

PART A

ADME: OVERVIEW AND CURRENT TOPICS

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REGULATORY DRUG DISPOSITION AND NDA PACKAGE INCLUDING MIST

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1.1 INTRODUCTION

Drug metabolism and pharmacokinetics (DMPK) plays an important and integral part in drug discovery and development. In drug discovery, during lead optimization and drug candidate identification, metabolism studies are conducted to screen a large number of compounds with potential liabilities. The emphasis is on generating data efficiently and in a timely fashion related to a compound's absorption, distribution, metabolism, and excretion (ADME) characteristics. Advances in analytical technologies, drug metabolism, and transporter biology has enabled drug metabolism scientists develop *in vitro* and *in vivo* tools to screen a large number of compounds efficiently and incorporate ADME information in lead optimization and identification. This early characterization for metabolic and pharmacokinetic (PK) properties is an essential element of lead optimization and candidate selection [1, 2]. Metabolism studies in the early drug discovery stages often involve evaluating a series of compounds to help identify and select a candidate for further development. The main purposes of these studies are to see if the compound has adequate metabolic stability, has low potential for any drug–drug interactions due to cytochrome P450 (CYP) induction or inhibition, and is not metabolized by polymorphic enzymes (such as CYP2D6) or exclusively by a single enzyme. In addition, the metabolites are characterized to assess if any reactive

metabolites of safety concern are generated. Recent survey suggests that the DMPK role in early optimization has resulted in reduced attrition due to PK-related issues from 40% (1990s) to <10% (2000s) [3]. Once the compound is selected for clinical development, detailed PK/ADME studies are conducted to characterize the bioavailability, metabolic properties, distribution, and excretion and elimination of the drug. These studies provide information to assess safety and provide data for registration. The focus of this chapter is to describe the various DMPK studies performed at various stages of drug development and how they are captured when filing a new drug application (NDA). Figure 1.1 shows the various DMPK studies conducted at different stages of drug development. The schematic is intended as an illustration, but the precise timing of the studies depends not only on the drug and its properties but also on the intended therapeutic benefit and target population. There are a number of regulatory guidance documents issued by regulatory authorities (U.S. Food and Drug Administration [FDA], European Medicines Agency [EMA], etc.), and these guidelines are expected to be adhered to during the conduct of *in vitro* and *in vivo* metabolism studies. In addition, DMPK provides bioanalytical support for safety (toxicology and first-in-human) and efficacy (proof of concept or pivotal clinical) studies. The method development, validation, and sample analysis are expected to be conducted according to the guidelines issued by regulatory

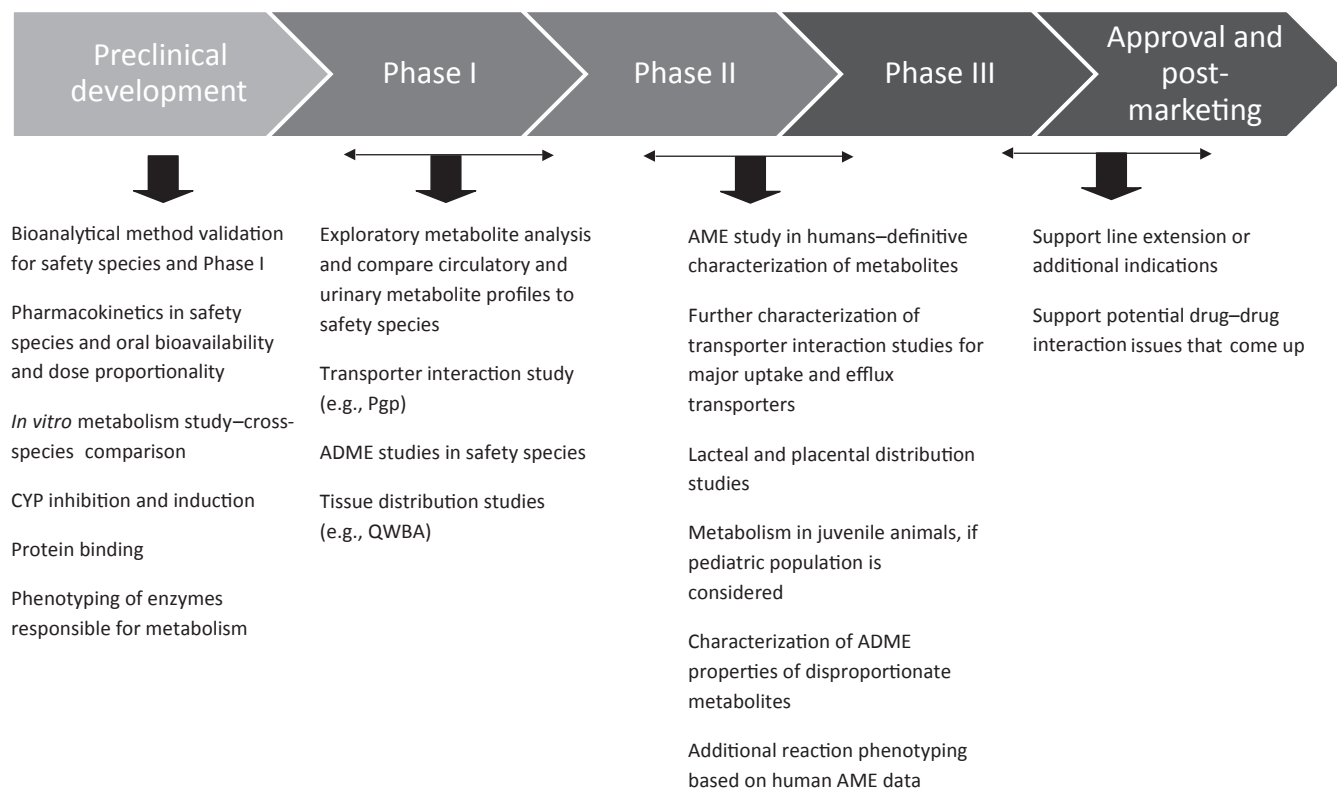


FIGURE 1.1. Various DMPK studies conducted at different stages of drug development.

agencies worldwide, and any sample analysis conducted under good laboratory practice (GLP) guidelines is expected to meet the standards set forth under these guidelines. The metabolism data generated at various stages of development need to be integrated and summarized for regulatory filing and approval. The agreement to assemble all the quality, safety, and efficacy information in a common format (Common Technical Document or CTD) and the technical requirements for the registration of pharmaceuticals has been harmonized by the International Committee on Harmonization (ICH) process. This has revolutionized the regulatory review process and harmonized electronic submissions. Table 1.1 shows the table of contents for the drug metabolism contributions in a CTD for an NDA. The objectives of this chapter are to discuss DMPK studies conducted during development and to reference the regulatory guidance documents that apply to various studies. The purposes are to integrate the information across studies and species and to present information related to safety and efficacy in an unambiguous and transparent manner to help regulators evaluate the content and main features of the drug.

TABLE 1.1. DMPK Summaries in the Sections of the Common Technical Document (CTD)

2.4 Nonclinical overview
2.6 Nonclinical written/tabulated summaries
2.6.4 Pharmacokinetic written summary
• Brief summary
• Methods of analysis
• Absorption
• Distribution
• Metabolism (including interspecies comparison)
• Excretion
• Pharmacokinetic drug interactions
• Other pharmacokinetic studies
2.6.5 Pharmacokinetic tabulated summary
Module 4 (Reports of studies summarized in Sections 2.6.4 and 2.6.5)
4.2.2.1 Analytical methods and validation reports
4.2.2.2 Absorption
4.2.2.3 Distribution
4.2.2.4 Metabolism
4.2.2.5 Excretion
4.2.2.6 Pharmacokinetic drug interactions
4.2.2.7 Other pharmacokinetic studies
4.3 Literature references (single PDF/reference)

1.2 NONCLINICAL OVERVIEW

Nonclinical overview presents the summary of information related to pharmacology, PK/ADME, and toxicology. Nonclinical PK information in this section is not intended to contain a summary of each study conducted. The purposes are to integrate drug metabolism information generated across studies and to provide a summary of findings that may have safety and efficacy implications. Taking the pharmacology, PK, and toxicology results into account, the implications of the nonclinical findings for safe human use of the pharmaceutical should be discussed. It should briefly describe the PK/toxicokinetic (TK) data in safety species, metabolism characteristics *in vitro* and *in vivo* and any interspecies differences, tissue distribution properties, and relevance to safety or efficacy. In addition, any inconsistencies and limitations of the data should be discussed.

1.3 PK

Section 2.2 of Module 2 describes the nonclinical PK information. This section gives a brief list of all ADME studies and methods of analysis. It also gives a brief summary of principal findings. The information is summarized in approximately three to four pages.

The “Methods of Analysis” section contains a brief summary of analytical methods employed for analysis of biological samples. Bioanalytical laboratories support a range of regulatory studies ranging from early toxicology studies through clinical studies (Phases I, II, and III) to definitive bioequivalence and bioavailability studies in support of the marketed product. PK/TK data aid the interpretation of efficacy and safety information, and therefore the quality of PK/TK data is directly related to the quality of bioanalytical data and the methods employed to generate such data. Therefore, the method validation and sample analysis should be carried out as per GLP regulatory guidelines [4, 5]. Although bioanalytical methods are validated to ensure that they function and perform as intended, the actual study or “incurred” samples from animals and human subjects may differ in composition and could potentially differ in their behavior compared with standard or quality control samples. This has resulted in a recommendation to analyze a fraction of study samples for reproducibility. The current regulatory expectations is such that many companies routinely conduct incurred sample reanalysis (ISR) to ensure the reproducibility and quality of study sample data. The detailed discussion of regulatory requirements is not within the scope of this chapter but the reader is referred to recent reviews in this area [6–8]. The bioanalytical methods used in toxicology studies should describe the species, detection and quantitation limits, and validation and stability of data.

1.4 ABSORPTION

Early in drug development, PK studies are conducted in rodent (rats and mice) and nonrodent (dogs or monkeys) species to assess absorption (extent and rate of absorption), bioavailability (%*F*), dose proportionality, and kinetic parameters (C_{\max} , AUC, and $t_{1/2}$). The choice of species is dependent on the metabolic similarities between animal species and humans based on *in vitro* and *in vivo* metabolism studies. It is important to ensure that metabolites generated in humans are covered at least in one of the species used in toxicology testing. In addition, it is important to ensure that adequate exposures are obtained in the dose range tested for safety assessment. Any nonlinearity in PK should be assessed and should aid in dose selection for safety studies. Typically, absorption and exposure assessment is done in male and female animals to evaluate any gender differences. This information should be taken into consideration when selecting doses for toxicology studies.

The route of administration should be based on the intended route of delivery for humans. Although many small-molecule drugs are orally delivered, intravenous PK are also determined to characterize the absolute bioavailability, clearance, and volume of distribution of molecule for better understanding of oral kinetics. It is important to carry out PK characterization with adequately characterized compound for stability and purity. The salt and crystalline form of the compound should be the same as the one that is used for safety studies. The dose selection should cover the dose range where the compound was found effective in pharmacology models to 30- to 50-fold of the efficacious dose for safety studies. The selected doses would generate exposure data across the dose range for dose proportionality assessment and help to assess any metabolism issues at higher doses. It is important that PK studies employ dose volumes and acceptable formulations used in toxicology studies so the data obtained in PK studies can be useful for carrying out safety studies. Formulation strategies and approaches used in preclinical setting are discussed in detail in Chapter 31. Although nonclinical PK studies are not required to be conducted as per GLP, they are often carried out under the spirit of GLP guidelines. The bioanalysis of study samples employs robust and reproducible methods. The PK analysis typically employs noncompartmental methods and describes PK information such as C_{\max} , T_{\max} , AUC, CL, V_{ss} , $t_{1/2}$, and %*F*. It is important to determine half-life accurately so

that any potential accumulation could be understood before repeat dose studies are conducted.

The absorption data are summarized in Section 2.6.4. The written summaries describe the test system (species, gender, number of replicated animals), the route of administration, formulations used, and analytical and data analysis methodologies used. The summary of findings should describe the absorption (rate and extent of absorption), percent oral availability, linearity of kinetics, and gender differences, if any.

1.5 DISTRIBUTION

Distribution is a process by which drug and its metabolites partition in and out of various cells and body tissues. Distribution of a compound into tissues requires the compound to permeate cell membranes, and this primarily occurs via passive diffusion. However, there are specialized barriers such as the blood–brain barrier (BBB) that express transporter proteins (e.g., P-glycoprotein [Pgp]) to prevent or minimize access to tissue. Drug distribution into tissues depends on the physicochemical properties of the compound (log P, pK_a , molecular weight, etc.), protein binding, permeability, and transporter activity [9, 10].

1.5.1 Plasma Protein Binding

Plasma protein binding dictates the rate and extent of distribution of drugs in the body. It is generally thought that the free drug is considered pharmacologically and toxicologically relevant [11, 12]. It is the free fraction and not the bound fraction that is available for distribution into tissues. Drugs bind to plasma protein albumin, alpha-acid glycoprotein (AGP), and lipoproteins. Binding and transport of exogenous and endogenous are important functions of plasma proteins. Drugs that have high lipophilicity tend to bind to a greater extent to plasma proteins. A more in-depth discussion of techniques and issues related to plasma protein binding are presented in Chapters 9 and 12.

As outlined above, the plasma protein binding is an important determinant for the pharmacodynamics (PD)/toxicodynamics of drugs, and the free fraction dictates the drug distribution and also influences clearance and half-life. Therefore, understanding the extent of protein binding across species is an important consideration for interpretation of efficacy and safety. It is important to take into consideration any difference in protein binding across species in explaining the effects observed in pharmacology or toxicology studies. In addition, range of concentrations that are observed in efficacy and safety studies across various species need

to be evaluated to determine if the protein binding is linear or saturable. This may have an important role in explaining the effects of drugs at higher concentrations if the binding is saturable. Furthermore, it is known that disease conditions, age, and pregnancy alter the levels of plasma proteins, thereby influencing the free drug concentrations, clearance, and distribution [13]. For example, AGP levels are known to increase during pregnancy and this needs to be taken into consideration for drugs that are highly bound to this plasma protein [13]. Although protein binding is determined *ex vivo* using plasma from healthy animals and humans, sometimes the plasma protein binding is also determined in disease populations to ensure that protein binding is similar.

In addition to plasma protein binding, blood-to-plasma ratio is also determined for drugs. This is particularly important for drugs that are distributed into blood cells and bind to cellular components [14]. In such cases where blood-to-plasma partitioning is much greater than one, the plasma PK profile alone will not adequately capture the PK of the drugs. Therefore, it is important to understand the blood partitioning of the compound early on so that PK will be assessed in relevant matrix.

Plasma protein and blood-to-plasma partitioning is captured under “Distribution” in Section 2.6.4 of the written summaries. The species, matrix, concentration range, and techniques used are described. The results should describe the extent of binding across species and highlight any species differences. In addition, it will discuss the observation of any saturable binding and the relevance of this to the interpretation of PK/TK. There are no specific guidances that address plasma protein binding alone, although it is referred in some guidances.

1.5.2 Tissue Distribution

Tissue distribution studies are essential in understanding the distribution of a compound and its metabolites into tissues and any potential for accumulation. Typically, tissue distribution studies are conducted as single-dose studies with radiolabeled compounds (e.g., ^{14}C) via the intended route of administration [15]. Upon administration of the compound, the tissues of interest are sampled over a time course (up to 1–2 weeks) depending on PK properties. The samples are analyzed by a combination of techniques using tissue homogenization, combustion of tissue homogenate, and determination of radioactivity using liquid scintillation counting. This traditional method is labor intensive and permits limited understanding of distribution as not all tissues are sampled for practical consideration. Increasingly, the quantitative whole-body autoradiography (QWBA) technique is

used for determination of tissue distribution for its ease and comprehensive nature [16]. Once the radiolabeled drug is administered to an animal, the animal is sacrificed at specified time points, frozen and embedded in a matrix, and the carcass sliced into thin sections for determination of radioactivity by an imaging technique. This allows to capture concentrations virtually in all tissues and the sections are available for any further investigations if questions arise later during the drug development. A limitation of QWBA studies is that only small animals can be accommodated and any studies with large animals may have to use a more traditional method described above. Another limitation is that the concentration data obtained from these studies are based on total radioactivity and not on the parent compound. Therefore, care must be exercised when interpreting concentration data and should be combined with other PK information before making any inferences.

The purposes of conducting tissue distribution are to understand the extent of distribution of drug-related material (parent and metabolites), to determine the potential for accumulation in any tissues, to understand distribution into tissues with special barriers (e.g., CNS) and binding to melanin, and to provide dosimetry analysis and guidance for conducting radiolabeled human absorption, metabolism, and excretion (AME) studies.

1.5.3 Lacteal and Placental Distribution Studies

In addition to the tissue distribution studies, drug distribution into milk and placenta is assessed during development to understand the potential exposure and risk for breastfeeding infant and fetus, respectively. These studies are carried out much later in the drug development as a part of the registration package and typically conducted in species used for reproductive and developmental toxicology studies. There is some guidance regarding placental and lacteal transfer studies. Both FDA and EMEA guidances on reproductive and developmental toxicity make statements that imply that the distribution of drug and/or its metabolites into milk and placenta (fetus) be evaluated [17–19]. For example, the EMEA draft guidance (“Guideline on Risk Assessment of Medicinal Products on Human Reproduction and Lactation,” 2006) states that “information about the excretion into milk of the active substance and/or metabolites should be available.” In addition, the guidance also states that “the exposure in pregnant animals measured by plasma concentrations of the compound and/or metabolites should be assessed.” Similarly, ICH guidance (ICH S3A) refers to the need for assessment of exposure in newborns, dams, or fetuses and states that “secretion in milk may be assessed to define its role in the exposure to newborns” [20]. In addition to the

studies stated above, specialized distribution studies such as distribution into semen may need to be considered. For some drugs, there is a possibility of drug eliciting potential effects in the female subjects when the drug is administered to male due to excretion into semen and subsequent exposure to females. This is not routinely assessed for many drugs and there is no regulatory guidance specifically requiring a study. However, many drugs have been shown to be excreted into semen to some extent and if there is concern for reproductive developmental toxicity, then it may be prudent to evaluate this proactively. The compound properties, techniques used for evaluation, and *in vitro* and *in vivo* models used for determination of lacteal and placental transfer are discussed in great detail in Chapter 17.

The milk and placental information is summarized under “Distribution” in Section 2.6.4 and the summary data is captured in the tabulated summaries under Section 2.6.5. The summary details the species, strain, the analytical techniques and methods, exposure or concentration data, and milk-to-plasma or placenta- or fetus-to-plasma concentration ratio. This data is useful for interpretation and risk assessment for infants or fetuses.

1.6 METABOLISM

Majority of the drugs are biotransformed to metabolites before being excreted in urine and feces. Early on in drug discovery, compounds are characterized and optimized for their metabolic properties leading to a compound with desired PK properties. When elimination of a drug occurs via metabolism, the rate and routes of metabolism can significantly affect a drug’s safety and efficacy. Therefore, a number of *in vitro* and *in vivo* metabolism studies are conducted to fully understand the metabolic profile of a compound during drug development. A thorough understanding is required to address potential metabolism-based issues such as reactive metabolites of safety concern, potential drug–drug interactions due to CYP induction or inhibition, PK variability due to polymorphism, and potential active metabolites influencing PK-PD understanding and intellectual property rights. The information generated from these *in vitro* and *in vivo* metabolism studies constitutes an integral part of an NDA/marketing authorization application (MAA).

1.6.1 *In vitro* Metabolism Studies

In vitro drug metabolism studies are conducted to identify the metabolic rates and routes and to help understand the potential safety and efficacy issues related to drug or metabolites. The purposes of these studies are

as follows: (1) to identify and characterize metabolites in safety species and humans in order to identify and select relevant species for safety assessment; (2) to identify any potential for drug–drug interactions; (3) to identify the enzymes responsible and to determine any genetic polymorphisms that may influence the PK, PD, and safety of the drug; and (4) to identify any potential reactive or genotoxic structural alerts that require further assessment in safety studies. Liver subcellular fractions such as microsomes or S9 and hepatocytes are predominantly used for *in vitro* metabolism studies. For most drugs that are metabolized by the CYP system, the liver microsomes are the test system of choice. However, the hepatocyte system provides a full complementary of metabolic enzymes. Regardless of the system used, it is important to use appropriate controls to ensure the viability of the system; employing known marker activity controls and sensitive analytical systems to detect and profile metabolites. The choice of metabolic system and the advantages and disadvantages of each system are discussed in greater detail in Chapter 9. Chapters 19 and 20 describe various mass spectrometry methods and strategies employed in drug metabolism studies.

Regulatory agencies have issued guidance documents regarding the conduct of *in vitro* metabolism and drug–drug interaction studies during development [21–24]. Both FDA and Health Canada documents outline the various model systems, probes, and considerations for experiments (choice of concentration and time course). It is apparent from these guidelines that the test system needs to be shown as viable and is well characterized to investigate new chemical entities or drug products. The choice of test system should be justified based on the metabolic pathways of the drug product from preliminary experimentation. Recombinant test systems may be used where applicable to further characterize if polymorphic enzymes are involved in the metabolism. The guidance document states that *in vitro* studies should be conducted at concentrations similar to those seen *in vivo*. This is particularly important if a metabolic pathway is saturable. Depending on the concentration used, the metabolic rate and relative abundance of metabolites may be different. These guidances emphasize that the *in vitro* studies should be confirmed with *in vivo* studies and cannot replace *in vivo* studies. They should be used as guidance and be confirmed with *in vivo* data.

The description of metabolite profiling, identification, and cross-species comparison, and major metabolic pathways should be captured in Module 2.6.4 under the “Metabolism” section. The data should be tabulated under the “Metabolism” section in Section 2.6.5. The summaries should describe the experimental conditions used (test system, concentration, time of incubation, detection system, etc.), relative rates of metabolism,

major metabolic pathways and identification, and cross-species comparison. Summaries should highlight if there is any unique metabolism in a particular species. The study reports pertaining to metabolism studies are submitted in Module 4, Section 4.2 under “Metabolism.”

1.6.2 Drug–Drug Interaction Studies

1.6.2.1 CYP Inhibition Metabolism is the major route of clearance for most drugs, and CYP plays a major role in metabolism with approximately 75% of the drugs metabolized by this family of enzymes [25]. The major CYP enzymes involved in drug metabolism are CYP3A4, CYP2D6, CYP2C9, CYP2C19, CYP1A2, CYP2E1, CYP2B6, and CYP2A6. Inhibition of these enzymes can result in altered clearance and PK of drugs leading to adverse effects. This is particularly important considering that the aging population is often on multiple drugs (polypharmacy) for various ailments. Therefore, evaluation of drug interactions is part of drug effectiveness and safety. A detailed discussion of methods, conditions for incubation, probe substrates, and liquid chromatography-mass spectrometry (LC-MS) technologies are presented in Chapters 9 and 14.

1.6.2.2 CYP Induction Induction of drug metabolism enzymes refers to a process where the activity of enzymes increase upon administration of a compound through increased expression and synthesis or by stabilization of enzyme. Most oxidative and conjugative enzymes and drug transporters are inducible to a varying degree. However, the induction of CYP enzymes is of most concern during drug development due to their prominent role in the metabolism of drugs. There are three principle nuclear receptors, namely, the pregnane X receptor (PXR), the constitutive androstane receptor (CAR), and the aryl hydrocarbon receptor (AhR), that regulate the induction of CYP enzymes [26]. Drug binding to receptors leads to a series of molecular events resulting in increased mRNA expression and synthesis of enzyme. Increased levels of enzyme increases clearance and reduces exposure of drugs that are metabolized by the induced enzyme(s). Enzyme induction leads to reduced PD activity due to lowered exposures, although there is potential for manifestation of toxicity due to increased levels of metabolite(s) that are of safety concern.

The detailed discussion of the mechanisms of induction is not within the scope of this chapter. However, Chapter 15 provides a detailed account of *in vitro* and *in vivo* techniques and their merit, strategies, modeling and simulation, and risk assessment.

Regulatory agencies have issued guidance for the assessment of induction potential for drugs during drug development, and it is expected that this information is available at the time of NDA filing. Many companies

routinely conduct this evaluation prior to going into Phase III due to a large number of patients involved in a clinical trial and potential for drug–drug interactions due to induction. The FDA guidance recommends using a positive control inducer in the experiments to account for variability between individual hepatocyte preparations. The positive control inducers should produce at least >2-fold induction at the recommended concentrations. The guidance provides recommended substrates for conducting induction studies. The guidance recommends using three concentrations spanning maximal concentration observed at therapeutic dose and an order of magnitude higher than average plasma concentrations. The hepatocyte preparations should be treated for at least 2–3 days for induction to occur. Following treatment, enzymatic activity of CYP3A, CYP2B6, and CYP1A2 should be evaluated using recommended probe substrates. Although enzymatic activity determination is most reliable, other means of induction evaluation such as immunoquantitation of enzymes, mRNA determination, and reporter gene assays are also acceptable. However, recent survey by Pharmaceutical Research and Manufacturers of America (PhRMA) recommends enzymatic as well as mRNA determination as the most reliable [27]. If a drug produces a change in catalytic activity or mRNA that is >40% of the positive control, then it is considered as an inducer and further evaluation in clinic is warranted.

1.6.2.3 Transporter Interaction Studies Transporters can be major determinants for absorption, distribution, and disposition of drugs. Transporters are expressed at key physiological barriers to limit the distribution of drugs or facilitate excretion. Recent advances in biochemical and molecular biological techniques have led not only to identify many transporters but also to clone and characterize function and distribution in tissues. Transporters are broadly classified into solute carriers (SLC) or ATP binding cassette (ABC) transporters. Chapter 3 describes in detail the transporter family, their role in drug transport and disposition, and techniques used to characterize and understand the role of transporters in drug absorption and disposition. Modulation of the function of these transporters can result in drug–drug interactions and it is well documented in the literature (e.g., statins, digoxin, and cephalosporin antibiotics) [28, 29].

Regulatory agencies worldwide recognized the issue of transporter-based drug interactions and addressed this in their respective documents. For example, the FDA draft guidance issued in 2006 lists some of the major human transporters and known substrates, inhibitors, and inducers. The guidance states that Pgp is the best studied of all the transporters and it is appropriate to evaluate during drug development. Pgp is expressed at the intestinal barrier limiting the absorption of drugs.

It is also expressed in the kidney and liver playing a critical role in excretion of drugs and their metabolites. Pgp also plays a critical role in limiting distribution of drugs into the CNS and fetus due to its expression in these barriers [29]. Any interference in the function of Pgp can lead to altered levels of drugs in circulation and tissues and compromise the safety and efficacy of drugs. Therefore, studies should be conducted to determine if a drug candidate is a substrate and inhibitor of Pgp. This can be accomplished with Caco-2 cells or other engineered cell lines that overexpress Pgp. Irrespective of test system used, the experimentation should include known substrates and inhibitors to demonstrate the suitability of test systems. Bidirectional transport measurements are preferred and net flux should be calculated for interpretation of results. If the compound has a net flux ratio of >2, then it is considered a substrate and further evaluation should be carried out. If the test compound has an efflux ratio <2, then it is not a Pgp substrate; and follow-up interaction studies are not needed. The range of concentrations (e.g., 1, 10, and 100 μM) should be considered during evaluation and the selection depends on maximal concentrations observed in the plasma and at the intestinal barrier and on the solubility limitation of the drug. A test compound should also be tested for its inhibition, irrespective of whether or not it is a substrate. Again, a wide range of concentrations should be considered to generate inhibitory data (IC_{50} or K_i). The draft guidance provides a list of acceptable probe substrate and positive control inhibitors. If the test compound has an $\text{I}/\text{IC}_{50} > 0.1$, then it is an inhibitor of Pgp and an *in vivo* interaction study should be conducted with digoxin as a probe. If the ratio is <0.1, then the test compound is a weak inhibitor and an *in vivo* interaction study is not needed. The potential for Pgp induction is also discussed in the guidance document and it is recommended that if the drug is shown not to induce CYP3A *in vivo*, then no further test of Pgp induction *in vivo* is necessary. However, if the *in vivo* CYP3A induction test is positive, then an additional study of the investigation drug's effect on a Pgp probe substrate is recommended. Similar guidance is also provided by Japan's Ministry of Health, Labor, and Welfare (MHLW) under the name of "Methods for Drug Interaction Studies." This document discusses in some parts the guidance for assessing transporter-mediated interactions at the intestinal barrier, tissue distribution, and elimination into urine and bile.

1.6.2.4 Identification of Enzymes Responsible for Metabolism Most drugs are metabolized and cleared from the body and it has been reported that 75% of the marketed drugs are primarily metabolized by the CYP system. Although many CYP enzymes exhibit overlapping substrate specificity, in most cases a single

isoform seem to contribute to the majority of the metabolism. Therefore, the goal early on in the development is to identify if the drug is metabolized by a single enzyme or multiple enzymes or different family of enzymes. It is ideal to have equal contribution from multiple enzymes to make less susceptible for drug interactions when coadministered with a potent inhibitor or inducer of that particular enzyme. Another goal of phenotyping is to identify if a polymorphic enzyme is involved in metabolism. Recent FDA guidance addresses the reaction phenotyping in detail and states that if human *in vivo* data indicate that CYP enzymes contribute >25% of a drug's clearance, studies to identify drug metabolizing CYP enzymes *in vitro* should be conducted [22]. This recommendation includes cases in which oxidative metabolism is followed by transferase reactions because a drug–drug interaction that inhibits oxidation of the parent compound can result in elevated levels of the parent compound. The guidance suggests that clinically relevant concentrations of the drug should be considered for *in vitro* experiments. Preliminary experiments should be conducted to assess linearity of metabolite formation and protein concentrations, and time course should be optimized. Reliable and robust analytical methods should be used to monitor the formation of metabolites, and analytical methods should have acceptable sensitivity to measure the percent of inhibition over the range of concentrations tested. The guidance outlines three principal ways, namely, use of CYP isoform chemical inhibitors or selective antibodies, recombinant enzyme systems, and correlation analysis with a bank of microsomes whose activities were characterized with known isoform selective probe substrates. Chapter 13 discusses in detail various aspects of CYP and non-CYP enzymes involved in drug metabolism.

Identification of enzymes responsible for metabolism should be summarized under the “Metabolism” section in Section 2.6.4. The summary should describe the test systems used, conditions of incubations, analytical methods, and the results. In summary, the data should also speak about the consequences of the findings, for example, state that if exclusively metabolized by particular enzymes, potential for interaction exists if coadministered with strong inhibitors of that particular enzymes. It is also important to convey a consistent message across documents such as written summaries, overviews, and investigator brochures.

1.6.3 *In vivo* Metabolism (ADME) Studies

Biodisposition studies or ADME of drugs play a critical role in its initial selection as well as in its subsequent clinical development. *In vivo* ADME studies

provide important information regarding the absorption of the drug, distribution of the drug-related material into the target tissues, important metabolic pathways, and the eventual excretion of the drug-related material from the body [30, 31]. The information generated from these studies is helpful in understanding the outcomes of the safety studies and efficacy of pharmacology studies [32]. Therefore, *in vivo* metabolism studies with radiolabeled tracers have become a crucial component of the drug development package for regulatory submissions.

Typically, *in vivo* drug metabolism and disposition studies are conducted with a radiolabel (^{14}C or ^3H) material to provide quantitative information on the rate and extent of metabolism, routes of excretion for parent compound, and its metabolites and circulating metabolites. The nonclinical ADME studies are conducted in two species, rodents (rats or mice) and nonrodents (dogs or monkeys), and the choice is based on the species used in toxicology studies. The selection of dose, route of administration, and formulations should mimic the safety studies. These studies are typically single-dose studies with sample collection up to a week; however, longer duration of sample collection may be needed if the drug has a long half-life. Plasma, urine, and fecal samples are collected during the duration of the study to analyze for radioactivity and for metabolite profiling. Tissue samples may be collected if there is a particular issue to address safety or efficacy concerns. Metabolic profiles of plasma, urine, and fecal samples are generated by a combination of techniques (e.g., high-performance liquid chromatography [HPLC] radioactivity detection, liquid chromatography [LC] fractionation followed by scintillation counting, and LC-MS coupled to radioactivity detection). The reader is referred to Chapter 22 for a detailed description of the radioactivity profiling techniques and recent advances. Also of interest are Chapter 19 and Chapter 20, which describe mass spectrometry methods for metabolite identification.

Metabolic profiles of urine and fecal samples generated in ADME studies provide information about the extent of metabolism and the routes of excretion for the parent and metabolites. This will enable one to assess the important metabolic pathways, the importance of organs in the elimination, the total amount of drug substance absorbed, and if any metabolites of safety or activity concern are generated. Metabolic profiles of plasma samples collected over the time course enable one to calculate exposure for metabolites, understanding major circulatory metabolites, and the half-life of metabolites that could potentially result in accumulation upon repeat administration. In addition, it provides information about metabolites that would enable comparison between toxicology species and humans.

1.6.3.1 Metabolites in Safety Testing (MIST) One of the most debated and controversial guidances in the area of metabolism was issued by the FDA in 2005 related to MIST [33]. This guidance made recommendations on when to identify and characterize metabolites. This guidance followed an earlier publication by the pharmaceutical industry on the role of metabolites as potential mediators of adverse effects, which itself was discussed extensively in the literature [34]. Following an extensive discussion at scientific meetings, workshops, and in the literature, the FDA has issued a final guidance in 2008 [35]. While there were some differences between the draft and the final guidance, the final guidance stated that the disproportionate metabolites, present at >10% of the parent exposure, need to be considered for safety assessment. The guidance recommends conducting drug metabolite profiling and identification early in development such that any metabolites of safety concern (disproportionate or unique human metabolites) are addressed before exposing a large number of patients in pivotal clinical studies. The guidance states that coverage of disproportionate metabolites observed in humans must be demonstrated in at least one of the species used for toxicology studies. It is noteworthy that the guidance does not state sensitive species but rather one of the species. The guidance had great impact on the generation of quantitative and qualitative profiles early on in development so that comparisons of metabolic profiles can be made in human and safety species. This has led to conducting and completing radiolabeled studies so that all necessary information is available before initiation of Phase III studies. There are a number of publications on the timing and the different approaches for this data early on in development with and without the use of radiolabeled compound. For any disproportionate metabolites observed in humans but not present in any of the safety species, further testing needs to be done with the metabolite. The FDA recommends conducting further studies. These include (1) general toxicology studies with the metabolite at the exposure equivalent to those obtained in humans; (2) *in vitro* genotoxicity testing in an acceptable test; (3) embryo-fetal developmental studies if the drug is intended for women with child-bearing potential; and (4) carcinogenicity studies with the metabolite. The metabolic characterization and issues are complex and the guidance speaks the need to consider issues case by case. The guidance also states that certain conjugative metabolites (O-glucuronides and sulfate conjugates) other than acyl glucuronides are pharmacologically inactive and not of safety concern even when they occur at greater than the 10% cutoff. Although the FDA guidance considers >10% of parent exposure as disproportionate metabolite, the ICH guidance differs by stating that major metabolites are >10%

drug-related exposure [36]. This may have great impact for drugs that extensively metabolized and may reduce the burden to monitor a number of metabolites if based on total drug-related material rather than parent. Precisely for these reasons, the FDA guidance provides examples of case studies and highlights the complexity of issues and the need for considering metabolism in safety testing on a case-by-case basis.

1.7 EXCRETION

As stated in previous sections, ADME studies provide important information regarding metabolism and excretion of compounds into urine and feces. The purpose of these studies is to understand not only the metabolism but also how the parent and metabolites are eliminated. The information generated from these studies is useful in evaluating if the kidney or liver is an important organ of elimination and if there is any safety concern in hepatic or renal-impaired populations. In addition, the excretion data also sheds light on the role of transporters. For example, if the renal excretion of parent or metabolite is greater than the glomerular filtration, then it is likely that an active secretion may be occurring and further studies need to be conducted to evaluate potential issues. In nonclinical studies early in the development, excretion studies are conducted in intact and bile duct-cannulated rodent and nonrodent species to understand the excretion of drug and metabolites. This enables to understand the metabolic pathways and rate of excretion. The reader is referred to Chapter 18 for a detailed discussion of bile collection in animals and humans. Also, Chapter 16 discusses various animal models used to study drug metabolism and transporters.

The information generated regarding excretion should be captured in Module 2.6.4 and tabulated summaries should contain summary data in Module 2.6.5 under the “Excretion” section. The written summary should discuss if the parent is excreted intact or metabolized, the relative contribution of liver and kidney for excretion, and the species comparison of excretory profiles of parent and metabolites.

1.8 IMPACT OF METABOLISM INFORMATION ON LABELING

As described in the previous sections, drug metabolism and disposition data is generated to fully understand the metabolic pathways, clearance, drug–drug interaction potential, and changes in exposure in diseased or special populations. The results from the studies described above should be integrated and included in the NDA. This integration of data not only helps reviewers

evaluate drug for approval but also summarizes the key aspects of drug in the prescription label for prescribers and patients. The FDA also recommended that when the information has important implications for the safe and effective use of the drug and the drug metabolism information results in recommendations for dosage adjustments, contraindications, or warnings, this information should be included in the appropriate sections, such as boxed warning, dosage and administration, contraindications, and drug interactions. The drug metabolism information is captured in the “Clinical Pharmacology” section of the prescription label under the subsections of Pharmacokinetics, Absorption, Distribution, Metabolism, and Excretion. The information is brief and conveys important information to the prescriber to either adjust the dose or avoid prescribing the medicine when the patient is taking concomitant interacting medications.

1.9 CONCLUSIONS

Drug metabolism plays a central role from drug discovery through drug development and approval. A number of studies are conducted early in lead optimization and candidate selection. With advances in analytical technologies and *in vitro* and *in vivo* metabolism tools, rapid screening methods can be employed to speed up discovery. However, once a compound is nominated, detailed metabolism studies need to be conducted in a timely fashion and staged according to the development of a product candidate. Most importantly, drug metabolism scientists should be aware of the regulatory guidances and expectations for the conduct of these studies so that best practices can be followed in generation of data.

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