

## Title page

### Review

#### **Activation/deactivation of anticancer drugs by CYP3A4: influencing factors for personalized cancer therapy**

Fengling Wang, Xue Zhang, Yanyan Wang, Yunna Chen, Huiyu Lu, Xiangyun Meng, Xi Ye, Weidong Chen  
Department of Pharmacy, Hefei Hospital Affiliated to Anhui Medical University (The Second People's  
Hospital of Hefei), Hefei 230011, Anhui, China (F.W., X.M., X.Y.); School of Pharmacy, Anhui Medical  
University, Hefei 230032, Anhui, China (F.W.); School of Pharmacy, Anhui University of Chinese  
Medicine, Hefei 230012, Anhui, China (F.W., X.Z., Y.W., Y.C., H.L., W.C.); The Second People's Hospital  
of Hefei, Affiliated to Bengbu Medical College, Hefei 230011, Anhui, China (F.W., X.M., X.Y.);  
MOE-Anhui Joint Collaborative Innovation Center for Quality Improvement of Anhui Genuine Chinese  
Medicinal Materials, Hefei 230012, Anhui, China (W.C.); Institute of Pharmaceutics, School of  
Pharmaceutical Sciences, Anhui University of Chinese Medicine, Hefei 230012, Anhui, China (X.Z., H.L.,  
W.C.)

## Running Title Page

### Activation/ inactivation of anticancer drugs by CYP3A4

**Corresponding author: Fengling Wang**

**Co-corresponding author: Weidong Chen**

Address: Department of Pharmacy, Hefei Hospital Affiliated to Anhui Medical University (The Second People's Hospital of Hefei), Hefei 230011, Anhui, China; E-mail: syx050686wfl@163.com (Fengling Wang), wdchen@ahtcm.edu.cn (Weidong Chen); Telephone: +86 0551 62965779

**Fengling Wang and Xue Zhang contributed equally to this work.**

**Number of text pages: 55**

**Number of figures: 2**

**Number of tables: 3**

**Number of references: 197**

**Number of words in abstract: 237**

**Word count:** 7590 words not including abstract, abbreviations, references, figure legends, and table title and footnotes

#### **Abbreviations**

AUC, area under the curve; CKD, chronic kidney disease;  $C_{max}$ , maximum plasma concentration; CRP, C-reactive protein; CYP: cytochrome P450; CYP3A4, Cytochrome P450 3A4; DDI: drug-drug interaction; DMEs, drug metabolizing enzymes; FDA, Food and Drug Administration; HCC, hepatocellular carcinoma; HLM, human liver microsomes; IL-6, Interleukin 6; NF- $\kappa$ B, nuclear factor- $\kappa$ B; PBPK, physiologically

based pharmacokinetic; TDM, therapeutic drug monitoring; TKI, tyrosine kinase inhibitor; SJW, St. John's wort; SNP, single nucleotide polymorphism.

**Abstract:** Cytochrome P450 3A4 (CYP3A4), one of the most important members of the cytochrome P450 subfamily, is a crucial catalyst in the metabolism of numerous drugs. As it catalyzes numerous processes for drug activation or inactivation, the pharmacological activities and clinical outcomes of anticancer drugs metabolized by CYP3A4 are highly dependent on the enzyme's activity and expression. Due to the complexity of tumor microenvironments and various influencing factors observed in human *in vitro* models and clinical studies, the pharmacokinetics of most anticancer drugs are influenced by the extent of induction or inhibition of CYP3A4-mediated metabolism, and these details are not fully recognized and highlighted. Therefore, this interindividual variability due to genetic and nongenetic factors, together with the narrow therapeutic index of most anticancer drugs, contributes to their unique set of exposures and responses, which have important implications for achieving the expected efficacy and minimizing adverse events of chemotherapy for cancer in individuals. To elucidate the mechanisms of CYP3A4-mediated activation/inactivation of anticancer drugs associated with personalized therapy, this review focuses on the underlying determinants that contribute to differences in CYP3A4 metabolic activity and provides a comprehensive and valuable overview of the significance of these factors, which differs from current considerations for dosing regimens in cancer therapy. We also discuss knowledge gaps, challenges and opportunities to explore optimal dosing regimens for drug metabolic activation/inactivation in individual patients, with particular emphasis on pooling and analyzing clinical information that affects CYP3A4 activity.

**Keywords:** Anticancer drugs, CYP3A4-mediated metabolism, Interindividual variability, Activation, Inactivation, Personalized cancer therapy

**Significance statement:** This review focuses on anticancer drugs that are activated/deactivated by CYP3A4 and highlights outstanding factors affecting the interindividual variability of CYP3A4 activity in order to gain a detailed understanding of CYP3A4-mediated drug metabolism mechanisms. A systematic analysis of available information on the underlying genetic and non-genetic determinants leading to variation in CYP3A4 metabolic activity to predict therapeutic response to drug exposure, maximize efficacy and avoid unpredictable adverse events has clinical implications for the identification and development of CYP3A4-targeted cancer therapeutics.

## Introduction

Currently, chemotherapy remains a common and powerful tool to eliminate malignant cells. However, the efficacy of anticancer drugs is compromised by the frequent emergence of adverse effects, which may result in therapeutic failure (Bins et al., 2019; Oyaga-Iriarte et al., 2019). Metabolism, one of the critical aspects of pharmacokinetics, can alter the chemical structure of drugs and facilitate their elimination through various drug metabolizing enzymes (DMEs) *in vivo*. This process is indispensable in the determination of an optimum window between the drug therapeutic potential and its safety parameters (Purnapatre et al., 2008). It is important to note that the human cytochrome P450 3A (CYP3A) subfamily of cytochrome P450 enzymes (CYPs), which is part of the hemoglobin superfamily, is directly engaged in the phase I metabolic reactions (i.e., oxidation, reduction and hydrolysis) of compounds *in vivo*. CYP3A4, in particular, is a vital isoform of the CYP3A subfamily identified as being involved in the metabolism of more than half of therapeutic drugs. It is predominantly expressed in the liver and intestine (two major

organs for drug oxidation and elimination), accounting for approximately 30% of total hepatic CYP protein and 80% of total small intestinal CYP content (Paine et al., 2006; Wang et al., 2008). A variety of approved anticancer drugs with different chemical structures can be metabolized by CYP3A4 based on the differences in action mechanism and molecular characteristics, including antiestrogens-tamoxifen and exemestane (Desta et al., 2004; Kamdem et al., 2011), nitrogen mustards-cyclophosphamide and ifosfamide (Huang et al., 2000; Schmidt et al., 2004), taxanes-paclitaxel, docetaxel (Hirth et al., 2000; Martínez et al., 2002); topoisomerase inhibitors-irinotecan, etoposide, and teniposide (Santos et al., 2000; de Graan et al., 2013); vinca alkaloids-vincristine, vinblastine, vindesine, and vinorelbine (Kajita et al., 2000); and tyrosine kinase inhibitors (TKIs), such as imatinib, gefitinib, sunitinib, and sorafenib (Towles et al., 2016; Wulkersdorfer et al., 2016; Amaya et al., 2018; Huang et al., 2020; Thomson et al., 2021). Drug efficacy and adverse effects are relevant to changes in the metabolic behavior of the parent compound and plasma or tissue concentrations of its active/inactive metabolites. Generally, the metabolism of CYP3A4 involved in numerous anticancer drugs leads to two pharmacokinetics (drug exposure) and pharmacodynamics (efficacy/toxicity) outcomes. One is the conversion of prodrugs into active or more active metabolites, which may result in higher potency or toxicity; these prodrugs are substances with little or no activity against the desired pharmacological target (e.g., cyclophosphamide, ifosfamide, etoposide, teniposide, tamoxifen and some TKIs). The other biotransformation pathway is deactivated by CYP3A4 into its inactive form, such as taxanes, irinotecan, and a few TKIs (see **Table 1**). Consequently, the pharmacological activity and toxicity of both the parent drug and its metabolite(s) largely depend on the effect of CYP3A4. For instance, if the parent compound is a prodrug, inhibition or induction of metabolism may result in

a decrease or increase in therapeutic efficacy and adverse events. Conversely, if the parent chemical is more active than its metabolite, inhibition or induction of metabolism increases or decreases the exposure to the drug and its therapeutic and/or harmful effects.

The interindividual variability in CYP3A4 expression and activity are very high in the liver (>100-fold) and extrahepatic tissues (Zanger and Schwab, 2013), which is likely dictated by a multitude of factors, including genetic variation and enzyme inhibition or induction due to different patient characteristics (e.g., age, sex), concomitant diseases (e.g., hepatic or renal dysfunction), and inflammation that often accompany tumors; therefore, drug dosage adjustments are frequently required (Kivistö et al., 1995; Kolwankar et al., 2007; Klampfer, 2011; Ahmed et al., 2016; Yamamoto et al., 2018; Al-Zoughbi and Hoefler, 2020). Moreover, considering that cancer patients are commonly prescribed multiple medications, it is essential to evaluate the effects of co-administered CYP3A4 inducers or inhibitors on drug pharmacokinetics and metabolism, as altered metabolic behaviors are closely related to drug exposure, multiple-drug resistance, and unpredictable risk for developing adverse events (Haddad et al., 2007; Tanaka et al., 2011; Filppula et al., 2012). The actual pharmacological and toxic effects of anticancer medications that patients experience are quite unpredictable due to the narrow therapeutic index of the majority of anticancer drugs and the considerable interindividual data variation (Wulkersdorfer et al., 2016; Zhou et al., 2022). Previous reviews have summarized drug-drug interactions (DDIs) mediated by anticancer drugs as CYP3A4 substrates or modulators during cancer therapy, which have increased clinicians' awareness of risk, but have not listed and analyzed the factors that contribute to interindividual variability of CYP3A4 and how clinicians

should assess patients' CYP3A4 activity based on these factors to adjust dosage (Tian et al., 2014).

Despite recent advancements in the characterization of CYP3A4 expression, function, and regulation in cancer, definitive recommendations on dose adjustments for the individual patient by taking into account alterations in CYP3A4 activity remain scarce. To our knowledge, the regulatory mechanisms of CYP3A4 in complex tumor environments, as well as changes in CYP3A4 activity, are directly related to specific anticancer drug exposure and pharmacological outcomes, which are important for selecting individualized treatment regimens (**Fig. 1**). In light of this, this review focuses on anticancer drugs primarily metabolized by CYP3A4 in clinical settings and highlights the prominent factors of the variability in CYP3A4-activated/deactivated anticancer drugs to gain a detailed understanding of the alteration profile of CYP3A4 and predict therapeutic response to drug exposure. In addition, understanding the CYP3A4-mediated drug metabolizing mechanisms will have clinical implications for the identification and development of CYP3A4-targeted cancer therapeutics with the goals of maximizing efficacy and avoiding unpredictable adverse events.

#### **CYP3A4 metabolically deactivated anticancer drugs**

**Taxanes.** Taxanes exhibit an anticancer effect by stimulating tubulin polymerization and suppressing depolymerization. CYP3A4 plays an essential role in the metabolism of taxanes. Both paclitaxel and docetaxel are chemical structural analogs with O-tetracyclic structures at positions 4 and 5 that can be metabolized by CYP3A4, which lowers their bioavailability. A minority of paclitaxel is deactivated *in vivo* by the conversion of CYP3A4 to 3'-p-hydroxypaclitaxel and 6a, 3'-p-dihydroxypaclitaxel, and the remaining paclitaxel is metabolized by CYP2C8. Docetaxel is

metabolized by CYP3A4 to inactive hydroxylated derivatives. Patients with low CYP3A4 activity are at risk of reduced clearance, resulting in increased toxicity of paclitaxel and docetaxel (Shou et al., 1998; Hirth et al., 2000; de Graan et al., 2013; Nieuweboer et al., 2015).

**Topoisomerase inhibitors.** Anticancer drugs targeting topoisomerases I and II have been clinically approved, and several topoisomerase inhibitors in the market to treat or at least slow the progression of cancer have proven to be extremely effective (Dehshahri et al., 2020). Irinotecan, a selective topoisomerase I inhibitor, has been extensively used for more than two decades to treat a range of solid tumors globally, and it remains an essential anticancer drug for many patients (Bailly, 2019). This prodrug generally undergoes two metabolic pathways: the primary metabolic pathway that is currently known is to convert it into an active metabolite SN-38, which is 100~1000 times more active than the parent compound, through carboxylesterase *in vivo* to exert its anticancer effect. Another metabolic pathway requires conversion by hepatic CYP3A4 into two inactive carbonyloxycamptothecin metabolites, APC and NPC, of which NPC can be almost completely hydrolyzed to SN-38 by carboxylesterase, and CYP3A4 indirectly influences the concentration of the active metabolite SN-38. Therefore, when CYP3A4 activity is restrained, the generation of the inactive oxidative products APC and NPC is decreased, while the production of the active metabolite SN-38 is increased, and the therapeutic impact is boosted. In contrast, the elevation of CYP3A4 activity evoked a significant reduction in the pathway for the production of SN-38 in irinotecan-treated patients. In the absence of irinotecan dose adjustment, elevated CYP3A4 activity will place the individual at undesirable risk (Santos et al., 2000; Mathijssen et al., 2002).



**Vinca alkaloids.** Vinca alkaloids, as natural or semisynthetic chemicals, are clinically pivotal antimicrotubule chemotherapeutic agents, such as vincristine, vinblastine, vindesine, and vinorelbine, which have had extensive clinical applications in the treatment of a wide range of cancers over the last several decades (Risinger et al., 2009). The liver provides the primary route for the metabolism of these drugs, and their high interindividual variability in pharmacokinetic properties has been linked to CYP3A4-mediated metabolism (Zhou-Pan et al., 1993; Zhou et al., 1993; Kajita et al., 2000; Yao et al., 2000). *In vitro* data from human liver microsomes (HLM) verified the involvement of CYP3A4 in the biotransformation of vindesine (a vinblastine alkaloid derived from vinblastine), suggesting that this deacetylvinblastine amide can also be converted into a major metabolite (M) (Zhou et al., 1993). Similar to other vinca alkaloids, vincristine undergoes substantial hepatic elimination in humans. CYP3A4 facilitates the metabolism of vincristine, and CYP3A4 inducers increase the systemic clearance of vincristine in patients with brain tumors (Villikka et al., 1999).

**TKIs.** TKIs, a class of small molecule anticancer drugs that target membrane-bound receptors and cytoplasmic tyrosine kinases, can cross the cell membrane to hinder the phosphorylation of tyrosine residues in cancer cells, thereby blocking the transmission of downstream signaling pathways in cells, inhibiting the growth and metastasis of cancer cells and having a negligible effect on normal cells. Since the first TKI drug imatinib was approved for marketing in 2001, more than sixty drugs have been successfully approved by the Food and Drug Administration (FDA) to date, and oncology therapeutics have embraced TKIs as important therapeutic drugs. CYP3A4 is the major metabolic enzyme implicated in the metabolism of almost all TKIs, including the clinically representative TKIs imatinib, erlotinib, gefitinib, asciminib, etc. (Ghassabian et al., 2012; Scheers et al., 2015; Towles et al., 2016; Deeks et al., 2022). A series of TKIs can be deactivated by CYP3A4 into less or inactive metabolite(s).

The metabolism of sorafenib occurs primarily in the liver, and CYP3A4 is the prominent enzyme mediating its metabolism (Keating and Santoro, 2009). It is oxidized selectively by human CYP3A4-mediated oxidation pathways to the principal N-oxide and N-hydroxymethyl metabolites (Ghassabian et al., 2012). Sorafenib N-oxide, which accounts for 17% of circulating analytes in plasma, is the major metabolite of CYP3A4 and has less pharmacological activity. Additional metabolites of CYP3A4 are N-methylhydroxylation acylation, N-oxidation plus N-methylhydroxylation, N-demethylation and N-oxidation plus N-demethylation methylation. It has been observed that coadministration of felodipine can inhibit the activity of CYP3A4 and increase the plasma concentration of sorafenib in patients with hepatocellular carcinoma (HCC) (Gomo et al., 2011). In addition, the CYP3A4 inducer prednisolone facilitated sorafenib metabolism (Noda et al., 2013). Consequently, variations in CYP3A4 activity may influence the metabolism of sorafenib and the susceptibility of these malignancies, thereby considerably altering its therapeutic efficiency and toxicity.

In addition to sorafenib, hepatic CYP3A4 is also implicated in imatinib N-demethylation. During this metabolic process, imatinib forms a metabolite with the same intrinsic affinity and longer half-life but less pharmacological activity (N-desmethylimatinib, CGP74588) (Manley et al., 2013). According to pharmacokinetics studies, the CYP3A4 inhibitor ketoconazole considerably affected the bioavailability of imatinib and its primary metabolite, N-desmethylimatinib, as well as its absorption and metabolic rate. Grape seed and green tea extracts reduce CYP3A4 activity, decreasing imatinib and N-desmethylimatinib exposure and increasing clearance in a dose-dependent manner (Darweesh et al., 2020).

Likewise, nilotinib is metabolized in the liver predominantly through CYP3A4-mediated oxidation and hydroxylation pathways, and its hydroxymethylphenyl and nitroxide metabolites are poorly active as BCR-ABL1 inhibitors. A drug interaction study of nilotinib in combination with a strong CYP3A4 inducer

(rifampicin) or inhibitor (ketoconazole) in healthy subjects indicated that nilotinib exposure decreased [64% decrease in maximum plasma concentration ( $C_{max}$ ), 80% decrease in area under the curve (AUC)] or increased (3-fold increase in AUC), requiring adjustment dosage of nilotinib (Tanaka et al., 2011).

The biotransformation of gefitinib is primarily metabolized by CYP3A4, including morpholineepoxidation and oxidative defluorination, to form two hydroxyaniline metabolites in humans. The predominant plasma metabolite is O-desmethyl gefitinib. It inhibited EGFR-stimulated cell proliferation 14 times less effectively than gefitinib (McKillop et al., 2005). Coadministration of gefitinib with a CYP3A4 inducer (rifampicin) or inhibitor (itraconazole) in healthy male volunteers showed that rifampicin decreased gefitinib plasma levels, while itraconazole increased gefitinib plasma levels (Swaisland et al., 2005).

Dasatinib is extensively metabolized by CYP3A4 into five major metabolites in humans, including para-hydroxylation and C5-methyl hydroxylation of the chloromethylbenzene ring and partial dealkylation of the hydroxyethyl group. The active metabolite formed by CYP3A4 has similar potency to dasatinib, although it only contributes approximately 5% of the AUC of dasatinib, and it has a weak pharmacological effect (Li et al., 2009a).

### **CYP3A4 metabolically activated anticancer drugs**

***Cyclophosphamide and Ifosfamide.*** Cyclophosphamide and ifosfamide are the most broadly used alkylating agent antineoplastic prodrugs for the treatment of various malignancies, including solid and hematological malignancies. Cyclophosphamide needs to be catalyzed by hepatic CYPs (including CYP2B6, CYP3A4/3A5 and CYP2C9) to exhibit cytotoxic activity *in vivo*, and its main activation of the metabolic pathway therefore depends on the catalysis of CYP3A4 to form the active metabolite 4-hydroxycyclophosphamide (4-OHCP) (accounting for 70% to 80%). Cyclophosphamide undergoes

dechloroethylation, predominantly catalyzed by CYP3A4, to form the inactive metabolite 2-chloroethylcyclophosphamide and an equimolar byproduct chloroacetaldehyde (Huang et al., 2000). This minor metabolic pathway accounts for approximately 19% of the total metabolic clearance of cyclophosphamide. In addition, 4-OHCP can undergo secondary metabolism by CYP3A4 to form inactive 4-ketocyclophosphamide (Jounaidi et al., 1998; Patterson and Murray, 2002). Treatment with CYP3A4 inhibitors could produce a metabolic inhibitory effect, and cyclophosphamide produced nephrotoxic and neurotoxic chloroacetaldehyde exposure, thereby reducing the side effects caused by cyclophosphamide metabolism to chloroacetaldehyde (Yang et al., 2018). Ifosfamide is metabolized by CYP3A4 to generate active 4-hydroxy derivatives to exert its cytotoxic effect (Huang et al., 2000). Factors that affect CYP3A4 activity, however, can disturb the balance between activation (4-hydroxylation) and inactivation (N-dichloroethylation) of the two metabolic pathways, leading to changes in the pharmacokinetics and pharmacodynamics of such drugs. By activating hypoxia-inducible factor-1 activity in DAOY medulloblastoma cells, its expression was suppressed, and the prevention of the conversion of cyclophosphamide and ifosfamide to their active forms reduced the cytotoxicities (Valencia-Cervantes et al., 2019).

**Tamoxifen.** Tamoxifen, a prodrug with relatively low affinity for the estrogen receptor, has been approved by the FDA for the treatment and prevention of breast and ovarian cancer. CYP3A4 is the main enzyme subtype that metabolizes tamoxifen, and its metabolites endoxifen and 4-hydroxytamoxifen have a high affinity for estrogen receptors. Both metabolites are 30 to 100 times stronger than tamoxifen in antiestrogenic activity. Moreover, these metabolites suppress estrogen-dependent cell proliferation and gene expression more effectively than tamoxifen (Desta et al., 2004). Since the plasma concentration of endoxifen far exceeds that of 4-hydroxytamoxifen,

endoxifen is considered to be the most important active metabolite due to its efficacy. In breast cancer patients (all of which are extensive metabolizers) treated with tamoxifen, when moderate or strong CYP3A4 inhibitors (e.g., amiodarone, ciprofloxacin, clarithromycin, diltiazem, fluconazole, fusidic acid) were concomitantly used, endoxifen levels were observed to be significantly lower than in patients not receiving concomitant inhibitors (Puszek et al., 2019a).

**Natural compounds.** Novel derivatives of the antineoplastic agents vinblastine and vincristine are derived from the *Madagascar vinca* plant, and these vinca alkaloids have improved therapeutic properties. Previous studies have confirmed that CYP3A4 is involved in the metabolism of vinblastine and vinorelbine (Zhou-Pan et al., 1993; Yao et al., 2000; de Graeve et al., 2008). According to *in vitro* results obtained from HLM and recombinant CHO cells, CYP3A4-mediated metabolism converts vinblastine to a major metabolite (desacetyl vinblastine), and this compound is more biologically active than the parent drug (Zhou-Pan et al., 1993; Yao et al., 2000). Vinorelbine is a semisynthetic compound, CYP3A4 is the predominant enzyme involved in its biotransformation in humans, 4-O-deacetyl vinorelbine has been demonstrated to be the main active metabolite, and its antitumor activity is similar to vinorelbine (Kajita et al., 2000; de Graeve et al., 2008). Another antineoplastic natural product, epipodophyllotoxin, is a lignan extracted from the roots and rhizomes of *Podophyllum* species. Over the years, its semisynthetic chemotherapeutic derivatives etoposide and teniposide have already been applied for cancer therapy and were shown to have high clinical importance. CYP3A4 also plays a critical role in the metabolism of these two DNA topoisomerase II inhibitors, which undergo O-demethylation to produce catechol (Relling et al., 1994; Zhuo et al., 2004).

**TKIs.** CYP3A4 can convert some TKIs into their active metabolites (see Table 1). *In vivo*, sunitinib is largely metabolized by CYP3A4 to create the principal active metabolite N-desethyl sunitinib, which has

anticancer activity comparable to that of sunitinib. Therefore, the anticancer activity of sunitinib is achieved by the parent drug in combination with the active metabolite (Amaya et al., 2018). Quantitative reactive phenotyping experiments demonstrated a relatively important contribution of the hepatic CYP3A4 enzyme correlated with the metabolic activation of lapatinib, which is O-dealkylated and subsequently oxidized to a reactive and potentially hazardous quinoneimine (Towles et al., 2016; Bissada et al., 2019). Interindividual variability in CYP3A4 activity likely influences exposure to the reactive metabolite quinoneimine. This reactive metabolite was associated with hepatotoxicity and may covalently modify the CYP3A4 apoprotein and/or heme moiety, potentially leading to inactivation mediated by the CYP3A4 metabolic mechanism (Teng et al., 2010; Takakusa et al., 2011). CYP3A4 inducers (dexamethasone and rifampin) increased lapatinib's cytotoxicity in HepaRG cells, which was strongly related to increased reactive metabolite generation (Hardy et al., 2014). Furthermore, debenzylated lapatinib was more cytotoxic to HepaRG cells than the parent agent (Hardy et al., 2014). In breast cancer patients, concomitant use of a CYP3A4 inducer (dexamethasone) and lapatinib increased the risk of lapatinib-induced hepatotoxicity compared with lapatinib alone (Teo et al., 2012). Accordingly, an integral part of successful individualized therapy is to optimize lapatinib exposure based on metabolic mechanisms without adverse toxicity.

### **Activity and expression of CYP3A4 in tumor tissues**

In addition to being expressed in the liver, CYP3A4 is expressed in a variety of extrahepatic tissues, including malignant tissues. Since the toxicity of a given agent is closely linked to its metabolic fate in the target tissue, the profile of CYP3A4 in target tumor tissue potentially has a significant impact on the *in situ* metabolism of anticancer drugs mediated by this enzyme (Kapucuoglu et al., 2003; Schmidt et al., 2004; van Eijk et al., 2019). Each tumor tissue possesses a unique characteristic of CYPs that, by and large, determines the sensitivity to a given drug,

therefore leading to a tumor tissue-selective therapeutic response to drug exposure (Ding and Kaminsky, 2003). For instance, CYP3A4 protein is expressed in approximately 20% to 55% of breast cancer tissue, in which CYP3A4 mediates the metabolic mechanism of chemotherapeutic drugs (Schmidt et al., 2004). The expression level of CYP3A4 in breast cancer tissue is directly correlated with clinical outcomes in patients treated with docetaxel (Miyoshi et al., 2002). Patients with high CYP3A4 mRNA levels in breast cancer tissue were significantly less chemosensitive to docetaxel treatment than patients with low CYP3A4 mRNA levels (Miyoshi et al., 2002). Furthermore, clinical data showed that low CYP3A4 expression in primary breast cancer lesions had significantly better response and clinical benefit rates to docetaxel treatment, indicating that assessing the expression of CYP3A4 in breast cancer tissue is an effective method for predicting tumor response to docetaxel treatment (Sakurai et al., 2011). According to the results of the aforementioned studies, the high levels of CYP3A4 mRNA in tumor tissues could accelerate the metabolism of docetaxel, hence limiting its efficacy or potentially contributing to resistance.

Moreover, tissue-specific expression and activity of CYP3A4 differ from those found in normal or paracancerous tissues, influencing the local activation or inactivation of intratumoral drugs. A prospective ColoCare study showed that CYP3A4 expression was significantly downregulated in colorectal tumors relative to normal mucosa tissues (Beyerle et al., 2020). The presence of CYP3A activity in colorectal cancer cells could also influence tumor sensitivity to paclitaxel, and inhibition of CYP3A4 in colorectal cancer patients treated with paclitaxel significantly decreased intratumoral paclitaxel inactivation (Martínez et al., 2002). Similarly, CYP3A4 is expressed both in normal prostate tissue and prostate cancer tissue. Reduced CYP3A4 expression in prostate

cancer cells relative to normal prostatic tissue may result in differing therapeutic responses to taxanes (Mitsiades et al., 2012). Likewise, both mRNA and protein expression of CYP3A4 were decreased in the tumor tissue of HCC patients compared with nontumor liver tissues. This may be caused by special metabolic reorganization and changes in the *in vivo* environment (Flannery et al., 2020). As a result, CYP3A4 inhibition in tumor tissues of HCC patients was linked to a marked decrease in CYP3A4-mediated drug metabolism. For example, due to a remarkable decrease in CYP3A4 expression, sorafenib metabolism was significantly reduced in the HLM and tumor tissue of HCC patients (Ye et al., 2014; Hsieh et al., 2020). It should be noted that CYP3A4 expression levels may vary depending on the stage of tumor development. Unfortunately, the factors that contribute to the difference in CYP3A4 expression levels between tumor and normal tissues remain largely unknown.

#### **Factors influencing CYP3A4 activity and expression in personalized cancer therapy**

***Impact of physiological conditions on CYP3A4-activated/deactivated anticancer drugs.*** With respect to nongenetic factors, a series of studies have reported a sex difference in CYP3A4 activity. It has been confirmed that CYP3A4 activity is higher in women than in men by analyzing plasma 4 $\beta$ -hydroxycholesterol (an endogenous CYP3A marker) in subjects from different populations (Diczfalusy et al., 2011). The protein and mRNA levels as well as the activity of CYP3A4 in HLMs and hepatocytes were higher in females than in males (Liu et al., 2021). Conversely, studies in HLM from diabetes and nonalcoholic fatty liver disease donors suggested an insignificant sex difference in CYP3A4 activity and protein expression (Jamwal et al., 2018). As a result, the effects of sex on CYP3A4 metabolic activity under various conditions need to be further elucidated.



There are significant age differences in the activity and expression of CYPs due to age and age-related factors (e.g., height, weight, body surface area, body fat, serum albumin, total body water), which potentially affect intrinsic drug metabolism and disposition (Maagdenberg et al., 2018; Liu et al., 2021). In children aged from 1 to 18 years, the activity of CYP3A4 per gram of liver decreased slightly with age, as measured by a physiological population pharmacokinetic modeling method, indicating that the activity of CYP3A4 is affected by age (Brussee et al., 2018). However, no age-related differences in CYP3A4 activity were observed when comparing samples in HLMs and hepatocytes from the 20- to 60-year-old age group with the over 60-year-old age group (Parkinson et al., 2004; Jamwal et al., 2018).

Although information on the physiological conditions for CYP3A4-mediated metabolism appears to be easily obtained for guiding clinicians in selecting appropriate dose adjustments, in the majority of studies, the effect of sex and age on CYP3A4 metabolic activity remains to be verified in a subpopulation of specific cancers, which may have implications for cancer therapy. In most cases, the fact that cancer patients usually have other diseases and confounding factors makes it difficult to obtain specific data on the CYP3A4 profile of these patients.

***Impact of tumor-associated inflammation on CYP3A4 and drug responses.*** For decades, inflammation has been considered a pivotal factor for interindividual variability of CYP3A4 metabolism, which should be taken into account in drug development and clinical practice to avoid undesired drug responses and toxicities (Lenoir et al., 2021). Within the tumor microenvironment, elevated plasma concentrations of the acute-phase reactant C-reactive protein (CRP) and proinflammatory cytokines, particularly interleukin 6 (IL-6), were proven to play important roles in the occurrence and development of a variety of malignancies (e.g., tumor cell proliferation, invasion, progression and metastasis) (Heikkilä et al., 2008; Allin and Nordestgaard, 2011; Taniguchi and Karin, 2014). Convincing evidence has revealed that the effects of

tumor-derived CRP and IL-6 are closely associated with reduced mRNA and protein synthesis of hepatic CYP3A4 and attenuated enzymatic activity in tumors derived from different patients, directly leading to decelerated CYP3A4-mediated metabolism and enhanced toxicity to docetaxel and vinorelbine (Rivory et al., 2002; Charles et al., 2006; Kacevska et al., 2008). Consistent with clinical observations, the repressed transcriptional level of the CYP3A4 gene was greatly associated with increased production of these inflammatory markers in human primary hepatocytes and different animal models of extrahepatic cancer xenografts, and the concentration-inhibition relationship between IL-6 and CYP3A4 appears to be the strongest among the proinflammatory cytokines and CYP isozymes (Charles et al., 2006; Aitken and Morgan, 2007). These observations indicate that the inhibitory effect of proinflammatory cytokines on CYP3A4 expression and activity is recognized as a common feature of tumor-derived inflammatory responses, which thus impairs the biotransformation of a wide range of (pro) drugs that are metabolized through the CYP3A4 enzyme (Kacevska et al., 2008). A physiologically based pharmacokinetic model was recently developed to quantify the effect of inflammatory responses on CYP3A4 activity to predict the pharmacokinetic behavior of its substrates based on systemic CRP (Simon et al., 2021). Mechanistically, previous work revealed that a network signal pathway of transcription factors, including the nuclear receptor-mediated regulatory pathways, nuclear factor-kappaB (NF- $\kappa$ B) signaling pathways, and several other liver-enriched transcription factors, was responsible for tumor-derived proinflammatory cytokines and likely to be involved in inflammation-altered CYP3A4 repression (Goodwin et al., 1999; Goodwin et al., 2002; Charles et al., 2006; Zhou et al., 2006; Martínez-Jiménez et al., 2007). Recent studies suggest that the pentose phosphate pathway contributes to IL-6-mediated CYP3A decrease and that miR-155 and other miRNAs cause coordinated CYP3A4 downregulation in the context of inflammation (Kugler et al., 2020; Liu et al., 2020).

Furthermore, obesity is a low-grade chronic inflammatory condition that has been linked to tumor-promoting effects (Olefsky and Glass, 2010; Iyengar et al., 2016). It has been reported that CYP3A4 activity and protein expression is suppressed *in vitro* and in obese humans (Kotlyar and Carson, 1999; van Rongen et al., 2018). Taken together, these studies imply that tumor-mediated inflammation has the potential to affect CYP3A4-mediated drug responses and toxicities. Thus, it is biologically and therapeutically important to pay close attention to the critical relationship between tumor-related inflammation and CYP3A4 activity and expression, as well as to conduct intervention studies to improve the pharmacokinetics and efficacy of these chemotherapeutic drugs.

***Impact of hepatic or renal impairment on CYP3A4 and drug responses.*** Hepatic and renal function are considered to be major determinants of drug exposure (Krens et al., 2019). Unfortunately, renal impairment and hepatic impairment in cancer patients are common comorbidities either because of the disease itself or as a result of previous toxic anticancer regimens.

The liver is known to be the primary organ involved in the metabolism and disposal of the vast majority of drugs. Because the activity of CYPs in the liver has a significant impact on the pharmacokinetics and therapeutic effects of the majority of anticancer drugs, when the main organs are damaged, homeostasis is disrupted, and the expression and activity of hepatic CYPs may change. In most cases, the release of circulating proinflammatory cytokines caused by infection or inflammation, for example, can result in altered intrahepatic and extrahepatic CYP expression and decreased CYP activities. Accordingly, liver dysfunction is associated with decreased CYP3A4 activity (Kolwankar et al., 2007). The catalytic activity and protein expression of CYP3A4 were significantly lower than those in healthy individuals quantified by using proteomic methods and HLM, which may alter drug clearance, thereby influencing the efficacy and safety of CYP3A4-metabolized drug treatments (Jamwal et al., 2018).

Moreover, CYP3A4 activity in the liver decreased with increased fat content, and protein content decreased with increased severity of steatosis in humans (Kolwankar et al., 2007). The etiology (noncholestatic vs. cholestatic) and severity of liver disease are closely related to decreased CYP3A4 activity, and the decreased CYP3A4 activity contributes to an impaired ability of the liver to eliminate therapeutic drugs metabolized by CYP3A4 among patients with hepatic cirrhosis, which potentially affects subsequent therapy response (Furlan et al., 1999; Chalasani et al., 2001; Yang et al., 2003). Prior pharmacokinetics studies have demonstrated that the metabolic clearance of CYP3A4 substrates (lignocaine, quinidine, midazolam, etc.) in patients with liver cirrhosis is significantly reduced (Stanek et al., 1997; Orlando et al., 2003; Vuppalachchi et al., 2013). In HCC patients with a certain degree of liver damage, the altered pharmacokinetics of sorafenib and bosutinib resulted in an unpredictable inhibition-plasma concentration relationship between CYP3A4 and drug exposure *in vivo* (Fucile et al., 2015; Abbas and Hsyu, 2016).

In addition to hepatic impairment, care should also be given to the activity and expression of CYP3A4 in renal impairment. It is estimated that approximately 55% of cancer patients have a glomerular filtration rate below 90 mL/min, and approximately 15% have a glomerular filtration rate below 60 mL/min (Launay-Vacher et al., 2007). In patients with chronic kidney disease (CKD), the activity and/or expression of DMEs may be downregulated or directly inhibited by accumulated toxins, and drug metabolic clearance may be altered, which may lead to increased drug exposure at normally administered doses. Renal impairment reduces not only renal clearance but also hepatic clearance of CYP3A4 substrates. CYP3A4 activity is significantly attenuated in patients with severe CKD, which could have a substantial impact on drug therapy. Therefore, for patients with moderate to severe renal impairment, it is necessary to adjust the dose through therapeutic drug monitoring (TDM) to ensure that patients with CKD receive the maximum benefit from drug treatments while minimizing potential toxicity (Yamamoto et al., 2018). For

patients with mild renal or hepatic impairment, asciminib and dabrafenib are generally well tolerated, and no dose adjustment or special monitoring is required (Puszkiel et al., 2019b; Hoch et al., 2021). Additionally, the effects of uremic toxins on CYP3A4 activity were diminished in end-stage renal disease patients on maintenance hemodialysis, whereas there was no discernible effect on the pharmacokinetics of CYP3A4-metabolized drugs. However, caution is recommended when administering and performing appropriate TDM (Pai et al., 2019).

***Concomitant drugs involving inhibition or induction of CYP3A4-activated/deactivated anticancer drugs.***

In most cases, cancer patients receive combination chemotherapy along with other drugs (such as antiemetics) as needed, which might induce or inhibit CYP3A4 activity, thereby altering the metabolic balance of activation and inactivation of some anticancer drugs. The risk of clinical safety issues is high under these conditions. Therefore, care should be taken to avoid potential DDIs of anticancer drugs, which may modulate the activity of this metabolic pathway in the liver and extrahepatic tissues. Comedication with CYP3A4 inducers might enhance the activity of this enzyme and promote the activation/inactivation of CYP3A4-metabolized anticancer drugs, possibly resulting in enhanced/reduced efficacy and toxicity. Conversely, inhibitors and substrates of CYP3A4 may interfere with the activation/inactivation of these drugs (Kivistö et al., 1995). For example, the CYP3A4 inducer St. John's wort (SJW) could increase docetaxel metabolism, and the effect of SJW on the pharmacokinetics of irinotecan was stronger than that of docetaxel (Komoroski et al., 2005). The co-administered SJW in patients treated with irinotecan reduced the concentration of active metabolites in plasma and affected the efficacy (Mathijssen et al., 2002). Therefore, it is theoretically possible that patients with CYP3A4-metabolized drug treatments would experience increased drug toxicities when co-administration of CYP3A4 inhibitors. However, a

retrospective study did not indicate an increase in irinotecan-induced toxicity when co-administered with clarithromycin (a CYP3A4 inhibitor) in patients with colorectal cancer (Makihara et al., 2017), possibly due to uridine diphosphate glucuronosyltransferase isoform 1A1, organic anion transporter 1B1, genetic variation factors such as irinotecan-induced wide interindividual variability in drug response and toxicity (Riera et al., 2018). More detailed studies on individualized dose adjustment for cancer patients in clinical practice may be accomplished by developing a combined population pharmacokinetic model of irinotecan and its metabolites to prevent adverse effects caused by excessive drug/metabolite accumulation (Oyaga-Iriarte et al., 2019).

The pharmacokinetic consequences of TKIs are frequently affected by comedication with known CYP3A4 inducers or inhibitors in clinical practice (Teo et al., 2015). Co-administration of erlotinib with a CYP3A4 inhibitor (ketoconazole) significantly increased erlotinib exposure, but pre- or contemporaneous treatment with a powerful CYP3A4 inducer (rifampicin) decreased erlotinib exposure (Rakhit et al., 2008; Hamilton et al., 2014). Hence, dosage adjustment of erlotinib may be necessary when CYP3A4 inducers and inhibitors are concomitantly used. It has been reported that the anti-acute myeloid leukemia mediated by different TKIs (sorafenib, quizatinib, and gilletitinib) in bone marrow mesenchymal stem cells is affected by CYP3A4 activity. Inhibition of CYP3A4 activity by clarithromycin reduced the resistance of bone marrow mesenchymal stem cells to TKIs (Chang et al., 2019). Possible interactions between imatinib and CYP3A4 inhibitors or inducers may result in alterations in the plasma concentration of imatinib and co-administered drugs. Two open-label, fixed-sequence studies showed that co-administration of imatinib with SJW in healthy adult volunteers significantly affected the pharmacokinetics of imatinib (reduced imatinib exposure by 30% ~ 40% and increased oral clearance by 44%), which may compromise imatinib's clinical efficacy (Frye et al., 2004; Smith et al., 2004). Aprepitant (a moderate CYP3A4 inhibitor), a drug

that prevents chemotherapy-induced nausea and vomiting, largely increased the AUC and  $C_{max}$  (99% and 53%, respectively) following a single dose of bosutinib in healthy volunteers (Hsyu et al., 2017). Another study showed that sorafenib increased plasma concentration in HCC patients by inhibiting CYP3A4 in combination with felodipine (Gomo et al., 2011), and combined with prednisolone reduced the plasma concentration by inducing CYP3A4 (Noda et al., 2013). In addition, enhanced exposure to sunitinib or pazopanib was detected in patients co-administered with CYP3A4 inhibitors (amlodipine, amiodarone, diltiazem), hence increasing the risk of adverse events (Azam et al., 2020).

Increased attention should be given to CYP3A4-metabolized anticancer drugs with narrow therapeutic indices that are also CYP3A4 inhibitors and/or inducers in the clinic (see **Table 2**). For instance, tamoxifen and its active metabolites desmethyltamoxifen and 4-hydroxytamoxifen inhibit CYP3A4 enzyme-mediated metabolic activity; therefore, tamoxifen and its metabolites may cause DDIs by inhibiting the metabolic activity of CYP3A4, particularly when used in combination with other CYP3A4-metabolized substrates (Bekaii-Saab et al., 2004). In addition, some TKIs, such as imatinib, dasatinib, and gefitinib, have different degrees of inhibitory effects on CYP3A4 activity (Teo et al., 2015). These TKIs may result in pathway-dependent inhibition of paclitaxel hydroxylation by reducing the ratio of paclitaxel metabolites, and such differential metabolism has been linked to paclitaxel-induced neurotoxicity in cancer patients (Wang et al., 2014). Imatinib has a moderate inhibitory effect on CYP3A4 activity and can competitively inhibit the metabolism of CYP3A4 substrates (Filppula et al., 2012). Dasatinib is a weak time-dependent inhibitor of CYP3A4, which is also subject to DDIs with CYP3A4 inhibitors or inducers. In clinical DDI studies, when co-administered with a potent CYP3A4 inhibitor (ketoconazole) in patients with advanced solid tumors, its exposure increased nearly 5-fold, and the terminal half-life was reached within 3.3 h~8.7 h (Johnson et al., 2010). Concomitant apatinib administration resulted in significant increases in systemic

exposure to nifedipine (CYP3A4 substrate) (Zhu et al., 2020), and it greatly hindered the metabolism of gefitinib *in vitro* and *in vivo* (Wang et al., 2021).

These available pharmacokinetic-pharmacodynamic DDI data suggest that co-administered inducers or inhibitors of CYP3A4 may have a considerable impact on drug exposure and overall clinical outcomes. Especially for anticancer medicines with low therapeutic indices and steep dose–response curves, even small alterations in the pharmacokinetic profile might significantly affect the clinical effectiveness of drugs. Due to the increased risk of toxicity, any metabolic interactions resulting from concurrent therapy with CYP3A4 inhibitors or inducers should be recognized and avoided as soon as possible. If necessary, the daily dose must be lowered (Teo et al., 2012; Noda et al., 2013; Azam et al., 2020).

***Impact of genetic polymorphisms on CYP3A4 and drug responses in cancer therapy.*** The presence of single nucleotide polymorphisms (SNPs) is another predominant factor in CYP3A4 expression and activity, causing sharp variations in drug susceptibility and leading to differences in CYP3A4-dependent drug response, pharmacological activity, toxicity and clearance among individuals and ethnicities (Ozdemir et al., 2000; Rahmioglu et al., 2011). According to an investigation of nucleotide diversity and spectrum, CYP3A4 is predominantly selectively distributed among Africans, Caucasians and Chinese (Chen et al., 2009; Hu et al., 2017). The current Human CYP Allele Nomenclature Database shows that 15 distinct CYP3A4 allelic variations can modify its function (<https://www.pharmvar.org/gene/CYP3A4>). Variations in CYP3A4 expression and activity can be elicited by some CYP3A4 SNPs, hence affecting the metabolism of CYP3A4 substrate drugs (summarized in **Table 3**) and the clinical and pharmacokinetic consequences of CYP3A4 gene variants. The predominant CYP3A4 genotype is the wild type (CYP3A4\*1/\*1). Multiple SNP mutations are associated with alterations in the



expression and activity of the CYP3A4 gene, resulting in substantial racial disparities. Among these alleles, the mutation frequency of CYP3A4\*1G (g.20230G>A, rs2242480) in intron 10 of the noncoding region of CYP3A4 is high in Chinese, Asian Caucasian and African individuals (Du et al., 2007). This allele acts as an enhancer and promoter to increase the activity of CYP3A4, enhancing the metabolic capacity (Du et al., 2007). Another gene, CYP3A4\*1B (rs2740574A>G), has considerable regional differences in distribution, and the allele frequency in the gene's promoter region is relatively prominent and less distributed in Asians, while it is more distributed in Africans. To date, the reported results of pharmacokinetics and drug exposure-safety association studies of CYP3A4\*1B gene variants have been inconsistent, and it remains controversial whether CYP3A4\*1B exhibits altered enzymatic transcription rates and activity (Spurdle et al., 2002; Atasilp et al., 2020; Torres Espíndola et al., 2020). In non-African populations, CYP3A4\*1B mutation moderately increased CYP3A4 expression and activity (Schirmer et al., 2006), whereas this genetic variant had no profound effect on the pharmacokinetics and drug toxicity of erlotinib and its major metabolite OSI-420 in young children with brain tumors (Reddick et al., 2019). Similarly, the CYP3A4\*1B allele was marginally correlated with high-grade toxicity in erlotinib-treated patients. Individuals with higher CYP3A4 expression (G/G and A/G) were less likely to develop rash (Rudin et al., 2008).

Some frequent allelic variations (e.g., CYP3A4\*18 and \*16 alleles) can alter catalytic activities depending on different substrate characteristics *in vitro* and *in vivo*, leading to interindividual differences in the pharmacokinetics and pharmacodynamics of these drugs. CYP3A4\*18 (rs169068 T>C) results in a shift from leucine to proline at codon 293 due to a variant allele in exon 10 (Leu293Pro). Three genotypes exist: wild type (T/T), heterozygous mutant (T/C), and homozygous mutant (C/C) (Hu et al., 2005). CYP3A4\*18 is primarily distributed among East

Asians, such as Chinese, Japanese, Koreans, and Malaysians, but not in Caucasians. The existence of CYP3A4\*18 SNP loci was associated with an increase in the level of CYP3A4 activity (Fukushima-Uesaka et al., 2004), which could lead to a significant reduction in systemic plasma exposure of CYP3A4 substrates (Zeng et al., 2009). However, no correlation was observed between the increased toxicity of docetaxel and CYP3A4\*18 in patients. Similarly, a recent study suggested that the CYP3A4\*18 allele is not significantly associated with irinotecan-induced severe neutropenia (Atasilp et al., 2020). Likewise, another study found no significant connection between CYP3A4\*18 variation and imatinib mesylate response in Malaysian patients with chronic myeloid leukemia (Maddin et al., 2016). CYP3A4\*16 has been commonly observed in Japanese, Korean and Mexican populations (Lamba et al., 2002; Ruzilawati et al., 2007; Chen et al., 2011; Hu et al., 2017). For paclitaxel and irinotecan, CYP3A4\*16 showed a considerable reduction in enzymatic activity (more than 60%) toward paclitaxel and irinotecan compared to the wild type, while CYP3A4\*18 exhibited a moderate reduction in its catalytic activity (by 34% ~ 52%) (Maekawa et al., 2010). In a clinical study of 235 Japanese cancer patients treated with paclitaxel, a significant reduction (20%) in paclitaxel metabolite 3-p-hydroxypaclitaxel/paclitaxel exposure was observed in heterozygous CYP3A4\*16 carriers compared to wild-type CYP3A4\*1/\*1 carriers, suggesting a lower metabolic activity of CYP3A4\*16 (Nakajima et al., 2006). In contrast, the catalytic activity of CYP3A4\*16 toward docetaxel was retained, indicating that this allele has no substantial impact on docetaxel metabolism *in vivo* (Maekawa et al., 2010).

Several variant alleles are associated with reduced hepatic CYP3A4 gene transcription, and attenuation of enzymatic activity therefore reduces drug clearance and increases the risk of paclitaxel-induced grade 3 neurotoxicity (de Graan et al., 2013; Hannachi et al., 2020). For example, CYP3A4\*20 allele

(rs67666821) is a rare variant in which enzyme activity is absent. It is found in 1.2% of the Spanish population while harboring a low frequency in most Asian, European, and African populations (0.22%, 0.06%, and 0.26%, respectively) (Apellániz-Ruiz et al., 2015a). This allelic variant is characterized by the insertion of an adenine residue (c.1461\_1462insA), which carries a premature stop codon (p. P488Tfs\*494) and thus synthesizing a truncated and inactive protein (Westlind-Johnsson et al., 2006). Similar to CYP3A4\*20, the CYP3A4\*22 variant allele (rs35599367 C>T) is a frequently investigated allele variant of CYP3A4 that is mainly due to a downregulation of CYP3A4 mRNA expression and activity both *in vivo* and *in vitro*, with an incidence of 0.083 in Caucasians and 0.043 in Asian and African populations (Elens et al., 2011). A population-pharmacokinetic model was recently developed to investigate pazopanib systemic exposure; its clearance rate in CYP3A4\*22 mutant patients was significantly lower (35%) than that in wild-type patients, and the incidence of severe toxicity in patients was increased, requiring dose adjustment according to CYP3A4\*22 status (Bins et al., 2019). A multivariate analysis indicated that the clearance of another TKI, sunitinib, was reduced by 22.5% in 114 patients with metastatic renal cell carcinoma or gastrointestinal stromal tumors who had the CYP3A4\*22 genotype compared to the clearance in CYP3A4\*22/\*22 participants (Diekstra et al., 2014). Similarly, erlotinib clearance was reduced in people carrying the CYP3A4\*22 allele, and CYP3A4\*22 heterozygotes were associated with severe early onset myelosuppression in patients with sunitinib-induced renal cell carcinoma (Patel et al., 2018). A pharmacokinetic-pharmacogenetic evaluation showed that the CYP3A4\*22 genotype was closely related to the metabolism and efficacy of tamoxifen in 730 breast cancer patients who received tamoxifen adjuvant therapy, resulting in increased plasma concentrations of endoxifen (Puszkiel et al., 2019a).

In addition, some low-frequency/rare alleles (e.g., CYP3A4\*2, CYP3A4\*4,\*5, \*6, \*8, \*11, \*12, \*13, \*17 and \*26 alleles) have been reported to be linked to reduced enzymatic activity (Hsieh et al., 2001; García-Martín et al., 2002; Lamba et al., 2002; Chen et al., 2011; Werk and Cascorbi, 2014). Few studies have investigated the impact of such genetic variants on the pharmacokinetic consequences of anticancer drugs specifically metabolized by CYP3A4. There are no changes between the wild-type gene and other gene variants in the levels of expression and enzyme activity, such as CYP3A4\*3,\*7, \*9 and \*10 (Dai et al., 2001; Eiselt et al., 2001).

On the whole, the high frequency of these genetic variants may alter enzyme activity in the context of genotype-phenotype association. The genotype or actual phenotype of different populations and substrate drug-dependent characteristics should be elucidated before treatment to predict drug response and toxic effects. Prior pharmacogenetic testing may assist individualize clinical treatment and improve the benefit/risk ratio of pharmaceuticals.

### **Current Challenges, Knowledge Gaps, and Future Perspectives**

CYP3A4 is involved in the metabolism of numerous anticancer drugs, and several CYP3A4-metabolized drugs also act as inhibitors or inducers of this enzyme. The pharmacological activity of both the parent drug and its metabolite(s) depend on the activity of CYP3A4. Due to the large interindividual variation in CYP3A4 activity and narrow therapeutic window of most anticancer drugs, understanding the factors (e.g., age, gender, concomitant disease, inflammation, concomitant drugs, and genetic variation) that affect CYP3A4 activation/inactivation of anticancer drugs based on metabolic mechanisms is of great significance for tailoring pharmacotherapy to improve therapeutic efficacy and minimize adverse drug effects. Liver or renal impairment can reduce the expression and activity of CYP3A4 to vary degrees. Tumor-derived inflammatory responses are associated with repression of CYP3A4 expression and activity.

Particular caution should be recommended for co-administration of CYP3A4 substrates with its inhibitors and inducers, especially for individuals with a low therapeutic index, as induction or inhibition of CYP3A4 activity may lead to changes in drug response, systemic exposure, and efficacy. Moreover, mutants at various SNP locations can change CYP3A4 catalytic activity and gene expression, which, in turn, directly lead to differences in drug exposure and response. Besides, the metabolic activity of CYP3A4 in tumor sites is different from that in normal or adjacent tissues owing to the special living environment of tumor tissues, which inevitably affects local drug response and efficacy/toxicity.

However, several knowledge gaps for comprehensive evaluation of CYP3A4-activated/deactivated anticancer drugs were identified. Current standard of care involves consideration of P450 polymorphisms, DDIs, etc. Almost no information is available on how the synergy of these contributing factors to CYP3A4 activity and expression in personalized cancer therapy. For a comprehensive and reliable assessment of a patient's CYP3A4 activity, not only information on the patient's body surface area, genetic variants, and DDI needs to be obtained, but other factors (e.g., age, sex, concomitant disease, inflammation, tissue-specific expression and activity of CYP3A4) should also be considered as quantitative indicators for predicting drug concentration-response relationships to aid in dose adjustment, which are distinct from current considerations for dosing regimens in cancer therapy. Another knowledge gap is the lack of information on the relationship between the metabolic characteristics of CYP3A4 and effective concentrations of different substrates in tumor tissues. The influences of the structural diversity of different CYP3A4-metabolized substrates and varying protein-binding properties on CYP3A4 catalytic activity should be considered. Generally, the use of TDM approaches to estimate drug concentration is primarily dependent on the drug partition in whole blood. Another issue arises whether the active metabolites can reach effective concentrations in the tumor cells if bioactivation occurs predominantly in the liver. As a

result of the physicochemical properties of the compound or poor vascularization of the tumor, delivery of the drug or active metabolite into the tumor is possibly inadequate, and their plasma concentration may not reflect the actual concentration of tumor tissue. Therefore, determining the local concentration of drugs in tumor cells is warranted. Unfortunately, since few studies have investigated and quantified the concentrations of these anti-cancer drugs metabolized by CYP3A4 in the tumor versus in blood, specific data are scarcely available on tumor and blood concentrations of these anticancer drugs.

To address the aforementioned knowledge gaps, a population pharmacokinetic (PK)/pharmacodynamic (PD) model can be developed to serve as a tool to characterize the relationship between anticancer drug concentration (dose)–response, incorporating various individual factors including ethnicity, age, gender, concomitant disease, genetic polymorphisms and concomitant treatment into personalized therapy. On the one hand, a complete simulation model will include many aspects such as baseline response, concentration (dose)–response, temporal response, variability, covariate response, disease progression, sample number and compliance aspects. On the other hand, hundreds of individual patients' data collected by clinicians and a series of different clinical trial designs and covariates can be used to develop and further optimize a PK/PD model. Lastly, a well-defined model and simulations may predict trial outcomes based on detailed clinical information, which is useful in achieving personalized treatment. For anticancer drugs that depend on CYP3A4 activation or inactivation, in-depth quantitative assessment is critical to further develop such model to accurately evaluate CYP3A4-mediated metabolism. Detection and analysis of differences in drug concentrations in different tissue and blood samples is a prerequisite for corroborating the model. If possible, direct testing of CYP3A4 activity and function, may be more promising to achieve personalized therapy.

For example, the use of endogenous biomarkers to assess CYP3A4 activity can closely reflect drug exposure or efficacy/toxicity in individual patients. It was published that both urinary 6 $\beta$ -hydroxycortisol to cortisol ratio and plasma 4 $\beta$ -hydroxycholesterol showed a good dynamic response range upon strong CYP3A4 induction with rifampicin (Dutreix et al., 2014). Additionally, plasma 4 $\beta$ -hydroxycholesterol to cholesterol ratio was comparable to midazolam clearance as a marker of CYP3A4 induction by rifampicin (Björkhem-Bergman et al., 2013). These evaluation methods may be a reliable way to monitor CYP3A4 activity in the patients. It is important to note that appropriate biological samples should be selected. The majority of substances in the body are excreted in urine, which is more concentrated and easy to collect. However, there may be differences in metabolic pathways between individuals, as well as differences in renal clearance rates. The concentrations of endogenous biomarkers are relatively stable in plasma, but some substances may be too low due to rapid metabolism. Therefore, depending on the purpose of the trial, a quick, easy and accurate method can be chosen to determine CYP3A4 activity in biological samples. There are interindividual variations in the concentrations of endogenous probes, and it is recommended that baseline values be collected and measured in clinical studies to eliminate the effect of individual differences on the evaluation of CYP3A4 activity. Currently, few markers are available for accurate evaluation of CYP3A4 activity, and individual differences and half-life need to be further explored. Hence, the challenge in the field is the lack of well-designed clinical trials of anticancer drugs and appropriate numbers of subjects. Clinical trials should focus on the effect of CYP3A4 activity on drug activation/inactivation based on drug exposure and response, and specific clinical data on multiple metabolic and non-metabolic determinants associated with exposure parameters should be included in the inclusion and exclusion criteria for subjects, which will provide reliable clinical data to support the development and validation of

anticancer drug concentration–response relationship prediction models and may be a future area of focus for investment.

Furthermore, novel drug formulations, such as nanocarrier-based drug delivery systems, have been designed to encapsulate CYP3A4 inhibitor-encapsulated poly (lactic-co-glycolic acid) (PLGA) nanoparticles to target hepatocytes by utilizing the mechanism of liver clearance of nanocarriers. The anticancer effect of docetaxel was significantly enhanced by preinjection of PLGA nanoparticles with hepatocyte targeting and CYP3A4 inhibition. This targeted strategy minimizes toxicity to normal cells and enables increased delivery of active drug to tumor cells while reducing the dosage of docetaxel (Paolini et al., 2017). Apparently, whether the activity of CYP3A4 needs to be inhibited or enhanced by targeted prodrug delivery systems depends on different types of cancer, as well as the profile of CYP3A4-metabolized drugs. Hence, anticancer prodrugs can be designed for various purposes by enhancing or inhibiting the activity of CYP3A4 in target tissues, such as improving pharmacokinetics, reducing side effects or specifically recognizing tumor tissues or cells, and implementing individualized treatment regimens for patients (**Fig. 2**). Currently, the practical translation of metabolically based drug-delivery optimization will be challenged to develop novel therapeutic strategies.

## **Conclusions**

The evaluation of clinical consequences should be based on information available on the relationship between exposure and efficacy/safety. This article discusses the effects of interindividual variability in CYP3A4 expression and activity on anticancer drugs from the two aspects of metabolic activation and inactivation, and various confounding factors affecting the expression and activity of CYP3A4 in cancer patients are highlighted, such as age, sex, concomitant disease, inflammation, concomitant drugs, and genetic variation. Data on these factors are indispensable for a comprehensive evaluation of



CYP3A4-activated/deactivated drugs in personalized cancer therapy. Therefore, future research should prospectively identify the added value of individualized drug treatment based on the aforementioned genetic, physiological and pathological factors, rather than only rough estimates based on the cancer patient's body surface area and existing dosing guidelines. If possible, a validated anticancer drug concentration–response relationship model based on relevant exposure parameters should be developed for evaluation of the clinical significance of interindividual variability in CYP3A4 activity and translation into treatment recommendations.

#### **Conflict of interest**

No potential conflicts of interest were disclosed.

#### **Author contributions**

*Participated in research design:* F.L. Wang, X. Zhan, and W.D. Chen.

*Wrote or contributed to the writing of the manuscript:* F.L. Wang, X. Zhang, Y.Y. Wang, W.D. Y.N. Chen, Lu, Meng, Ye, and W.D. Chen.

#### **Acknowledgments**

We thank American Journal Experts (AJE) for their professional English language editing services.

## References

- Abbas R and Hsyu P-H (2016) Clinical Pharmacokinetics and Pharmacodynamics of Bosutinib. *Clin Pharmacokinet* **55**:1191-1204.
- Abumiya M, Mita A, Takahashi S, Yoshioka T, Kameoka Y, Takahashi N, and Miura M (2018) Effects of polymorphisms in NR112, CYP3A4, and ABC transporters on the steady-state plasma trough concentrations of bosutinib in Japanese patient with chronic myeloid leukemia. *Med Oncol* **35**:90.
- Ahmed S, Zhou Z, Zhou J, and Chen SQ (2016). Pharmacogenomics of Drug Metabolizing Enzymes and Transporters: Relevance to Precision Medicine. *Genomics Proteomics Bioinformatics* **14**:298-313.
- Aitken AE and Morgan ET (2007) Gene-specific effects of inflammatory cytokines on cytochrome P450 2C, 2B6 and 3A4 mRNA levels in human hepatocytes. *Drug Metab Dispos* **35**:1687-1693.
- Al-Zoughbi W and Hoefler G (2020) Tumor macroenvironment: An Update. *Pathobiology* **87**:58-60.
- Albertella MR, Loadman PM, Jones PH, Phillips RM, Rampling R, Burnet N, Alcock C, Anthony A, Vjaters E, Dunk CR, Harris PA, Wong A, Lalani AS, and Twelves CJ (2008) Hypoxia-selective targeting by the bioreductive prodrug AQ4N in patients with solid tumors: results of a phase I study. *Clin Cancer Res* **14**:1096-1104.
- Allin KH and Nordestgaard BG (2011) Elevated C-reactive protein in the diagnosis, prognosis, and cause of cancer. *Crit Rev Clin Lab Sci* **48**:155-170.
- Amaya GM, Durandis R, Bourgeois DS, Perkins JA, Abouda AA, Wines KJ, Mohamud M, Starks SA, Daniels RN, and Jackson KD (2018) Cytochromes P450 1A2 and 3A4 Catalyze the Metabolic Activation of Sunitinib. *Chem Res Toxicol* **31**:570-584.
- Apellániz-Ruiz M, Inglada-Pérez L, Naranjo MEG, Sánchez L, Mancikova V, Currás-Freixes M, de Cubas AA, Comino-Méndez I, Triki S, Rebai A, Rasool M, Moya G, Grazina M, Opocher G, Cascón A, Taboada-Echalar P, Ingelman-Sundberg M, Carracedo A, Robledo M, Llerena A, and Rodríguez-Antona

- C (2015a) High frequency and founder effect of the CYP3A4\*20 loss-of-function allele in the Spanish population classifies CYP3A4 as a polymorphic enzyme. *Pharmacogenomics J* **15**:288-292.
- Apellániz-Ruiz M, Lee M-Y, Sánchez-Barroso L, Gutiérrez-Gutiérrez G, Calvo I, García-Estévez L, Sereno M, García-Donás J, Castelo B, Guerra E, Leandro-García LJ, Cascón A, Johansson I, Robledo M, Ingelman-Sundberg M, and Rodríguez-Antona C (2015b) Whole-exome sequencing reveals defective CYP3A4 variants predictive of paclitaxel dose-limiting neuropathy. *Clin Cancer Res* **21**:322-328.
- Assis J, Pereira D, Gomes M, Marques D, Marques I, Nogueira A, Catarino R, and Medeiros R (2013) Influence of CYP3A4 genotypes in the outcome of serous ovarian cancer patients treated with first-line chemotherapy: implication of a CYP3A4 activity profile. *Int J Clin Exp Med* **6**:552-561.
- Atasilp C, Chansriwong P, Sirachainan E, Reungwetwattana T, Sirilertrakul S, Chamnanphon M, Puangpetch A, and Sukasem C (2020) Effect of drug metabolizing enzymes and transporters in Thai colorectal cancer patients treated with irinotecan-based chemotherapy. *Sci Rep* **10**:13486.
- Azam C, Claraz P, Chevreau C, Vinson C, Cottura E, Mourey L, Pouessel D, Guibaud S, Pollet O, Le Goff M, Bardies C, Pelagatti V, Canonge J, and Puisset F (2020) Association between clinically relevant toxicities of pazopanib and sunitinib and the use of weak CYP3A4 and P-gp inhibitors. *Eur J Clin Pharmacol* **76**:579-587.
- Bailly C (2019) Irinotecan: 25 years of cancer treatment. *Pharmacol Res* **148**:104398.
- Baxter SD, Teft WA, Choi Y-H, Winqvist E, and Kim RB (2014) Tamoxifen-associated hot flash severity is inversely correlated with endoxifen concentration and CYP3A4\*22. *Breast Cancer Res Treat* **145**:419-428.
- Bekaii-Saab T, Perloff M, Weemhoff J, Greenblatt D, von Moltke L (2004) Interactions of tamoxifen,

- N-desmethyltamoxifen and 4-hydroxytamoxifen with P-glycoprotein and CYP3A. *Biopharm Drug Dispos* **25**:283-289.
- Beyerle J, Holowatyj AN, Haffa M, Frei E, Gigic B, Schrotz-King P, Boehm J, Habermann N, Stiborova M, Scherer D, Kölsch T, Skender S, Becker N, Herpel E, Schneider M, Ulrich A, Schirmacher P, Chang-Claude J, Brenner H, Hoffmeister M, Haug U, Owen RW, and Ulrich CM (2020) Expression Patterns of Xenobiotic-Metabolizing Enzymes in Tumor and Adjacent Normal Mucosa Tissues among Patients with Colorectal Cancer: The ColoCare Study. *Cancer Epidemiol Biomarkers Prev* **29**:460-469.
- Bins S, Huitema A, Laven P, Bouazzaoui S, Yu H, van Erp N, van Herpen C, Hamberg P, Gelderblom H, Steeghs N, Sleijfer S, van Schaik R, Mathijssen R, and Koolen S (2019) Impact of CYP3A4\*22 on Pazopanib Pharmacokinetics in Cancer Patients. *Clin Pharmacokinet* **58**:651-658.
- Bissada JE, Truong V, Abouda AA, Wines KJ, Crouch RD, and Jackson KD (2019) Interindividual Variation in CYP3A Activity Influences Lapatinib Bioactivation. *Drug Metab Dispos* **47**:1257-1269.
- Björkhem-Bergman L, Bäckström T, Nylén H, Rönquist-Nii Y, Bredberg E, Andersson TB, Bertilsson L, and Diczfalusy U (2013). Comparison of endogenous 4 $\beta$ -hydroxycholesterol with midazolam as markers for CYP3A4 induction by rifampicin. *Drug Metab Dispos* **41**:1488-1493.
- Boyer SH, Sun Z, Jiang H, Esterbrook J, Gómez-Galeno JE, Craigo W, Reddy KR, Ugarkar BG, MacKenna DA, and Erion MD (2006) Synthesis and characterization of a novel liver-targeted prodrug of cytosine-1-beta-D-arabinofuranoside monophosphate for the treatment of hepatocellular carcinoma. *J Med Chem* **49**:7711-7720.
- Brussee J, Yu H, Krekels E, Palić S, Brill M, Barrett J, Rostami-Hodjegan A, de Wildt S, and Knibbe CJPr (2018) Characterization of Intestinal and Hepatic CYP3A-Mediated Metabolism of Midazolam in Children Using a Physiological Population Pharmacokinetic Modelling Approach. *Pharm Res* **35**:182.

- Chalasani N, Gorski JC, Patel NH, Hall SD, and Galinsky RE (2001) Hepatic and intestinal cytochrome P450 3A activity in cirrhosis: effects of transjugular intrahepatic portosystemic shunts. *Hepatology (Baltimore, Md)* **34**:1103-1108.
- Chang Y, Hernandez D, Alonso S, Gao M, Su M, Ghiatur G, Levis M, and Jones R (2019) Role of CYP3A4 in bone marrow microenvironment-mediated protection of FLT3/ITD AML from tyrosine kinase inhibitors. *Blood Adv* **3**:908-916.
- Charles KA, Rivory LP, Brown SL, Liddle C, Clarke SJ, and Robertson GR (2006) Transcriptional repression of hepatic cytochrome P450 3A4 gene in the presence of cancer. *Clin Cancer Res* **12**:7492-7497.
- Chen CH, Wang SC, Tsou HH, Ho IK, Tian JN, Yu CJ, Hsiao CF, Chou SY, Lin YF, Fang KC, Huang CL, Su LW, Fang YC, Liu ML, Lin KM, Hsu YT, Liu SC, Chen A, and Liu YL (2011) Genetic polymorphisms in CYP3A4 are associated with withdrawal symptoms and adverse reactions in methadone maintenance patients. *Pharmacogenomics* **12**:1397-1406.
- Chen X, Wang H, Zhou G, Zhang X, Dong X, Zhi L, Jin L, and He F (2009) Molecular population genetics of human CYP3A locus: signatures of positive selection and implications for evolutionary environmental medicine. *Environ Health Perspect* **117**:1541-1548.
- Dai D, Tang J, Rose R, Hodgson E, Bienstock RJ, Mohrenweiser HW, and Goldstein JA (2001) Identification of variants of CYP3A4 and characterization of their abilities to metabolize testosterone and chlorpyrifos. *J Pharmacol Exp Ther* **299**:825-831.
- Darweesh R, El-Elimat T, Zayed A, Khamis T, Babaresh W, Arafat T, and Al Sharie A (2020) The effect of grape seed and green tea extracts on the pharmacokinetics of imatinib and its main metabolite, N-desmethyl imatinib, in rats. *BMC Pharmacol Toxicol* **21**:77.

- Deeks ED. (2022) Asciminib: First Approval. *Drugs*. **82**: 219-226.
- de Graan A, Elens L, Sprowl J, Sparreboom A, Friberg L, van der Holt B, de Raaf P, de Bruijn P, Engels F, Eskens F, Wiemer E, Verweij J, Mathijssen R, and van Schaik R (2013) CYP3A4\*22 genotype and systemic exposure affect paclitaxel-induced neurotoxicity. *Clin Cancer Res* **19**:3316-3324.
- de Graeve J, van Heugen JC, Zorza G, Fahy J, and Puozzo C (2008) Metabolism pathway of vinorelbine (Navelbine®) in human: Characterisation of the metabolites by HPLC–MS/MS. *J Pharm Biomed Anal* **47**:47-58.
- Dehshahri A, Ashrafizadeh M, Ghasemipour Afshar E, Pardakhty A, Mandegary A, Mohammadinejad R, and Sethi G (2020) Topoisomerase inhibitors: Pharmacology and emerging nanoscale delivery systems. *Pharmacol Res* **151**:104551.
- Demurtas S, La Verde N, Rota S, Casazza G, Montrasio C, Cheli S, Cona MS, Dalu D, Fasola C, Ferrario S, Filipazzi V, Gambaro A, Tosca N, and Clementi E (2021) Single nucleotide polymorphisms to predict taxanes toxicities and effectiveness in cancer patients. *Pharmacogenomics J* **21**:491-497.
- Desta Z, Ward B, Soukhova N, and Flockhart D (2004) Comprehensive evaluation of tamoxifen sequential biotransformation by the human cytochrome P450 system in vitro: prominent roles for CYP3A and CYP2D6. *J Pharmacol Exp Ther* **310**:1062-1075.
- Dickinson PA, Cantarini MV, Collier J, Frewer P, Martin S, Pickup K, and Ballard P (2016) Metabolic disposition of osimertinib in rats, dogs, and humans: insights into a drug designed to bind covalently to a cysteine residue of epidermal growth factor receptor. *Drug Metab Dispos* **44**:1201-1212.
- Diczfalusy U, Nylén H, Elander P, and Bertilsson L (2011) 4β-Hydroxycholesterol, an endogenous marker of CYP3A4/5 activity in humans. *Br J Clin Pharmacol* **71**:183-189.
- Diekstra M, Klümpen H, Lolkema M, Yu H, Kloth J, Gelderblom H, van Schaik R, Gurney H, Swen J,

- Huitema A, Steeghs N, and Mathijssen R (2014) Association analysis of genetic polymorphisms in genes related to sunitinib pharmacokinetics, specifically clearance of sunitinib and SU12662. *Clin Pharmacol Ther* **96**:81-89.
- Diekstra MH, Belaustegui A, Swen JJ, Boven E, Castellano D, Gelderblom H, Mathijssen RH, García-Donas J, Rodríguez-Antona C, Rini BI, and Guchelaar HJ (2017) Sunitinib-induced hypertension in CYP3A4 rs4646437 A-allele carriers with metastatic renal cell carcinoma. *Pharmacogenomics J* **17**:42-46.
- Ding X and Kaminsky L (2003) Human extrahepatic cytochromes P450: function in xenobiotic metabolism and tissue-selective chemical toxicity in the respiratory and gastrointestinal tracts. *Annu Rev Pharmacol Toxicol* **43**:149-173.
- Du J, Yu L, Wang L, Zhang A, Shu A, Xu L, Xu M, Shi Y, Li X, Feng G, Xing Q, and He L (2007) Differences in CYP3A41G genotype distribution and haplotypes of CYP3A4, CYP3A5 and CYP3A7 in 3 Chinese populations. *Clin Chim Acta* **383**:172-174.
- Dutreix C, Lorenzo S, and Wang Y (2014). Comparison of two endogenous biomarkers of CYP3A4 activity in a drug-drug interaction study between midostaurin and rifampicin. *Eur J Clin Pharmacol* **70**: 915-920.
- Eiselt R, Domanski TL, Zibat A, Mueller R, Presecan-Siedel E, Hustert E, Zanger UM, Brockmoller J, Klenk HP, Meyer UA, Khan KK, He YA, Halpert JR, and Wojnowski L (2001) Identification and functional characterization of eight CYP3A4 protein variants. *Pharmacogenetics* **11**:447-458.
- Ekhart C, Doodeman VD, Rodenhuis S, Smits PHM, Beijnen JH, and Huitema ADR (2009) Polymorphisms of drug-metabolizing enzymes (GST, CYP2B6 and CYP3A) affect the pharmacokinetics of thiotepa and tepla. *Br J Clin Pharmacol* **67**:50-60.



- Elens L, Becker ML, Haufroid V, Hofman A, Visser LE, Uitterlinden AG, Stricker B, and van Schaik RH (2011) Novel CYP3A4 intron 6 single nucleotide polymorphism is associated with simvastatin-mediated cholesterol reduction in the Rotterdam Study. *Pharmacogenet Genomics* **21**:861-866.
- Ewesuedo RB and Dolan ME (2000) Pharmacokinetics of oral O6-benzylguanine and evidence of interaction with oral ketoconazole in the rat. *Cancer Chemother Pharmacol* **46**:150-155.
- Filppula A, Laitila J, Neuvonen P, and Backman J (2012) Potent mechanism-based inhibition of CYP3A4 by imatinib explains its liability to interact with CYP3A4 substrates. *Br J Pharmacol* **165**:2787-2798.
- Flannery P, Abbott K, and Pondugula S (2020) Correlation of PPM1A Downregulation with CYP3A4 Repression in the Tumor Liver Tissue of Hepatocellular Carcinoma Patients. *Eur J Drug Metab Pharmacokinet* **45**:297-304.
- Frye RF, Fitzgerald SM, Lagattuta TF, Hruska MW, and Egorin MJ (2004) Effect of St John's Wort on imatinib mesylate pharmacokinetics. *Clin Pharmacol Ther* **76**:323-329.
- Fucile C, Marengo S, Bazzica M, Zuccoli M, Lantieri F, Robbiano L, Marini V, Di Gion P, Pieri G, Stura P, Martelli A, Savarino V, Mattioli F, and Picciotto A (2015) Measurement of sorafenib plasma concentration by high-performance liquid chromatography in patients with advanced hepatocellular carcinoma: is it useful the application in clinical practice? A pilot study. *Med Oncol* **32**:335.
- Fukushima-Uesaka H, Saito Y, Watanabe H, Shiseki K, Saeki M, Nakamura T, Kurose K, Sai K, Komamura K, Ueno K, Kamakura S, Kitakaze M, Hanai S, Nakajima T, Matsumoto K, Saito H, Goto Y, Kimura H, Katoh M, Sugai K, Minami N, Shirao K, Tamura T, Yamamoto N, Minami H, Ohtsu A, Yoshida T, Saijo N, Kitamura Y, Kamatani N, Ozawa S, and Sawada J (2004) Haplotypes of CYP3A4 and their close linkage with CYP3A5 haplotypes in a Japanese population. *Human Mutation* **23**:100.
- Furlan V, Demirdjian S, Bourdon O, Magdalou J, and Taburet AM (1999) Glucuronidation of drugs by

hepatic microsomes derived from healthy and cirrhotic human livers. *J Pharmacol Exp Ther* **289**:1169-1175.

García-Martín E, Martínez C, Pizarro RM, García-Gamito FJ, Gullsten H, Raunio H, and Agúndez JAG (2002) CYP3A4 variant alleles in white individuals with low CYP3A4 enzyme activity. *Clin Pharmacol Ther* **71**:196-204.

Ghassabian S, Rawling T, Zhou F, Doddareddy MR, Tattam BN, Hibbs DE, Edwards RJ, Cui PH, and Murray M (2012) Role of human CYP3A4 in the biotransformation of sorafenib to its major oxidized metabolites. *Biochem Pharmacol* **84**:215-223.

Goey AK, With Md, Agema BC, Hoop EO-D, Singh RK, van der Veldt AA, Mathijssen RH, van Schaik RH, and Bins S (2019) Effects of pharmacogenetic variants on vemurafenib-related toxicities in patients with melanoma. *Pharmacogenomics* **20**:1283-1290.

Goldinger SM, Rinderknecht J, Dummer R, Kuhn FP, Yang K-H, Lee L, Ayala RC, Racha J, Geng W, Moore D, Liu M, Joe AK, Bazan SPG, and Grippo JF (2015) A single-dose mass balance and metabolite-profiling study of vemurafenib in patients with metastatic melanoma. *Pharmacol Res Perspect* **3**:e00113.

Gomo C, Coriat R, Faivre L, Mir O, Ropert S, Billefont B, Dauphin A, Tod M, Goldwasser F, and Blanchet B (2011) Pharmacokinetic interaction involving sorafenib and the calcium-channel blocker felodipine in a patient with hepatocellular carcinoma. *Invest New Drugs* **29**:1511-1514.

Goodwin B, Hodgson E, D'Costa DJ, Robertson GR, and Liddle C (2002) Transcriptional regulation of the human CYP3A4 gene by the constitutive androstane receptor. *Mol Pharmacol* **62**:359-365.

Goodwin B, Hodgson E, and Liddle C (1999) The orphan human pregnane X receptor mediates the transcriptional activation of CYP3A4 by rifampicin through a distal enhancer module. *Mol Pharmacol*

56:1329-1339.

Gor PP, Su HI, Gray RJ, Gimotty PA, Horn M, Aplenc R, Vaughan WP, Tallman MS, Rebbeck TR, and DeMichele A (2010) Cyclophosphamide-metabolizing enzyme polymorphisms and survival outcomes after adjuvant chemotherapy for node-positive breast cancer: a retrospective cohort study. *Breast Cancer Res* **12**:R26.

Haddad A, Davis M, and Lagman R (2007) The pharmacological importance of cytochrome CYP3A4 in the palliation of symptoms: review and recommendations for avoiding adverse drug interactions. *Support Care Cancer* **15**:251-257.

Hamilton M, Wolf JL, Drolet DW, Fettner SH, Rakhit AK, Witt K, and Lum BL (2014) The effect of rifampicin, a prototypical CYP3A4 inducer, on erlotinib pharmacokinetics in healthy subjects. *Cancer Chemother Pharmacol* **73**:613-621.

Hannachi I, Ben Fredj N, Chadli Z, Ben Fadhel N, Ben Romdhane H, Touitou Y, Boughattas NA, Chaabane A, and Aouam K (2020) Effect of CYP3A4\*22 and CYP3A4\*1B but not CYP3A5\*3 polymorphisms on tacrolimus pharmacokinetic model in Tunisian kidney transplant. *Toxicol Appl Pharmacol* **396**:115000.

Hardy KD, Wahlin MD, Papageorgiou I, Unadkat JD, Rettie AE, and Nelson SD (2014) Studies on the role of metabolic activation in tyrosine kinase inhibitor-dependent hepatotoxicity: induction of CYP3A4 enhances the cytotoxicity of lapatinib in HepaRG cells. *Drug Metab Dispos* **42**:162-171.

Heikkilä K, Ebrahim S, and Lawlor DA (2008) Systematic review of the association between circulating interleukin-6 (IL-6) and cancer. *Eur J Cancer* **44**:937-945.

Hertz DL, Kidwell KM, Seewald NJ, Gersch CL, Desta Z, Flockhart DA, Storniolo AM, Stearns V, Skaar TC, Hayes DF, Henry NL, and Rae JM (2017) Polymorphisms in drug-metabolizing enzymes and

steady-state exemestane concentration in postmenopausal patients with breast cancer.

*Pharmacogenomics J* **17**:521-527.

Hirth J, Watkins P, Strawderman M, Schott A, Bruno R, and Baker L (2000) The effect of an individual's cytochrome CYP3A4 activity on docetaxel clearance. *Clin Cancer Res* **6**:1255-1258.

Hoch M, Sato M, Zack J, Quinlan M, Sengupta T, Allepuz A, Aimone P, and Hourcade-Potelleret F (2021) Pharmacokinetics of Asciminib in Individuals With Hepatic or Renal Impairment. *J Clin Pharmacol* **61**:1454-1465.

Hsieh C, Chen Y, Tsai T, Wang L, Tai H, Huang H, and Huang Y (2020) Robust combination of liver stereotactic body radiotherapy modulates pharmacokinetics of sorafenib toward preferable parameters. *Sci Rep* **10**:9575.

Hsieh KP, Lin YY, Cheng CL, Lai ML, Lin MS, Siest JP, and Huang JD (2001) Novel mutations of CYP3A4 in Chinese. *Drug Metab Dispos* **29**:268-273.

Hsyu PH, Pignataro DS and Matschke K (2017). Effect of aprepitant, a moderate CYP3A4 inhibitor, on bosutinib exposure in healthy subjects. *Eur J Clin Pharmacol* **73**:49-56.

Hu GX, Dai DP, Wang H, Huang XX, Zhou XY, Cai J, Chen H, and Cai JP (2017) Systematic screening for CYP3A4 genetic polymorphisms in a Han Chinese population. *Pharmacogenomics* **18**:369-379.

Hu Y-F, He J, Chen G-L, Wang D, Liu Z-Q, Zhang C, Duan L-F, and Zhou H-H (2005) CYP3A5\*3 and CYP3A4\*18 single nucleotide polymorphisms in a Chinese population. *Clin Chim Acta* **353**:187-192.

Huang L, Jiang S, and Shi Y (2020) Tyrosine kinase inhibitors for solid tumors in the past 20 years (2001-2020). *J Hematol Oncol* **13**:143.

Huang Z, Roy P, and Waxman D (2000) Role of human liver microsomal CYP3A4 and CYP2B6 in catalyzing N-dechloroethylation of cyclophosphamide and ifosfamide. *Biochem Pharmacol* **59**:961-972.

- Indra R, Pompach P, Martínek V, Takácsová P, Vavrová K, Heger Z, Adam V, Eckschlagler T, Kopečková K, Arlt VM, and Stiborová M (2019) Identification of Human Enzymes Oxidizing the Anti-Thyroid-Cancer Drug Vandetanib and Explanation of the High Efficiency of Cytochrome P450 3A4 in its Oxidation. *Int J Mol Sci* **20**:3392.
- Iyengar NM, Gucalp A, Dannenberg AJ, and Hudis CA (2016) Obesity and Cancer Mechanisms: Tumor Microenvironment and Inflammation. *J Clin Oncol* **34**:4270-4276.
- Jacobson PA, Green K, Birnbaum A, and Rimmel RP (2002) Cytochrome P450 isozymes 3A4 and 2B6 are involved in the in vitro human metabolism of thiotepa to TEPA. *Cancer Chemother Pharmacol* **49**:461-467.
- Jamwal R, de la Monte S, Ogasawara K, Adusumalli S, Barlock B, and Akhlaghi F (2018) Nonalcoholic Fatty Liver Disease and Diabetes Are Associated with Decreased CYP3A4 Protein Expression and Activity in Human Liver. *Mol Pharm* **15**:2621-2632.
- Johnson FM, Agrawal S, Burris H, Rosen L, Dhillon N, Hong D, Blackwood-Chirchir A, Luo FR, Sy O, Kaul S, and Chiappori AA (2010) Phase 1 pharmacokinetic and drug-interaction study of dasatinib in patients with advanced solid tumors. *Cancer* **116**:1582-1591.
- Jounaidi Y, Hecht JE, and Waxman DJ (1998) Retroviral transfer of human cytochrome P450 genes for oxazaphosphorine-based cancer gene therapy. *Cancer Res* **58**:4391-4401.
- Kacevska M, Robertson GR, Clarke SJ, and Liddle C (2008) Inflammation and CYP3A4-mediated drug metabolism in advanced cancer: impact and implications for chemotherapeutic drug dosing. *Expert Opin Drug Metab Toxicol* **4**:137-149.
- Kajita J, Kuwabara T, Kobayashi H, and Kobayashi S (2000) CYP3A4 is mainly responsible for the metabolism of a new vinca alkaloid, vinorelbine, in human liver microsomes. *Drug Metab Dispos*

28:1121-1127.

Kamdem LK, Flockhart DA, and Desta Z (2011) In vitro cytochrome P450-mediated metabolism of exemestane. *Drug Metab Dispos* **39**:98-105.

Kapucuoglu N, Coban T, Raunio H, Pelkonen O, Edwards RJ, Boobis AR, and Iscan M (2003) Expression of CYP3A4 in human breast tumour and non-tumour tissues. *Cancer Lett* **202**:17-23.

Keating GM and Santoro A (2009) Sorafenib: a review of its use in advanced hepatocellular carcinoma. *Drugs* **69**:223-240.

Kivistö KT, Kroemer HK, and Eichelbaum M (1995) The role of human cytochrome P450 enzymes in the metabolism of anticancer agents: implications for drug interactions. *Br J Clin Pharmacol* **40**:523-530.

Klumpfer L (2011) Cytokines, inflammation and colon cancer. *Curr Cancer Drug Targets* **11**:451-464.

Kolwankar D, Vuppalanchi R, Ethell B, Jones DR, Wrighton SA, Hall SD, and Chalasani N (2007) Association between nonalcoholic hepatic steatosis and hepatic cytochrome P-450 3A activity. *Clin Gastroenterol Hepatol* **5**:388-393.

Komoroski B, Parise R, Egorin M, Strom S, and Venkataramanan R (2005) Effect of the St. John's wort constituent hyperforin on docetaxel metabolism by human hepatocyte cultures. *Clin Cancer Res* **11**:6972-6979.

Kotlyar M and Carson SW (1999) Effects of obesity on the cytochrome P450 enzyme system. *Int J Clin Pharmacol Ther* **37**:8-19.

Krens SD, Lassche G, Jansman FGA, Desar IME, Lankheet NAG, Burger DM, van Herpen CML, and van Erp NP (2019) Dose recommendations for anticancer drugs in patients with renal or hepatic impairment. *Lancet Oncol* **20**:e200-e207.

Kugler N, Klein K, and Zanger UM (2020) MiR-155 and other microRNAs downregulate drug

metabolizing cytochromes P450 in inflammation. *Biochem Pharmacol* **171**:113725.

Lacy S, Hsu B, Miles D, Aftab D, Wang R, and Nguyen L (2015) Metabolism and Disposition of Cabozantinib in Healthy Male Volunteers and Pharmacologic Characterization of Its Major Metabolites.

*Drug Metab Dispos* **43**:1190-1207.

Lamba JK, Lin YS, Thummel K, Daly A, Watkins PB, Strom S, Zhang J, and Schuetz EG (2002) Common allelic variants of cytochrome P4503A4 and their prevalence in different populations. *Pharmacogenetics*

**12**:121-132.

Launay-Vacher V, Oudard S, Janus N, Gligorov J, Pourrat X, Rixe O, Morere J-F, Beuzeboc P, and Deray G (2007) Prevalence of Renal Insufficiency in cancer patients and implications for anticancer drug management: the renal insufficiency and anticancer medications (IRMA) study. *Cancer* **110**:1376-1384.

Lenoir C, Rollason V, Desmeules JA, and Samer CF (2021) Influence of Inflammation on Cytochromes P450 Activity in Adults: A Systematic Review of the Literature. *Front Pharmacol* **12**:733935.

Li X, He Y, Ruiz C, Koenig M, Cameron M, and Vojtkovsky T (2009a) Characterization of dasatinib and its structural analogs as CYP3A4 mechanism-based inactivators and the proposed bioactivation pathways.

*Drug Metab Dispos* **37**:1242-1250.

Li X, Kamenecka TM, and Cameron MD (2009b) Bioactivation of the epidermal growth factor receptor inhibitor gefitinib: implications for pulmonary and hepatic toxicities. *Chem Res Toxicol* **22**:1736-1742.

Liu J, Chen Z, Chen H, Hou Y, Lu W, He J, Tong H, Zhou Y, and Cai W (2017) Genetic Polymorphisms Contribute to the Individual Variations of Imatinib Mesylate Plasma Levels and Adverse Reactions in Chinese GIST Patients. *Int J Mol Sci* **18**:603

Liu J, Jin X, Zhou F, Chen H, Wang W, Liu Y, Wang G, Hao K, and Zhang J (2020) Disrupted hepatic pentose phosphate pathway directly participates in and indirectly promotes CYP3A reduction: A new

- strategy for CYP3A-mediated drug hepatotoxicity. *Br J Pharmacol* **177**:1538-1555.
- Liu J, Lu Y, Corton J, and Klaassen C (2021) Expression of cytochrome P450 isozyme transcripts and activities in human livers. *Xenobiotica* **51**:279-286.
- Maagdenberg H, Bierings MB, van Ommen CH, van der Meer FJ, Appel IM, Tamminga RY, Cessie SL, Swen JJ, der Straaten TV, Boer A, Maitland-van der Zee AH (2018) Effects of age and genetic variations in VKORC1, CYP2C9 and CYP3A4 on the phenprocoumon dose in pediatric patients. *Pharmacogenomics* **19**:1195-1202.
- Maddin N, Husin A, Gan SH, Aziz BA, and Ankathil R (2016) Impact of CYP3A4\*18 and CYP3A5\*3 Polymorphisms on Imatinib Mesylate Response Among Chronic Myeloid Leukemia Patients in Malaysia. *Oncol Ther* **4**:303-314.
- Maekawa K, Harakawa N, Yoshimura T, Kim SR, Fujimura Y, Aohara F, Sai K, Katori N, Tohkin M, Naito M, Hasegawa R, Okuda H, Sawada J, Niwa T, and Saito Y (2010) CYP3A4\*16 and CYP3A4\*18 alleles found in East Asians exhibit differential catalytic activities for seven CYP3A4 substrate drugs. *Drug Metab Dispos* **38**:2100-2104.
- Makihara K, Nakamura S, Miyagi K, Ueno H, and Nakata I (2017) Clarithromycin co-administration does not increase irinotecan (CPT-11) toxicity in colorectal cancer patients. *Cancer Chemother Pharmacol* **80**:527-533.
- Manley P, Blasco F, Mestan J, and Aichholz R (2013) The kinetic deuterium isotope effect as applied to metabolic deactivation of imatinib to the des-methyl metabolite, CGP74588. *Bioorg Med Chem* **21**:3231-3239.
- Martínez-Jiménez CP, Jover R, Donato MT, Castell JV, and Gómez-Lechón MJ (2007) Transcriptional regulation and expression of CYP3A4 in hepatocytes. *Curr Drug Metab* **8**:185-194.



- Martínez C, García-Martín E, Pizarro RM, García-Gamito FJ, and Agúndez JAG (2002) Expression of paclitaxel-inactivating CYP3A activity in human colorectal cancer: implications for drug therapy. *Br J Cancer* **87**:681-686.
- Mathijssen R, Verweij J, de Bruijn P, Loos W, and Sparreboom AJ (2002) Effects of St. John's wort on irinotecan metabolism. *J Natl Cancer Inst* **94**:1247-1249.
- McKillop D, McCormick A, Millar A, Miles G, Phillips P, and Hutchison M (2005) Cytochrome P450-dependent metabolism of gefitinib. *Xenobiotica* **35**:39-50.
- Mitsiades N, Sung C, Schultz N, Danila D, He B, Eedunuri V, Fleisher M, Sander C, Sawyers C, and Scher H (2012) Distinct patterns of dysregulated expression of enzymes involved in androgen synthesis and metabolism in metastatic prostate cancer tumors. *Cancer Res* **72**:6142-6152.
- Miyoshi Y, Ando A, Takamura Y, Taguchi T, Tamaki Y, and Noguchi S (2002) Prediction of response to docetaxel by CYP3A4 mRNA expression in breast cancer tissues. *Int J Cancer* **97**:129-132.
- Mross K, Frost A, Steinbild S, Hedbom S, Büchert M, Fasol U, Unger C, Krätzschmar J, Heinig R, Boix O, and Christensen O (2012) A phase I dose-escalation study of regorafenib (BAY 73-4506), an inhibitor of oncogenic, angiogenic, and stromal kinases, in patients with advanced solid tumors. *Clin Cancer Res* **18**:2658-2667.
- Mullard A (2018) 2017 FDA drug approvals. *Nat Rev Drug Discov* **17**:81-85.
- Nakajima Y, Yoshitani T, Fukushima-Uesaka H, Saito Y, Kaniwa N, Kurose K, Ozawa S, Aoyagi N, Kamatani N, Yamamoto N, Kunitoh H, Ohe Y, Tamura T, Yoshida T, Minami H, Saijo N, Katori N, and Sawada J (2006) Impact of the haplotype CYP3A4\*16B harboring the Thr185Ser substitution on paclitaxel metabolism in Japanese patients with cancer. *Clin Pharmacol Ther* **80**:179-191.
- Narjot C, Cessot A, Thomas-Schoemann A, Golmard JL, Huillard O, Boudou-Rouquette P, Behouche A,

- Taieb F, Durand JP, Dauphin A, Coriat R, Vidal M, Tod M, Alexandre J, Lorient MA, Goldwasser F, and Blanchet B (2015) Role of the lean body mass and of pharmacogenetic variants on the pharmacokinetics and pharmacodynamics of sunitinib in cancer patients. *Invest New Drugs* **33**:257-268.
- Nieuweboer AJ, de Morrée ES, de Graan AJ, Sparreboom A, de Wit R, and Mathijssen RH (2015) Inter-patient variability in docetaxel pharmacokinetics: A review. *Cancer Treat Rev* **41**:605-613.
- Noda S, Shioya M, Hira D, Fujiyama Y, Morita SY, and Terada T (2013) Pharmacokinetic interaction between sorafenib and prednisolone in a patient with hepatocellular carcinoma. *Cancer Chemother Pharmacol* **72**:269-272.
- Olefsky JM and Glass CK (2010) Macrophages, inflammation, and insulin resistance. *Annu Rev Physiol* **72**:219-246.
- Orlando R, Piccoli P, De Martin S, Padriani R, and Palatini P (2003) Effect of the CYP3A4 inhibitor erythromycin on the pharmacokinetics of lignocaine and its pharmacologically active metabolites in subjects with normal and impaired liver function. *Br J Clin Pharmacol* **55**:86-93.
- Oyaga-Iriarte E, Insausti A, Sayar O, and Aldaz A (2019) Population pharmacokinetic model of irinotecan and its metabolites in patients with metastatic colorectal cancer. *Eur J Clin Pharmacol* **75**:529-542.
- Ozdemir V, Kalow W, Tang BK, Paterson AD, Walker SE, Endrenyi L, and Kashuba AD (2000) Evaluation of the genetic component of variability in CYP3A4 activity: a repeated drug administration method. *Pharmacogenetics* **10**:373-388.
- Ozeki T, Nagahama M, Fujita K, Suzuki A, Sugino K, Ito K, and Miura M (2019) Influence of CYP3A4/5 and ABC transporter polymorphisms on lenvatinib plasma trough concentrations in Japanese patients with thyroid cancer. *Sci Rep* **9**:5404.
- Pai S, Chaikin P, and Berg J (2019) Pharmacokinetics of Lapatinib, a Nonrenally Cleared Drug, in Patients

- With End-Stage Renal Disease on Maintenance Hemodialysis. *J Clin Pharmacol* **59**:1379-1383.
- Paine MF, Hart HL, Ludington SS, Haining RL, Rettie AE, and Zeldin DC (2006). The human intestinal cytochrome P450 "pie". *Drug Metab Dispos.* **34**: 880-886.
- Paolini M, Poul L, Berjaud C, Germain M, Darmon A, Bergère M, Pottier A, Levy L, and Vibert E (2017) Nano-sized cytochrome P450 3A4 inhibitors to block hepatic metabolism of docetaxel. *Int J Nanomedicine* **12**:5537-5556.
- Parkinson A, Mudra D, Johnson C, Dwyer A, and Carroll K (2004) The effects of gender, age, ethnicity, and liver cirrhosis on cytochrome P450 enzyme activity in human liver microsomes and inducibility in cultured human hepatocytes. *Toxicol Appl Pharmacol* **199**:193-209.
- Patel N, Chakraborty K, Messmer G, Krishnan K, and Bossaer JJJooppotISoOPP (2018) Severe sunitinib-induced myelosuppression in a patient with a CYP 3A4 polymorphism. *J Oncol Pharm Pract* **24**:623-626.
- Patterson HL and Murray IG (2002) Tumour Cytochrome P450 and Drug Activation. *Curr Pharm Des* **8**:1335-1347.
- Petros WP, Hopkins PJ, Spruill S, Broadwater G, Vredenburg JJ, Colvin OM, Peters WP, Jones RB, Hall J, and Marks JR (2005) Associations between drug metabolism genotype, chemotherapy pharmacokinetics, and overall survival in patients with breast cancer. *J Clin Oncol* **23**:6117-6125.
- Purnapatre K, Khattar SK, and Saini KS (2008) Cytochrome P450s in the development of target-based anticancer drugs. *Cancer Lett* **259**:1-15.
- Puszkiew A, Arellano C, Vachoux C, Evrard A, Le Morvan V, Boyer J, Robert J, Delmas C, Dalenc F, Debled M, Venat-Bouvet L, Jacot W, Suc E, Sillet-Bach I, Filleron T, Roché H, Chatelut E, White-Koning M, and Thomas F (2019a) Factors Affecting Tamoxifen Metabolism in Patients With

- Breast Cancer: Preliminary Results of the French PHACS Study. *Clin Pharmacol Ther* **106**:585-595.
- Puszkiet A, Noé G, Bellesoeur A, Kramkimel N, Paludetto M-N, Thomas-Schoemann A, Vidal M, Goldwasser F, Chatelut E, and Blanchet B (2019b) Clinical Pharmacokinetics and Pharmacodynamics of Dabrafenib. *Clin Pharmacokinet* **58**:451-467.
- Quintieri L, Geroni C, Fantin M, Battaglia R, Rosato A, Speed W, Zanovello P, and Floreani M (2005) Formation and antitumor activity of PNU-159682, a major metabolite of nemorubicin in human liver microsomes. *Clin Cancer Res* **11**:1608-1617.
- Rahmioglu N, Heaton J, Clement G, Gill R, Surdulescu G, Zlobecka K, Hodgkiss D, Ma Y, Hider RC, Smith NW, and Ahmadi KR (2011) Genetic epidemiology of induced CYP3A4 activity. *Pharmacogenet Genomics* **21**:642-651.
- Rakhit A, Pantze MP, Fettner S, Jones HM, Charoin J-E, Riek M, Lum BL, and Hamilton M (2008) The effects of CYP3A4 inhibition on erlotinib pharmacokinetics: computer-based simulation (SimCYP) predicts in vivo metabolic inhibition. *Eur J Clin Pharmacol* **64**:31-41.
- Reddick S, Campagne O, Huang J, Onar-Thomas A, Broniscer A, Gajjar A, and Stewart C (2019) Pharmacokinetics and safety of erlotinib and its metabolite OSI-420 in infants and children with primary brain tumors. *Cancer Chemother Pharmacol* **84**:829-838.
- Relling MV, Nemec J, Schuetz EG, Schuetz JD, Gonzalez FJ, and Korzekwa KR (1994) O-demethylation of epipodophyllotoxins is catalyzed by human cytochrome P450 3A4. *Mol Pharmacol* **45**:352-358.
- Riera P, Salazar J, Virgili A, Tobeña M, Sebio A, Gallano P, Barnadas A, and Páez D (2018) Relevance of CYP3A4\*20, UGT1A1\*37 and UGT1A1\*28 variants in irinotecan-induced severe toxicity. *Br J Clin Pharmacol* **84**:1389-1392.
- Risinger AL, Giles FJ, and Mooberry SL (2009) Microtubule dynamics as a target in oncology. *Cancer*

*Treat Rev* **35**:255-261.

Rivory LP, Slaviero KA, and Clarke SJ (2002) Hepatic cytochrome P450 3A drug metabolism is reduced in cancer patients who have an acute-phase response. *Br J Cancer* **87**:277-280.

Rudin CM, Liu W, Desai A, Karrison T, Jiang X, Janisch L, Das S, Ramirez J, Poonkuzhali B, Schuetz E, Fackenthal DL, Chen P, Armstrong DK, Brahmer JR, Fleming GF, Vokes EE, Carducci MA, and Ratain MJ (2008) Pharmacogenomic and pharmacokinetic determinants of erlotinib toxicity. *J Clin Oncol* **26**:1119-1127.

Ruzilawati AB, Suhaimi AW, and Gan SH (2007) Genetic polymorphisms of CYP3A4: CYP3A4\*18 allele is found in five healthy Malaysian subjects. *Clin Chim Acta* **383**:158-162.

Sai K, Saito Y, Fukushima-Uesaka H, Kurose K, Kaniwa N, Kamatani N, Shirao K, Yamamoto N, Hamaguchi T, Kunitoh H, Ohe Y, Tamura T, Yamada Y, Minami H, Ohtsu A, Yoshida T, Saijo N, and Sawada J (2008) Impact of CYP3A4 haplotypes on irinotecan pharmacokinetics in Japanese cancer patients. *Cancer Chemother Pharmacol* **62**:529-537.

Sakurai K, Enomoto K, Matsuo S, Amano S, and Shiono M (2011) CYP3A4 expression to predict treatment response to docetaxel for metastasis and recurrence of primary breast cancer. *Surg today* **41**:674-679.

Santos A, Zanetta S, Cresteil T, Deroussent A, Pein F, Raymond E, Vernillet L, Risse M, Boige V, Gouyette A, and Vassal G (2000) Metabolism of irinotecan (CPT-11) by CYP3A4 and CYP3A5 in humans. *Clin Cancer Res* **6**:2012-2020.

Scheers E, Leclercq L, de Jong J, Bode N, Bockx M, Laenen A, Cuyckens F, Skee D, Murphy J, Sukbuntherng J, and Mannens G (2015) Absorption, metabolism, and excretion of oral <sup>14</sup>C radiolabeled ibrutinib: an open-label, phase I, single-dose study in healthy men. *Drug Metab Dispos* **43**:289-297.

- Schirmer M, Toliat MR, Haberl M, Suk A, Kamdem LK, Klein K, Brockmöller J, Nürnberg P, Zanger UM, and Wojnowski L (2006) Genetic signature consistent with selection against the CYP3A4\*1B allele in non-African populations. *Pharmacogenet Genomics* **16**:59-71.
- Schmidt R, Baumann F, Knüpfer H, Brauckhoff M, Horn LC, Schönfelder M, Köhler U, and Preiss R (2004) CYP3A4, CYP2C9 and CYP2B6 expression and ifosfamide turnover in breast cancer tissue microsomes. *Br J Cancer* **90**:911-916.
- Seong SJ, Lim M, Sohn SK, Moon JH, Oh SJ, Kim BS, Ryoo HM, Chung JS, Joo YD, Bang SM, Jung CW, Kim DH, Park SY, Yoon SS, Kim I, Lee HG, Won JH, Min YH, Cheong JW, Park JS, Eom KS, Hyun MS, Kim MK, Kim H, Park MR, Park J, Kim CS, Kim HJ, Kim YK, Park EK, Zang DY, Jo DY, Lee HW, and Yoon YR (2013) Influence of enzyme and transporter polymorphisms on trough imatinib concentration and clinical response in chronic myeloid leukemia patients. *Ann Oncol* **24**:756-760.
- Shou M, Martinet M, Korzekwa KR, Krausz KW, Gonzalez FJ, and Gelboin HV (1998) Role of human cytochrome P450 3A4 and 3A5 in the metabolism of taxotere and its derivatives: enzyme specificity, interindividual distribution and metabolic contribution in human liver. *Pharmacogenetics* **8**:391-401.
- Sim S, Bergh J, Hellström M, Hatschek T, and Xie H (2018) Pharmacogenetic impact of docetaxel on neoadjuvant treatment of breast cancer patients. *Pharmacogenomics* **19**:1259-1268.
- Simon F, Gautier-Veyret E, Truffot A, Chenel M, Payen L, Stanke-Labesque F, and Tod M (2021) Modeling Approach to Predict the Impact of Inflammation on the Pharmacokinetics of CYP2C19 and CYP3A4 Substrates. *Pharm Res* **38**:415-428.
- Smith P, Bullock JM, Booker BM, Haas CE, Berenson CS, and Jusko WJ (2004) The influence of St. John's wort on the pharmacokinetics and protein binding of imatinib mesylate. *Pharmacotherapy* **24**:1508-1514.

- Spurdle AB, Goodwin B, Hodgson E, Hopper JL, Chen X, Purdie DM, McCredie MR, Giles GG, Chenevix-Trench G, and Liddle C (2002) The CYP3A4\*1B polymorphism has no functional significance and is not associated with risk of breast or ovarian cancer. *Pharmacogenetics* **12**:355-366.
- Stanek EJ, Simko RJ, DeNofrio D, and Pavri BB (1997) Prolonged quinidine half-life with associated toxicity in a patient with hepatic failure. *Pharmacotherapy* **17**:622-625.
- Stiborová M, Indra R, Moserová M, Černá V, Rupertová M, Martínek V, Eckschlager T, Kizek R, and Frei E (2012) Cytochrome b5 Increases Cytochrome P450 3A4-Mediated Activation of Anticancer Drug Ellipticine to 13-Hydroxyellipticine Whose Covalent Binding to DNA Is Elevated by Sulfotransferases and N,O-Acetyltransferases. *Chem Res Toxicol* **25**:1075-1085.
- Swaisland H, Ranson M, Smith R, Leadbetter J, Laight A, McKillop D, and Wild M (2005) Pharmacokinetic drug interactions of gefitinib with rifampicin, itraconazole and metoprolol. *Clin Pharmacokinet* **44**:1067-1081.
- Syed YY (2020) Zanubrutinib: First Approval. *Drugs* **80**:91-97.
- Takakusa H, Wahlin MD, Zhao C, Hanson KL, New LS, Chan EC, and Nelson SD (2011) Metabolic intermediate complex formation of human cytochrome P450 3A4 by lapatinib. *Drug Metab Dispos* **39**:1022-1030.
- Tanaka C, Yin O, Smith T, Sethuraman V, Grouss K, Galitz L, Harrell R, and Schran H (2011) Effects of rifampin and ketoconazole on the pharmacokinetics of nilotinib in healthy participants. *J Clin Pharmacol* **51**:75-83.
- Taniguchi K and Karin M (2014) IL-6 and related cytokines as the critical lynchpins between inflammation and cancer. *Semin Immunol* **26**:54-74.
- Teft WA, Gong IY, Dingle B, Potvin K, Younus J, Vandenberg TA, Brackstone M, Perera FE, Choi Y-H,

- Zou G, Legan RM, Tirona RG, and Kim RB (2013) CYP3A4 and seasonal variation in vitamin D status in addition to CYP2D6 contribute to therapeutic endoxifen level during tamoxifen therapy. *Breast Cancer Res Treat* **139**:95-105.
- Teng WC, Oh JW, New LS, Wahlin MD, Nelson SD, Ho HK, and Chan EC (2010) Mechanism-based inactivation of cytochrome P450 3A4 by lapatinib. *Mol Pharmacol* **78**:693-703.
- Teo YL, Saetaew M, Chanthawong S, Yap YS, Chan EC, Ho HK, and Chan A (2012) Effect of CYP3A4 inducer dexamethasone on hepatotoxicity of lapatinib: clinical and in vitro evidence. *Breast Cancer Res Treat* **133**:703-711.
- Teo YL, Ho HK, and Chan A (2015) Metabolism-related pharmacokinetic drug-drug interactions with tyrosine kinase inhibitors: current understanding, challenges and recommendations. *Br J Clin Pharmacol* **79**:241-253.
- Thomson RJ, Moshirfar M, and Ronquillo Y (2021). *Tyrosine Kinase Inhibitors*. In: *StatPearls*. Treasure Island (FL): StatPearls.
- Tian D and Hu Z (2014). CYP3A4-mediated pharmacokinetic interactions in cancer therapy. *Curr Drug Metab* **15**:808-817.
- Torres Espíndola L, Rojo-Serrato D, Álvaro-Heredia A, Castillejos López M, de Uña-Flores A, Pérez-García M, Zapata-Tarres M, Cárdenas-Cardos R, Granados J, Chávez-Pacheco J, Salinas-Lara C, de Arellano I, and Aquino-Gálvez A (2020) CYP450 Analysis of gene allelic variants can predict ifosfamide toxicity in Mexican paediatric patients. *Biomarkers* **25**:331-340.
- Towles JK, Clark RN, Wahlin MD, Uttamsingh V, Rettie AE, and Jackson KD (2016) Cytochrome P450 3A4 and CYP3A5-Catalyzed Bioactivation of Lapatinib. *Drug Metab Dispos* **44**:1584-1597.
- Valencia-Cervantes J, Huerta-Yepez S, Aquino-Jarquín G, Rodríguez-Enríquez S, Martínez-Fong D,



- Arias-Montaño J, and Dávila-Borja V (2019) Hypoxia increases chemoresistance in human medulloblastoma DAOY cells via hypoxia-inducible factor 1 $\alpha$ -mediated downregulation of the CYP2B6, CYP3A4 and CYP3A5 enzymes and inhibition of cell proliferation. *Oncol Rep* **41**:178-190.
- van Eijk M, Boosman RJ, Schinkel AH, Huitema ADR, and Beijnen JH (2019) Cytochrome P450 3A4, 3A5, and 2C8 expression in breast, prostate, lung, endometrial, and ovarian tumors: relevance for resistance to taxanes. *Cancer Chemother Pharmacol* **84**:487-499.
- van Rongen A, Brill MJE, Vaughns JD, Välitälo PAJ, van Dongen EPA, van Ramshorst B, Barrett JS, van den Anker JN, and Knibbe CAJ (2018) Higher Midazolam Clearance in Obese Adolescents Compared with Morbidly Obese Adults. *Clin Pharmacokinet* **57**:601-611.
- Villikka K, Kivistö KT, Mäenpää H, Joensuu H, and Neuvonen PJ (1999) Cytochrome P450-inducing antiepileptics increase the clearance of vincristine in patients with brain tumors. *Clin Pharmacol Ther* **66**:589-593.
- Vuppalanchi R, Liang T, Goswami CP, Nalamasu R, Li L, Jones D, Wei R, Liu W, Sarasani V, Janga SC, and Chalasani N (2013) Relationship between differential hepatic microRNA expression and decreased hepatic cytochrome P450 3A activity in cirrhosis. *PLoS One* **8**:e74471.
- Wang C, Ying L, Jin M, Zhang F, Shi D, Dai Y, and Zhou Z (2021) An investigation into possible interactions among four vascular epidermal growth factor receptor-tyrosine kinase inhibitors with gefitinib. *Cancer Chemother Pharmacol* **87**:43-52.
- Wang MZ, Wu JQ, Dennison JB, Bridges AS, Hall SD, Kornbluth S, Tidwell RR, Smith PC, Voyksner RD, Paine MF, and Hall JE (2008) A gel-free MS-based quantitative proteomic approach accurately measures cytochrome P450 protein concentrations in human liver microsomes. *Proteomics* **8**:4186-4196.

- Wang Y, Wang M, Qi H, Pan P, Hou T, Li J, He G, and Zhang H (2014) Pathway-dependent inhibition of paclitaxel hydroxylation by kinase inhibitors and assessment of drug-drug interaction potentials. *Drug Metab Dispos* **42**:782-795.
- Werk AN and Cascorbi I (2014) Functional gene variants of CYP3A4. *Clin Pharmacol Ther* **96**:340-348.
- Westlind-Johnsson A, Hermann R, Huennemeyer A, Hauns B, Lahu G, Nassr N, Zech K, Ingelman-Sundberg M, and von Richter O (2006) Identification and characterization of CYP3A4\*20, a novel rare CYP3A4 allele without functional activity. *Clin Pharmacol Ther* **79**:339-349.
- Wulkersdorfer B, Zeitlinger M, and Schmid M (2016) Pharmacokinetic Aspects of Vascular Endothelial Growth Factor Tyrosine Kinase Inhibitors. *Clin Pharmacokinet* **55**:47-77.
- Xu B, Yan M, Ma F, Hu X, Feng J, Ouyang Q, Tong Z, Li H, Zhang Q, Sun T, Wang X, Yin Y, Cheng Y, Li W, Gu Y, Chen Q, Liu J, Cheng J, Geng C, Qin S, Wang S, Lu J, Shen K, Liu Q, Wang X, Wang H, Luo T, Yang J, Wu Y, Yu Z, Zhu X, Chen C, and Zou J (2021) Pyrotinib plus capecitabine versus lapatinib plus capecitabine for the treatment of HER2-positive metastatic breast cancer (PHOEBE): a multicentre, open-label, randomised, controlled, phase 3 trial. *Lancet Oncol* **22**:351-360.
- Yamamoto Y, Usui N, Nishida T, Mori M, Takahashi Y, Imai K, Kagawa Y, and Inoue Y (2018) Influence of Renal Function on Pharmacokinetics of Antiepileptic Drugs Metabolized by CYP3A4 in a Patient With Renal Impairment. *Ther Drug Monit* **40**:144-147.
- Yang L-Q, Li S-J, Cao Y-F, Man X-B, Yu W-F, Wang H-Y, and Wu M-C (2003) Different alterations of cytochrome P450 3A4 isoform and its gene expression in livers of patients with chronic liver diseases. *World J Gastroenterol* **9**:359-363.
- Yang L, Yan C, Zhang F, Jiang B, Gao S, Liang Y, Huang L, and Chen W (2018) Effects of ketoconazole on cyclophosphamide metabolism: evaluation of CYP3A4 inhibition effect using the in vitro and in vivo

models. *Exp Anim* **67**:71-82.

Yao D, Ding S, Burchell B, Wolf CR, and Friedberg T (2000) Detoxication of vinca alkaloids by human

P450 CYP3A4-mediated metabolism: implications for the development of drug resistance. *J Pharmacol Exp Ther* **294**:387-395.

Ye L, Yang X, Guo E, Chen W, Lu L, Wang Y, Peng X, Yan T, Zhou F, and Liu Z (2014) Sorafenib

metabolism is significantly altered in the liver tumor tissue of hepatocellular carcinoma patient. *PLoS One* **9**:e96664.

Zanger UM and Schwab M (2013) Cytochrome P450 enzymes in drug metabolism: regulation of gene

expression, enzyme activities, and impact of genetic variation. *Pharmacol Ther* **138**:103-141.

Zeng Y, He YJ, He FY, Fan L, Zhou HH (2009) Effect of bifendate on the pharmacokinetics of

cyclosporine in relation to the CYP3A4\*18B genotype in healthy subjects. *Acta Pharmacol Sin* **30**:478-484.

Zhou-Pan XR, Séréé E, Zhou XJ, Placidi M, Maurel P, Barra Y, and Rahmani R (1993) Involvement of

human liver cytochrome P450 3A in vinblastine metabolism: drug interactions. *Cancer Res* **53**:5121-5126.

Zhou C, Tabb MM, Nelson EL, Grün F, Verma S, Sadatrafiei A, Lin M, Mallick S, Forman BM, Thummel

KE, and Blumberg B (2006) Mutual repression between steroid and xenobiotic receptor and NF-kappaB signaling pathways links xenobiotic metabolism and inflammation. *J Clin Invest* **116**:2280-2289.

Zhou D, Podoll T, Xu Y, Moorthy G, Vishwanathan K, Ware J, Slatter JG, and Al-Huniti N (2019)

Evaluation of the Drug-Drug Interaction Potential of Acalabrutinib and Its Active Metabolite, ACP-5862, Using a Physiologically-Based Pharmacokinetic Modeling Approach. *CPT Pharmacometrics Syst Pharmacol* **8**:489-499.

Zhou W, Deng Y, Zhang C, Liu Z, Zhang J, Zhou L, Shao L, and Zhang C (2022) Current status of therapeutic drug monitoring for methotrexate, imatinib, paclitaxel in China. *Clin Biochem* **104**:44-50.

Zhou XJ, Zhou-Pan XR, Gauthier T, Placidi M, Maurel P, and Rahmani R (1993) Human liver microsomal cytochrome P450 3A isozymes mediated vindesine biotransformation. *Metabolic drug interactions. Biochem Pharmacol* **45**:853-861.

Zhu Y-T, Teng Z, Zhang Y-F, Li W, Guo L-X, Liu Y-P, Qu X-J, Wang Q-R, Mao S-Y, Chen X-Y, and Zhong D-F (2020) Effects of Apatinib on the Pharmacokinetics of Nifedipine and Warfarin in Patients with Advanced Solid Tumors. *Drug Des Devel Ther* **14**:1963-1970.

Zhuo X, Zheng N, Felix CA, and Blair IA (2004) Kinetics and regulation of cytochrome P450-mediated etoposide metabolism. *Drug Metab Dispos* **32**:993-1000.

#### Footnotes

This work was supported by funding from the National Natural Science Foundation of China [Grants 82003849, 82073923] and Key project of Natural Science Foundation of Bengbu Medical College [Grant BYKY2019297ZD].

#### Legends for Figures

**Fig. 1.** Schematic diagram of the main factors affecting the activity, expression and function of CYP3A4 in the regulation of CYP3A4-mediated drug metabolism in tumor tissue. ↑ indicates increase; ↓ indicates decrease

**Fig. 2.** Design of targeted prodrugs based on the expression and activity of CYP3A4 in tumor tissues. The inhibitory or enhanced expression and activity of CYP3A4 in tumor tissues depends on different types of cancer, and targeted anticancer prodrugs that can specifically recognize tumor tissues are designed to achieve optimal therapeutic concentrations. The drug exposure and efficacy of these prodrugs and their

active metabolites depend on the oxidative and reductive metabolism of CYP3A4.

**Table 1** Overview of the significant effects of CYP3A4-mediated metabolism on the activity of anticancer drugs and their metabolites

Action type	Anticancer drug	Metabolite and activity	Phase	Ref.
CYP3A4-activated	Cyclophosphamide	Active 4- hydroxycyclophosphamide	Approved	(Huang et al., 2000)
	Ifosfamide	Active 4-hydroxy derivatives	Approved	(Huang et al., 2000)
	Etoposide	Etoposide catechol	Approved	(Relling et al., 1994)
	Teniposide	Teniposide catechol derivative	Approved	(Relling et al., 1994)
	Tamoxifen	Active endoxifen and 4-hydroxytamoxifen (>30-100-fold)	Approved	(Desta et al., 2004)
	Vinblastine	More biologically active desacetyl vinblastine	Approved	(Zhou-Pan et al., 1993)
	Vinorelbine	Active 4-O-deacetyl vinorelbine	Approved	(Kajita et al., 2000)
	AQ4N	Active AQ4	Phase 1 trial	(Albertella et al., 2008)
	Sunitinib	Active N-desethylsunitinib	Approved	(Amaya et al., 2018)
	Lapatinib	Active quinone-imine	Approved	(Towles et al., 2016; Bissada et al., 2019)
	Dabrafenib	Active carboxy-dabrafenib	Approved	(Puszek et al., 2019b)
	Acalabrutinib	Active acalabrutinib M27 metabolite (ACP-5862)	Approved	(Zhou et al., 2019)
	Regorafenib	Similar active M-2 (N-oxide) and M-5 (N-oxide and N-desmethyl)	Approved	(Mross et al., 2012)
	Neratinib	Active O-depicoline neratinib	Approved	(Mullard, 2018)

	Erlotinib	Active O-desmethyl-erlotinib (OSI-420)	Approved	(Reddick et al., 2019)
	Gefitinib	Active quinone-imine	Approved	(Li et al., 2009b)
	Osimertinib	Active AZ7550 and AZ5104	Approved	(Dickinson et al., 2016)
	O6-Benzylguanine	O6-benzyl-8-oxoguanine (an equally active, yet longer-lived metabolite)	Phase 3 trial	(Ewesuedo and Dolan, 2000)
	Methoxymorpholinyl doxorubicin (MMDX; nemorubicin)	Active PNU-159682 (>100-fold)	Phase 2 trial	(Quintieri et al., 2005)
	Cytosine-1- $\beta$ -D-arabinofuranoside Monophosphate	Active hydroxylated derivative (>28-120-fold)	Preclinical trial	(Boyer et al., 2006)
	Ellipticine	Active 13-hydroxy- and 12-hydroxyellipticine	Preclinical trial	(Stiborová et al., 2012)
	Icotinib	Oxidative metabolites M3 and M5	Phase 4 trial	(Huang et al., 2020)
	Crizotinib	PF-06260182	Approved	(Huang et al., 2020)
	Alectinib	M4	Approved	(Huang et al., 2020)
	Entrectinib	Similar active metabolite M5	Approved	(Huang et al., 2020)
CYP3A4-deactivated	Paclitaxel	Inactive 3'-p-hydroxypaclitaxel and 6a,	Approved	(Martínez et al., 2002)

	3'-p-dihydroxypaclitaxel		
Docetaxel	Inactive hydroxy-docetaxel	Approved	(Sim et al., 2018)
Irinotecan	Inactive APC and NPC	Approved	(Santos et al., 2000)
Thiotepa	Inactive teпа	Approved	(Jacobson et al., 2002)
Doxorubicin	Inactive doxorubicinol deoxyglycone	Approved	(Kivistö et al., 1995)
Exemestane	6-hydroxymethylexemestane	Approved	(Kamdem et al., 2011)
Vincristine	Inactive dihydro-hydroxycatharanthine vincristine derivative	Approved	(Villikka et al., 1999)
Vindesine	Unidentified	Approved	(Zhou et al., 1993)
Imatinib	Less active N-demethylated piperazine derivative	Approved	(Filppula et al., 2012)
Nilotinib	Less active oxidation and hydroxylation metabolites (<14-fold)	Approved	(Tanaka et al., 2011)
Dasatinib	Inactive hydroxylation at the para-position of the chloromethylphenyl ring (major metabolite), hydroxylation of the C5-methyl of the chloromethylphenyl ring, and N-dealkylation of the hydroxyethyl moiety	Approved	(Li et al., 2009a)
Sorafenib	Less active pyridostigmine	Approved	(Keating and Santoro, 2009)
Ibrutinib	Less active dihydrodiol metabolite (PCI-45227)	Approved	(Scheers et al., 2015)



Vandetanib	Less active N-desmethyl-vandetanib	Approved	(Indra et al., 2019)
Vemurafenib	Inactive OH-vemurafenib	Approved	(Goldinger et al., 2015)
Bosutinib	Less active oxydechlorinated bosutinib (M2) and N-desmethylated bosutinib (M5)	Approved	(Abbas and Hsyu, 2016)
Cabozantinib	Less active N-oxide metabolite (EXEL-1644)	Approved	(Lacy et al., 2015)
Zanubrutinib	Unidentified	Approved	(Syed, 2020)
Lenvatinib	O-desmethyl lenvatinib	Approved	(Ozeki et al., 2019)
Pyrotinib	O-depicolyl pyrotinib, O-depicolyl and pyrrolidine lactam pyrotinib, and pyrrolidine lactam pyrotinib	Phase 3 trial	(Xu et al., 2021)
Avapritinib	Oxidative metabolite M499	Approved	(Huang et al., 2020)
Brigatinib	Less active AP26123	Approved	(Huang et al., 2020)
Lorlatinib	Oxidative cleavage metabolite, M8	Approved	(Huang et al., 2020)
Larotrectinib	O-linked glucuronide metabolite	Approved	(Thomson et al., 2021)
Axitinib	Axitinib sulfoxide (M12)	Approved	(Huang et al., 2020)
Pazopanib	10 to 20-fold less active pazopanib M24	Approved	(Thomson et al., 2021)
Pralsetinib	Unidentified	Approved	(Huang et al., 2020)

Data source: [www.fda.gov](http://www.fda.gov), [www.drugs.com](http://www.drugs.com), and [www.clinicaltrials.gov](http://www.clinicaltrials.gov).

**Table 2** Representative CYP3A4-metabolized drugs are also CYP3A4 inhibitors and/or inducers in the clinic

Anticancer drug	CYP3A4 inhibitors	CYP3A inducers	Approved dose	Toxicity data
Cyclophosphamide*	No	YES	500~1000 mg/m <sup>2</sup>	LD <sub>50</sub> = 40 mg/kg (Dog, iv)
Ifosfamide*	YES	YES	1.2~2.5g/m <sup>2</sup>	LD <sub>50</sub> = 140 mg/kg (Rat, ip)
Etoposide*	YES	YES	60~150 mg/m <sup>2</sup>	LD <sub>50</sub> = 37 mg/kg (Rabbit, iv)
Teniposide*	YES	NO	30~100 mg/m <sup>2</sup>	LD <sub>50</sub> = 29.57 mg/kg (Mouse, iv)
Tamoxifen*	YES	YES	20 mg~40 mg/d	LD <sub>50</sub> = 4,100 mg/kg (Rat, oral)
Paclitaxel*	NO	YES	135~175 mg/m <sup>2</sup>	LD <sub>50</sub> = 32.53 mg/kg (Rat, ip)
Docetaxel*	YES	NO	60~100 mg/m <sup>2</sup>	LD <sub>50</sub> = 2.5 mg/kg (Dog, iv)
Doxorubicin*	YES	NO	60~75mg/m <sup>2</sup>	LD <sub>50</sub> = 10. 7 mg/kg (Rat, ip)
Irinotecan*	YES	NO	125~350 mg/m <sup>2</sup>	LD <sub>50</sub> = 40 mg/kg (Dog, iv)
Vinblastine*	YES	YES	150~250 µg/kg	LD <sub>50</sub> = 2 mg/kg (Rat, ip)
Imatinib*	YES	NO	400~800 mg/d	TDLO = 79.8 mg/kg/1w, intermittent (Human, oral)
Sunitinib*	YES	NO	25~75 mg/d	MTD = 50 mg/d (Human, oral)
Lapatinib	YES	NO	750~1250 mg/d	TDLO =1,198.4 ml/kg/8w, intermittent (Human, oral)
Dabrafenib*	YES	YES	100~300 mg/d	LD <sub>50</sub> >1000 mg/kg (Rat, oral)
Acalabrutinib*	YES	NO	100~400 mg/d	No data available
Regorafenib*	YES	NO	80~160 mg/d	MTD = 82 mg/m <sup>2</sup> /qd (Human, oral)
Nilotinib*	YES	NO	400~800 mg/d	LD <sub>50</sub> > 2,000 mg/kg (Rat, oral)
Erlotinib*	YES	NO	100~150 mg/d	TDLO = 9.8 mg/kg/8d, intermittent (Human, oral)

Dasatinib*	YES	NO	100~180 mg/d	LD <sub>50</sub> = 100 mg/kg (Rat, oral)
Gefitinib	YES	NO	250~500 mg/d	TDLO = 3.6 mg/kg (Human, oral)
Sorafenib*	YES	NO	400~800 mg/d	LD <sub>50</sub> > 2,000 mg/kg (Rat, oral)
Vemurafenib*	NO	YES	960~1920 mg/d	LD <sub>50</sub> > 2,500 mg/kg (Rat, oral)
Bosutinib*	YES	NO	300~500 mg/d	TDLO = 525 ml/kg/21d, intermittent (Mouse, oral)
Osimertinib*	YES	YES	40~80 mg/d	LD <sub>50</sub> = 14,500 mg/kg (Rat, oral)
Asciminib	YES	NO	80~400 mg/d	No data available
Lenvatinib	YES	YES	8~12 mg/d	MTD = 25 mg/d (Human, oral)
Ceritinib*	YES	NO	150~450 mg/d	MTD = 750 mg/d (Human, oral)
Pazopanib	YES	NO	200~800 mg/d	MTD = 600 mg/d (Human, oral)
Crizotinib*	YES	NO	250~500 mg/d	MTD = 165 mg/m <sup>2</sup> /bid (Human, oral)
Lorlatinib	YES	YES	50~100 mg/d	No data available
Pralsetinib	YES	YES	100~400 mg/d	MTD = 400 mg/d (Human, oral)

Information from the DrugBank database (<https://go.drugbank.com/drugs>) and ChemIDplus (<https://chem.nlm.nih.gov/chemidplus/>). These inducers and inhibitors may interact with any CYP3A4 substrate and lead to an increased risk of adverse events. Anticancer drugs with a narrow therapeutic range metabolized by CYP3A4 are marked with an asterisk based on information retrieved from the DrugBank database. LD<sub>50</sub>: lethal dose, 50%; MTD, maximum tolerated dose; ip, intraperitoneal; iv, intravenous; TDLO: lowest dose resulting in a toxic effect.

**Table 3** Clinical and pharmacokinetic consequences of CYP3A4 gene variants

Substrate	Number of patients	Cancer type	CYP3A4 allele	Clinical consequences	Ref.
Paclitaxel	261	Ovarian cancer	CYP3A4*22	5-year survival rate better than CYP3A expressers (in combination with CYP3A5 genotype)	(Assis et al., 2013)
Paclitaxel	261	Various cancers	CYP3A4*22	Increased risk of developing severe neurotoxicity in female carriers	(de Graan et al., 2013)
Paclitaxel nab-paclitaxel	125	Breast cancer and a few other cancers	CYP3A4*22	A trend of association with paclitaxel and nab-paclitaxel-induced toxicity	(Demurtas et al., 2021)
Paclitaxel	235	NSCLC and a few other cancers	CYP3A4*16	Reduced 3'-p-hydroxylation of paclitaxel and increased levels of 6 alpha-hydroxypaclitaxel	(Nakajima et al., 2006)
Paclitaxel	228	Breast and ovarian cancer	CYP3A4*20	Increased risk of paclitaxel-induced neuropathy	(Apellániz-Ruiz et al., 2015b)
Irinotecan	308	mCRC	CYP3A4*20	No association with irinotecan-induced toxicity	(Riera et al., 2018)
Irinotecan	177	Lung, colon and other	CYP3A4*16	Decreased metabolism of irinotecan to APC, and total clearance of irinotecan or toxicities (severe diarrhea and	(Sai et al., 2008)

		cancers		neutropenia) was not significant	
Sunitinib	114	Esophagus, ovary and a few other cancers	CYP3A4*22	Decreased clearance in CYP3A4 *1/*22, increased clearance in CYP3A4 *22/*22	(Diekstra et al., 2014)
Sunitinib	92	Renal cancer	CYP3A4*22	A high clearance in CYP3A4*22 on sunitinib	(Narjoz et al., 2015)
Sunitinib	287	mRCC	CYP3A4 rs4646437 A-allele	An increased risk for hypertension	(Diekstra et al., 2017)
Pazopanib	94	Advanced or metastatic solid tumors	CYP3A4*22	A substantial lower pazopanib clearance in CYP3A4*22 heterozygotes (35% lower clearance), potentially makes them more susceptible to (severe) toxicity.	(Bins et al., 2019)
Docetaxel	150	Breast cancer	CYP3A4*22	Increased risk for grade 3/4 adverse events	(Sim et al., 2018)
Vemurafenib	97	Melanoma	CYP3A4*22	Increased risk for several severe Vemurafenib-related toxicities, such as grade 3-4 nausea, grade 1-4 hyperbilirubinemia and cutaneous squamous cell carcinoma	(Goey et al., 2019)
Tamoxifen	132	Breast cancer	CYP3A4*22	Increased endoxifen concentration	(Baxter et al., 2014)
Tamoxifen	196	Breast cancer	CYP3A4*22	Significant predictor of endoxifen concentration	(Teft et al., 2013)

Tamoxifen	730	Breast cancer	CYP3A4*22	Increased plasma concentration of endoxifen	(Puszekiel et al., 2019a)
Exemestane	246	Breast cancer	CYP3A4*22	A 54% increase in steady-state exemestane concentration	(Hertz et al., 2017)
Imatinib	82	CML	CYP3A4*18	The pharmacokinetics and clinical response of imatinib were not affected	(Seong et al., 2013)
Lenvatinib	40	Thyroid cancer	CYP3A4*1G	Lower mean steady-state dose-adjusted plasma trough concentrations	(Ozeki et al., 2019)
Imatinib	68	GIST	CYP3A4*1G	Decreased imatinib mesylate plasma levels in Chinese gastrointestinal stromal tumor patients	(Liu et al., 2017)
Bosutinib	30	CML	CYP3A4*1G	No significant differences in the steady-state plasma trough concentrations of bosutinib	(Abumiya et al., 2018)
Ifosfamide	131	Soft tissue sarcoma, CNS tumor and other solid tumor	CYP3A4*1B	Increased the risk of severe leukopenia and neutropenia, and this toxicity has also been found to be associated with poor prognosis	(Torres Espíndola et al., 2020)
Erlotinib	80	Lung, head and neck, and ovarian cancer	CYP3A4*1B	CYP3A4*1B (G/G and A/G) was marginally associated with lower AUC and trough levels of erlotinib than that in a dominant model of the A allele (possibly higher CYP3A4 expression), and the odds of high-grade toxicity	(Rudin et al., 2008)

decreased					
Cyclophosphamide	350	Breast cancer	CYP3A4*1B	Significantly worse disease-free survival than those who were wild-type *1A/*1A	(Gor et al., 2010)
Cyclophosphamide	85	Breast cancer	CYP3A4*1B	Higher AUC of parent cyclophosphamide and significantly worse overall survival	(Petros et al., 2005)
Thiotepa	124	Breast cancer and germ cell cancer	CYP3A4*1B	Reduced clearance, which resulted in increased exposure to thiotepa of 26% and decreased exposure to tepa of 27% in homozygous patients.	(Ekhart et al., 2009)

Abbreviations: CML, chronic myeloid leukemia; CNS, central nervous system; CRC colorectal carcinoma; GIST, gastrointestinal stromal tumor; MTC, medullary thyroid cancer; mCRC, metastatic colorectal cancer; mRCC, metastatic renal cell carcinoma; NSCLC, non-small cell lung cancer.

Fig. 1

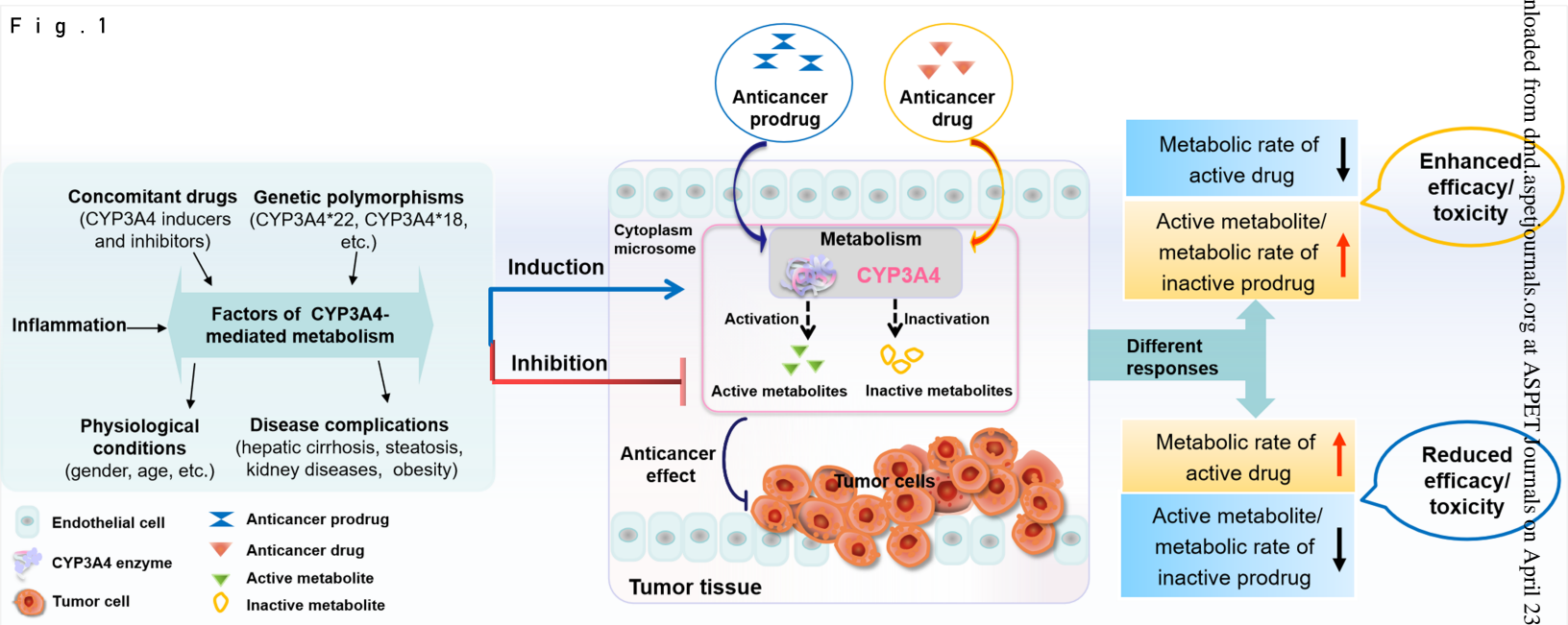




Fig.2

