APPENDIX A6.1.3.19 CYP1A2 - FACTSHEET

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Cytochrome P450

Cytochrome P450 (CYP) enzymes are a superfamily of heme-containing mono-oxygenases that are found in all kingdoms of life, and which show extraordinary diversity in their reaction chemistry. In mammals, these proteins are found primarily in endoplasmic reticulum of hepatocytes and other cell types, where they oxidize steroids, fatty acids and xenobiotics, and are important for the detoxification and clearance of various compounds, as well as for hormone synthesis and breakdown, cholesterol synthesis and vitamin D metabolism [1]. In plants, these proteins are important for the biosynthesis of several compounds such as hormones, defensive compounds and fatty acids. In bacteria, they are important for several metabolic processes, such as the biosynthesis of antibiotic erythromycin in *Saccharopolyspora erythraea (Streptomyces erythraeus)* [2]. The knowledge of the human P450s enzymes has gained a lot of importance in the context of drug development, as the fate of candidate drugs is largely influenced by the CYP metabolism [3]. CYP-mediated drug metabolism can thus lead to altered drug efficacies, through inactivation of an active drug, or on the opposite, activation of a prodrug [4]. Similarly, toxic compounds may be detoxified through CYP-catalyzed biotransformation, and inert xenobiotics can be activated to become toxicants.

Organisms produce many different cytochrome P450 enzymes (at least 58 in humans), which together with alternative splicing can provide a wide array of enzymes with different substrate and tissue specificities. Individual cytochrome P450 proteins follow the nomenclature: CYP (superfamily), followed by a number (family), then a letter (subfamily), and another number (protein); e.g., CYP1A2 is the second protein in family 1, subfamily A. In general, family members should share >40% identity, while subfamily members should share >55% identity [2].

Cytochrome P450 enzymes use ion-containing heme to oxidize their substrates, using protons derived from NADH or NADPH to split the oxygen so a single atom can be added to a substrate. They also require electrons, which they receive from a variety of redox partners. The most common reaction catalyzed by cytochromes P450 is a monooxygenase reaction, e.g., insertion of one atom of oxygen into the aliphatic position of an organic substrate (RH) while the other oxygen atom is reduced to water [1]:

$$RH + O_2 + NADPH + H^+ \rightarrow ROH + H_2O + NADP^+$$

In humans, only about a dozen enzymes belonging to the 1, 2, and 3 CYP-families are responsible for the metabolism of the majority of drugs and other xenobiotics [5]. These CYP enzymes are controlled by a number of different factors, including sex, age, ethnicity, disease, and diurnal influences [5].

CYP1A2 - Expression

In humans, CYP1A2 is constitutively expressed at higher levels only in liver and represents about 4–16% of the total hepatic P450 pool [6]. It has also been detected at low levels in the stomach, duodenum, urinary bladder, seminal vesicle and skin [7]. Its expression in the respiratory tissues has not been so much documented, however, its mRNA has been detected at least in the lung [8]. CYP1A2 mRNA content in the liver has

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shown up to a 40-fold variability between individuals, with corresponding variability in the enzyme activity and drug metabolism [9].

CYP1A2 – activity

The CYP1A1 and CYP1A2 genes are oriented head-to-head, sharing a 23 kb bi-directional promoter, which contains at least 13 Ah-receptor (i.e., aryl hydrocarbon receptor; AhR) response elements, some of which appear to regulate transcription of both genes coordinately [5]. The AhR response elements are ligand-activated transcription factors that control the expression of a diverse set of genes. Consequently, both genes are highly inducible by numerous xenobiotics which act as AhR ligands such as methylcholanthrene and other polycyclic aromatic hydrocarbons, dioxins, β-naphthoflavon [5].

The elimination of a large list of drugs depends mainly on the CYP1A2 activity, being predominantly metabolized by this enzyme (Table 1). CYP1A2 is also important for the metabolism of endogenous substrates (e.g., melatonin, estrone and estradiol, bilirubin and uroporphyrinogen [10], and for the elimination of environmental xenobiotics. Through its activity, it can additionally activate carcinogens, such as dietary heterocyclic amines, mycotoxins, tobacco-specific nitrosamines, and aryl amines [11].

Heterocyclic and aromatic amines are initially N-oxidized by the action of cytochrome CYP1A2 in the liver [12]. The resulting N-hydroxy heterocyclic amine can be O-acetylated in the liver, then detoxified by conjugation with glutathione (GSH) by the action of GSH transferases (GST)s or glucuronidated by the action of UDP-glucuronosyltransferases. Alternatively, the N-hydroxy-heterocyclic amines can enter the bloodstream and be transported to target organs, where they can be O-acetylated in tissues containing N-acetyltransferase-2 NAT2. This N-acetoxy derivative can react with DNA to form covalent heterocyclic amine-DNA adducts [12]. The formation of DNA adducts and the extent of tumor induction is dependent on the amount of aromatic or heterocyclic amines which are converted to the reactive metabolite. The amount produced depends on the quantity and activity of the enzymes involved in its formation, CYP1A2 and NAT2, and also the enzymes involved in detoxification, such as GSTs and UDP-glucuronosyltransferases.

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CYP1A2 polymorphisms and variability in activity

CYP1A2 is a highly polymorphic gene. About 40 variants of the CYP1A2 protein, including the "predicted" haplotypes have been described (status November 2010), and few additional single nucleotide polymorphisms (SNPs) in the Home Page of the Human Cytochrome P450 Allele Nomenclature Committee (www.cypalleles.ki.se).

The activities of CYP1A2 exhibit polymorphic distributions in human populations, however the role of polymorphisms observed so far is still being discussed [9, 10, 12-17]. Some specific association studies support a role of CYP1A2 in the regulation of blood pressure, although further investigations will likely shed more light on the mechanistic aspects [18]. Polymorphisms can be detected by phenotyping assays. There is one substrate binding site on CYP1A2, which is quite rigid, thus one substrate is sufficient to test the enzymatic activity. Probe drugs or endogenous substances (such as phenacetin, caffeine, theophylline or melatonin, respectively) have all been successfully used to test CYP1A2 activity, however, caffeine is the most commonly used substance [9]. Measuring the ratio of caffeine metabolites reflects well the activities of CYP1A2 [19]. Individuals are classified as slow or rapid *N*-oxidizers, depending on their activity levels of CYP1A2 [19]. Slow metabolizer phenotype (or "poor" metabolizer), using substrate probes, has been identified in Australians (5%), Japanese (14%) and Chinese (5%) [10]. Asian and African populations have generally lower CYP1A2 activity compared with Caucasians [20]. Slow metabolizers can end up with increased in vivo drug concentrations and exaggeration of drug effects [9]. For example, higher dose-adjusted plasma levels of clozapine in women compared with men have been reported in different study populations [10]. Testing CYP1A2 activity before selection of drugs and initial doses would thus be an advantage for patients. Changes in diet, lifestyle, concomitant drug intake or environment should also be considered during the course of a treatment. Smoking cessation, and even a change in coffee consumption has occasionally led to adverse drug effects through elevated drug plasma concentrations [9].

Wide variations in human CYP1A2 activity have been reported in different populations, however, the variant alleles identified so far in the structural gene do not explain the activity range reported. Differences in activity can range from 40- to 130-fold [20]. Constitutional factors and enzyme induction/inhibition may explain these differences, but also basal levels of expression. Differences of up to 15-fold were indeed found in 12 different human liver samples s [21]. In vitro hepatic microsomal activity using different human samples showed differences in N-oxidation of 2-naphthylamine and 4-aminobiphenyl of up to 28- [22] and 44-fold [23], respectively.

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Paraxanthine/caffeine ratio as a measure of CYP1A2 activity

When comparing intravenous and oral caffeine doses, the absolute bioavailability of caffeine has been reported to be 100% [24]. It is rapidly and completely absorbed after oral administration, with the Cmax occurring at approximately 1 hour and its half-life is reported to be between 3-7 hours in healthy adults [24]. The primary pathways of caffeine metabolism involve N-3, N-1, and N-7 demethylation to form paraxanthine, theobromine, and theophylline [24]. Studies in vitro and in vivo provide conclusive evidence that CYP1A2 is almost solely responsible for caffeine N-3 demethylation that leads to the formation of paraxanthine [24]. CYP2E1 and CYP2D6 could play a minor role in caffeine metabolism, but it is believed to be minimal at normal dietary caffeine. Paraxanthine is not produced by plants and is only observed in nature as a metabolite of caffeine and theobromine in animals. Measuring the ratio of paraxanthine and caffeine thus provides an accurate assessment of CYP1A2 activity [24].

Saliva, plasma and serum are considered equally appropriate sampling matrices and typically the paraxanthine/caffeine concentration ratio at 4 h after a caffeine dose is considered to be an accurate estimate of CYP1A2 activity [24]. These parameters are preferable to urinary metrics of CYP1A2 activity due to the complex metabolism of caffeine, subsequent metabolism of caffeine metabolites and the effect of caffeine on urine flow, which in turn influences the renal excretion of its metabolites [24].

In PMI's studies, cytochrome P450 1A2 activity was calculated in plasma as the metabolic ratio of paraxanthine/caffeine, both expressed in molar equivalent (nmol/L).

A conversion factor was applied as follows:

Paraxanthine (PX): the molecular weight is 180.166 g/mol [25]. For the conversion of PX from ng/mL to nmol/L, the result in ng/mL was multiplied by 5.550.

Caffeine: the molecular weight is 194.193 g/mol [26]. For the conversion of CAF from ng/mL to nmol/L, the result in ng/mL was multiplied by 5.150.

The ratio is expressed in percent.

CYP1A2 – Substrates, inhibitors and inducers

Substrates of the CYP1A2 enzyme are characteristically planar molecules with polyaromatic rings (such as aromatic, polyaromatic and heterocyclic amides and amines [5]) that fit the narrow and planar active-site architecture of the enzyme [10].

About 40% of human drug metabolism is carried out by the CYP polymorphic enyzmes, which can result in altered drug pharmacokinetics [27]. CYP1A2 is a highly polymorphic gene. Given the existence of slow or rapid metabolizers [19], the different drugs that act either as CYP1A2 substrates, inducers or inhibitors need to be dosed with care. Since the metabolism of these drugs might differ between smokers and non-smokers [9, 28], the smoking status of patients has also to be taken in consideration. For example, the case of clozapine has been largely described [28-32]. This drug has a narrow therapeutic range, and adverse drug reactions have been observed in connection with elevated plasma concentrations after smoking cessation. Its disposition was found to be significantly

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correlated with CYP1A2 activity. The magnitude of change observed in CYP1A2 activity on smoking cessation corresponds well to differences reported between smokers and non-smokers with regard to clozapine pharmacokinetics. In a study conducted in 11 psychiatric patients allowed to smoke up to 14 cigarettes per day, mean plasma levels of clozapine increased by 57.4% on implementation of a hospital-wide nonsmoking policy [31]. A 1.5-fold clozapine dose reduction soon after smoking cessation has thus been proposed in a predictive model [33] and a difference between smokers (≥15 cigarettes per day) and non-smokers in clozapine maintenance doses needed to reach a given plasma concentration of approximately 50% has been found [30]. In the case of theophylline, abstinence from smoking for 1 week resulted in a 37.6% decrease in clearance [34].

Table 1 Non-exhaustive list of compounds interacting with CYP1A2, either as substrates, inhibitors or inducers [5, 35, 36]

CYP1A2 substrates	CYP1A2 inhibitors	CYP1A2 inducers
Acenocoumarol	Amiodarone	Aminoglutethimide
Alosetron	Cimetidine	Antipyrine
Betaxolol	Ciprofloxacin	Bilirubin
Caffeine	Diclofenac	Carbamazepine
Clomiprmaine	Desipramine	Coffee
Cyclobenzaprine	Disulfiram	Cruciferous vegetables (e.g. broccoli, cabbage, brussel sprouts)
Clozapine	Efavirenz	Nelfinavir
Decarbazine	Enoxacin	Omeprazole
Duloxetine	Fluoxetine	Phenobarbital and other barbiturates
Estradiol	Fluvoxamine	Phenytoin
Estrogens, conjugated A/synthetic	Fospropofol	Polychlorinated biphenyls
Estrogens, conjugated equine	Furafylline	Polycyclic aromatic hydrocarbons (tobacco; charbroiled meat)
Estrogens, esterified	Gemfibrozil	Primaquine
Estropipate	Isoniazid	Primidone
Flutamine	Ketoconazole	Rifampin
Fluvoxamine	Methoxsalen	Ritonavir
Guanabenz	Mexiletine	Sulfinpyrazone
Haloperidol	Mexiletine	2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)
Imipramine	Miconazole	
Mexiletine	Moricizine	

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CYP1A2 substrates	CYP1A2 inhibitors	CYP1A2 inducers
Mirtazapine	α-Naphthoflavone	
Nabumetone	Nifedipine	
Naproxen	Norfloxacin	
Olanzapine	Ofloxacin	
Pimozide	Oral contraceptives	
Propanolol	Primaquine	
Ramelteon	Propofol	
Rasagiline	Thiabendazole	
Riluzole	Ticlopidine	
Ropinirole	Tolfenamic acid	
Ropivacaine	Tranylcypromine	
Tacrine	Zileuton	
Theophylline		
Thiothixene		
Tizanidine		
Triamterene		
Trifluoperazine		
Zileuton		
Zolmitriptan		

CYP1A2 in smokers and after smoking cessation

Induction of CYP1A2 by smoking is well established and has been confirmed in *in vivo* studies [16, 37, 38]. Components of cigarette smoke, probably polycyclic aromatic hydrocarbons induce CYP1A2 activity to a different extent in various populations of smokers [19]. A marked racial difference in the effect of cigarette smoking on CYP1A2 activity has been found [39]. Caucasian smokers have CYP1A2 levels nearly twice that of non-smokers while African–American smokers have CYP1A2 activities approximating that of African American or Caucasian non-smokers. These results indicate genetically based differences in the response of CYP1A2 activity to xenobiotic exposure. Because a clear link between CYP1A2 activity and CYP1A2 gene variation had not been established [17], a study was initiated to investigate the relationship between CYP1A2 activity and the activities of other polymorphic enzymes that are involved in the metabolism of heterocyclic aromatic amines and polycyclic aromatic hydrocarbons [12]. The results showed that polymorphism of CYP1A1 and GSTM1 can impact the activity of CPY1A1. In that study, the effects of cigarette smoking on

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CYP1A2 activity was examined by measuring CYP1A2 function in smokers before and after three weeks of smoking cessation [12]. Caffeine phenotyping was performed at entry into the study while the subjects were still actively smoking, and was repeated at the end of the study, after three week of complete smoking cessation. Subjects had mean CYP1A2 activity of 17.8 (expressed as the urinary molar ratio of 1,7-dimethyl xanthine + 1,7-dimethyluric acid /1,3,7-trimethyl xanthine as an indicator of caffeine phenotyping) while smoking, which decreased to 10.9 three weeks after cessation of smoking.

The cytochrome P450 enzyme CYP1A2 mediates the rate-limiting step in the metabolism of many drugs including theophylline, clozapine, and tacrine as well as in the bioactivation of procarcinogens. CYP1A2 activity shows both pronounced intra- and interindividual variability, which is, among other factors, related to smoking causing enzyme induction, to drug intake and to dietary factors which may result in induction or inhibition. In contrast to these exogenous factors, genetic influences on enzyme activity seem to be less pronounced [9]. Genetic polymorphisms of cytochrome P450 oxidoreductase (POR), an electron transfer protein that catalyzes the activity of several cytochromes P450, have also been studied [40]. Data showed that POR polymorphisms influence the CYP1A2 basal but not the induced activity and do not seem to influence CYP1A2 inducibility.

Another smoking cessation study, established an apparent half-life of CYP1A2 activity decrease of 38.6 hours (27.4-54.4 hours) [29]. In this study, subjects were phenotyped for their CYP1A2 activity at 6, 4, and 1 day before smoking cessation and at 0, 1, 2, 3, 6, 8, 10, and 13 day thereafter by use of the paraxanthine-to-caffeine ratio in plasma, 6 hours after a 148-mg caffeine test dose. Changes in the individual activities were already significantly different after two days of cessation [29].

The effect of smoking cessation on the metabolism of drugs that are CYP1A2-dependent as has been discussed above, consists in a potential increase of plasma concentrations of those drugs.

CYP1A2 and other influencing factors

Expression of CYP1A2 appears to be induced by various dietary constituents. Cruciferous vegetables such as cabbages, cauliflower and broccoli are known to increase levels of CYP1A2 activity. Lower activity of CYP1A2 in South Asians appears to be due to cooking these vegetables in curries using ingredients such as cumin and turmeric (a plant from the ginger family), compounds known to inhibit the enzyme. Consumption of pan-fried or grilled meat containing high levels of heterocyclic aromatic amines, caffeine and heavy exercise also increase CYP1A2 activity [9, 41]. A study conducted with subjects given a diet with cooked meat showed that subjects had mean CYP1A2 activity of 9.01 after one week of consuming meat cooked at low temperature, but this value increased to 12.7 after one week of consuming meat cooked at high temperature [12].

Menstrual cycle can slightly affect CYP1A2 activity. Females in general have lower CYP1A2 activity than males, which is further decreased if oral contraceptives are taken [9].

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CYP1A2 polymorphism and link to tobacco-induced diseases

Fast metabolizers are more likely to get higher concentrations of CYP1A2-biotransformed metabolites, including those from inactive substances that become carcinogenic [9]. Along this line, CYP1A2 has been implicated as a risk factor in several types of cancers [39, 42-44].

CYP1A2 phenotyping has allowed the assessment of colorectal cancer risk by the determination CYP1A2 phenotype in relation to dietary exposure to heterocyclic amines from well-done cooked meat [39], and has supported the hypothesis that an individual's cancer risk is related to individual carcinogen exposure and to polymorphisms in levels of carcinogen metabolizing enzymes.

As CYP1A2 metabolizes some polycyclic aromatic hydrocarbons (PAHs) to carcinogenic intermediates, the higher CYP1A2 activity generates more carcinogenic metabolites and then is likely to enhance the risk of tobacco-related cancers [45]. In this context, it was suggested that CYP1A2 on chromosome 15q24 may be a causative gene candidate to increase the risk of bladder cancer. Moreover, CYP1A2∗1C an allele, which is known to have lower CYP1A2 activity [46], was indicated to reduce the disease risk [45].

List of abbreviations

CAF	Caffeine
FDA	Food and Drug Administration
GSTs	Glutathione S-transferase
HC	Health Canada
IARC	International Agency for Research on Cancer
IUPAC	International Union of Pure and Applied Chemistry
MW	Molecular weight
NA	Not applicable
NMR	Nicotine metabolic ratio
NRT	Nicotine replacement therapy
PMI	Philip Morris International
RDT	Reproductive or developmental toxicant
RT	Respiratory toxicant
WHO	World Health Organization

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