Distinction between drug-drug interactions mediated by CYP3A4 and CYP3A5 during pharmacokinetic assessment in drug registration procedures by the EMA: An in-depth analysis

Talhi N[1], Brandon EFA[2], Maliepaard M[2]

[1] Faculty of science, Utrecht University, Utrecht, The Netherlands

[2] Medicines Evaluation Board (MEB), Graadt van Roggenweg 500 3531AH Utrecht, The Netherlands

ABSTRACT

Background: The cytochrome P450 3A (CYP3A) subfamily members CYP3A4 and CYP3A5 play an important role in the metabolism of more than 50% of all marketed drugs, often leading to drug-drug interactions (DDIs). The European Medicines Agency (EMA) serves as the regulatory authority for the evaluation and approval of medicinal products during drug development in the European Union. The EMA guidelines outline systematic approaches, including *in vitro* and *in vivo* studies, to assess these potential DDIs. CYP3A5 expression, however, can vary among different ethnic groups. In Caucasians, only 20% possesses active CYP3A5 enzyme, leading to interindividual differences in DDI potential for one medicinal product. Low CYP3A5 expression in Caucasian subjects and evidence on differences in CYP3A4 and CYP3A5 substrate and inhibitor specificity raises uncertainty whether the specific role of CYP3A5 in DDIs is adequately studied.

Aim: To investigate whether medicinal products previously evaluated by EMA adequately assessed DDIs involving both CYP3A4 and CYP3A5, or if CYP3A5 was overlooked.

Methods: A retrospective dossier study was performed based on the medicine data for drugs registered by EMA between 2021 to 2023. Medicinal products were selected from a table downloaded from the EMA website that includes all medicines that have requested marketing authorisation by EMA. All human medicinal products providing new (pre-)clinical information and mentioning CYP3A in their European Public Assessment Report (EPAR) from 2021 to 2023 were included and information on provided *in vitro* and *in vivo* data including CYP3A4 and CYP3A5 was collected. The Summary of Products Characteristics (SmPC) of each of these products was also screened for information about CYP3A. The primary outcome was to determine how (extensively) CYP3A5 was investigated with regard to drug-drug interactions during the drug registration process of the EMA. Both the situation of a medicinal product being a CYP3A(4/5) substrate (victim) or an inhibitor/inducer (perpetrator) were considered. In addition, information on the selectivity of commonly used CYP3A inhibitors and substrates for 3A4 versus 3A5 was looked for in literature.

Results: A total of 73 medicinal products were included for screening of the EPAR and SmPC. The majority of this selection, 34.2%, belongs to the therapeutic subgroup of antineoplastic agents. While each EPAR mentioned CYP3A4, only 22% separately mentioned CYP3A5. Forty-five percent of the reports cited CYP3A4/5. CYP3A was considered potentially clinically relevant for 53 out of 73 products based on *in vitro* data in the EPAR. Twentynine out of these 53 (29%) mentioned CYP3A5 or CYP3A4/5 *in vitro,* next to CYP3A4. Only three out of 73 individual EPARs (4%) mentioned CYP3A5 in their *in vivo* data and all three anticipated clinically relevant effects depending on the presence or absence of CYP3A5 expression. According to their EPAR, all three medicinal products were victims of CYP3A, suggesting potential additional loss of efficacy in individuals expressing CYP3A5 in case of combination with an inhibitor of CYP3A4/5. However, none of the applicants performed an *in vivo* study to propose dosing recommendations for this CYP3A5 expressing subpopulation. Only one of the three products included information about effects of genetic polymorphisms of 3A5 in its SmPC.

Rifampicin, itraconazole, and midazolam were the most frequently used (*in vivo*) inducer, inhibitor, and substrate of CYP3A, respectively. All three interactants were predominantly referred to in the EPARs as CYP3A4 interactant, with no reference to CYP3A5 being made.

Conclusion: In most cases, the effect of CYP3A5 expression next to expression of CYP3A4 is expected to result in a loss of efficacy due to increased metabolism of, and therefore decreased exposure to the medicinal product. Due to expected decreased exposure, no increase in safety issues is expected in case of CYP3A5 expression. However, loss of efficacy, especially for drugs with a narrow therapeutic window and for medicinal products for life-threatening diseases, can have a significant impact on treatment outcome. Dosing recommendations authorised by EMA therefore need to be accurate to

ensure optimal treatment, as well as inclusive, given the diversity of ethnicities in Europe. Applicants should include various ethnic groups with different degrees of CYP3A5 expression and perform CYP3A5 genotyping in *in vivo* studies, in order to accurately propose dosing recommendations for CYP3A5 expressing patients. Additionally, studies describing differences in affinity of inhibitors and substrates for CYP3A4 vs 3A5 bring another issue to light: are the conclusions drawn regarding the (lack of) clinical significance of CYP3A5 accurate? Further research is necessary to ascertain the impact of these differences in specificity (*in vitro* and *in vivo*) on drug metabolism and therapeutic outcomes.

1. INTRODUCTION

The cytochrome P450 (CYP) superfamily is a group of haem-thiolate enzymes that play a crucial role in metabolism of many exogenous and endogenous compounds in the human body. (1) Based on their biochemical relatedness, the enzymes are assigned into families (e.g., CYP1, CYP2) with subgrouping (e.g., CYP1A, CYP2C). They are further distinguished by a specific number for each individual enzyme (e.g., CYP1A2, CYP2C19). (2) CYP3A subfamily members CYP3A4 and CYP3A5 are one of the most important CYP enzymes and metabolise beyond 50% of all marketed drugs. (3)(4) Enzymes of this subfamily are therefore often involved in drug-drug interactions (DDIs). Consequently, the European Medicines Agency (EMA) recommends investigating the impact of CYP3A enzymes on metabolism of (new) medicines, as well as their effect on CYP3A-mediated metabolism during drug development. (5)

Cytochrome P450 enzymes catalyse the oxidative, peroxidative and reductive metabolism of many endogenous (e.g. prostaglandins, steroids, fatty acids) and xenobiotic substrates (e.g. medicines, environmental pollutants). (1) These enzymes catalyse the conversion of lipid-soluble compounds into more water-soluble forms, thereby enabling their elimination from the body. Inhibitors of (a) CYP enzyme(s) can hinder these conversion processes, leading to reduced and delayed elimination of the substance/drug. This results in prolonged and higher exposure to that drug, which can lead to dangerously high exposure and therefore to an increased risk of toxicity and side effects, depending on the drug's therapeutic window. On the other hand, inducers of a CYP enzyme accelerate its catalytic activity and therefore elimination of that particular drug. This can lead to subtherapeutic plasma concentrations due to faster elimination of the drug, and to reduced treatment efficacy. For some drugs however, conversion by a specific CYP enzyme is necessary for the drug in order to exhibit its therapeutic function. These type of medicines are also known as pro-drugs. When CYP enzymes involved in the conversion and activation of prodrugs are inhibited, this process may be slowed down or reduced. This leads to a delayed onset of therapeutic action, reduced effectiveness and efficacy, and a possible increase of side effects due to accumulation of the inactivated drug. Inducers of these enzymes accelerate metabolism into the drug's active form and can therefore increase plasma concentrations and pharmacological response. Consequently, the co-administration of medicines with substances that inhibit or induce CYP enzymes can result in a drug-drug interaction: the effect of the drug is altered by another drug, supplement, or substance. DDI's require careful consideration in treatment management, as they can result in hospital admissions and, in the worst-case scenario, in fatal adverse events. An interaction may require dosage or dosing frequency adjustments, and sometimes even avoidance of the combination altogether. This applies especially for medicines with a narrow therapeutic window, as small changes in their dosage or exposure can have significant effects on the treatment outcome (efficacy or safety). Drug-drug interactions are becoming increasingly common due to polypharmacy and a growing number of authorised drugs, which shows the importance to carefully investigate these interactions during drug development and authorisation processes.

The CYP3A subfamily consist of 4 individual enzymes: CYP3A4, CYP3A5, CYP3A7, and CYP3A43. (4) CYP3A4 is highly expressed in the liver and intestine and accounts for up to 50% of total hepatic CYP enzymes. It stands out as one of the most notable CYP enzyme involved in drug-drug interactions. Grapefruit juice (a CYP3A4 inhibitor) and St John's Wort (a CYP3A4 inducer) are well-known examples of substances that can alter the therapeutic effects and increase side effects of medicinal products, metabolised by CYP3A4 (e.g. simvastatin, oral contraceptives). (6) In the Netherlands, pharmacies even include warnings about these interactions on the secondary packaging of involved drugs, contributing to increased awareness about CYP3A4.

On the other hand, CYP3A5 is a significantly less recognized isoform, despite its similarities to CYP3A4. To specify: the CYP3A5 protein exhibits an 85% gene sequence similarity with CYP3A4, and their substrate specificity also overlaps. (7) However, certain variations in catalytic properties of CYP3A5 have been identified. The intrinsic clearance of steroid hormones (e.g. testosterone, estradiol) by CYP3A5 is lower than that by CYP3A4. Conversely, the intrinsic clearance values of some anticancer drugs (e.g. vincristine, ifosfamide) by CYP3A5 are greater than those by CYP3A4. (8) Unlike CYP3A4, CYP3A5 is found to a lesser degree in the liver and more prominently found in the kidneys and lungs. (9) Among CYP3A enzymes, CYP3A43 is the least known isoform due to very low expression and limited functional and clinical significance. (10) CYP3A7 is the most prominent CYP enzyme expressed in the human foetal liver; however, its expression shifts to CYP3A4 after birth and therefore CYP3A7 enzyme activity completely disappears or falls to very low by the age of 1. (11) (12)

The overall CYP3A metabolism in adult patients is thus mainly dependent on CYP3A4 and CYP3A5 activity. Interindividual variability in activity of these enzymes may be caused by age, food, use of drugs that induce or repress their DNA transcription and translation, or genetic polymorphisms. CYP3A4 and CYP3A5 allele frequencies can vary among different ethnic groups due to the different expression of these genetic polymorphisms. In African populations for instance, approximately 90-100% has normal CYP3A4 metabolism and the majority, 74%, is also CYP3A5 expressor. Asians follow with 92-98% of their population having normal CYP3A4 metabolism, and 25-34% being CYP3A5 expressor. Among Caucasians however, up to 90% express two active CYP3A4 alleles and only 15-25% is CYP3A5 expressor. Consequently, a majority of the population in the Netherlands is CYP3A5 non-expressor, meaning that they do not have CYP3A5 activity. In these individuals, CYP3A5 does not contribute to their overall CYP3A metabolism. However, in the minority that does express functional CYP3A5, total CYP3A mediated metabolism can be significantly dependent on the contribution of this subfamily member, dependent on substrate characteristics. (13) CYP3A-mediated metabolism is therefore dependent on 3A4 and 3A5 expression and genetic differences can thus affect drug levels and activity, especially for drugs that are primarily metabolised by CYP3A. The genes responsible for coding for CYP3A4 and CYP3A5 can be attributed to many different alleles, but for both enzymes, only *1 is of importance. *1 is also known as the wild-type allele: this allele codes for a DNA sequence of the most common allele in a population, which leads to an active protein. (14) All other CYP3A alleles code for either an inactivated or non-functioning protein. As mentioned before, the majority of Caucasians and Africans express two active CYP3A4*1 alleles, resulting in normal CYP3A4 metabolism. Individuals carrying at least one CYP3A5*1 allele are assumed to have CYP3A5 activity that contributes to the total CYP3A metabolism. The frequencies of the CYP3A5*1 allele varies between different populations and suggest a potential impact of environmental factors: there is evidence for a positive correlation between higher CYP3A5*1 frequencies and decreased geographic distance from the equator. This may be evolutionary explained by the fact that CYP3A5 expression offers a selective advantage in warm climates, due to its role in retaining salt and water. (15)

Genotyping to identify genetic polymorphisms can help to predict an individual's ability to respond to specific medicines. The phenotype however, represents the actual physical and biochemical characteristics, determined by both genotype and environmental factors (e.g. concomitant medication use, diet, comorbidities). For some drugs CYP3A genotyping prior to starting a treatment is advised in the hospital setting because of interindividual responses and risk of overdosing (resulting in toxicity) or underdosing (compromising effectiveness). This approach ensures safety, minimizes side effects, and enhances treatment efficacy. According to pharmacogenetics expertise centre of the Erasmus Medical Centre, CYP3A5 is involved in metabolism of seven drugs. One of those drugs is tacrolimus, an immunosuppressive that is used after a kidney, liver, or heart transplantation. (16) Tacrolimus is primarily metabolised by both CYP3A4 and CYP3A5 and its dose recommendations depend on the

patient's CYP3A genotype. CYP3A5 non-expressors eliminate the drug slower than expressors and therefore have an increased risk of side effects and toxicity. Only this drug offers additional dose recommendations for homo- and heterozygote CYP3A5-expressors on the website of the Royal Dutch Pharmacists Association, which offers a drug database used by different parties in healthcare (including physicians and pharmacists). (17) The other six drugs (apixaban, fluoxetine, guanfacine, midazolam, vincristine, and vortioxetine) do not have additional CYP3A5 dosing advices or have not been assessed (yet). (18) (19)

As previously emphasised, CYP enzymes play a crucial role in the metabolism of many drugs. Regulatory agencies are actively involved in ensuring their appropriate assessment during drug development and approval processes. The European Medicines Agency serves as the regulatory authority for medicines within the European Union (EU) and is responsible for the evaluation, approval and monitoring of all medicines that are marketed in the region. (20) It has formulated guidelines that specifically address the assessment of drug-drug interactions, offering a systematic and mechanistic approach for the evaluation of the interaction potential of a medicine during development. These guidelines include specific requirements and recommendations to assess potential interactions between CYP enzymes and drugs, including *in vitro* and *in vivo* studies. In general, *in vitro* metabolism studies are first to be conducted to predict *in vivo* interactions with the new medicinal product. If relevant involvement of the drug is found *in vitro*, *in vivo* studies are recommended to be executed to confirm and quantify this interaction. If CYP enzymes are involved in the primary elimination pathways of the new drug, CYP enzyme inhibitors and/or inducers can influence pharmacokinetics of the new drug. In this case, the drug is a potential DDI victim. The EMA recommends to assess the pharmacokinetics both with and without concomitant administration of a potent enzyme inhibitor and inducer. If possible, the inhibitor and inducer should be selective and thus having no impact on other enzymes or transports involved in the drug elimination process. The guideline also states that if the candidate CYP enzyme is relatively little studied and is usually not included in enzyme inhibition/inducing screening of drugs, *in vitro* studies are considered sufficient to investigate the inhibitory or inducing effect on that enzyme of common concomitant used drugs. If the investigational drug inhibits or induces CYP enzymes, recommendations for *in vitro* and *in vivo* testing including specific marker substrates are given. Under these circumstances, the new drug can alter pharmacokinetics of other drugs/substrates of this CYP enzyme and is therefore called a potential DDI perpetrator.

CYP3A5, despite its potential relevance in subpopulations, is often conflated with CYP3A4, or even neglected. The U.S. Food and Drug Administration (FDA) table is used during drug development and provides *in vitro* and clinical index substrates, inducers, and inhibitors for specific CYP enzymes. In this table, no distinction is made between CYP3A4 and CYP3A5 and all interactants are labelled for CYP3A4/5. (21) (22) The same applies to the EMA guideline: it refers to CYP3A and makes no distinction between 3A4 and 3A5. (5) However, published research has shown that there is indeed a difference in affinity for some interactants. Itraconazole and ketoconazole are two frequently used CYP3A inhibitors for *in vitro* and *in vivo* experiments, but studies indicate that ketoconazole inhibits both CYP3A4 and CYP3A5, while itraconazole only inhibits 3A4. (23) This provokes consideration whether conclusions, drawn in assessment rapports regarding the (lack of) (pre-)clinical significance of CYP3A5, are accurate, potentially dependent on the inhibitor or substrate used. Another reason for neglect of CYP3A5 in studies, is the low expression of CYP3A5 in the Caucasian population. Since most DDI studies are performed in Caucasian subjects, and the majority of this population lacks CYP3A5 expression, the relevance of distinguishing CYP3A in CYP3A4 and CYP3A5 *in vitro* or genotyping *in vivo* is diminished. This raises the question whether previously evaluated drugs adequately assessed interactions involving CYP3A (and thus both CYP3A5 and CYP3A4), or if CYP3A5 was separately assessed at all. This analysis will investigate how these DDIs involving CYP3A have been assessed by the EMA in the time period

between 2021 and 2023. If possible and needed, the final goal of this research project is to propose specific instructions regarding assessment of CYP3A metabolism and interaction studies for inclusion in the EMA guideline.

2. METHODS

2.2 Study design and data-collection

A retrospective dossier study was performed based on European Medicines Agency's (EMA) medicine-related published data. The medicine data table was downloaded from the official website of the EMA on 7 December 2023. The Excel table format available for download on this webpage was last updated on 4 December 2023, due to update issues since relaunch of the website on 5 December 2023. The table contains a list of European public assessment reports (EPARs) for human and veterinary medicines of pharmaceutical companies that have applied for marketing authorisation on European Union level. This does not only include medicines that have been authorised or have been refused authorisation, but also those that have been suspended or withdrawn after approval. The data table includes drugs having a marketing authorisation date of up to October 1995. (24)

2.2 Exclusion criteria

Medicines in the downloaded table were excluded if they met the following criteria:

- Category ''veterinary'' medicines
- Marketing authorisation date before 01-01-2021
- Generic and hybrid medicines
- Biosimilars
- Monoclonal antibodies without small-molecule(s)
- Vaccines
- No mentioning of CYP3A, CYP3A4 or CYP3A5 in the EPAR
- At least one of the active substances of a combination product has been approved before 01-01-2021

The dossiers of generic and hybrid medicines for application for marketing authorisation must only demonstrate bioequivalence to a reference medicinal product. This reference product must already be authorised, for at least 8 years due to the period of data exclusivity, by a member state of the EMA. The (pre-)clinical and quality data of this reference medicine are cross-referred in the dossier of the new drug. Generic and hybrid drugs are therefore excluded due to lack of new pre-clinical and clinical data. (25) (26)

Biosimilar medicines are not generic to their reference medicine, but highly similar when it comes to safety, efficacy, and quality. Requirements for approval of a biosimilar are therefore to demonstrate no clinically relevant differences by comparative quality and (non-)clinical studies. This data is relative and deficient of new (pre-)clinical information and consequently excluded in this analysis. (27)

Monoclonal antibodies (MABs) are derived from immunoglobulins and are metabolised into peptides and amino acids by proteolysis. CYP enzymes are generally not involved in metabolism of MABs are therefore not included. (28) However, if the drug additionally contains a small-molecule (antibody-drug conjugate), it is not excluded. Targeted cancer therapies often include a MAB with a small molecule attached to specifically target cancer cells. (29) This small molecule can be metabolised by CYP enzymes and therefore antibody-drug conjugates are not excluded.

The active component in vaccines is either a weakened or inactive part of a virus or bacterium. These are not metabolised by CYP enzymes, but by different mechanisms like phagocytosis. Vaccines are therefore not taken into account for further analysis. (30)

2.3 Data collection

All European Public Assessment Reports and Summaries of Product Characteristics of the selected products were screened for specifics regarding CYP3A, CYP3A4, and CYP3A5. Furthermore, information on the selectivity of commonly used CYP3A inhibitors and substrates for 3A4 versus 3A5 was looked for in literature.

2.3.1 EPAR screening

European assessment reports of the selected medicines were screened for *in vivo* and *in vitro* data including CYP3A. If *in vitro* data was available, mentioning of CYP3A5 and the type of conducted study and outcome were specifically noted. Secondly, information about clinical (*in vivo*) CYP3A-related perpetrator and/or victim drug-drug interaction studies was identified and processed. This includes not only the type of enzyme inhibitors, inducers, and enzymes, but also the clinically relevance and conclusions regarding CYP3A, CYP3A4 and CYP3A5. The frequencies of mentioning of CYP3A, CYP3A4, CYP3A5, and CYP3A4/5 in the EPAR were also separately counted.

2.3.2 SmPC screening

The summary of product characteristics (SmPC) of all drugs was available on the website of the EMA and were also screened for specifics of CYP3A for the selected medicinal products. CYP3A4 victim and perpetrator interaction dependent warnings or dose recommendations were noted, including the corresponding paragraphs of the SmPC. Just like in the EPAR, the frequencies of mentioning of CYP3A, CYP3A4, CYP3A5, and CYP3A4/5 were separately counted.

2.4 Data analysis

The primary aim was to determine how (extensively) the investigation of CYP3A5 expression was conducted during the drug registration process of the EMA. The collected data above was incorporated in Excel to conduct a descriptive analysis. Additionally, an exploratory analysis was performed to identify patterns and possible causal relations.

3. RESULTS

3.1 Selection of medicines

The Excel table including all medicine data was downloaded on 7 December 2023 and was last updated on 4 December 2023. Figure 1 shows the order and process of selecting medicines after implementing the exclusion criteria outlined in section 2.2. The EMA has authorised 2050 medicines over the course of twenty-nine years, including both human and veterinary categories. First, 291 veterinary medicines were eliminated, leaving 1759 human medicinal products. This selection was reduced by 1512 after excluding drugs authorised before 01-01-2021. The remaining 247 medicines were than screened for generics/hybrids (46), biosimilars (23), monoclonal antibodies (33), and vaccines (11). 134 EPARs were analysed for mentioning of CYP3A and 51 of those medicines were not further included due to not fulfilling this criterium. During this process, it was noted that some combination therapies contained one or more active substance(s) that has/have already been authorised before 01-01-2021. These ten drugs were therefore also excluded. Finally, 73 different medicines out of 2050 have been assessed.

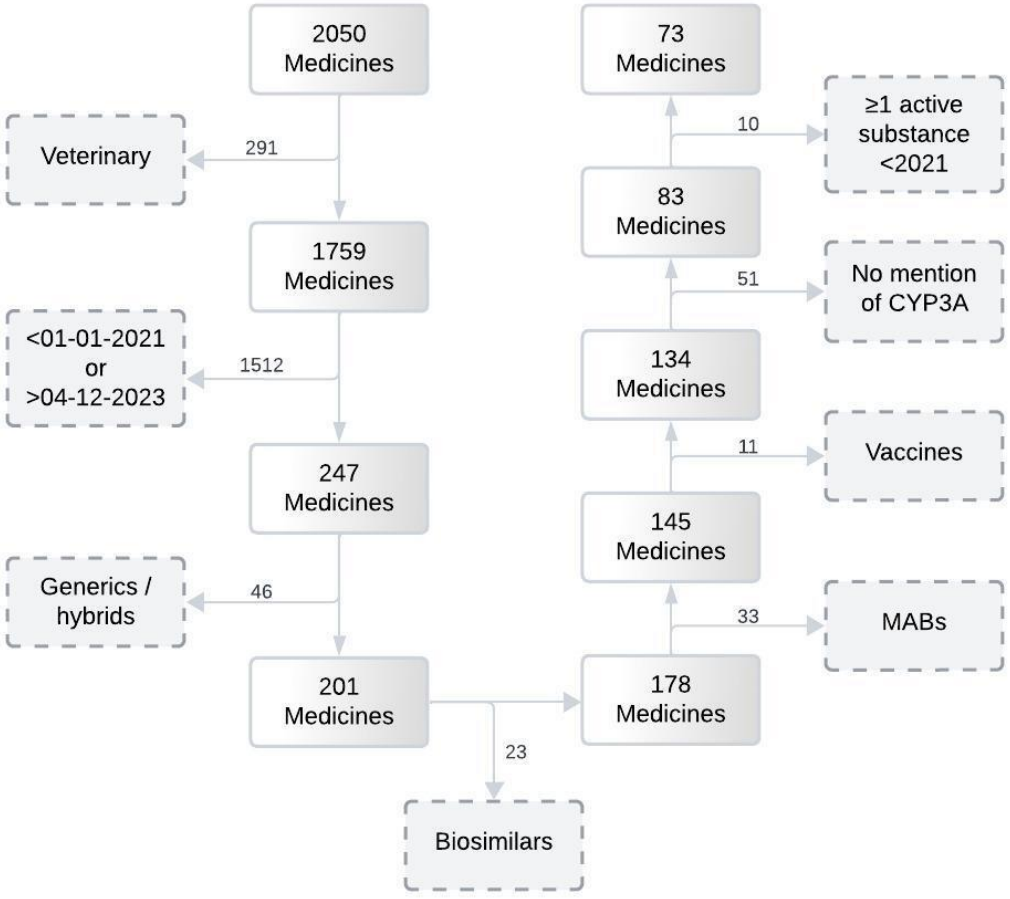

Figure 1. Selection of medicinal products included in the study.

3.2 Medicines characteristics

All medicines are classified into 14 groups based on their Anatomical Therapeutic Chemical (ATC) code. This code assigns drugs based on the system or organ it works on and how it works. Table 1 shows the characteristics of the 73 selected medicines based on this code and information available from the Excel sheet and EMA website. The majority (33 out of 73, 45.2%) of these drugs belong to group L: antineoplastic and immunomodulating agents. This therapeutic subgroup is therefore further specified. L01 has the largest contribution to subgroup L: it includes 25 antineoplastic agents. L04 includes six immunosuppressants and L02 includes two endocrine therapies, but L03 does not include any of the 73 drugs. The other medicines are divided into the remaining groups, but group G, R, and S do not include any drugs. The ATC code for two medicinal products was not provided in the SmPC or Excel table, but was available from website of the World Health Organisation Collaborating Centre for Drug Statistics Methodology. (31)

Some marketing authorisation processes are handled differently in case of special circumstances. Three of those processes have been highlighted in table 1. A form of marketing approval given to drugs for rare conditions or drugs that are unethical to collect full data for, is ''exceptional circumstances''. (32) 4 out of 73 medicines (5.4%) were approved by this process. If the drug is of major interest for the public health, the applicant is eligible for ''accelerated assessment''. (33) Only 2 of 73 medicines (2.7%) have been authorised using this procedure. ''Conditional marketing authorisation'' applies for drugs where the benefit of immediate availability outweighs the risk of lack of available additional data during the authorisation process. (34) Ten of the 73 selected products (13.7%) were granted a conditional marketing authorisation.

Table 1. Characteristics of 73 selected medicinal products.

Characteristics	**N**	**% of total**
Therapeutic area		
A Alimentary tract and metabolism	5	6.8%
B Blood and blood forming organs	6	8.2%
C Cardiovascular system	4	5.5%
D Dermatologicals	4	5.5%
G Genito urinary system and sex hormones	0	0%
H Systemic hormonal preparations, excl. sex hormones and insulins	3	4.1%
J Anti-infectives for systemic use	7	9.6%
L Antineoplastic and immunomodulating agents	33	45.2%
M Musculo-skeletal system	1	1.4%
N Nervous system	7	9.6%
P Antiparasitic products, insecticides and repellents	1	1.4%
R Respiratory system	0	0%
S Sensory organs	0	0%
V Various	2	2.7%

Therapeutical subgroup L		
L01 Antineoplastic agents	25	34.2%
L02 Endocrine therapy	2	2.7%
L03 Immunostimulants	0	0%
L04 Immunosuppressants	6	8.2%
Exceptional circumstances		
Yes	4	5.5%
No	69	94.5%
Accelerated assessment		
Yes	2	2.7%
No	71	97.3%
Conditional approval		
Yes	10	13.7%
No	63	86.3%
Total medicinal products	73	100%

3.3 CYP3A screening specifics

Information concerning CYP3A, CYP3A4, and CYP3A5 was obtained from two different sources: the European assessment reports and the summary of product characteristic (SmPC). Section 3.3.1 provides information regarding CYP3A in the EPARs, section 3.3.2 includes data from the SmPC.

3.3.1 EPAR

Mentioning of CYP3A4, CYP3A4/5, and CYP3A5 in EPAR

Mentioning of CYP3A in the EPAR was one of the inclusion criteria of selecting medicines for this analysis and all 73 drugs therefore meet this criterium. Mentioning of CYP3A4, CYP3A4/5, and CYP3A5 was also separately analysed and is visualised in figure 2. Each EPAR (100%) mentioned CYP3A4, 22% (16/73) of the EPARs mentioned CYP3A5. 45% (33/73) of the rapports cited CYP3A4/5.

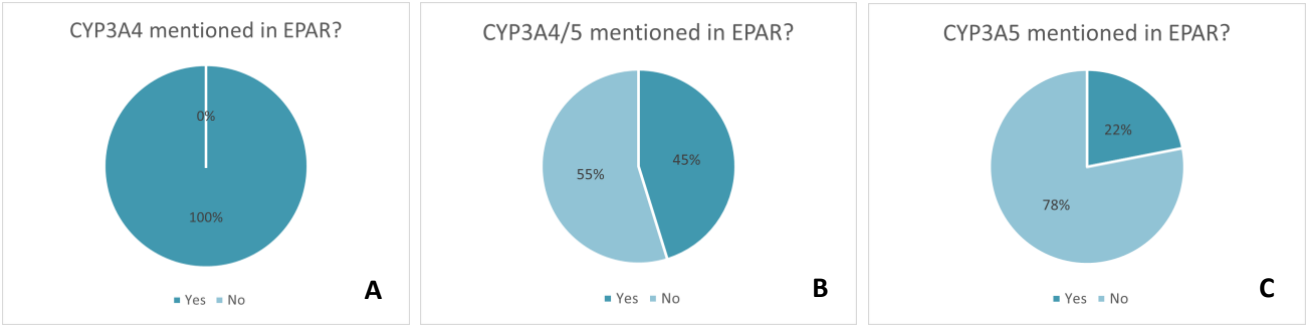

Figure 2. Mentioning of CYP3A4 (A), CYP3A4/5 (B), and CYP3A5 (C) in the EPARs of the selected 73 medicinal products

Frequencies of mentioning of CYP3A4, CYP3A4/5, and CYP3A5 in EPAR

Figure 3 illustrates the minimum, maximum and mean number of frequencies of mentioning of CYP3A4, CYP3A4/5, and CYP3A5 in the individual EPARs (either related to *in vitro* or *in vivo*). The mean number of mentioning of CYP3A4 in the EPARs is 19.91, with the highest frequency of mentioning of CYP3A4 reaching 87 in a single EPAR. For CYP3A4/5, the maximum frequency of mentioning was 21 and the mean number of mentioning was 1.6. The mean number of mentioning of CYP3A5 was 0.71 and the maximum number of mentioning of CYP3A5 in the reviewed EPARs was 18. The minimum frequency of mentioning of CYP3A4, CYP3A4/5, and CYP3A5 was zero.

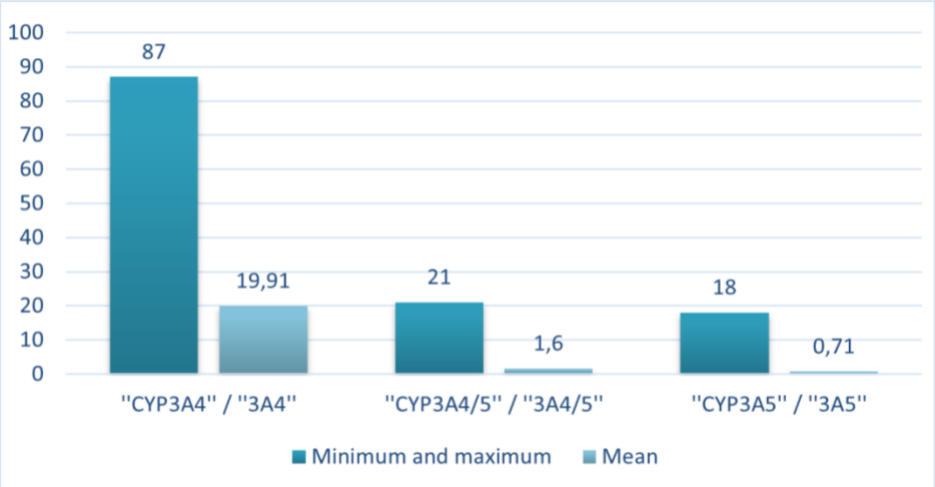

Figure 3. The minimum, maximum, and mean number of the frequencies of mentioning of CYP3A4, CYP3A4/5, and CYP3A5 in the EPARs of the selected 73 medicinal products.

CYP3A relevance *in vitro* and *in vivo*

Figure 4 visualises the process of screening the 73 European Public Assessment Reports for information regarding the clinical relevance of CYP3A. First, all EPARs were screened for presence of *in vitro* data concerning CYP3A, yielding 68 medicinal products. Subsequently, *in vitro* data in 68 separate EPARs were screened for specifics on their potential clinical relevance of CYP3A. CYP3A was considered potentially clinically relevant in 53 EPARs. *In vivo* data from clinical drug-drug interaction studies were also analysed for clinical relevance of CYP3A and for information concerning the drug being a perpetrator (5), victim (14), or both (18) in DDI studies. Perpetrators of medicinal products can be divided into three categories based on their effect on the metabolism of other drugs: inhibitors, inducers, or both. 23 of the 37 medicinal products for which CYP3A was considered relevant based on *in vivo* data acted as perpetrators, of which 65.2% (15/23) were inhibitors of CYP3A, 26.1% (6/23) were inducers of CYP3A, and 8.7% (2/23) were both inhibitor and inducer. The 32 victims of DDIs can also be divided in these three groups based on the effects of other substances on the metabolism of the investigated medicinal product. 12.5% (4/32) is affected by an inhibitor of CYP3A, 25% (8/32) is affected by an inducer of CYP3A, and 62.5% (20/32) is affected by both inhibitor and inducer.

Bar chart A, B, and C in figure 4 provide data on the CYP3A substrates, inhibitors, and inducers used *in vivo* in all conducted clinical DDI studies. Each bar chart shows the frequencies of the used interactants and different types within the three separate groups. Some studies used more than one type of inhibitor, inducer, or substrate. Midazolam (substrate), itraconazole (inhibitor), and rifampicin (inducer) were the most frequently used CYP3A interactants in these clinical studies.

Out of the 73 medicinal products under review, the applicant accepted the recommendation to conduct an *in vivo* study concerning CYP3A as a post-authorisation measure for 12 of them, as mentioned in the

EPAR. In the case of five of these products, warnings have been included in the SmPC until the study results become available.

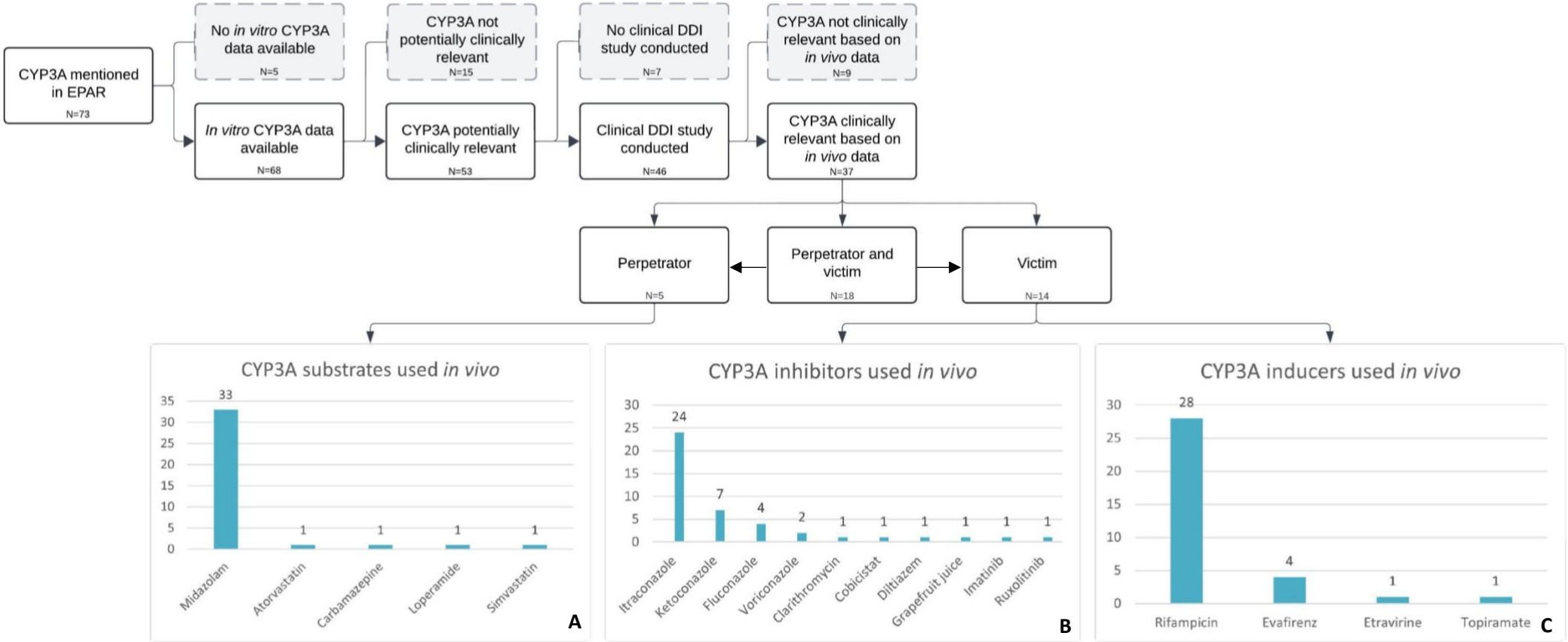

Figure 4. The process of screening 73 European Public Assessment Reports for information regarding the clinical relevance of CYP3A. In vitro data in each separate EPAR were screened for potential clinical relevance of CYP3A. In vivo data from clinical drug-drug interaction studies were also analysed for clinical relevance of CYP3A and for information concerning the drug being a victim (inhibitor ± inducer), perpetrator (inhibitor ± inducer), or both in DDI studies. Bar chart A, B, and C provide data as for the CYP3A substrates, inhibitors, and inducers used in vivo in all conducted clinical DDI studies. Each bar chart shows the frequencies of the used interactants and different types within the three separate groups.

CYP3A5 *in vitro* and *in vivo*

Out of the 73 medicinal products assessed, 35 mentioned either CYP3A5 or CYP3A4/5 in the *in vitro* data in their assessment reports. Among these 35, three EPARs mentioned CYP3A5 eventually in their final *in vivo* conclusions (e.g. interactions, dose recommendations) regarding CYP3A. CYP3A5 was involved in metabolism of all three drugs, and presence or absence of this isoform could potentially have a significant impact on the pharmacokinetics (e.g. C_{max} and AUC) of the products. Based on *in vitro* data, a relevant effect of CYP3A5 expression cannot be excluded. No additional *in vivo* studies have been conducted to refute or validate these findings.

Midazolam, itraconazole, and rifampicin

In all 46 conducted *in vivo* studies, midazolam, itraconazole, and rifampicin emerged as the most frequently used CYP3A substrate, inhibitor, and inducer, respectively. Each EPAR presented varied references to the enzyme affected by these interactants. Figure 5 illustrates the frequencies with which each report cited CYP3A, CYP3A4, CYP3A4/5, or CYP3A5 as the enzyme affected by the interactant. Rifampicin was identified as a CYP3A4 inducer 20 out of 28 times (71.4%); the remaining eight occurrences (28.6%) identified it as a CYP3A inducer (both CYP3A4 and 3A5 inducer). Itraconazole was reported as a CYP3A4 inhibitor fourteen times (58.3%) and as a CYP3A inhibitor (both CYP3A4 and 3A5 inhibitor) ten times (41.7%). Regarding midazolam, 25 out of 33 reports (75.8%) referred to it as a CYP3A4 substrate (both CYP3A4 and 3A5 substrate), while seven reports (21.2%) labeled it as a CYP3A substrate, and one EPAR (3%) referred to it as a CYP3A4/5 substrate. Notably, CYP3A5 was not once mentioned for midazolam, itraconazole, and rifampicin.

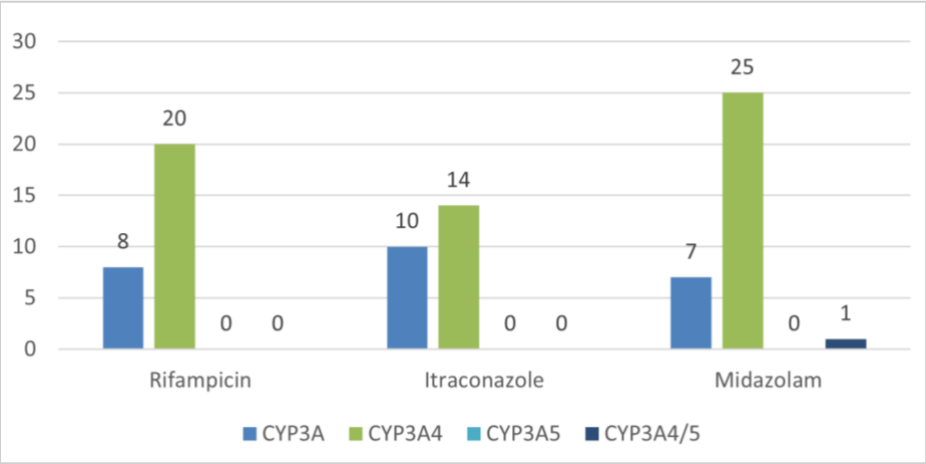

Figure 5. Frequency at which each EPAR cited CYP3A, CYP3A4, CYP3A5, and CYP3A4/5 as the enzyme affected by the most used in vivo interactants rifampicin (inducer), itraconazole (inhibitor, (inhibitor) and midazolam (substrate).

3.3.2 SmPC

Mentioning of CYP3A4, CYP3A4/5, and CYP3A5 in SmPC

Mentioning of CYP3A4, CYP3A4/5, and CYP3A5 in the SmPCs of the 73 included medicinal products has been separately analysed and is visualised in figure 6. 81% of the SmPCs mentioned CYP3A4, 15% of the SmPCs mentioned CYP3A4/5, and 12% of the SmPCs cited CYP3A5.

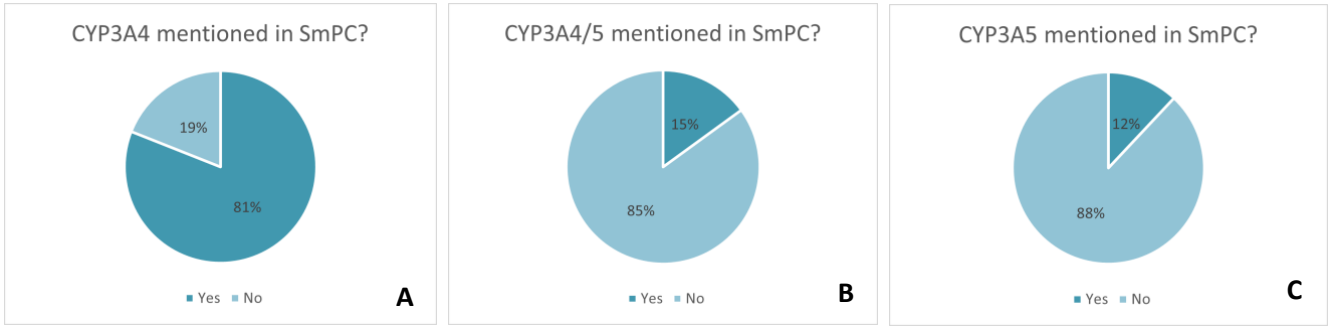

Figure 6. Mentioning of CYP3A4 (A), CYP3A4/5 (B), and CYP3A5 (C) in the Summary of Product Characteristics for the 73 selected medicinal products.

Frequencies of mentioning of CYP3A4, CYP3A4/5, and CYP3A5 in SmPC

Figure 7 illustrates the minimum, maximum and mean number of frequencies of mentioning of CYP3A4, CYP3A4/5, and CYP3A5 in the corresponding SmPC of all 73 medicines. The mean number of mentioning of CYP3A4 per SmPC is 8, with the highest frequency of mentioning of CYP3A4 reaching 44 in a single SmPC. For CYP3A4/5, the maximum frequency of mentioning was two and the mean number of mentioning was 0.19. The mean number of mentioning of CYP3A5 was 0.23 and the maximum amount of mentioning of CYP3A5 in the reviewed SmPCs was three. The minimum frequency for mentioning of CYP3A4, CYP3A4/5, and CYP3A5 was zero.

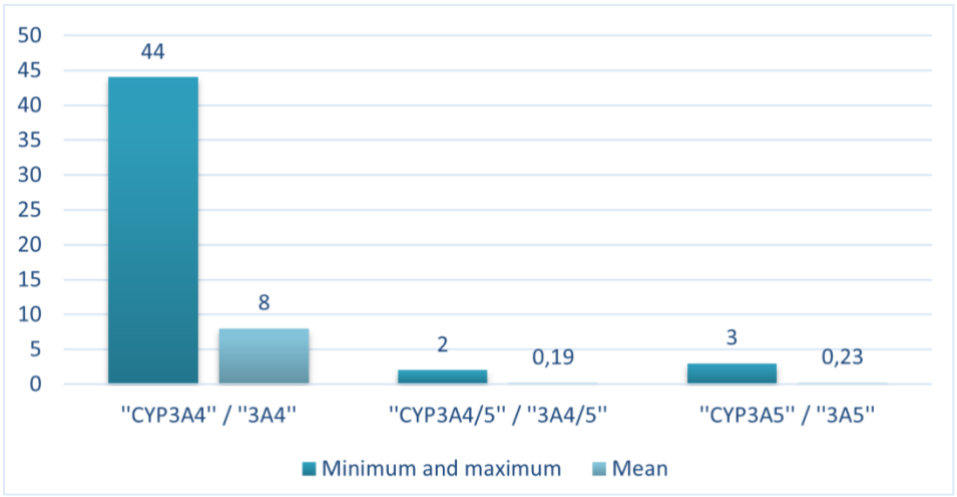

Figure 7. The minimum, maximum, and mean number of mentioning of CYP3A4, CYP3A4/5, and CYP3A5 in the 73 corresponding SmPCs.

SmPC sections

SmPCs are divided into several sections to provide relevant information about its medicinal product. While every section is important for a comprehensive understanding of the product, sections 4.2, 4.3, 4.4, and 4.5 are most essential to describe interactions or dosing recommendations due to interactions or genetic polymorphisms. Section 4.2 (Posology and method of administration) includes information about dosage instructions and administration routes. Section 4.3 (Contraindications) describes situations in which the drug should not be used due to risks for the patient. 4.4 (Special warnings and precautions for use) offers additional information regarding potential risks or precautions when using the product. Section 4.5 (Interaction with other medicinal products and other forms of interaction) provides information about potential interactions between the medicinal product and other substances.

A total of 32 out of 73 SmPCs provided dose recommendations or interaction information related to interactions with CYP3A as a victim (inhibitor ± inducer). Of these, fourteen were solely victim, while eighteen medicinal products acted as both perpetrator and victim. Figure 8 illustrates how many of those SmPCs mentioned CYP3A (once or more) in section 4.2-4.5. Most mentions of CYP3A information were found in section 4.5 (32), followed by sections 4.4 (18) and 4.2 (18), while CYP3A was least mentioned in 4.3 (7).

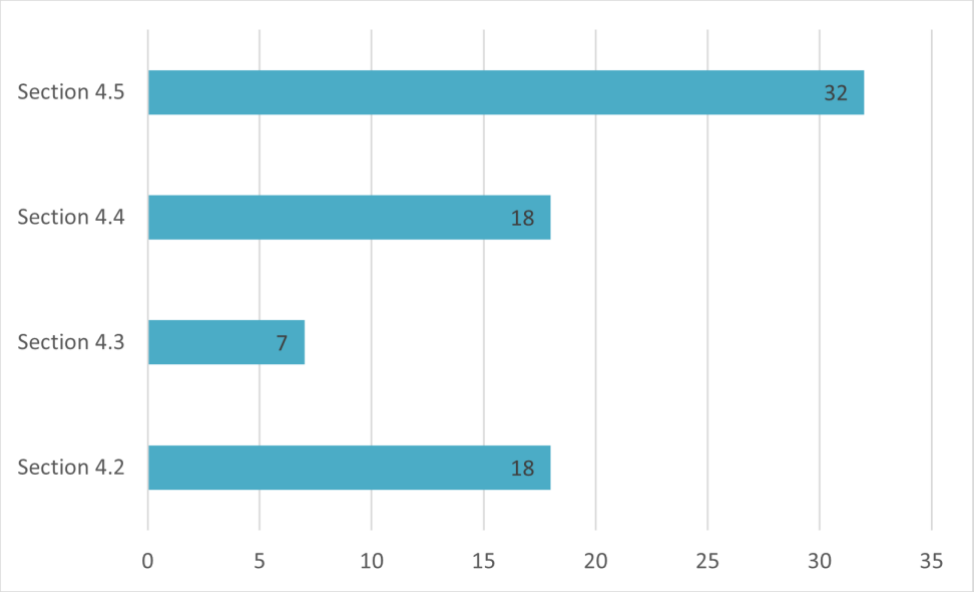

Figure 8. The number of mentioning of CYP3A (CYP3A, 3A4, 3A5, 3A4/5 related) victim dose recommendations or interactions in section 4.2-4.5 of the 32 SmPCs.

A total of 23 out of 73 SmPCs provided dose recommendations or interaction information related to interactions with the medicinal product as a perpetrator (inhibitor ± inducer) of CYP3A. Of these, five were solely perpetrators, while eighteen medicinal products acted as both perpetrator and victim. Figure 9 demonstrates how many of those SmPCs mentioned CYP3A (once or more) in sections 4.2-4.5. All 23 medicinal products mentioned CYP3A victim information in SmPC section 4.5 (23). Most mentions of CYP3A information were found in section 4.5 (23), followed by SmPC sections 4.4 (7) and 4.3 (1), while CYP3A was not once mentioned in SmPC section 4.2 (0).

In all SmPCs in which CYP3A was clinically relevant (37), CYP3A5 was mentioned in seven SmPCs. CYP3A5 was not once mentioned in sections 4.2-4.5. Six medicinal products mentioned CYP3A5 under the heading 'biotransformation' in SmPC section 5.2, which describes the pharmacokinetic properties

of the drug. One medicinal product also mentioned CYP3A5 in SmPC section 5.2, but under the heading 'CYP2D6 phenotypes and CYP3A polymorphism'.

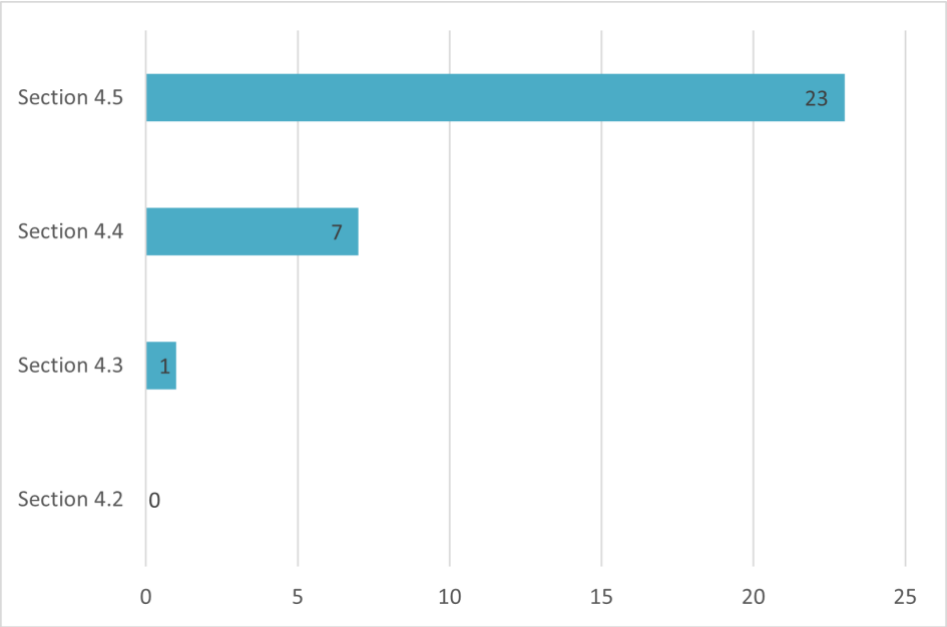

Figure 9. The number of mentioning of CYP3A (CYP3A, 3A4, 3A5, 3A4/5 related) perpetrator dose recommendations or interactions in section 4.2-4.5 of the 23 SmPCs.

4. DISCUSSION

The aim of this study was to investigate whether medicinal products previously evaluated by the European Medicines Agency adequately assessed DDIs involving both CYP3A4 and CYP3A5, or if CYP3A5 was overlooked. Two findings responsible for questioning whether CYP3A5 had been sufficiently studied were (1) the low CYP3A5 expression in Caucasian subjects and (2) the evidence on differences in CYP3A4 and CYP3A5 substrate and inhibitor specificity.

4.1 CYP3A5 *in vitro* and *in vivo* studies

CYP3A5 is expressed polymorphically and contributes to total CYP3A metabolism if expressed. CYP3A5*1 carriers therefore have an increased total CYP3A metabolism which, in the case of CYP3A substrate medicinal products (victims), either results in increased CYP3A elimination of a drug or in increased CYP3A activation of a prodrug as compared to CYP3A5 non-expressors. Increased elimination leads to lower blood plasma concentrations of and lower exposure to the medicinal product, which may decrease efficacy. Increased activation of a prodrug enhances pharmacological activity of the medicinal product, which can result in excessive exposure and an increase in side effects or even toxicity. In case the drug is an inducer (perpetrator) of CYP3A, CYP3A5-expression could lead to greater induction of total CYP3A metabolism, consequently resulting in a further increased metabolism of the victim drug as compared to CYP3A5 non-expressors. If the medicinal product is an inhibitor (perpetrator) of CYP3A, CYP3A5-expression would lead to increased inhibition of total CYP3A metabolism (depending on the binding affinity for CYP3A4 and 3A5), consequently resulting in decreased metabolism of the victim drug as compared to CYP3A5 non-expressors. All effects for CYP3A victims and perpetrators however depend on the affinity of the medicinal product for CYP3A5: the higher the affinity for CYP3A5 (compared to CYP3A4) of the medicinal product/substance (substrate, inhibitor, and/or inducer of CYP3A), the more CYP3A5 expressor pharmacokinetics deviate from the pharmacokinetics of CYP3A5 non-expressors. This means that CYP3A5 expressors can have a different pharmacokinetic effect of substrates, inhibitors, and/or inducers of CYP3A for the same medicinal products compared to CYP3A5 non-expressors. This should therefore be taken into consideration during development of medicinal products, in order to adequately research efficacy and safety for this subpopulation.

CYP3A5 was mentioned relatively little in both EPAR (22% of the EPARs) and SmPC (12% of the SmPCs) in comparison with CYP3A4 in the EPAR (100%) and SmPC (81%). The mean number of mentionings of CYP3A5 per document was also lower in the EPAR (0.71) and SmPC (0.23) than CYP3A4 in the EPAR (19.91) and SmPC (8). Less than half of all 73 included medicinal products (35 out of 73) mentioned CYP3A5 or CYP3A4/5 in the *in vitro* data of their EPARs. This shows that CYP3A5 in often not investigated in *in vitro* studies, while the EMA guideline on the investigation of drug interactions states that *in vitro* studies provide important information that is necessary for the extrapolation to human preclinical safety data, including DDI potential. The guideline also remarks that if indicated by *in vitro* studies, particular *in vivo* studies are recommended to be executed to confirm and quantify interactions found *in vitro*. (5) If the *in vitro* conclusions are inaccurate or complete, this can consequently impact the selection of *in vivo* studies that will be conducted and therefore the final conclusions regarding dose recommendations or contraindications. For the majority (38 out of 73 EPARs) of included medicinal products, it remains unclear whether CYP3A5 could have been potentially clinically relevant *in vitro* and whether this effect would be significant in following *in vitro* studies due to lack of investigating. The conclusions regarding the absence of clinical relevance of CYP3A5 could therefore be questioned, especially for products where CYP3A4 has been deemed clinically relevant. CYP3A4 and CYP3A5 share an 85% gene sequence similarity, suggesting that if CYP3A4 is involved, it is likely CYP3A5 is involved,

and vice versa. Particularly in the EPARs of medicinal products where CYP3A4 is deemed clinically relevant, CYP3A5 should have been investigated *in vitro* and *in vivo*, as individuals expressing CYP3A5 could experience a different clinical effect in these cases.

Only three out of 37 medicinal products, where CYP3A was deemed clinically relevant based on *in vitro* and *in vivo* data in their EPAR, included information regarding CYP3A5 in their final *in vivo* conclusions in their EPAR. In all three cases (CYP3A victims), CYP3A5-expression was expected to result in a loss of efficacy due to increased metabolism and elimination by CYP3A5, without increasing safety issues. For none of the three medicinal product, *in vivo* studies were conducted to refute or validate these findings, and only two of the three medicinal products mentioned this potential reduction of efficacy in their SmPC. Loss of efficacy, especially for medicinal products with a narrow therapeutic window and/or medicinal products for life-threatening diseases, can have a significant impact on treatment outcome. This impact on this treatment outcome can be different for various groups of medicines. 33 of the 73 included medicinal products belonged to the therapeutic subgroup of antineoplastic and immunomodulating agents. This includes medication for the treatment of cancer and autoimmune disorders. Medicinal products within this therapeutic subgroup often have a narrow therapeutic window, which means that their effectiveness is dependent on blood plasma concentration levels. Lower levels can result in an inadequate treatment of their condition and exposes the patient to all associated consequences of the illness, including potentially fetal outcomes. The lack of thorough investigation of CYP3A5 *in vitro* and *in vivo* studies undoubtedly brings consequences that cannot be ignored.

As discussed in the introduction, CYP3A5 expression varies among different ethnic groups. Approximately 90-100% of Africans is CYP3A5 expressor, while only 20% of Caucasians expresses active CYP3A5. Not one EPAR included CYP3A5 genotyping information of subjects, not one EPAR or SmPC (section 5.2) included information regarding potential CYP3A racial effects, and in not one EPAR an *in vivo* DDI study was performed specifically in CYP3A5-expressing subjects. In most DDI studies, the majority of subjects are Caucasian, which represents in most cases the worst-case scenario. This implies that this predominantly CYP3A5 non-expressing group is subjected to the highest increase in exposure of a medicinal product metabolised by CYP3A, due to lack of CYP3A5 contribution to total CYP3A metabolism. CYP3A5 expressors would thus potentially be under-treated, since the dose reduction in case of co-treatment with a CYP3A4 inhibitor is too high or the dose increase in case of co-treatment with a CYP3A inducer is too low. A total of 37 medicinal products (perpetrator ± victims) provided CYP3A dose recommendations or interaction information in (one or more) section 4.2-4.5 of their SmPC. CYP3A5 was not once mentioned in these sections and all conclusions in these sections may therefore not be accurate for CYP3A5-expressors. Given the diversity of ethnicities in Europe, dose recommendations and interaction information provided by the EMA should be inclusive, also with respect to CYP3A5 expression, to ensure optimal treatment for all patients. This inclusivity of racial CYP3A expression deserves greater attention in DDI studies and during the assessment of study effects.

The information above demonstrates that CYP3A5 has not been sufficiently studied in the 73 included medicinal products: not only did more than half of the EPARs not include or mention CYP3A5 *in vitro* studies, but not one EPAR included an *in vivo* study specifically on CYP3A5 expressors. This raises concerns whether previously assessed reports of medicinal products by the EMA adequately include dose recommendations and/or contraindications that are suitable for the subpopulation of CYP3A5 expressors, taking both efficacy and safety into account. The three assessment reports of medicinal products that did predict an effect of CYP3A5 *in vivo*, show irregularities concerning drawing conclusions in their EPAR and SmPC, as well as a lack of conducting *in vivo* studies in CYP3A5 expressing populations. The current EMA and FDA guidelines make no distinction between the two isoforms and

only mention CYP3A or CYP3A4, while studies show that CYP3A5 can have a significant impact on treatment outcome in CYP3A5 expressing subpopulations. (5) (22) This shows the need for a more clear EMA guideline considering both CYP3A4 and CYP3A5 in *in vitro* and *in vivo* interaction studies. This includes studies investigating whether CYP3A5 is involved in the metabolism of the new medicinal product and if the drug has inducing or inhibiting interaction potential of CYP3A5, next to CYP3A4. The guidelines should also incorporate recommendations regarding the inclusion of diverse ethnic groups with varying levels of CYP3A5 expression and standard CYP3A5 genotyping in *in vivo* studies for medicinal products where CYP3A may be clinically relevant. This is essential for applicants to accurately research and propose dosing recommendations for patients expressing CYP3A5. A thorough study begins with the execution of the appropriate *in vitro* tests, which subsequently guide the selection of *in vivo* tests. Consequently, only the most important information regarding these tests will eventually be included in the SmPC of the medicinal products. This underscores the critical importance of conducting the *correct in vitro and in vivo* tests, as they ultimately determine the content (e.g. for patient and physician) of the provided literature in the SmPC.

4.2 Differences in CYP3A4 and CYP3A5 substrate and inhibitor specificity

The differences in interactant specificity of CYP3A4 and CYP3A5 can shed light on the variations in drug responses among individuals. Knowledge about these differences is essential to predict drug-drug interactions and minimizing side effects of medicinal products, ultimately leading to safer and more effective treatments. The most *in vivo* used 3A substrate and inhibitor were midazolam and itraconazole, respectively. Information on the affinity of these two interactants for 3A4 versus 3A5 was looked for in literature. All included EPARs alternately mention midazolam, itraconazole, and rifampicin either as a CYP3A4 or CYP3A interactant, and do not refer to CYP3A5. However, studies have shown that affinity of substrates and inhibitors of CYP3A can be different for CYP3A4 or CYP3A5.

''Youscript'' is a medication management system that predicts responses regarding metabolism of and interactions with (a combination of) medicines based on genetic information of an individual and is used by healthcare providers prescribing medication. It combines information from published studies for its predictions. (35) Itraconazole is in this system referred to as a strong CYP3A4 inhibitor, with no mention of CYP3A5. This implicates that itraconazole inhibits specifically CYP3A4. A study by Lukkari et al. indeed highlights that itraconazole inhibits CYP3A4 more potently (38% of activity left) than CYP3A5 (67% of activity left). (36) Togashi et al. discovered in their study that the inhibitory potency of itraconazole was approximately nine times higher for CYP3A4 than for CYP3A5. (37) These findings indicate that itraconazole has a different affinity for CYP3A4 and CYP3A5. If this frequently used inhibitor specifically inhibits CYP3A4 and to a much lesser extent CYP3A5, this can have several impacts on the conclusions drawn regarding *in vitro* and *in vivo* studies on the effect of CYP3A4/5. The conducted *in vitro* studies with itraconazole may be biased if only focused on CYP3A4 if conducted in microsomes (not if conducted in enzyme specific systems): this could lead to an incomplete understanding of how this inhibitor affects metabolism of substances metabolised by both CYP3A4 and CYP3A5. Regarding the impact *in vivo*: If itraconazole primarily inhibits CYP3A4 and has an inferior effect on CYP3A5, it could impact the interpretation of *in vivo* results. For example, if a medicinal product that is primarily metabolised by both CYP3A4 and 3A5 is tested and concomitant administered with itraconazole, the degree of inhibition may be different than expected in CYP3A5 expressors compared to non-expressors based on *in vitro* data alone, since 3A5 is not inhibited by itraconazole. Additionally, the effect of itraconazole does not predict the effect inhibitors of both CYP3A4 and CYP3A5. If CYP3A5 would contribute to total CYP3A metabolism for a medicinal product, itraconazole would have a relatively lower inhibitory effect on metabolism of CYP3A5 expressors than non-expressors, which could result in higher blood plasma levels of the medicinal product than expected based on the

itraconazole DDI study. Consequently, dosage recommendations and potential drug-interactions related to CYP3A(4) for this CYP3A5 expressing subgroup may not be adequate.

Midazolam is referred to as a substrate for both CYP3A4 (major path size) and CYP3A5 (minor path size) in Youscript. (38) However, Huang et al. has performed research *in vitro* on phenotyped human liver microsomes and observed higher CYP3A5 metabolic activity towards midazolam than for CYP3A4, indicating that CYP3A5 metabolises midazolam more potently than CYP3A4, which contradicts the conclusion drawn by Youscript. (39) Soars et al. on the other hand, noted a similar in vitro clearance of CYP3A4 and CYP3A5 of midazolam. (40) These different findings in the three sources show that there are irregularities, and therefore uncertainties, regarding substrate specificity of midazolam towards CYP3A4 and 3A5. These differences can have a significant impact on conclusions drawn regarding the effect of CYP3A4/5 on metabolism of midazolam. Variations in affinity for these two isoforms could result in different metabolic pathways for midazolam, depending on CYP3A5 expression and on its affinity towards midazolam. If it is uncertain whether midazolam is metabolised by CYP3A4, CYP3A5, or both, and to what extent each enzyme contributes to its metabolism, it can lead to uncertain conclusions regarding its pharmacokinetic parameters such as clearance rate and bioavailability, as well as and drug-drug interactions. Differences in affinity may cause varying degrees of inhibition or induction by other substances interacting with CYP3A4/5, potentially affecting the efficacy and safety of the CYP3A substrate and co-administered medicinal products. Without clear understanding of the role of each enzyme in metabolism of a medicinal product, assessments of potential drug-drug interactions and provided dosing recommendations may be less accurate.

Inducers (e.g. rifampicin) of CYP3A typically function by binding and activating the transcription factor pregnane X receptor (PXR), which upregulates the synthesis of CYP3A enzymes. (41) In individuals expressing CYP3A5, CYP3A inducers not only increase CYP3A4 synthesis, but also CYP3A5 synthesis. This may result in a relatively greater increase in total CYP3A expression among CYP3A5 expressors compared to non-expressors. Consequently, this increased enzyme activity in CYP3A5 expressors can influence the metabolism of other substances that are metabolised by CYP3A, potentially leading to lower blood plasma concentrations than in CYP3A5 non-expressors. This difference in enzyme induction effect may result in a different drug interaction potential in CYP3A5 expressors and non-expressors, thus again showing the need for specific dosage recommendations for individuals expressing CYP3A5.

Figure 5 illustrates the inconsistency in how midazolam, itraconazole, and rifampicin are referred to within EPARs, sometimes being labeled (within one EPAR) as an interactant of CYP3A (which includes both CYP3A4 and CYP3A5) and other times specifically as a CYP3A4 interactant. This can lead to confusion regarding the interpretation of study outcomes, which can potentially affect the consistency, reliability, and clarity of the data presented. For instance, if a study reports interactions with CYP3A, without specifying whether it refers to both isoforms or only CYP3A4, it is unclear whether the conclusions (e.g. dose recommendations, drug-drug interactions) drawn also apply for CYP3A5. Addressing these semantic issues is essential to ensure the accuracy and comprehensiveness of information provided in the EPARs, and eventually in their SmPCs. Therefore, clear and consistent terminology regarding CYP3A4 and CYP3A5 is necessary.

The discrepancies in documenting of specificities of CYP3A4 and 3A5 concerning widely used substrate (midazolam) and inhibitor (itraconazole) and inconsistencies in referral in the EPARs to midazolam, itraconazole, and rifampicin underscore the necessity for further research into the precise affinities of these and more interactants for both 3A isoforms. These interacting compounds play a crucial role in conducting *in vitro* and *in vivo* testing of potential drug-drug interactions. Overestimating or underestimating these effects can significantly impact treatment safety and/or efficacy. Current FDA

and EMA guidelines categorize all CYP3A interactants as either CYP3A or CYP3A4/5 inhibitor or substrate, implying no distinction in affinity between the two isoforms. However, scientific evidence indicates otherwise. Therefore, additional research focusing on the specificity of inhibitors and inducers for CYP3A4 and CYP3A5 is necessary to refine and expand the guidelines to include specific inhibitors/inducers of either CYP3A4, CYP3A5, or both. This will contribute to more consistent terminology regarding CYP3A4/5 and to more accurate drug interaction assessments (reports).

4. CONCLUSION

To conclude, previously evaluated medicinal product did not assess interactions regarding CYP3A4 and CYP3A5 adequately. The minority of EPARs investigated CYP3A5 and only three out of 73 EPARs mentioned CYP3A5 in their *in vivo* conclusions. In these three cases, the effect of CYP3A5 expression next to expression of CYP3A4 was expected to result in a loss of efficacy due to increased metabolism of, and therefore decreased exposure to the medicinal product. Due to expected decreased exposure, no increase in safety issues is expected in case of CYP3A5 expression. However, loss of efficacy, especially for drugs with a narrow therapeutic window and for medicinal products for life-threatening diseases, can have a significant impact on treatment outcome. Dosing recommendations authorised by EMA therefore need to be accurate to ensure optimal treatment, as well as inclusive, given the diversity of ethnicities in Europe. Applicants should include various ethnic groups with different degrees of CYP3A5 expression and perform CYP3A5 genotyping in *in vivo* studies, in order to accurately propose dosing recommendations for CYP3A5 expressing patients.
Additionally, studies describing differences in affinity of inhibitors and substrates for CYP3A4 vs 3A5 bring another issue to light: are the conclusions drawn regarding the (lack of) clinical significance of CYP3A5 accurate? Further research is necessary to ascertain the impact of these differences in specificity (*in vitro* and *in vivo*) on drug metabolism and therapeutic outcomes.

The EMA guideline should be expanded to include recommendations for the investigation of CYP3A4 and CYP3A5 both *in vitro* and *in vivo*. Additionally, it should include information regarding differences in specificity of CYP3A substrates and inhibitors. These additions are necessary for applicants to thoroughly assess potential drug-drug interactions and propose appropriate dosing recommendations for both CYP3A5 expressors and non-expressors.

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