maximize benefit to risk ratio? | Goirand F, Lemaitre F, Launay M, Tron C, Chatelut E, Boyer JC, Bardou M, Schmitt A. | Expert Opin Drug Metab Toxicol. 2018 Dec;14(12):1303-1313. doi: 10.1080/17425255.2018.1550484. Epub 2018 Nov 23. Review. | 2018 | No |
| Implementing routine pre-emptive DPD testing with adaptive dosing to secure 5-FU administration: Performance in digestive and head and neck cancer patients | Launay M., Sébastien S., Dahan L., Seitz J.F., Dufaud F., Lacarelle B., Ciccolini J. | Fundamental and Clinical Pharmacology (2018) 32 Supplement 1 (33). Date of Publication: 1 Jun 2018 | 2018 | No |
| Pharmacogenetic analyses of 2183 patients with advanced colorectal cancer; potential role for common dihydropyrimidine dehydrogenase variants in toxicity to chemotherapy. | Madi A, Fisher D, Maughan TS, Colley JP, Meade AM, Maynard J, Humphreys V, Wasan H, Adams RA, Idziaszczyk S, Harris R, Kaplan RS, Cheadle JP. | Eur J Cancer. 2018 Oct;102:31-39. doi: 10.1016/j.ejca.2018.07.009. Epub 2018 Aug 13. | 2018 | No |
| Predicting 5-Fluorouracil related severe toxicity with DPD functional tests in plasma, fresh saliva and dried saliva samples | Schwartsmann G., Franzoi M.A.B., Alves G.V., Antunes M.V., Neto O., Artmann A., Raymundo S., Tegner M., Muller V., Hahn R.Z., Linden R. | Journal of Clinical Oncology (2018) 36:15 Supplement 1. Date of Publication: 1 May 2018 | 2018 | No |
| Preliminary Evidence for Enhanced Thymine Absorption: A Putative New Phenotype Associated With Fluoropyrimidine Toxicity in Cancer Patients. | Duley JA, Ni M, Shannon C, Norris RL, Sheffield L, Cowley D, Harris M, van Kuilenburg ABP, Helsby N, George R, Charles BG. | Ther Drug Monit. 2018 Aug;40(4):495-502. doi: 10.1097/FTD.0000000000000532. | 2018 | No |
| Successful use of uridine triacetate (Vistogard) three weeks after capecitabine in a patient with homozygous dihydropyrimidine dehydrogenase mutation: A case | Zurayk M, Keung YK, Yu D, Hu EH. | J Oncol Pharm Pract. 2019 Jan;25(1):234-238. doi: 10.1177/1078155217732141. Epub 2017 Sep 26. Review. | 2019 | No |

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| report and review of the literature. | | | | |
| The clinical relevance of multiple DPYD polymorphisms on patients candidate for fluoropyrimidine based-chemotherapy. An Italian case-control study. | Iachetta F, Bonelli C, Romagnani A, Zamponi R, Tofani L, Farnetti E, Nicoli D, Damato A, Banzi M, Casali B, Pinto C. | Br J Cancer. 2019 Apr;120(8):834-839. doi: 10.1038/s41416-019-0423-8. Epub 2019 Mar 12. | 2019 | No |
| The correlation between DPYD*9A (c.85T > C) genotype and dihydropyrimidine dehydrogenase deficiency phenotype in patients with gastrointestinal malignancies treated with fluoropyrimidines: Updated analysis | Maharjan A.S., McMillin G.A., Patel G.K., Awan S., Taylor W.R., Pai S., Frankel A.E., Nelson C., Wang B., Hosein P.J., Singh A., Khushman M.M. | Journal of Clinical Oncology (2019) 37 Supplement 4. Date of Publication: 1 Feb 2019 | 2019 | No |
| The impact of liver resection on the dihydrouracil:uracil plasma ratio in patients with colorectal liver metastases | Jacobs B.A.W., Snoeren N., Samim M., Rosing H., de Vries N., Deenen M.J., Beijnen J.H., Schellens J.H.M., Koopman M., van Hillegersberg R. | European Journal of Clinical Pharmacology (2018) 74:6 (737-744). Date of Publication: 1 Jun 2018 | 2018 | No |
| The safety of oral uracil-tegafur plus leucovorin in the treatment of colorectal cancer in patients with partial DPD deficiency | Cubero D.D., Del Giglio A. | Journal of Clinical Oncology (2010) 28:15 SUPPL. 1. Date of Publication: 20 May 2010 | 2010 | No |

## Annex I – Description of the machine learning methods used to estimate the number cases with a reaction profile similar to DPD cases

***General description***

Supervised machine learning methods for classification were applied to EudraVigilance data. The machine learning models learn patterns in the reaction profile of known cases of DPD. In other words, the algorithm detects terms, which were reported in cases where the patient is known to be DP deficient. The model was then used to identify cases not reported to be DP deficient, which have similar reaction profiles.

***Definition of true positives***

True positive cases were case reports that reported dihydropyrimidine dehydrogenase deficiency as a reaction preferred term, as medical history, or that had a test result suggestive of DPD.

***Definition of true negatives***

True negative cases were defined as case reports of medicinal products used in similar indications as fluorouracil and fluorouracil related substances, but which do not have a dihydropyrimidine dehydrogenase interaction. Trastuzumab, pembrolizumab, docetaxel and irinotecan were used. Cases were randomly selected on a 1:1:1:1:1 relation. This means that for each true positive, four true negatives were assigned.

Cases that reported the true negative drugs as well as fluorouracil or related products were removed from the sample, except if they were DP deficient, in which case they were assigned to the true positives.

***Features***

The features used were age, gender and adverse reaction reported at HLT level.

***Training and testing set***

The combined observations in the true positives and true negatives resulted in a data frame, that was split. A training set had 75% of the sample size and the testing set 25%.

***Analytics***

Distributed random forests, gradient boosting machines, generalised linear models and deep learning algorithms were applied using H2O for R. H2O is an open source lightweight in-memory machine learning platform written in Java. It was chosen because of its simplicity, ease of reproducibility, interactivity with R and Python and, especially, for being lightweight, which means it can run in most laptops.

***Code***

The annotated code and the H2O Flow are available for consulation.