

Special Section – New Models in Drug Metabolism and Transport—Minireview

P450-Humanized and Human Liver Chimeric Mouse Models for Studying Xenobiotic Metabolism and Toxicity

Karl-Dimiter Bissig, Weiguo Han, Mercedes Barzi, Nataliia Kovalchuk, Liang Ding, Xiaoyu Fan, Francis P. Pankowicz, Qing-Yu Zhang, and Xinxin Ding

Baylor College of Medicine, Houston, Texas (K.-D.B., M.B., F.P.P.); and Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, Arizona (W.H., N.K., L.D., X.F., Q.-Y.Z., X.D.)

Received June 25, 2018; accepted August 3, 2018

ABSTRACT

Preclinical evaluation of drug candidates in experimental animal models is an essential step in drug development. Humanized mouse models have emerged as a promising alternative to traditional animal models. The purpose of this mini-review is to provide a brief survey of currently available mouse models for studying human xenobiotic metabolism. Here, we describe both genetic

humanization and human liver chimeric mouse models, focusing on the advantages and limitations while outlining their key features and applications. Although this field of biomedical science is relatively young, these humanized mouse models have the potential to transform preclinical drug testing and eventually lead to a more cost-effective and rapid development of new therapies.

Introduction

Translating a drug from discovery to human therapy relies heavily on data from preclinical studies in animal models. However, many drug candidates fail during clinical trials because the experimental animal models used in preclinical studies poorly predict human xenobiotic metabolism. Such failures are caused in part by species differences in drug-metabolizing enzymes (DMEs), which have evolved and adapted to the different metabolic conditions of each species. Mice, for instance, have an expanded set of DMEs referred to as members of the cytochrome P450 (P450) family. In mice, these DMEs are encoded by 72 functional genes, whereas humans possess only 27 (Nelson et al., 2004). Thus, drugs are often metabolized differently in mice and in humans. Reactive drug metabolites drive toxicity, and their presence or absence is determined by the species-specific set of DMEs. Therefore, preclinical testing using a “humanized” system would be desirable before starting a clinical trial with a new drug.

There is growing interest and practice in utilizing humanized mouse models to overcome species differences in drug metabolism; the list of available humanized mouse models is rapidly expanding. Thus, although

the topic has been frequently reviewed (Strom et al., 2010; Shen et al., 2011; Yoshizato et al., 2012; Kitamura and Sugihara, 2014; Sanoh and Ohta, 2014; Scheer and Wolf, 2014; Stiborová et al., 2014; Gonzalez et al., 2015; Scheer and Wilson, 2016; Yamazaki et al., 2016), it is worthwhile to revisit and update in the context of this special issue on humanized models of xenobiotic metabolism and toxicity.

It is necessary to stress that humanized mouse models are not humans. Though many of the available mouse models are valuable for revealing important aspects of human xenobiotic metabolism and response, none can perfectly replicate the human response. The purpose of this mini-review is to provide a brief survey of currently available mouse models for studying human xenobiotic metabolism by outlining their key features, limitations, and application notes, in hopes of helping the readers to identify the appropriate models for their specific application. Efforts are made to discuss P450-humanized mouse models, where one or more members of the mouse P450 family are replaced by relevant members of the human P450 family, together with human liver chimeric mouse models, where much of the mouse liver is replaced by human hepatocytes, given the current debate on the relative merits of the two distinct types of models.

P450-Humanized Mouse Models

P450 humanization in mice typically involves cross-breeding of a human *CYP*-transgenic mouse model with a relevant mouse-*Cyp* knockout mouse model or knocking in the human genes to replace the mouse genes. The human transgene fragment may include a single *CYP* gene or multiple neighboring *CYP* genes that are in close proximity and may share regulatory

Q.-Y.Z. and X.D. are supported in part by the National Institutes of Health (NIH) [Grants CA092596, ES020867, and GM082978]. K.-D.B. is supported by the NIH [Grants HL134510 and DK115461] and the Texas Hepatocellular Carcinoma Consortium (THCCC) (CPRIT #RP150587). K.-D.B., M.B., F.P.P. have a financial interest in Avachrome Inc. No other potential conflicts of interest relevant to this article are reported.

<https://doi.org/10.1124/dmd.118.083303>

ABBREVIATIONS: BAC, bacterial artificial chromosome; BaP, benzo(a)pyrene; CAR, constitutive androstane receptor; DME, drug-metabolizing enzyme; FAH, fumarylacetoacetate hydrolase; FIAU, fialuridine; HAC, human artificial chromosome; HSVtk, herpes simplex virus type 1 thymidine kinase; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; P450, cytochrome P450; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; PXR, pregnane X receptor; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; uPA, urokinase-type plasminogen activator; WT, wild-type.

elements. The relevant knockout mouse may have one or multiple mouse *Cyp* genes deleted. The tissue specificity and expression levels of the human CYP transgene are not always identical to the situation in humans for a given transgenic mouse model. Thus, an effective application of a P450-humanized mouse model for studying xenobiotic metabolism or toxicity requires detailed knowledge of the characteristics of each transgenic model as well as the mouse-*Cyp* knockout model used. Unfortunately, some of the reported mouse models have not been characterized as thoroughly as others, which limits their usefulness for broad application.

Most human P450 family members that are involved in xenobiotic metabolism, including members of the *CYP1-4* gene families, have been introduced into the mouse genome as a transgene, as shown in Table 1. The reported characteristics of these human *CYP*-transgenic mouse models are summarized here, including the origin of the human transgene used for model generation, the nature of the promoter and regulatory components that control the expression of the transgene, and the main tissue sites of reported transgene expression. Additional information, such as on transgene inducibility, occurrence of sexual dimorphism, and means of humanization, that is relevant to the usefulness or limitation of a given model is further considered. Where available, applications of P450-humanized mouse

models in xenobiotic metabolism or toxicity studies are briefly described. Discussions are organized by *CYP* gene families and subfamilies.

CYP1

CYP1A1 and *CYP1A2* are neighboring genes arranged in a head-to-head orientation, which made it practical and necessary to produce a transgenic mouse that harbors both human genes (Jiang et al., 2005). Given the use of a bacterial artificial chromosome (BAC) clone containing ample regulatory sequences of the human genes, both *CYP1A1* and *CYP1A2* are expressed in the same tissues in mice as in humans and demonstrate the expected inducibility by aryl hydrocarbon receptor ligands (Jiang et al., 2005; Dragin et al., 2007; Shi et al., 2008). Humanization was made possible by crossing the *CYP1A*-transgenic mouse to the *Cyp1a1/1a2*-null mouse. Expression of the transgene supported effective disposition of oral benzo(a)pyrene (BaP) and provided partial rescue of BaP-induced toxicological phenotypes in the null mice (Dragin et al., 2007). This humanized mouse model has also been used to demonstrate the ability of the human *CYP1A* transgenes to mediate PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; a dietary carcinogen)-induced colon carcinogenesis; the

TABLE 1
Human P450-transgenic and P450-humanized mouse models

Model	Human P450 Transgene Structure	Promoter	Mouse Gene Knockout for Humanization	References
CYP1A1/1A2-transgenic	Gene (BAC)	Authentic	None	Jiang et al. (2005)
CYP1A1/1A2 humanized	Gene (BAC)	Authentic	<i>Cyp1a1_1a2</i> -null	Dragin et al. (2007)
CYP1B1-transgenic	cDNA	Tetracycline-regulated promoter	None	Hwang et al. (2001)
CYP1B1 humanized	Gene (BAC)	Authentic	<i>Cyp1b1</i> -null	Li et al. (2014a)
CYP2A6-transgenic	cDNA	Mouse transthyretin promoter and enhancer	None	Zhang et al. (2005)
CYP2A13/2B6/2F1-transgenic	Gene (BAC)	Authentic	None	Wei et al. (2012)
CYP2A13/2B6/2F1 humanized	Gene (BAC)	Authentic	<i>Cyp2a5</i> -null <i>Cyp2f2</i> -null <i>Cyp2abfgs</i> -null <i>Cyp2abfgs</i> -null	Megaraj et al. (2014) Cruzan et al. (2013) Li et al. (2014b) Jia et al. (2014)
CYP2A13-humanized	Gene (modified BAC)	Authentic	<i>Cyp2abfgs</i> -null	Lee et al. (2010)
CYP2C8-transgenic	cDNA	Mouse Tie2 promoter and enhancer	None	Lee et al. (2010)
CYP2C9-humanized	cDNA	Mouse albumin promoter	<i>Cyp2c</i> -null	Scheer et al. (2012a)
CYP2C18/2C19-transgenic	Gene (BAC)	Authentic	None	Löfgren et al. (2008)
CYP2D6-transgenic	Gene (PAC)	Authentic	None	Corchero et al. (2001)
CYP2D6-transgenic	Gene (PAC)	Authentic	None	Cheng et al. (2013)
CYP2D6.N-humanized	Gene (BAC)	Authentic	<i>Cyp2d</i> -null	Scheer et al. (2012b)
CYP2D6.1-humanized	Gene (modified BAC)	Authentic	<i>Cyp2d</i> -null	Scheer et al. (2012b)
CYP2D6.2-humanized	Gene (modified BAC)	Authentic	<i>Cyp2d</i> -null	Scheer et al. (2012b)
CYP2E1-transgenic	cDNA	Mouse albumin promoter/enhancer	None	Morgan et al. (2002)
CYP2E1-humanized	Gene (BAC)	Authentic	<i>Cyp2e1</i> -null	Cheung et al. (2005)
CYP2J2-transgenic	cDNA	Mouse Tie2 promoter and enhancer	None	Lee et al. (2010)
CYP3A4-transgenic	Gene (BAC)	Authentic	None	Granvil et al. (2003)
CYP3A4-transgenic	cDNA	Human ApoE promoter	None	van Herwaarden et al. (2005)
CYP3A4-humanized	cDNA	Human ApoE promoter or mouse villin promoter	<i>Cyp3a</i> -null	van Herwaarden et al. (2007)
CYP3A7-transgenic	cDNA	Mouse MT-1 promoter	None	Li et al. (1996)
CYP3A4/3A7-transgenic	Gene (BAC)	Authentic	None	Cheung et al. (2006)
CYP3A4/3A7-transgenic	Gene (BAC)	Authentic (PXR humanized)	None	Ma et al. (2008)
CYP3A4/3A7-humanized	Gene (modified BAC)	Authentic (PXR and CAR humanized)	<i>Cyp3a</i> -null (except for <i>Cyp3a13</i>)	Hasegawa et al. (2011a)
CYP3A4/3A5*3/3A7/3A43-humanized	Gene (HAC)	Authentic (CYP3A5 not expressed)	<i>Cyp3a</i> -null	Kazuki et al. (2013)
CYP3A4/3A5*1/3A7/3A43-humanized	Gene (modified HAC)	Authentic (CYP3A5 expressed)	<i>Cyp3a</i> -null	Abe et al. (2017)
CYP4A11-transgenic	Gene (BAC)	Authentic	None	Savas et al. (2009)
CYP4B1-transgenic	cDNA	Human ApoE promoter	None	Imaoka et al. (2001)
CYP4F2-transgenic	cDNA (expressing His-tag)	Mouse KAP promoter	None	Liu et al. (2009)

Models that are derived via cross-breeding between two different human *CYP*-transgenic models are not included. Authentic, original human *CYP* promoter was contained in the transgene; KAP, kidney androgen-regulated protein; PAC, P1 phage artificial chromosome.

humanized model showed colon tumorigenesis under conditions that did not yield detectable tumors in wild-type (WT) mice (Cheung et al., 2011). More recently, the same model was used to show the role of human CYP1As in PhIP-induced prostate carcinogenesis (Li et al., 2012) and the inhibitory effects of different forms of tocopherol on prostate cancer development (Chen et al., 2016).

CYP1A1/CYP1A2-humanized mice have also been applied to studying drug metabolism. In one study utilizing the human-CYP1A1/1A2-transgenic/*Cyp1a2*-null mouse (Derkenne et al., 2005), the role of the human P450 family members in theophylline disposition was demonstrated. Although the necessity to preinduce transgene expression with a CYP1A inducer [2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)] makes the study complicated to conduct and interpret, the fact that human CYP1A-specific metabolites are formed in vivo illustrated the potential utility of the model for in vivo drug metabolism studies. Two recent articles (Lin et al., 2017; MacLeod et al., 2018) also described the utility of a newly generated CYP1A1/CYP1A2-humanized model in studying the disposition of anticancer drugs; however, details regarding the mouse model were not provided.

Several issues may complicate the data interpretation or limit the application of the current CYP1A1/CYP1A2-humanized mouse model. Because of technical difficulties in separating human CYP1A1 and CYP1A2 proteins on immunoblots, the absolute levels of these proteins in various tissues of the transgenic or humanized mice have not been determined. The *Cyp1a1*-null mice were reportedly hyper-responsive to BaP-induced expression of *Cyp1b1*, which was not overcome by the transgenic expression of the human CYP1As (Dragin et al., 2007); it is unclear whether this property extends to the induction of *Cyp1b1* by other inducers. Given the overlapping substrate specificity between CYP1A1 and CYP1A2 and their known common responses to inducers, it would also be advantageous to have mouse models that express only one human CYP1A.

Although a human *CYP1B1* transgenic mouse with an inducible tetracycline promoter was reported many years ago (Hwang et al., 2001), the model was not used for studies on CYP1B1 function. A CYP1B1-humanized mouse model was recently generated by cross-breeding between a human *CYP1B1*-BAC transgenic mouse and a *Cyp1b1*-null mouse (Li et al., 2014a). Although this model contained the endogenous human promoter, constitutive expression of human CYP1B1 mRNA in the mouse liver and extrahepatic tissues was much lower than that of mouse CYP1B1; but human CYP1B1 protein was detectable in the liver after induction with TCDD (Maden et al., 2017). The transgenic expression of human CYP1B1 was insufficient to drive dibenzo[def,p]chrysene-induced transplacental carcinogenesis according to comparisons of lung tumor data from WT, *Cyp1b1*-null, and CYP1B1-humanized mice (Maden et al., 2017). Further characterization of the constitutive and inducible expression of the human *CYP1B1* transgene, particularly in extrahepatic target tissues, would facilitate application of the model in xenobiotic metabolism and toxicity studies.

CYP2

A *CYP2A6*-transgenic mouse model was developed using the *CYP2A6* cDNA driven by a liver-specific murine transthyretin promoter/enhancer (Zhang et al., 2005). The *CYP2A6* protein level in the transgenic mouse liver was within the range observed in human liver microsomes; however, the use of a surrogate promoter prohibits the utility of the mouse model to study the transcriptional regulation of the *CYP2A6* gene. The transgenic mouse liver displayed significantly higher coumarin 7-hydroxylation activities than WT mice, both in vitro and in vivo. Efforts are underway in the X. Ding laboratory to humanize *CYP2A6*, by intercrossing the *CYP2A6*-transgenic mouse with the *Cyp2abfgs*-null mouse, which has all *Cyp* genes in the mouse *Cyp2a*,

Cyp2b, *Cyp2f*, *Cyp2g*, and *Cyp2s* subfamilies deleted (Li et al., 2014b). The resultant *CYP2A6*-humanized mouse should be valuable for studying the function of hepatic *CYP2A6* in drug metabolism and chemical toxicity. Nonetheless, a *CYP2A6*-BAC transgenic mouse that demonstrates human-like *CYP2A6* expression in hepatocytes as well as extrahepatic tissues such as the lung is still needed.

A *CYP2A13/CYP2B6/CYP2F1*-transgenic mouse model was developed using a BAC clone containing the three closely situated genes *CYP2A13*, *CYP2B6*, and *CYP2F1* (Wei et al., 2012). The tissue distribution of transgene expression closely resembled the known profile in humans, with a respiratory tract-selective expression of *CYP2A13* and *CYP2F1* and an hepatic expression of *CYP2B6*. Three humanized versions of this mouse model have been described. The initial study of a humanized model on a *Cyp2a5*-null background revealed the remarkable ability of the human transgenes to mediate lung tumorigenesis induced by the carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (Megaraj et al., 2014). The same model was used to demonstrate the ability of lung inflammation to suppress *CYP2A13* expression in vivo (Wu et al., 2013; Liu et al., 2015). In contrast, studies on styrene lung toxicity, utilizing a *CYP2A13/CYP2B6/CYP2F1*-humanized model on the *Cyp2f2*-null background, suggested that the human transgenes did not play a significant role (Cruzan et al., 2013).

A newer version of the humanized mice was prepared on *Cyp2abfgs*-null background; this null mouse was resistant to NNK-induced lung tumorigenesis (Li et al., 2014b). Studies using the *CYP2A13/CYP2B6/CYP2F1*-humanized model on the *Cyp2abfgs*-null background confirmed that human *CYP2F1* is functional in the bioactivation of naphthalene, an acute lung toxicant and lung and nasal carcinogen, and that *CYP2A13* and *CYP2F1* are capable of mediating naphthalene toxicity in the lung and nasal mucosa in vivo (Li et al., 2017).

The polygenic nature of the *CYP2A13/CYP2B6/CYP2F1*-humanized model may limit the ability to identify specific roles of each of the *CYP* transgenes in the metabolism or toxicity of a given compound. This was overcome in part by the generation of a *CYP2A13* transgenic mouse model, in which the neighboring *CYP2F1* and *CYP2B6* genes were inactivated through targeted mutations in the BAC genomic clone, while preserving the regulatory sequences (Jia et al., 2014). A comparison of the *CYP2A13*-humanized model and *CYP2A13/CYP2B6/CYP2F1*-humanized model provided definitive data to support the role of *CYP2A13* in NNK bioactivation in the lung (Jia et al., 2014) and the respective activities of *CYP2A13* and *CYP2F1* toward naphthalene (Li et al., 2017).

The hepatic expression, induction, and activity of *CYP2B6* have also been characterized in the *CYP2A13/CYP2B6/CYP2F1*-humanized mouse models (Liu et al., 2015). The transgenic *CYP2B6* was highly induced by phenobarbital and dexamethasone, which are known inducers of *CYP2B6* in human liver, although a sexual dimorphism in the induction was observed, with males being more responsive to the inducers. The transgenic *CYP2B6* was active in the metabolism of bupropion, a known *CYP2B6* substrate drug; it also contributed to systemic clearance of nicotine. Notably, constitutive expression of *CYP2B6* in the liver is low in this model (Li et al., 2018); studies on the in vivo roles of hepatic *CYP2B6* in xenobiotic metabolism and toxicity should examine both naive and inducer-treated mice.

Three different human *CYP2C*-transgenic mouse models have been reported. One was a *CYP2C8*-transgenic mouse, which, together with a human *CYP2J2*-transgenic mouse, was developed using human cDNA driven by the murine endothelium-specific *Tie2* promoter (Lee et al., 2010). These mouse models, which were generated to study the role of renal P450 epoxygenase function in the regulation of blood pressure, have not been humanized (i.e., the corresponding mouse P450 family members have not been removed). A *CYP2C9*-humanized mouse model

was generated on a *Cyp2c*-null background; the *CYP2C9* transgene was driven by the liver-specific albumin promoter, whereas 14 of the 15 functional mouse *Cyp2c* genes (except *Cyp2c44*) were deleted (Scheer et al., 2012a). The transgenic *CYP2C9* was active toward the known *CYP2C9* substrates tolbutamide and diclofenac (Scheer et al., 2012a). A *CYP2C18/CYP2C19*-transgenic mouse was generated using a BAC genomic clone containing the complete *CYP2C18/CYP2C19* genes (Löfgren et al., 2008). *CYP2C18* and *CYP2C19* mRNA expression exhibited sexual dimorphism in the transgenic mouse, in an apparent tissue-specific manner (Löfgren et al., 2008), and both genes were subject to regulation by androgen and growth hormones (Löfgren et al., 2009). Functional *CYP2C19* protein was expressed mainly in the male mouse liver, as evidenced by microsomal immunoblot analysis and activity toward two known *CYP2C19* substrates, *R*-omeprazole and *S*-mephenytoin; *CYP2C18* protein was not detected in either liver or kidney. It is anticipated that a humanized version of the *CYP2C18/CYP2C19*-transgenic mouse would be useful for in vivo studies of *CYP2C19*-mediated xenobiotic metabolism or toxicity in male mice.

Several *CYP2D6* transgenic mouse models have been reported. The first *CYP2D6*-transgenic mouse was generated using a phage genomic clone containing the entire *CYP2D6* gene and flanking sequences (Corchero et al., 2001). The model was referred to as a humanized mouse, since the mouse *CYP2D* enzymes have little activity toward some of the human *CYP2D6* substrates (e.g., debrisoquine). Transgenic *CYP2D6* was expressed in multiple mouse tissues, including liver, small intestine, and kidney, as in humans; but, unlike in humans, the transgenic *CYP2D6* was not expressed in the brain (Corchero et al., 2001; Miksys et al., 2005). This mouse model was used extensively to study the in vivo role of *CYP2D6* in the metabolism of drugs, such as debrisoquine, and of various endogenous compounds, particularly the role of *CYP2D6* in the metabolism of amines like serotonin (Yu et al., 2004; Cheung and Gonzalez, 2008; Shen and Yu, 2009; Wu et al., 2009; Winter et al., 2011). It has also been used to study in vivo regulation of *CYP2D6* expression (Koh et al., 2014; Pan and Jeong, 2015; Pan et al., 2015, 2017; Kent and Jeong, 2017). A second *CYP2D6*-transgenic mouse model was generated using a P1 artificial chromosome clone containing the complete human *CYP2D* locus; it was found to have *CYP2D6* expression in the brain as well as in other tissues (Cheng et al., 2013). However, as far as we know, this model has not been used for the study of xenobiotic metabolism. Neither model has been crossed to a *Cyp2d*-null background.

A series of three *CYP2D6*-humanized mice (encoding *CYP2D6.1*, *CYP2D6.2*, and a novel *CYP2D6* variant, *CYP2D6.N*) were produced on a *Cyp2d*-null background, using an expression cassette derived from a *CYP2D6* BAC clone; the cassette consisted of 9 kilobase pairs from promoter region and the 3'-untranslated region, as well as all exons and introns, from a novel *CYP2D6* allele (Scheer et al., 2012b). The *CYP2D6.1* and *CYP2D6.2* expression cassettes were produced via coding region modifications of the *CYP2D6.N* cassette. The *CYP2D6.1* strain was characterized only on a heterozygous *Cyp2d*-null background, but the other two strains, particularly *CYP2D6.2*, were more thoroughly characterized. All three transgenic proteins were expressed in liver and intestine and metabolized known *CYP2D6* substrates bufuralol or debrisoquine. One of these strains, *CYP2D6.2*, was used to study the role of *CYP2D6* in the metabolism of primaquine (Potter et al., 2015) and the interaction between tamoxifen and several antidepressants, such as paroxetine, that are both substrates and inhibitors of *CYP2D6* (MacLeod et al., 2017).

Two different human *CYP2E1*-transgenic mice have been reported. The first model was developed using a *CYP2E1* cDNA driven by the murine albumin enhancer/promoter (Morgan et al., 2002). Given the known regulation of *CYP2E1* expression by post-translational

mechanisms, it was not surprising that the transgenic protein was induced by chronic alcohol treatment. This overexpression model was used for studying the role of *CYP2E1* in alcohol-induced liver injury and other pathologic changes (Butura et al., 2009; Kathirvel et al., 2009), but the model has not been humanized. Another *CYP2E1*-transgenic mouse model was developed using a BAC clone consisting of the complete *CYP2E1* gene; the model was then humanized on the *Cyp2e1*-null background (Cheung et al., 2005). The humanized model was used to demonstrate the ability of human *CYP2E1* in mediating acetaminophen-induced liver toxicity (Cheung et al., 2005) and ethanol-induced liver injury (Cederbaum, 2010; Lu et al., 2010).

CYP3

Several versions of human *CYP3A*-transgenic mice have been reported. A *CYP3A4*-transgenic mouse model was developed using a BAC clone consisting of the complete *CYP3A4* gene (Granvil et al., 2003). *CYP3A4* was expressed mainly in the small intestines of adult male mice in this model, which was used to demonstrate the importance of intestinal *CYP3A4* in the first-pass metabolism of midazolam and the interaction between midazolam and ketoconazole (Granvil et al., 2003). Hepatic *CYP3A4* expression was largely influenced by age and sex in this model (Yu et al., 2005), a phenomenon also observed in a BAC *CYP3A4/CYP3A7*-transgenic mouse (Cheung et al., 2006; Felmler et al., 2008) and an intercrossed *CYP3A4/CYP2D6*-double transgenic mouse (Felmler et al., 2008). The BAC clone that was used for the generation of the above *CYP3A4*-transgenic mice was further used to generate a new mouse model that harbored the *CYP3A4* and *CYP3A7* genes and are humanized for pregnane X receptor (PXR) (Ma et al., 2008). Notably, hepatic *CYP3A4* expression in this mouse model still showed age-dependent suppression in adult males. Nonetheless, the incorporation of PXR humanization added a new dimension for application in drug metabolism research (Cheng et al., 2009, 2011; Holmstock et al., 2013).

None of the above *CYP3A4*-transgenic strains was on a *Cyp3a*-null background, in contrast to another type of model where all or most of the endogenous mouse *Cyp3a* genes have been removed, such as the "multiple humanized" *Cyp3a*-null (except for *Cyp3a13*) mouse model—the *CYP3A4/CYP3A7/PXR/constitutive androstane receptor (CAR)*-humanized mouse (Hasegawa et al., 2011a). In a further improvement, humanized *CYP2C9*, *CYP2D6*, and *CYP3A4/7* models were combined with humanized PXR and CAR through sequential cross-breeding (Scheer et al., 2015). Both models have seen effective application (MacLeod et al., 2015; Chang et al., 2016; Ly et al., 2017; McMillan et al., 2018).

The transgenic expression and function of *CYP3A7*, which is restricted to the embryo in humans, have not been studied as extensively as those of *CYP3A4*. An early model using *CYP3A7* cDNA driven by the mouse metallothionein promoter supported *CYP3A7* expression in adult tissues and demonstrated apparent activity toward aflatoxin B₁ (Li et al., 1996). The transgenic *CYP3A7* from the *CYP3A4/CYP3A7* BAC transgenic mouse was developmentally regulated by glucocorticoids (Pang et al., 2012). However, few studies have examined the role of *CYP3A7* in fetal drug metabolism using these models.

In a different approach than BAC transgenesis, two similar lines of *CYP3A4*-transgenic mice were generated using *CYP3A4* cDNA driven by the human Apolipoprotein E promoter (van Herwaarden et al., 2005). Transgenic *CYP3A4*, which was active toward cyclosporin and midazolam, was selectively expressed in the liver and, at lower levels, the kidney. A complementary model developed using *CYP3A4* cDNA driven by the mouse villin promoter was also generated, which expressed *CYP3A4* selectively in duodenum, jejunum, ileum, and colon (van Herwaarden et al., 2007). Both models were humanized on the *Cyp3a*-null background. The two humanized models are not amenable

for studies of transcriptional regulation of CYP3A4 or drug-drug interactions at the gene regulation step, as the CYP3A4 expression is controlled by heterologous promoters, rather than by the authentic human *CYP3A4* promoter. However, they are valuable for studying the respective contributions of CYP3A4 in the two organs for the disposition of a variety of drugs, as well as the impact of the interactions among the drugs on CYP3A4 enzyme function (van Herwaarden et al., 2007; van Waterschoot et al., 2009, 2010; Mitsui et al., 2014; Choo et al., 2015; Zhang et al., 2016).

A *CYP3A* (*CYP3A4*, *CYP3A5*, *CYP3A7*, *CYP3A43*) [human artificial chromosome (HAC)] transgenic mouse, humanized on a *Cyp3a*-null background, has also been reported; the human transgenes showed human-like tissue specificity and developmental regulation, as well as an ability to metabolize marker substrates for CYP3A4 and CYP3A7 (Kazuki et al., 2013). Notably, although this model does not show the age-dependent hepatic CYP3A4 suppression that was found in male *CYP3A4*-BAC transgenic mice (Yu et al., 2005), the CYP3A4 expression level was significantly lower in males than in females (Kobayashi et al., 2017). The fetal expression of functional CYP3A7 in this mouse model allowed it to be used for demonstrating the ability of the human transgenes to support thalidomide-induced embryonic toxicity (Kazuki et al., 2016). However, the *CYP3A5* transgene was of the nonexpressed *3 allele, which has since been converted using gene-editing technology to the *1 allele, yielding the first *CYP3A*-humanized mouse model with functional CYP3A5 protein in the liver and intestine (Abe et al., 2017).

CYP4

Few models of human *CYP4* transgenic mice have been reported. Two lines of *CYP4A11* transgenic mouse were generated using a BAC clone (Savas et al., 2009); the model has been used to study CYP4A11 regulation and protein modification, as well as the role of CYP4A11 in 20-hydroxyecosatetraenoic acid-dependent hypertension (Savas et al., 2016; Albertolle et al., 2017). A *CYP4B1* transgenic mouse was prepared using CYP4B1 cDNA driven by the human apolipoprotein E gene promoter for hepatic expression; the transgenic *CYP4B1* was active toward lauric acid and 2-aminofluorene (Imaoka et al., 2001). A *CYP4F2*-transgenic mouse was prepared using CYP4F2 cDNA driven by a kidney androgen-regulated protein promoter for the production of an His-tagged CYP4F2 protein in the renal proximal tubule epithelial cells; the protein was active in production of 20-hydroxyecosatetraenoic acid (Liu et al., 2009). None of these have been humanized or used for studies of drug metabolism.

Challenges of Genetic Humanization

Besides the many obvious advantages and successful applications, as described above, humanization by exchanging murine genes with their human orthologs (genetic humanization) also has some potential limitations.

Species Difference in Regulation of the Human P450 Transgene. Despite the efforts to humanize some relevant nuclear factors, such as PXR and CAR, the human *P450* transgenes may be regulated by other factors, some of which we may not know or may not be able to humanize. Such differences in regulation may lead to species-specific drug-drug interactions that result from drug-induced changes in P450 expression in humanized mice, but not in humans, or vice versa.

Incomplete Humanization of All Pathways and Events Related to Drug Absorption, Distribution, Metabolism, and Disposition. Species differences in competing phase I metabolism pathways, phase II metabolism, or transporters can affect in vivo pharmacokinetic outcome.

Challenges in Studying Genetic Variants. Genetic polymorphisms of human *P450* genes can be studied in the mouse models, but it may not

be cost effective. With the rapid advancement of the gene-editing technology, it is easier than ever to generate mice that produce variant P450 proteins for functional analysis. However, the large number of these variants, the lack of complete understanding of the role of noncoding sequences in transgene expression, and the need to characterize each model once generated, can be cost and time intensive.

Lack of Complete Characterization. Most currently available models are incompletely characterized or documented. Some of these models were produced for a purpose other than xenobiotic metabolism, which impacted the extent to which the model was analyzed. Critical information regarding potential compensatory changes in the mouse DME genes is incompletely analyzed at best. At a minimum, the tissue distribution, levels of transgene protein expression, and inducibility at major sites of transgene expression should be determined and compared with those of humans. Information on developmental expression and sex differences in transgene expression, as well as activity of the transgenic human P450 toward known substrates, are also important to document.

Understanding the properties of a given model under study, including its limitations, will ensure proper experimental design. The factors to consider may include the selection of control mouse strains, the selection of proper genetic background, the inclusion of necessary in vitro studies and in vivo pharmacokinetic analysis of test compounds for efficacy studies, and the inducibility of the transgene by test compounds. Furthermore, the incorporation of the pharmacological inhibition approach for validation, as well as the confirmation of transgene expression in the specific conditions used for testing xenobiotic metabolism/toxicity are also useful. It may also be advisable to validate key findings in additional mouse models, such as the human liver chimeric mouse models described below.

Human Liver Chimeric Mouse Models

Development of Human Liver Chimeric Mice

An interesting alternative to the problems faced by genetic humanization is to develop human liver chimeric mice. Transplantation of entire human hepatocytes, instead of only replacing the gene of interest in mouse hepatocytes, leads to a cellular chimerism in the liver and circumvents some of the aforementioned challenges. Hepatocyte transplantation is usually performed via splenic injection of mice (Ponder et al., 1991). The hepatocytes migrate from the spleen into the liver where they integrate into the hepatic plates of the murine liver. Hepatocytes have also been transplanted into sites other than the liver such as the lymph nodes (Komori et al., 2012) or peritoneal cavity (Strom et al., 1999), but the need for proper drainage of the bile limits the long-term benefit of ectopic hepatocyte transplantation. Three different models of human liver chimeric mice have been described.

The *alb-uPA* Mouse. The principles of human liver chimerism were established using the transgenic *alb-uPA* mouse (urokinase-type plasminogen activator expressed under the albumin promoter), which was the first mouse model in which the liver could be repopulated with human hepatocytes (Dandri et al., 2001; Mercer et al., 2001). The *alb-uPA* mouse was developed as a coagulation model by the laboratories of Ralph Brinster (University of Pennsylvania) and Richard Palmiter (Howard Hughes Medical Institute and University of Washington) (Heckel et al., 1990). As expected, many of the *alb-uPA* mice died neonatally because of bleeding. About half of the transgenic pups survived but underwent liver failure a few weeks after birth. Interestingly, a few mice survived this second crisis and urokinase-type plasminogen activator (uPA) serum levels gradually returned to normal levels within 2 months. Analysis of these mice revealed healthy looking nodules within the liver, which expanded over months and eventually completely replaced the sick liver. Molecular analyses demonstrated

a genetic rearrangement that partially excised the toxic transgene (*alb-uPA*) within the nodules, which lead to clonal expansion of transgene-deficient hepatocytes (Sandgren et al., 1991). This natural selection phenomenon was subsequently recognized as a promising approach to efficiently expand transplanted healthy hepatocytes in a diseased liver. Rhim et al. (1994) were the first to prove this hypothesis in the *alb-uPA* mouse using healthy murine hepatocytes as a donor source. Eventually, two independent groups generated the first human liver chimeric mice, both using the *alb-uPA* strain (Dandri et al., 2001; Mercer et al., 2001). The *alb-uPA/SCID* mouse, which is also immune deficient to prevent donor cell rejection, can reach high human chimerism (Tateno et al., 2004).

The pioneering work done in the *alb-uPA* strain opened the door to hepatocyte repopulation and particularly to human liver chimerism. However, the *alb-uPA* mouse has several disadvantages, (see below and Table 2); therefore, updated versions of this strain (Weglaz et al., 2000; Suemizu et al., 2008; Tesfaye et al., 2013; Tateno et al., 2015) as well as new mouse genotypes amenable to human hepatocyte transplantation were developed (Washburn et al., 2011; Borel et al., 2017). We will limit this mini-review to human liver chimeric mice with a high degree of human chimerism (>70% of the liver), which is essential for many in vivo applications, including drug metabolism.

The *Fah^{-/-}/Il2rg^{-/-}/Rag2^{-/-}* Mouse. Repopulation with nodules of healthy hepatocytes has also been observed in patients with hereditary tyrosinemia type I (Kvittingen et al., 1993). In this case, the point mutation underlying the genetic disorder in the fumarylacetoacetate hydrolase (*FAH*) gene reverted to the WT allele (Kvittingen et al., 1994) by somatic mutation. Analogous to the rearranged *uPA* transgenic hepatocytes, the corrected *FAH* hepatocytes possess a growth advantage over the mutant cells and clonally expand to form nodules. This information in hand, it was apparent that the *Fah^{-/-}* mouse could be repopulated with WT murine hepatocytes (Overturf et al., 1996), as shown previously for the *uPA* mouse (Rhim et al., 1994). Although *Fah^{-/-}* mice were similarly neonatally lethal, they had an advantage over the transgenic *uPA* predecessors: the toxicity resulting from the mutated *Fah* gene could be regulated by the small-molecule drug nitisinone. After *Fah^{-/-}* mice receive transplantations, nitisinone is

withdrawn to apply selection pressure for the transplanted, *FAH*-positive hepatocytes. In 2007, two groups independently established the *Fah^{-/-}/Il2rg^{-/-}/Rag2^{-/-}* (FRG) mouse strain as a human liver chimeric mouse model (Azuma et al., 2007; Bissig et al., 2007). Their results were comparable and demonstrated robust repopulation of adult FRG mice with both fresh and cryopreserved human hepatocytes. In addition, Azuma et al. (2007) showed serial transplantation of human hepatocytes in FRG mice, whereas Bissig et al. (2007) demonstrated neonatal hepatocyte transplantation and viral transduction of transplanted human hepatocytes. Humanized FRG mice were healthy and could achieve high human chimerism (<95% human) (Bissig et al., 2010).

The TK-NOG Mouse. More recently, the TK-NOG mouse has been developed (Hasegawa et al., 2011b) for human liver chimerism. This strain contains the transgenic herpes simplex virus type 1 thymidine kinase (*HSVtk*) under the albumin promoter. HSVtk phosphorylates the drug ganciclovir, which leads to intoxication of the cell, whereas human hepatocytes without the transgene are not sensitive to the drug. Ganciclovir is administered before the hepatocyte transplantation, inducing intoxication of murine hepatocytes. In principle, this murine-specific intoxication could be used repeatedly even after the human hepatocytes are transplanted; however, none of the publications on the TK-NOG mouse describe such a repeated application. This mouse strain has also been shown to reach high human chimerism both by immunostaining and by human albumin levels in the murine serum (Hasegawa et al., 2011b).

Utility of Human Liver Chimeric Mice for Drug Metabolism

Since the primary site of drug metabolism is the liver, human liver chimeric mice are an attractive alternative to conventional animal models when it comes to in vivo validation of a new drug. All three human liver chimeric mouse models discussed here have been used for drug studies: human toxicity (Yamamoto et al., 2007; Sato et al., 2008; Foster et al., 2012; Kakuni et al., 2012; Samuelsson et al., 2014; Xu et al., 2014, 2015); human drug metabolism profiling (Katoh et al., 2007; Inoue et al., 2008; Lootens et al., 2009a,b,c, 2011; Pozo et al., 2009; Samuelsson et al., 2012, 2014; Sanoh et al., 2012b,c; Schulz-Utermoehl et al., 2012; Yamazaki et al., 2012; Nishimura et al., 2013; Tanoue et al.,

TABLE 2
Comparisons among the three human liver chimeric mouse models

Strain	uPA	FRG	TK-NOG
Development/genotype	Dandri et al. (2001) ¹ – <i>alb-uPA/Rag2^{-/-}</i> Mercer et al. (2001) ² – <i>alb-uPA/SCID/Bg</i> Tefsaye et al. (2013) ³ – <i>mup-uPA/SCID/Bg</i> Tateno et al. (2015) ⁴ – <i>alb-cDNA-uPA/SCID</i>	Bissig et al. (2007) Azuma et al. (2007) FRG	Hasegawa et al. (2011b) <i>Alb-HSVtk/SCID/Il2rg^{-/-}</i>
Mechanism of murine liver injury	Intoxication by liver overexpression of <i>uPA</i> ¹⁻⁴	Accumulation of toxic tyrosine catabolites	Generation of a toxic ganciclovir metabolite by expression of HSVtk in the liver
Advantages	Most experience	Inducible system	Inducible system
Drawbacks	Noninducible murine liver injury due to a constitutive <i>uPA</i> expression ¹⁻⁴ Narrow window of hepatocyte transplantation ^{1,2,4} High postnatal mortality due to internal bleeding ^{1,2,4} Spontaneous deletion of the <i>uPA</i> transgene decreases human hepatocyte repopulation and increases liver tumors incidence ^{1,2} Kidney disorders ^{1,2} Small body size ^{1,2} Female reproductive disorders ^{1,2} High human hepatocyte repopulation requires inhibition of host innate immune response and/or the human complement ^{1,2}	Hepatocyte transplantation at any age Human hepatocyte repopulation requires small molecular drug (nitisinone) Kidney disease due to <i>FAH</i> deficiency Cancer model with frequent liver tumors	Hepatocyte transplantation at any age Repopulation efficiency more hepatocyte donor dependent than other models Selection pressure only applied before hepatocyte transplantation Low breeding efficiency due to male sterility

2013; Bateman et al., 2014; Kamimura et al., 2015; Nakada et al., 2016; Wilson et al., 2018); drug-drug interaction (Hasegawa et al., 2012; Nishimura et al., 2013; Yamazaki et al., 2013; Suzuki et al., 2017; Uchida et al., 2018); human pharmacogenetic studies (Hu et al., 2013; Nishiyama et al., 2015); or other DME studies (Katoh and Yokoi, 2007; Inoue et al., 2009; Sanoh et al., 2012a, 2015; Schulz-Utermoehl et al., 2012; Tsukada et al., 2013; Suemizu et al., 2014; Nishiyama et al., 2015; Utoh et al., 2016; Kamimura et al., 2017; Shimizu et al., 2017; Yamazaki-Nishioka et al., 2018). A detailed analysis of all these studies is beyond the scope of this review; we will confine our review to a few examples illustrating the utility of human liver chimeric mice for drug studies.

A recent study by Xu et al. (2014) exemplifies the advantages of using human liver chimeric mouse models for hepatotoxicity over current preclinical models. In this study, the authors tested the dose response of the drug fialuridine (FIAU) in highly humanized TK-NOG mice. FIAU is a nucleoside analog that was developed to treat hepatitis B viral infections. FIAU did not present any toxicity in preclinical animal models but was terminated in phase II clinical trials because of lactic acidosis in patients after long-term exposure to the drug, which led to liver complications and pancreatitis (McKenzie et al., 1995). The authors showed a dose-dependent liver toxicity in the human liver chimeric mouse group as opposed to the nonhumanized control group. After 4 days of treatment with the highest dose of FIAU (400 mg/kg), only the chimeric group appeared lethargic and presented lactate and alanine aminotransferase elevations in plasma. Histologically, the liver tissue of these mice presented an accumulation of lipids in the human regions but not in the corresponding mouse areas. Furthermore, analysis by electron microscopy demonstrated mitochondrial abnormalities in the human hepatocytes, which were previously demonstrated to be the cause of liver failure in humans.

Another study by Nakada (2017) showed that chimeric mouse models were able to generate specific human metabolites that are not present in other species. The glucuronide metabolite M1 of YM543, a selective inhibitor of the sodium-glucose cotransporter, was detected only in the blood and urine of humans and humanized uPA/SCID mice, but not the other species tested (cynomolgus monkey, rhesus monkey, marmoset, beagle dog, NZW rabbit, Hartley guinea pig, golden hamster, and ICR mouse). Moreover, *in vitro* studies incubating human hepatocytes with YM543 did not show the presence of M1 metabolite, which demonstrates the ability of chimeric mouse models to predict human drug metabolites beyond those detected in cell culture platforms.

These two studies highlight the utility of human liver chimeric mice and their ability to identify “human-like” profiles for metabolites, toxicity, and drug-drug interactions. There is optimism that human liver chimeric mouse technology will become an essential element in preclinical drug development, since such models have several distinct advantages. In contrast to clinical trials, the effect of drugs can be tested in an isogenic context in human liver chimeric mice, meaning that the control groups are repopulated with hepatocytes from the same donor. Hence, the experimental readout is not biased by polymorphisms in P450 family members or other drug-processing enzymes. Alternatively, donor hepatocytes can be selected based on specific P450 family member profiles of particular relevance to the tested drug. From a practical standpoint, human liver chimeric mice offer the potential for gathering some unique data that are not available from clinical trials, in a controlled environment. The mice can be exposed to very high doses of an experimental drug, and all the while the human liver tissue can be harvested and analyzed at any time after exposure—both settings are unlikely to be approved in clinical trials from an ethical perspective, yet deliver valuable information for moving a drug forward into the clinic. Despite these advantages, the field of human liver chimeric mice is still young and there is a lot of room for optimization and standardization.

Challenges of Human Liver Chimeric Mice

In addition to the obvious benefits of using human liver chimeric mice for studying drug metabolism and toxicity, there are some general and model-specific limitations.

Variability within Human Liver Chimeric Mice. Contrary to inbred mice with a defined and well-characterized genotype and phenotype, human liver chimeric mice suffer from an increased variability. The variability is particularly related to the degree of human chimerism. It is difficult to control the degree of chimerism since many variables influence this outcome. Rigorous standard operating procedures can control some of this variation, but there is still much room for improvement in this area.

Remaining Murine Liver and Other Drug Metabolizing Murine Organs. Current evidence suggests that a 100% humanization of the murine liver is not possible. We hypothesize that species incompatibilities are the main reason for this phenomenon (e.g., multiple ligand receptor systems that cannot communicate across the species barrier). Differences in fibroblast growth factor (Naugler et al., 2015) or growth hormone (Masumoto et al., 2007) signaling have been shown, with many more species-specific protein interactions likely. Irrespectively, the consequence of this limitation is that the remaining murine liver cells express the full set of mouse DMEs. This is a significant problem since murine DMEs could be upregulated or have higher affinities for certain drugs than their human counterparts. Also, some DMEs, such as the P450 family members, have a high functional capacity; a reduction of the murine liver mass to 10% does not mean that the drug-metabolizing capacity of the murine liver component is reduced to 10%. Additionally, all three chimeric mouse models (particularly the FRG mouse) have a higher incidence of murine liver cancer than WT mice. Although there might be little normal murine liver remaining in older mice, large areas of neoplastic tissue can contribute to drug metabolism in liver toxicity assays. Extrahepatic murine drug metabolism can also be a confounding problem, depending on the DMEs involved in the metabolism of a given drug.

Immune Deficiency in Human Liver Chimeric Mice. The murine immune system has to be compromised to generate human liver chimeric mice. However, there are differences between mouse models. It seems clear that for all human liver chimeric mouse models T- and B-cell function needs to be eliminated, whereas there is a little bit more flexibility with natural killer cell function. For instance, the *alb-uPA* mouse has been crossed with the *Rag2^{-/-}* (Dandri et al., 2001) strain (only T- and B-cell deficient) to successfully engraft human hepatocytes. This is in contrast to the *Fah^{-/-}* mouse, which also requires the depletion of natural killer cells (*IL2rg^{-/-}*) for humanization (Bissig et al., 2007).

Many adverse drug reactions are mediated by the immune system. To detect such an event, a dual humanization with the hematopoietic system would be required. There are a few reports of such systems (summarized in Bissig et al., 2016); but further optimization is required to use these humanized mice for drug metabolism.

Strain-Specific Differences and Limitations. Human liver chimeric mice are bred on different genetic backgrounds. The importance of the mouse genetic background has been well documented and elaborated for human hematopoietic xenograft models; however, the situation is not clear for human liver chimerism.

The model-specific limitations are related to the knockout gene or inserted transgene. Table 2 summarizes these challenges, which are often technical. Every model has limitations, and no one model is clearly superior to another. There has been only one group comparing two different human liver chimeric mouse models (uPA and FRG) using the same drug, troglitazone (Schulz-Utermoehl et al., 2012; Samuelsson et al., 2014). That comparison was far from ideal, as the transplanted human hepatocytes were not from the same donor, the

human liver chimeric mice were produced by different groups, and, most importantly, the mean human liver chimerism was different in the two models. Nonetheless, analysis of troglitazone metabolites gave comparable results in humanized uPA and FRG mice (Schulz-Utermoehl et al., 2012; Samuelsson et al., 2014).

All of these challenges, in combination with reduced scalability, appear to dampen the enthusiasm to broadly implement human liver chimeric mice in the pharmaceutical industry. Nevertheless, there have been several promising advancements in recent years, and we expect that these kind of mouse models will increasingly be used.

Next Generation Human Liver Chimeric Mice

Increasing human chimerism is essential when using human liver chimeric mice for drug metabolism studies; but, as discussed above, there are natural boundaries. Another way of increasing human over murine drug metabolism is to functionally inactivate mouse DME without reducing the murine liver tissue mass. A first such attempt was recently taken by crossing the *Cyp3a* knockout mouse to the alb-uPA/SCID mouse (Kato et al., 2015; Nakada et al., 2016). Unfortunately, these mice upregulate the expression of several other CYP gene clusters, which defeats the purpose of the model. Therefore, we recently introduced a new humanized mouse strain in which we functionally inactivated all murine microsomal P450 family members. This was achieved by using a conditional knockout of the NADPH-P450 oxidoreductase (*Por^{cl}*) (Gu et al., 2003; Henderson et al., 2003; Wu et al., 2003), the electron donor for all microsomal P450 family members. When combined with the *Il2rg*^{-/-}, *Rag2*^{-/-}, and *Fah*^{-/-}, the novel PIRF strain was generated (Barzi et al., 2017). Humanized PIRF mice demonstrate higher levels of human-specific drug metabolites when compared with humanized FRG or nonhumanized PIRF mice (Barzi et al., 2017). Ideally, the *Por^{cl}* technology would be combined with new mouse strains that do not have a higher incidence of murine liver cancer, but for the time being this is a safe way of inactivating neoplastic and residual mouse liver drug metabolism.

Perspectives

Advancements in genetics and techniques to manipulate the mouse embryo have allowed the generation of genetically humanized mouse models. Many P450 family member clusters have been humanized and used for a better understanding and prediction of human drug metabolism. The transcriptional and post-transcriptional regulation of these transgenic human P450 family members is complex and may not be identical to their regulation in human cells, even though some improved mouse models (with the inclusion of humanized transcriptional regulators) have been generated. In addition to further optimized P450-humanized mouse models that will be developed as a result of continued efforts to increase the degree of genetic humanization, human liver chimeric mice are emerging as a promising alternative. These cellular chimeras have a very similar DME expression profile as human livers (Tateno et al., 2004); but their utility will be at the cost of other technical challenges, particularly the high experimental variability, even in the hands of specialists. Nevertheless, overcoming these challenges is possible and a broader implementation of this technology seems likely.

In summary, there is clearly a need for better models for predicting human drug metabolism and toxicity. Currently available mouse models of genetic humanization and cellular chimeras both have their own advantages and limitations, and their usage should be determined by the scientific question under study. Recent advancements in gene cloning and editing technologies have accelerated the rate at which these models can be improved, and it is possible that humanized mouse models will transform preclinical drug testing in the near future.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Bissig, Han, Barzi, Kovalchuk, L. Ding, Fan, Pankowicz, Zhang, and X. Ding.

References

- Abe S, Kobayashi K, Oji A, Sakuma T, Kazuki K, Takehara S, Nakamura K, Okada A, Tsukazaki Y, Senda N, et al. (2017) Modification of single-nucleotide polymorphism in a fully humanized CYP3A mouse by genome editing technology. *J Biol Chem* **292**:11230–11242.
- Albertolle ME, Kim D, Nagy LD, Yun CH, Pozzi A, Savas Ü, Johnson EF, and Guengerich FP (2017) Heme-thiolate sulfenylation of human cytochrome P450 4A11 functions as a redox switch for catalytic inhibition. *J Biol Chem* **292**:11230–11242.
- Azuma H, Paulk N, Ranade A, Dorrell C, Al-Dhalimy M, Ellis E, Strom S, Kay MA, Finegold M, and Grompe M (2007) Robust expansion of human hepatocytes in *Fah*^{-/-}/*Rag2*^{-/-}/*Il2rg*^{-/-} mice. *Nat Biotechnol* **25**:903–910.
- Barzi M, Pankowicz FP, Zorman B, Liu X, Legras X, Yang D, Borowiak M, Bissig-Choisat B, Sumazin P, Li F, et al. (2017) A novel humanized mouse lacking murine P450 oxidoreductase for studying human drug metabolism. *Nat Commun* **8**:39.
- Bateman TJ, Reddy VG, Kakuni M, Morikawa Y, and Kumar S (2014) Application of chimeric mice with humanized liver for study of human-specific drug metabolism. *Drug Metab Dispos* **42**:1055–1065.
- Bissig KD, Le TT, Woods NB, and Verma IM (2007) Repopulation of adult and neonatal mice with human hepatocytes: a chimeric animal model. *Proc Natl Acad Sci USA* **104**:20507–20511.
- Bissig KD, Paust S, and Barzi M (2016) Liver is liver and blood is blood, and finally the twain have met. *J Hepatol* **65**:245–248.
- Bissig KD, Wieland SF, Tran P, Isogawa M, Le TT, Chisari FV, and Verma IM (2010) Human liver chimeric mice provide a model for hepatitis B and C virus infection and treatment. *J Clin Invest* **120**:924–930.
- Borel F, Tang Q, Gernoux G, Greer C, Wang Z, Barzel A, Kay MA, Shultz LD, Greiner DL, Flotte TR, et al. (2017) Survival advantage of both human hepatocyte xenografts and genome-edited hepatocytes for treatment of α -1 antitrypsin deficiency. *Mol Ther* **25**:2477–2489.
- Buttara A, Nilsson K, Morgan K, Morgan TR, French SW, Johansson I, Schuppe-Koistinen I, and Ingelman-Sundberg M (2009) The impact of CYP2E1 on the development of alcoholic liver disease as studied in a transgenic mouse model. *J Hepatol* **50**:572–583.
- Cederbaum AI (2010) Role of CYP2E1 in ethanol-induced oxidant stress, fatty liver and hepatotoxicity. *Dig Dis* **28**:802–811.
- Chang JH, Chen J, Liu L, Messick K, and Ly J (2016) Rifampin-mediated induction of tamoxifen metabolism in a humanized PXR-CAR-CYP3A4/3A7-CYP2D6 mouse model. *Drug Metab Dispos* **44**:1736–1741.
- Chen JX, Li G, Wang H, Liu A, Lee MJ, Reuhl K, Suh N, Bosland MC, and Yang CS (2016) Dietary tocopherols inhibit PhIP-induced prostate carcinogenesis in CYP1A-humanized mice. *Cancer Lett* **371**:71–78.
- Cheng J, Ma X, and Gonzalez FJ (2011) Pregnane X receptor- and CYP3A4-humanized mouse models and their applications. *Br J Pharmacol* **163**:461–468.
- Cheng J, Ma X, Krausz KW, Idle JR, and Gonzalez FJ (2009) Rifampicin-activated human pregnane X receptor and CYP3A4 induction enhance acetaminophen-induced toxicity. *Drug Metab Dispos* **37**:1611–1621.
- Cheng J, Zhen Y, Miksys S, Beyoğlu D, Krausz KW, Tyndale RF, Yu A, Idle JR, and Gonzalez FJ (2013) Potential role of CYP2D6 in the central nervous system. *Xenobiotica* **43**:973–984.
- Cheung C and Gonzalez FJ (2008) Humanized mouse lines and their application for prediction of human drug metabolism and toxicological risk assessment. *J Pharmacol Exp Ther* **327**:288–299.
- Cheung C, Loy S, Li GX, Liu AB, and Yang CS (2011) Rapid induction of colon carcinogenesis in CYP1A-humanized mice by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine and dextran sodium sulfate. *Carcinogenesis* **32**:233–239.
- Cheung C, Yu AM, Chen CS, Krausz KW, Byrd LG, Feigenbaum L, Edwards RJ, Waxman DJ, and Gonzalez FJ (2006) Growth hormone determines sexual dimorphism of hepatic cytochrome P450 3A4 expression in transgenic mice. *J Pharmacol Exp Ther* **316**:1328–1334.
- Cheung C, Yu AM, Ward JM, Krausz KW, Akiyama TE, Feigenbaum L, and Gonzalez FJ (2005) The *cyp2e1*-humanized transgenic mouse: role of *cyp2e1* in acetaminophen hepatotoxicity. *Drug Metab Dispos* **33**:449–457.
- Choo EF, Woolsey S, DeMent K, Ly J, Messick K, Qin A, and Takahashi R (2015) Use of transgenic mouse models to understand the oral disposition and drug-drug interaction potential of cobimetinib, a MEK inhibitor. *Drug Metab Dispos* **43**:864–869.
- Corchero J, Granvil CP, Akiyama TE, Hayhurst GP, Pimprale S, Feigenbaum L, Idle JR, and Gonzalez FJ (2001) The CYP2D6 humanized mouse: effect of the human CYP2D6 transgene and HNF4alpha on the disposition of debrisoquine in the mouse. *Mol Pharmacol* **60**:1260–1267.
- Cruzan G, Bus J, Hotchkiss J, Sura R, Moore C, Yost G, Banton M, and Sarang S (2013) Studies of styrene, styrene oxide and 4-hydroxystyrene toxicity in CYP2F2 knockout and CYP2F1 humanized mice support lack of human relevance for mouse lung tumors. *Regul Toxicol Pharmacol* **66**:24–29.
- Dandri M, Burda MR, Török E, Pollok JM, Iwanska A, Sommer G, Rogiers X, Rogler CE, Gupta S, Will H, et al. (2001) Repopulation of mouse liver with human hepatocytes and in vivo infection with hepatitis B virus. *Hepatology* **33**:981–988.
- Derkenne S, Curran CP, Shertzer HG, Dalton TP, Dragin N, and Nebert DW (2005) Theophylline pharmacokinetics: comparison of *Cyp1a1*(^{-/-}) and *Cyp1a2*(^{-/-}) knockout mice, humanized hCYP1A1_1A2 knock-in mice lacking either the mouse *Cyp1a1* or *Cyp1a2* gene, and *Cyp1a1*(+/+) wild-type mice. *Pharmacogenet Genomics* **15**:503–511.
- Dragin N, Uno S, Wang B, Dalton TP, and Nebert DW (2007) Generation of 'humanized' hCYP1A1_1A2_1A2_1A2_1A2(-/-) mouse line. *Biochem Biophys Res Commun* **359**:635–642.
- Felmllee MA, Lon HK, Gonzalez FJ, and Yu AM (2008) Cytochrome P450 expression and regulation in CYP3A4/CYP2D6 double transgenic humanized mice. *Drug Metab Dispos* **36**:435–441.
- Foster JR, Jacobsen M, Kenna G, Schulz-Utermoehl T, Morikawa Y, Salmu J, and Wilson ID (2012) Differential effect of troglitazone on the human bile acid transporters, MRP2 and BSEP, in the PxB hepatic chimeric mouse. *Toxicol Pathol* **40**:1106–1116.
- Gonzalez FJ, Fang ZZ, and Ma X (2015) Transgenic mice and metabolomics for study of hepatic xenobiotic metabolism and toxicity. *Expert Opin Drug Metab Toxicol* **11**:869–881.
- Granvil CP, Yu AM, Elizondo G, Akiyama TE, Cheung C, Feigenbaum L, Krausz KW, and Gonzalez FJ (2003) Expression of the human CYP3A4 gene in the small intestine of

- transgenic mice: in vitro metabolism and pharmacokinetics of midazolam. *Drug Metab Dispos* **31**:548–558.
- Gu J, Weng Y, Zhang QY, Cui H, Behr M, Wu L, Yang W, Zhang L, and Ding X (2003) Liver-specific deletion of the NADPH-cytochrome P450 reductase gene: impact on plasma cholesterol homeostasis and the function and regulation of microsomal cytochrome P450 and heme oxygenase. *J Biol Chem* **278**:25895–25901.
- Hasegawa M, Kapelyukh Y, Tahara H, Seibler J, Rode A, Krueger S, Lee DN, Wolf CR, and Scheer N (2011a) Quantitative prediction of human pregnane X receptor and cytochrome P450 3A4 mediated drug-drug interaction in a novel multiple humanized mouse line. *Mol Pharmacol* **80**:518–528.
- Hasegawa M, Kawai K, Mitsui T, Taniguchi K, Monnai M, Wakui M, Ito M, Suematsu M, Peltz G, Nakamura M, et al. (2011b) The reconstituted 'humanized liver' in TK-NOG mice is mature and functional. *Biochem Biophys Res Commun* **405**:405–410.
- Hasegawa M, Tahara H, Inoue R, Kakuni M, Tateno C, and Ushiki J (2012) Investigation of drug-drug interactions caused by human pregnane X receptor-mediated induction of CYP3A4 and CYP2C subfamilies in chimeric mice with a humanized liver. *Drug Metab Dispos* **40**:474–480.
- Heckel JL, Sandgren EP, Degen JL, Palmer RD, and Brinster RL (1990) Neonatal bleeding in transgenic mice expressing urokinase-type plasminogen activator. *Cell* **62**:447–456.
- Henderson CJ, Otto DM, Carrie D, Magnuson MA, McLaren AW, Rosewell I, and Wolf CR (2003) Inactivation of the hepatic cytochrome P450 system by conditional deletion of hepatic cytochrome P450 reductase. *J Biol Chem* **278**:13480–13486.
- Holmstock N, Gonzalez FJ, Baes M, Annaert P, and Augustijns P (2013) PXR/CYP3A4-humanized mice for studying drug-drug interactions involving intestinal P-glycoprotein. *Mol Pharm* **10**:1056–1062.
- Hu Y, Wu M, Nishimura T, Zheng M, and Peltz G (2013) Human pharmacogenetic analysis in chimeric mice with 'humanized livers'. *Pharmacogenet Genomics* **23**:78–83.
- Hwang DY, Chae KR, Shin DH, Hwang JH, Lim CH, Kim YJ, Kim BJ, Goo JS, Shin YY, Jang IS, et al. (2001) Xenobiotic response in humanized double transgenic mice expressing tetracycline-controlled transactivator and human CYP1B1. *Arch Biochem Biophys* **395**:32–40.
- Imaoka S, Hayashi K, Hiroi T, Yabusaki Y, Kamataki T, and Funae Y (2001) A transgenic mouse expressing human CYP4B1 in the liver. *Biochem Biophys Res Commun* **284**:757–762.
- Inoue T, Nitta K, Sugihara K, Horie T, Kitamura S, and Ohta S (2008) CYP2C9-catalyzed metabolism of S-warfarin to 7-hydroxywarfarin in vivo and in vitro in chimeric mice with humanized liver. *Drug Metab Dispos* **36**:2429–2433.
- Inoue T, Sugihara K, Ohshita H, Horie T, Kitamura S, and Ohta S (2009) Prediction of human disposition toward S-3H-warfarin using chimeric mice with humanized liver. *Drug Metab Pharmacokinet* **24**:153–160.
- Jia K, Li L, Liu Z, Hartog M, Kluetzman K, Zhang QY, and Ding X (2014) Generation and characterization of a novel CYP2A13-transgenic mouse model. *Drug Metab Dispos* **42**:1341–1348.
- Jiang Z, Dalton TP, Jin L, Wang B, Tsunooka Y, Shertzer HG, DeKa R, and Nebert DW (2005) Toward the evaluation of function in genetic variability: characterizing human SNP frequencies and establishing BAC-transgenic mice carrying the human CYP1A1_CYP1A2 locus. *Hum Mutat* **25**:196–206.
- Kakuni M, Morita M, Matsuo K, Katoh Y, Nakajima M, Tateno C, and Yokoi T (2012) Chimeric mice with a humanized liver as an animal model of troglitazone-induced liver injury. *Toxicol Lett* **214**:9–18.
- Kamimura H, Ito S, Chijiwa H, Okuzono T, Ishiguro T, Yamamoto Y, Nishinoaki S, Ninomiya SI, Mitsui M, Kalgutkar AS, et al. (2017) Simulation of human plasma concentration-time profiles of the partial glucokinase activator PF-04937319 and its dispropionate N-demethylated metabolite using humanized chimeric mice and semi-physiological pharmacokinetic modeling. *Xenobiotica* **47**:382–393.
- Kamimura H, Ito S, Nozawa K, Nakamura S, Chijiwa H, Nagatsuka S, Kuronuma M, Ohnishi Y, Suemizu H, and Ninomiya S (2015) Formation of the accumulative human metabolite and human-specific glutathione conjugate of diflofenac in TK-NOG chimeric mice with humanized livers. *Drug Metab Dispos* **43**:309–316.
- Kathirvel E, Morgan K, French SW, and Morgan TR (2009) Overexpression of liver-specific cytochrome P4502E1 impairs hepatic insulin signaling in a transgenic mouse model of non-alcoholic fatty liver disease. *Eur J Gastroenterol Hepatol* **21**:973–983.
- Kato K, Ohbuchi M, Hamamura S, Ohshita H, Kazuki Y, Oshimura M, Sato K, Nakada N, Kawamura A, Usui T, et al. (2015) Development of murine Cyp3a knockout chimeric mice with humanized liver. *Drug Metab Dispos* **43**:1208–1217.
- Katoh M, Sawada T, Soeno Y, Nakajima M, Tateno C, Yoshizato K, and Yokoi T (2007) In vivo drug metabolism model for human cytochrome P450 enzyme using chimeric mice with humanized liver. *J Pharm Sci* **96**:428–437.
- Katoh M and Yokoi T (2007) Application of chimeric mice with humanized liver for predictive ADME. *Drug Metab Rev* **39**:145–157.
- Kazuki Y, Akita M, Kobayashi K, Osaki M, Satoh D, Ohta R, Abe S, Takehara S, Kazuki K, Yamazaki H, et al. (2016) Thalidomide-induced limb abnormalities in a humanized CYP3A mouse model. *Sci Rep* **6**:21419.
- Kazuki Y, Kobayashi K, Aueviriyavit S, Oshima T, Kuroiwa Y, Tsukazaki Y, Senda N, Kawakami H, Ohtsuki S, Abe S, et al. (2013) Trans-chromosomal mice containing a human CYP3A cluster for prediction of xenobiotic metabolism in humans. *Hum Mol Genet* **22**:578–592.
- Kent R and Jeong H (2017) Effects of fenofibrate on the expression of small heterodimer partner (SHP) and cytochrome P450 (CYP) 2D6. *Drug Metab Lett* **11**:68–72.
- Kitamura S and Sugihara K (2014) Current status of prediction of drug disposition and toxicity in humans using chimeric mice with humanized liver. *Xenobiotica* **44**:123–134.
- Kobayashi K, Abe C, Endo M, Kazuki Y, Oshimura M, and Chiba K (2017) Gender difference of hepatic and intestinal CYP3A4 in CYP3A humanized mice generated by a human chromosome-engineering technique. *Drug Metab Lett* **11**:60–67.
- Koh KH, Pan X, Shen HW, Arnold SL, Yu AM, Gonzalez FJ, Isoherranen N, and Jeong H (2014) Altered expression of small heterodimer partner governs cytochrome P450 (CYP) 2D6 induction during pregnancy in CYP2D6-humanized mice. *J Biol Chem* **289**:3105–3113.
- Komori J, Boone L, DeWard A, Hoppo T, and Lagasse E (2012) The mouse lymph node as an ectopic transplantation site for multiple tissues. *Nat Biotechnol* **30**:976–983.
- Kvittingen EA, Rootwelt H, Berger R, and Brandtzaeg P (1994) Self-induced correction of the genetic defect in tyrosinemia type I. *J Clin Invest* **94**:1657–1661.
- Kvittingen EA, Rootwelt H, Brandtzaeg P, Bergan A, and Berger R (1993) Hereditary tyrosinemia type I. Self-induced correction of the fumarylacetoacetase defect. *J Clin Invest* **91**:1816–1821.
- Lee CR, Imig JD, Edin ML, Foley J, DeGraff LM, Bradbury JA, Graves JP, Lih FB, Clark J, Myers P, et al. (2010) Endothelial expression of human cytochrome P450 epoxigenases lowers blood pressure and attenuates hypertension-induced renal injury in mice. *FASEB J* **24**:3770–3781.
- Li F, Jiang C, Larsen MC, Bushkofsky J, Krausz KW, Wang T, Jefcoate CR, and Gonzalez FJ (2014a) Lipidomics reveals a link between CYP1B1 and SCD1 in promoting obesity. *J Proteome Res* **13**:2679–2687.
- Li G, Wang H, Liu AB, Cheung C, Reuhl KR, Bosland MC, and Yang CS (2012) Dietary carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine-induced prostate carcinogenesis in CYP1A-humanized mice. *Cancer Prev Res (Phila)* **5**:963–972.
- Li L, Carratt S, Hartog M, Kovalchik N, Jia K, Wang Y, Zhang QY, Edwards P, Winkle LV, and Ding X (2017) Human CYP2A13 and CYP2F1 mediate naphthalene toxicity in the lung and nasal mucosa of CYP2A13/2F1-humanized mice. *Environ Health Perspect* **125**:067004.
- Li L, Megaraj V, Wei Y, and Ding X (2014b) Identification of cytochrome P450 enzymes critical for lung tumorigenesis by the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK): insights from a novel Cyp2abfgs-null mouse. *Carcinogenesis* **35**:2584–2591.
- Li L, Zhang QY, and Ding X (2018) A CYP2B6-humanized mouse model and its potential applications. *Drug Metab Pharmacokinet* **33**:2–8.
- Li Y, Yokoi T, Kitamura R, Sasaki M, Gunji M, Katsuki M, and Kamataki T (1996) Establishment of transgenic mice carrying human fetus-specific CYP3A7. *Arch Biochem Biophys* **329**:235–240.
- Lin D, Kostov R, Huang JT, Henderson CJ, and Wolf CR (2017) Novel pathways of ponatinib disposition catalyzed by CYP1A1 involving generation of potentially toxic metabolites. *J Pharmacol Exp Ther* **363**:12–19.
- Liu X, Zhao Y, Wang L, Yang X, Zheng Z, Zhang Y, Chen F, and Liu H (2009) Overexpression of cytochrome P450 4F2 in mice increases 20-hydroxyecosatetraenoic acid production and arterial blood pressure. *Kidney Int* **75**:1288–1296.
- Liu Z, Megaraj V, Li L, Sell S, Hu J, and Ding X (2015) Suppression of pulmonary CYP2A13 expression by carcinogen-induced lung tumorigenesis in a CYP2A13-humanized mouse model. *Drug Metab Dispos* **43**:698–702.
- Löfgren S, Baldwin RM, Carlerös M, Terelius Y, Fransson-Steen R, Mwynyi J, Waxman DJ, and Ingelman-Sundberg M (2009) Regulation of human CYP2C18 and CYP2C19 in transgenic mice: influence of castration, testosterone, and growth hormone. *Drug Metab Dispos* **37**:1505–1512.
- Löfgren S, Baldwin RM, Hiratsuka M, Lindqvist A, Carlberg A, Sim SC, Schülke M, Snaith M, Edenro A, Fransson-Steen R, et al. (2008) Generation of mice transgenic for human CYP2C18 and CYP2C19: characterization of the sexually dimorphic gene and enzyme expression. *Drug Metab Dispos* **36**:955–962.
- Lootens L, Meuleman P, Leroux-Roels G, and Van Eenoo P (2011) Metabolic studies with progesterone, methylclostebol and methasterone in the uPA+/+SCID chimeric mice. *J Steroid Biochem Mol Biol* **127**:374–381.
- Lootens L, Meuleman P, Pozo OJ, Van Eenoo P, Leroux-Roels G, and Delbeke FT (2009a) uPA+/+SCID mouse with humanized liver as a model for in vivo metabolism of exogenous steroids: methandienone as a case study. *Clin Chem* **55**:1783–1793.
- Lootens L, Van Eenoo P, Meuleman P, Leroux-Roels G, and Delbeke FT (2009b) The uPA (+/+)SCID mouse with humanized liver as a model for in vivo metabolism of 4-androstene-3,17-dione. *Drug Metab Dispos* **37**:2367–2374.
- Lootens L, Van Eenoo P, Meuleman P, Pozo OJ, Van Renterghem P, Leroux-Roels G, and Delbeke FT (2009c) Steroid metabolism in chimeric mice with humanized liver. *Drug Test Anal* **1**:531–537.
- Lu Y, Wu D, Wang X, Ward SC, and Cederbaum AI (2010) Chronic alcohol-induced liver injury and oxidant stress are decreased in cytochrome P4502E1 knockout mice and restored in humanized cytochrome P4502E1 knock-in mice. *Free Radic Biol Med* **49**:1406–1416.
- Ly JQ, Messick K, Qin A, Takahashi RH, and Choo EF (2017) Utility of CYP3A4 and PXR-CAR-CYP3A4/3A7 transgenic mouse models to assess the magnitude of CYP3A4 mediated drug-drug interactions. *Mol Pharm* **14**:1754–1759.
- Ma X, Cheung C, Krausz KW, Shah YM, Wang T, Idle JR, and Gonzalez FJ (2008) A double transgenic mouse model expressing human pregnane X receptor and cytochrome P450 3A4. *Drug Metab Dispos* **36**:2506–2512.
- MacLeod AK, Lin D, Huang JT, McLaughlin LA, Henderson CJ, and Wolf CR (2018) Identification of novel pathways of osimertinib disposition and potential implications for the outcome of lung cancer therapy. *Clin Cancer Res* **24**:2138–2147.
- MacLeod AK, McLaughlin LA, Henderson CJ, and Wolf CR (2015) Activation status of the pregnane X receptor influences vemurafenib availability in humanized mouse models. *Cancer Res* **75**:4573–4581.
- MacLeod AK, McLaughlin LA, Henderson CJ, and Wolf CR (2017) Application of mice humanized for CYP2D6 to the study of tamoxifen metabolism and drug-drug interaction with antidepressants. *Drug Metab Dispos* **45**:17–22.
- Madeen EP, Löhr CV, You H, Siddens LK, Krueger SK, Dashwood RH, Gonzalez FJ, Baird WM, Ho E, Bramer L, et al. (2017) Dibenzo[def]chrysenes transplacental carcinogenesis in wild-type, Cyp1b1 knockout, and CYP1B1 humanized mice. *Mol Carcinog* **56**:163–171.
- Masumoto N, Tateno C, Tachibana A, Utoh R, Morikawa Y, Shimada T, Momisako H, Itamoto T, Asahara T, and Yoshizato K (2007) GH enhances proliferation of human hepatocytes grafted into immunodeficient mice with damaged liver. *J Endocrinol* **194**:529–537.
- McKenzie R, Fried MW, Sallie R, Conjeevaram H, Di Bisceglie AM, Park Y, Savarese B, Kleiner D, Tsokos M, Luciano C, et al. (1995) Hepatic failure and lactic acidosis due to fialuridine (FIAU), an investigational nucleoside analogue for chronic hepatitis B. **333**:1099–1105.
- McMillan JM, Cobb DA, Lin Z, Banoub MG, Dagur RS, Branch Woods AA, Wang W, Makarov E, Koehler T, Joshi PS, et al. (2018) Antiretroviral drug metabolism in humanized PXR-CAR-CYP3A-NOG mice. *J Pharmacol Exp Ther* **365**:272–280.
- Megaraj V, Zhou X, Xie F, Liu Z, Yang W, and Ding X (2014) Role of CYP2A13 in the bioactivation and lung tumorigenicity of the tobacco-specific lung procarcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone: in vivo studies using a CYP2A13-humanized mouse model. *Carcinogenesis* **35**:131–137.
- Mercer DF, Schiller DE, Elliott JF, Douglas DN, Hao C, Rinfret A, Addison WR, Fischer KP, Churchill TA, Lakey JR, et al. (2001) Hepatitis C virus replication in mice with chimeric human livers. *Nat Med* **7**:927–933.
- Miksys SL, Cheung C, Gonzalez FJ, and Tyndale RF (2005) Human CYP2D6 and mouse CYP2D6: organic distribution in a humanized mouse model. *Drug Metab Dispos* **33**:1495–1502.
- Mitsui T, Nemoto T, Miyake T, Nagao S, Ogawa K, Kato M, Ishigai M, and Yamada H (2014) A useful model capable of predicting the clearance of cytochrome 3A4 (CYP3A4) substrates in humans: validity of CYP3A4 transgenic mice lacking their own Cyp3a enzymes. *Drug Metab Dispos* **42**:1540–1547.

- Morgan K, French SW, and Morgan TR (2002) Production of a cytochrome P450 2E1 transgenic mouse and initial evaluation of alcoholic liver damage. *Hepatology* **36**:122–134.
- Nakada N, Kawamura A, Kamimura H, Sato K, Kazuki Y, Kakuni M, Ohbuchi M, Kato K, Tateno C, Oshimura M, et al. (2016) Murine Cyp3a knockout chimeric mice with humanized liver: prediction of the metabolic profile of nefazodone in humans. *Biopharm Drug Dispos* **37**:3–14.
- Nakada N (2017) Evaluation of the utility of chimeric mice with humanized livers for the characterization and profiling of the metabolites of a selective inhibitor (YM543) of the sodium-glucose cotransporter 2. *Pharm Res* **34**:874–886.
- Naugler WE, Tarlow BD, Fedorov LM, Taylor M, Pelz C, Li B, Darnell J, and Grompe M (2015) Fibroblast growth factor signaling controls liver size in mice with humanized livers. *Gastroenterology* **149**:728–740.e15.
- Nelson DR, Zeldin DC, Hoffman SM, Maltais LJ, Wain HM, and Nebert DW (2004) Comparison of cytochrome P450 (CYP) genes from the mouse and human genomes, including nomenclature recommendations for genes, pseudogenes and alternative-splice variants. *Pharmacogenetics* **14**:1–18.
- Nishimura T, Hu Y, Wu M, Pham E, Suemizu H, Elazar M, Liu M, Idilman R, Yurdaydin C, Angus P, et al. (2013) Using chimeric mice with humanized livers to predict human drug metabolism and a drug-drug interaction [published correction appears in *J Pharmacol Exp Ther* (2013) 345:327]. *J Pharmacol Exp Ther* **344**:388–396.
- Nishiyama S, Suemizu H, Shibata N, Guengerich FP, and Yamazaki H (2015) Simulation of human plasma concentrations of thalidomide and primary 5-hydroxylated metabolites explored with pharmacokinetic data in humanized TK-NOG mice. *Chem Res Toxicol* **28**:2088–2090.
- Overturf K, Al-Dhalimy M, Tanguay R, Brantly M, Ou CN, Finegold M, and Grompe M (1996) Hepatocytes corrected by gene therapy are selected in vivo in a murine model of hereditary tyrosinaemia type I. *Nat Genet* **12**:266–273.
- Pan X and Jeong H (2015) Estrogen-induced cholestasis leads to repressed CYP2D6 expression in CYP2D6-humanized mice. *Mol Pharmacol* **88**:106–112.
- Pan X, Kent R, Won KJ, and Jeong H (2017) Cholic acid feeding leads to increased CYP2D6 expression in CYP2D6-humanized mice. *Drug Metab Dispos* **45**:346–352.
- Pan X, Lee YK, and Jeong H (2015) Farnesoid X receptor agonist represses cytochrome P450 2D6 expression by upregulating small heterodimer partner. *Drug Metab Dispos* **43**:1002–1007.
- Pang XY, Cheng J, Kim JH, Matsubara T, Krausz KW, and Gonzalez FJ (2012) Expression and regulation of human fetal-specific CYP3A7 in mice. *Endocrinology* **153**:1453–1463.
- Ponder KP, Gupta S, Leland F, Darlington G, Finegold M, DeMayo J, Ledley FD, Chowdhury JR, and Woo SL (1991) Mouse hepatocytes migrate to liver parenchyma and function indefinitely after intrasplenic transplantation. *Proc Natl Acad Sci U S A* **88**:1217–1221.
- Potter BM, Xie LH, Vuong C, Zhang J, Zhang P, Duan D, Luong TL, Bandara Herath HM, Dharmika Nanayakkara NP, Tekwani BL, et al. (2015) Differential CYP 2D6 metabolism alters primaquine pharmacokinetics. *Antimicrob Agents Chemother* **59**:2380–2387.
- Pozo OJ, Van Eenop P, Deventer K, Lootens L, Van Thuyne W, Parr MK, Schänzer W, Sancho JV, Hernández F, Meuleman P, et al. (2009) Detection and characterization of a new metabolite of 17alpha-methyltestosterone. *Drug Metab Dispos* **37**:2153–2162.
- Rhim JA, Sandgren EP, Degen JL, Palmer RD, and Brinster RL (1994) Replacement of diseased mouse liver by hepatic cell transplantation. *Science* **263**:1149–1152.
- Samuelsson K, Pickup K, Sarda S, Foster JR, Randall K, Abrahamsson A, Jacobsen M, Weidolf L, and Wilson I (2014) Troglitazone metabolism and transporter effects in chimeric mice: a comparison between chimeric humanized and chimeric murinized FRG mice. *Xenobiotica* **44**:186–195.
- Samuelsson K, Pickup K, Sarda S, Swales JG, Morikawa Y, Schulz-Utermoehl T, Hutchison M, and Wilson ID (2012) Pharmacokinetics and metabolism of midazolam in chimeric mice with humanized livers. *Xenobiotica* **42**:1128–1137.
- Sandgren EP, Palmer RD, Heckel JL, Daugherty CC, Brinster RL, and Degen JL (1991) Complete hepatic regeneration after somatic deletion of an albumin-plasminogen activator transgene. *Cell* **66**:245–256.
- Sanoh S, Horiguchi A, Sugihara K, Kotake Y, Tayama Y, Ohshita H, Tateno C, Horie T, Kitamura S, and Ohta S (2012a) Prediction of in vivo hepatic clearance and half-life of drug candidates in human using chimeric mice with humanized liver. *Drug Metab Dispos* **40**:322–328.
- Sanoh S, Horiguchi A, Sugihara K, Kotake Y, Tayama Y, Uramaru N, Ohshita H, Tateno C, Horie T, Kitamura S, et al. (2012b) Predictability of metabolism of ibuprofen and naproxen using chimeric mice with human hepatocytes. *Drug Metab Dispos* **40**:2267–2272.
- Sanoh S, Naritomi Y, Fujimoto M, Sato K, Kawamura A, Horiguchi A, Sugihara K, Kotake Y, Ohshita H, Tateno C, et al. (2015) Predictability of plasma concentration-time curves in humans using single-species allometric scaling of chimeric mice with humanized liver. *Xenobiotica* **45**:605–614.
- Sanoh S, Nozaki K, Murai H, Terashita S, Teramura T, and Ohta S (2012c) Prediction of human metabolism of FK3453 by aldehyde oxidase using chimeric mice transplanted with human or rat hepatocytes. *Drug Metab Dispos* **40**:76–82.
- Sanoh S and Ohta S (2014) Chimeric mice transplanted with human hepatocytes as a model for prediction of human drug metabolism and pharmacokinetics. *Biopharm Drug Dispos* **35**:71–86.
- Sato Y, Yamada H, Iwasaki K, Tateno C, Yokoi T, Yoshizato K, and Horii I (2008) Human hepatocytes can repopulate mouse liver: histopathology of the liver in human hepatocyte-transplanted chimeric mice and toxicologic responses to acetaminophen. *Toxicol Pathol* **36**:581–591.
- Savas U, Machemer DE, Hsu MH, Gaynor P, Lasker JM, Tukey RH, and Johnson EF (2009) Opposing roles of peroxisome proliferator-activated receptor alpha and growth hormone in the regulation of CYP4A11 expression in a transgenic mouse model. *J Biol Chem* **284**:16541–16552.
- Savas Ü, Wei S, Hsu MH, Falck JR, Guengerich FP, Capdevila JH, and Johnson EF (2016) 20-Hydroxyeicosatetraenoic acid (HETE)-dependent hypertension in human cytochrome P450 (CYP) 4A11 transgenic mice: normalization of blood pressure by sodium restriction, hydrochlorothiazide, or blockade of the type 1 angiotensin ii receptor. *J Biol Chem* **291**:16904–16919.
- Scheer N, Kapelyukh Y, Chatham L, Rode A, Buechel S, and Wolf CR (2012a) Generation and characterization of novel cytochrome P450 Cyp2c gene cluster knockout and CYP2C9 humanized mouse lines. *Mol Pharmacol* **82**:1022–1029.
- Scheer N, Kapelyukh Y, McEwan J, Beuger V, Stanley LA, Rode A, and Wolf CR (2012b) Modeling human cytochrome P450 2D6 metabolism and drug-drug interaction by a novel panel of knockout and humanized mouse lines. *Mol Pharmacol* **81**:63–72.
- Scheer N, Kapelyukh Y, Rode A, Oswald S, Busch D, McLaughlin LA, Lin D, Henderson CJ, and Wolf CR (2015) Defining human pathways of drug metabolism in vivo through the development of a multiple humanized mouse model. *Drug Metab Dispos* **43**:1679–1690.
- Scheer N and Wilson ID (2016) A comparison between genetically humanized and chimeric liver humanized mouse models for studies in drug metabolism and toxicity. *Drug Discov Today* **21**:250–263.
- Scheer N and Wolf CR (2014) Genetically humanized mouse models of drug metabolizing enzymes and transporters and their applications. *Xenobiotica* **44**:96–108.
- Schulz-Utermoehl T, Sarda S, Foster JR, Jacobsen M, Kenna JG, Morikawa Y, Salmu J, Gross G, and Wilson ID (2012) Evaluation of the pharmacokinetics, biotransformation and hepatic transporter effects of troglitazone in mice with humanized livers. *Xenobiotica* **42**:503–517.
- Shen HW, Jiang XL, Gonzalez FJ, and Yu AM (2011) Humanized transgenic mouse models for drug metabolism and pharmacokinetic research. *Curr Drug Metab* **12**:997–1006.
- Shen HW and Yu AM (2009) Difference in desipramine metabolic profile between wild-type and CYP2D6-humanized mice. *Drug Metab Lett* **3**:234–241.
- Shi Z, Chen Y, Dong H, Amos-Kroohs RM, and Nebert DW (2008) Generation of a 'humanized' hCYP1A1_1A2_Cyp1a1/1a2(-/-)_Ahrd mouse line harboring the poor-affinity aryl hydrocarbon receptor. *Biochem Biophys Res Commun* **376**:775–780.
- Shimizu M, Suemizu H, Mitsui M, Shibata N, Guengerich FP, and Yamazaki H (2017) Metabolic profiles of pomalidomide in human plasma simulated with pharmacokinetic data in control and humanized-liver mice. *Xenobiotica* **47**:844–848.
- Stiborová M, Frei E, Arlt VM, and Schmeiser HH (2014) Knockout and humanized mice as suitable tools to identify enzymes metabolizing the human carcinogen aristolochic acid. *Xenobiotica* **44**:135–145.
- Strom SC, Chowdhury JR, and Fox IJ (1999) Hepatocyte transplantation for the treatment of human disease. *Semin Liver Dis* **19**:39–48.
- Strom SC, Davila J, and Grompe M (2010) Chimeric mice with humanized liver: tools for the study of drug metabolism, excretion, and toxicity. *Methods Mol Biol* **640**:491–509.
- Suemizu H, Hasegawa M, Kawai K, Taniguchi K, Monnai M, Wakui M, Suematsu M, Ito M, Peltz G, and Nakamura M (2008) Establishment of a humanized model of liver using NOD/Shi-scid IL2Rgnull mice. *Biochem Biophys Res Commun* **377**:248–252.
- Suemizu H, Sota S, Kuronuma M, Shimizu M, and Yamazaki H (2014) Pharmacokinetics and effects on serum cholinesterase activities of organophosphorus pesticides acephate and chlorpyrifos in chimeric mice transplanted with human hepatocytes. *Regul Toxicol Pharmacol* **70**:468–473.
- Suzuki E, Koyama K, Nakai D, Goda R, Kuga H, and Chiba K (2017) Observation of clinically relevant drug interaction in chimeric mice with humanized livers: the case of valproic acid and carbapenem antibiotics. *Eur J Drug Metab Pharmacokin* **42**:965–972.
- Tanoue C, Sugihara K, Uramaru N, Tayama Y, Watanabe Y, Horie T, Ohta S, and Kitamura S (2013) Prediction of human metabolism of the sedative-hypnotic zaleplon using chimeric mice transplanted with human hepatocytes. *Xenobiotica* **43**:956–962.
- Tateno C, Kawase Y, Tobita Y, Hamamura S, Ohshita H, Yokomichi H, Sanada H, Kakuni M, Shiota A, Kojima Y, et al. (2015) Generation of novel chimeric mice with humanized livers by using hemizygous cDNA-uPA/SCID mice. *PLoS One* **10**:e0142145.
- Tateno C, Yoshizane Y, Saito N, Kataoka M, Utoh R, Yamasaki C, Tachibana A, Soeno Y, Asahina K, Hino H, et al. (2004) Near completely humanized liver in mice shows human-type metabolic responses to drugs. *Am J Pathol* **165**:901–912.
- Tesfaye A, Stift J, Maric D, Cui Q, Dienes HP, and Feinstein SM (2013) Chimeric mouse model for the infection of hepatitis B and C viruses. *PLoS One* **8**:e77298.
- Tsukada A, Suemizu H, Murayama N, Takano R, Shimizu M, Nakamura M, and Yamazaki H (2013) Plasma concentrations of melengestrol acetate in humans extrapolated from the pharmacokinetics established in in vivo experiments with rats and chimeric mice with humanized liver and physiologically based pharmacokinetic modeling. *Regul Toxicol Pharmacol* **65**:316–324.
- Uchida M, Tajima Y, Kakuni M, Kageyama Y, Okada T, Sakurada E, Tateno C, and Hayashi R (2018) Organic anion-transporting polypeptide (OATP)-mediated drug-drug interaction study between rosuvastatin and cyclosporin A in chimeric mice with humanized liver. *Drug Metab Dispos* **46**:11–19.
- Utoh M, Suemizu H, Mitsui M, Kawano M, Toda A, Uehara S, Uno Y, Shimizu M, Sasaki E, and Yamazaki H (2016) Human plasma concentrations of cytochrome P450 probe cocktails extrapolated from pharmacokinetics in mice transplanted with human hepatocytes and from pharmacokinetics in common marmosets using physiologically based pharmacokinetic modeling. *Xenobiotica* **46**:1049–1055.
- van Herwaarden AE, Smit JW, Sparidans RW, Wagenaar E, van der Kruijssen CM, Schellens JH, Beijnen JH, and Schinkel AH (2005) Midazolam and cyclosporin a metabolism in transgenic mice with liver-specific expression of human CYP3A4. *Drug Metab Dispos* **33**:892–895.
- van Herwaarden AE, Wagenaar E, van der Kruijssen CM, van Waterschoot RA, Smit JW, Song JY, van der Valk MA, van Tellingen O, van der Hoorn JW, Rosing H, et al. (2007) Knockout of cytochrome P450 3A yields new mouse models for understanding xenobiotic metabolism. *J Clin Invest* **117**:3583–3592.
- van Waterschoot RA, Rooswinkel RW, Sparidans RW, van Herwaarden AE, Beijnen JH, and Schinkel AH (2009) Inhibition and stimulation of intestinal and hepatic CYP3A activity: studies in humanized CYP3A4 transgenic mice using triazolam. *Drug Metab Dispos* **37**:2305–2313.
- van Waterschoot RA, ter Heine R, Wagenaar E, van der Kruijssen CM, Rooswinkel RW, Huijtema AD, Beijnen JH, and Schinkel AH (2010) Effects of cytochrome P450 3A (CYP3A) and the drug transporters P-glycoprotein (MDR1/ABCB1) and MRP2 (ABCC2) on the pharmacokinetics of lopinavir. *Br J Pharmacol* **160**:1224–1233.
- Washburn ML, Bility MT, Zhang L, Kovalev GI, Buntzman A, Frelinger JA, Barry W, Ploss A, Rice CM, and Su L (2011) A humanized mouse model to study hepatitis C virus infection, immune response, and liver disease. *Gastroenterology* **140**:1334–1344.
- Weglarz TC, Degen JL, and Sandgren EP (2000) Hepatocyte transplantation into diseased mouse liver. Kinetics of parenchymal repopulation and identification of the proliferative capacity of tetraploid and octaploid hepatocytes. *Am J Pathol* **157**:1963–1974.
- Wei Y, Wu H, Li L, Liu Z, Zhou X, Zhang QY, Weng Y, D'Agostino J, Ling G, Zhang X, et al. (2012) Generation and characterization of a CYP2A13/2B6/2F1-transgenic mouse model. *Drug Metab Dispos* **40**:1144–1150.
- Wilson CE, Dickie AP, Schreiter K, Wehr R, Wilson EM, Bial J, Scheer N, Wilson ID, and Riley RJ (2018) The pharmacokinetics and metabolism of diclofenac in chimeric humanized and murinized FRG mice. *Arch Toxicol* **92**:1953–1967.
- Winter JC, Amorosi DJ, Rice KC, Cheng K, and Yu AM (2011) Stimulus control by 5-methoxy-N, N-dimethyltryptamine in wild-type and CYP2D6-humanized mice. *Pharmacol Biochem Behav* **99**:311–315.

- Wu C, Jiang XL, Shen HW, and Yu AM (2009) Effects of CYP2D6 status on harmaline metabolism, pharmacokinetics and pharmacodynamics, and a pharmacogenetics-based pharmacokinetic model. *Biochem Pharmacol* **78**:617–624.
- Wu H, Liu Z, Ling G, Lawrence D, and Ding X (2013) Transcriptional suppression of CYP2A13 expression by lipopolysaccharide in cultured human lung cells and the lungs of a CYP2A13-humanized mouse model. *Toxicol Sci* **135**:476–485.
- Wu L, Gu J, Weng Y, Kluetzman K, Swiatek P, Behr M, Zhang QY, Zhuo X, Xie Q, and Ding X (2003) Conditional knockout of the mouse NADPH-cytochrome p450 reductase gene. *Genesis* **36**:177–181.
- Xu D, Nishimura T, Nishimura S, Zhang H, Zheng M, Guo YY, Masek M, Michie SA, Glenn J, and Peltz G (2014) Fialuridine induces acute liver failure in chimeric TK-NOG mice: a model for detecting hepatic drug toxicity prior to human testing. *PLoS Med* **11**:e1001628.
- Xu D, Wu M, Nishimura S, Nishimura T, Michie SA, Zheng M, Yang Z, Yates AJ, Day JS, Hillgren KM, et al. (2015) Chimeric TK-NOG mice: a predictive model for cholestatic human liver toxicity. *J Pharmacol Exp Ther* **352**:274–280.
- Yamamoto T, Tomizawa K, Fujikawa M, Sato Y, Yamada H, and Horii I (2007) Evaluation of human hepatocyte chimeric mice as a model for toxicological investigation using panomic approaches—effect of acetaminophen on the expression profiles of proteins and endogenous metabolites in liver, plasma and urine. *J Toxicol Sci* **32**:205–215.
- Yamazaki H, Suemizu H, Mitsui M, Shimizu M, and Guengerich FP (2016) Combining chimeric mice with humanized liver, mass spectrometry, and physiologically-based pharmacokinetic modeling in toxicology. *Chem Res Toxicol* **29**:1903–1911.
- Yamazaki H, Suemizu H, Murayama N, Utoh M, Shibata N, Nakamura M, and Guengerich FP (2013) In vivo drug interactions of the teratogen thalidomide with midazolam: heterotropic cooperativity of human cytochrome P450 in humanized TK-NOG mice. *Chem Res Toxicol* **26**:486–489.
- Yamazaki H, Suemizu H, Shimizu M, Igaya S, Shibata N, Nakamura M, Chowdhury G, and Guengerich FP (2012) In vivo formation of dihydroxylated and glutathione conjugate metabolites derived from thalidomide and 5-hydroxythalidomide in humanized TK-NOG mice. *Chem Res Toxicol* **25**:274–276.
- Yamazaki-Nishioka M, Shimizu M, Suemizu H, Nishiwaki M, Mitsui M, and Yamazaki H (2018) Human plasma metabolic profiles of benzydamine, a flavin-containing monooxygenase probe substrate, simulated with pharmacokinetic data from control and humanized-liver mice. *Xenobiotica* **48**:117–123.
- Yoshizato K, Tateno C, and Utoh R (2012) Mice with liver composed of human hepatocytes as an animal model for drug testing. *Curr Drug Discov Technol* **9**:63–76.
- Yu AM, Fukamachi K, Krausz KW, Cheung C, and Gonzalez FJ (2005) Potential role for human cytochrome P450 3A4 in estradiol homeostasis. *Endocrinology* **146**:2911–2919.
- Yu AM, Idle JR, and Gonzalez FJ (2004) Polymorphic cytochrome P450 2D6: humanized mouse model and endogenous substrates. *Drug Metab Rev* **36**:243–277.
- Zhang J, Heimbach T, Scheer N, Barve A, Li W, Lin W, and He H (2016) Clinical exposure boost predictions by integrating cytochrome P450 3A4-humanized mouse studies with PBPK modeling. *J Pharm Sci* **105**:1398–1404.
- Zhang QY, Gu J, Su T, Cui H, Zhang X, D'Agostino J, Zhuo X, Yang W, Swiatek PJ, and Ding X (2005) Generation and characterization of a transgenic mouse model with hepatic expression of human CYP2A6. *Biochem Biophys Res Commun* **338**:318–324.

Address correspondence to: Dr. Xinxin Ding, Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ 85721. E-mail: xding@pharmacy.arizona.edu; or Karl-Dimiter Bissig, Baylor College of Medicine, Houston, TX 77030. E-mail: bissig@bcm.edu
