



transADMET™

Precise. Predictive. Powerful.

More predictive power for more productive research.

A collaboration between:

TaconicArtemis

CXR biosciences

Current preclinical models can be poorly predictive of absorption, distribution, metabolism, excretion and toxicology (ADMET) in man. This inability to translate animal results to man is driven by profound interspecies differences in the levels and functions of proteins involved in ADMET, and is a major reason for development failure in the pharmaceutical and chemical industries.

In response, CXR Biosciences and TaconicArtemis have developed the transADMET™ mice panel. Key murine ADMET genes have either been knocked out or exchanged for their human counterparts. The models are presented in three panels representing critical pathways in compound metabolism, disposition and safety:

- The cytochrome P450 panel
- The nuclear receptor panel
- The drug transporter panel

The first cytochrome P450 and nuclear receptor models are available now, with additional models to follow.

Publications

- Scheer et al. (2008) A novel panel of mouse models to evaluate the role of human pregnane X receptor and constitutive androstane receptor in drug response. *JCI* 118(9): 3228–3239.
- van Waterschoot RA et al. (2009) Inhibition and stimulation of intestinal and hepatic CYP3A activity: studies in humanized CYP3A4 transgenic mice using triazolam. *DMD* 37(12): 2305–13.

- Thummel (2007). Gut instincts: CYP3A4 and intestinal metabolism. *JCI* 117(11): p3173–3176.
- Herwaarden et al. (2007). Knockout of cytochrome P450 3A yields new mouse models for understanding xenobiotic metabolism. *JCI* 117 (11): 3583–3592.
- Herwaarden et al. (2005). Midazolam and cyclosporin A metabolism in transgenic mice with liver-specific expression of human CYP3A4. *DMD* 33 (7): 892–895.

The Cytochrome P450 Panel

Cytochrome P450-dependent monooxygenases (CYPs) are a group of enzymes that account for the Phase I metabolism of the majority of clinically used drugs. CYP3A4 is the most abundant hepatic and intestinal CYP in humans and catalyzes the metabolism of over 50% of drugs in clinical use. These enzymes have diverged significantly between species, both in their multiplicity and substrate specificity. This can mean altered drug metabolism profiles between animals and humans, leading to differences in pharmacokinetics, efficacy and toxicity. **The result: the outcomes of preclinical studies may not be predictive of the situation in man.**

In response, we have derived and sourced a series of humanized and knockout CYP3A4 mouse models. Depending on your needs, we can offer models with gut or liver-specific CYP3A4 expression, or models where expression is under control of the human promoter. Potential utilities of these models include:

Studying human-specific metabolites *in vivo*.

Recent FDA guidelines “encourage the identification of differences in drug metabolism between animals used in nonclinical safety assessments and humans as early as possible during the drug development process.” [1, 2]. In the worst-case scenario a full toxicity study on a human-specific metabolite (or metabolites which are produced at disproportionately higher levels in humans) may be required.

Although *in vitro* systems such as hepatocytes can be used to screen for human-specific metabolites, until now there has been no simple way of characterizing their effects on efficacy and toxicity *in vivo*.

[1] Guidance for Industry: Safety Testing of Drug Metabolites. US Food and Drug Administration, Center for Drug Evaluation and Research, February 2008.

[2] Powley MW, et al. (2009) Safety assessment of drug metabolites: implications of regulatory guidance and potential application of genetically engineered mouse models that express human P450s. *Chem Res Toxicol* 22:257-262.

Assessing the impact of gut vs. liver CYP3A4 metabolism on bioavailability.

Oral bioavailability is commonly limited by first-pass metabolism. The impact of first-pass hepatic metabolism has been accepted for many years. It is increasingly recognized that first-pass intestinal metabolism also plays a major role in limiting oral bioavailability. It is therefore desirable to compare and rank the impact of intestinal vs. hepatic first-pass metabolism in preclinical studies. However, existing techniques such as gut perfusion studies are expensive and require a high level of technical expertise.

Now, the Humanized Liver CYP3A4 Mouse and the Humanized Gut CYP3A4 Mouse can be used to demonstrate the influence of gut vs. liver metabolism on oral bioavailability:

Examining the *in vivo* effects of CYP3A4 inhibition

Inhibition of cytochrome P450 3A4 by a drug can result in higher levels of co-administered 3A4 substrates in the body, and is therefore a major cause of toxic drug-drug interactions. Now, using the transADMET™ mice, it is possible to study inhibition of human CYPs *in vivo* before entering the clinic [3].

[3] van Waterschoot RA et al. (2009) Inhibition and stimulation of intestinal and hepatic CYP3A activity: studies in humanized CYP3A4 transgenic mice using triazolam. *DMD* 37(12):2305-13.

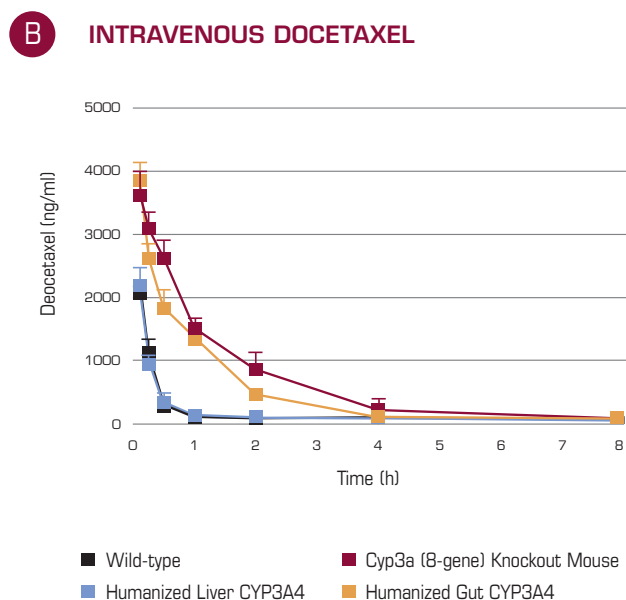
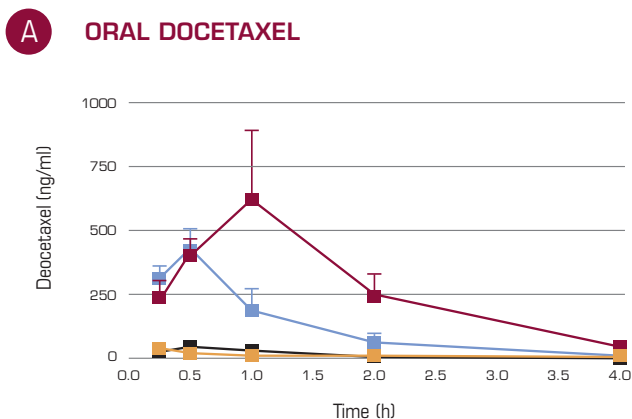
The Nuclear Receptor Panel

Nuclear receptors play a major role in regulating the body's response to chemical exposure.

The Pregnane X Receptor (PXR) and Constitutive Androstane Receptor (CAR) have the ability to bind a wide range of exogenous and endogenous ligands and, as a consequence, to control the level of expression of genes highly relevant to compound metabolism, such as the cytochrome P450s and drug transporters. Sequence variation in PXR and CAR between animals and man results in differences in the ability of exogenous ligands to interact and activate these transcription factors. Experimental data obtained in traditional animal models, or *in vitro* test systems, may therefore not reflect the interactions which occur in man.

In order to circumvent this problem we have created novel mouse models in which we have exchanged the murine genes for their human counterparts. Corresponding knockout models are also available.

◀ Figures A & B: Pharmacokinetic parameters estimated for docetaxel (10 mg/kg) in wild type and transADMET™ mice. In this example, after oral administration, docetaxel plasma exposure in the Humanized Gut CYP3A4 Mouse was reduced to levels comparable to those in wild-type mice, demonstrating the dominant impact of intestinal CYP3A4 rather than liver CYP3A4 on oral bioavailability.



Key utilities of these models include:

Demonstrating the relevance of rodent non-genotoxic carcinogenicity to man.

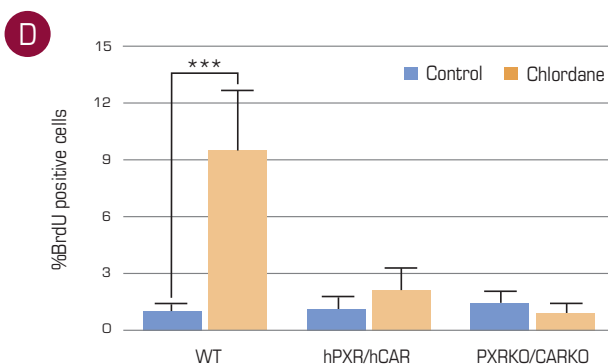
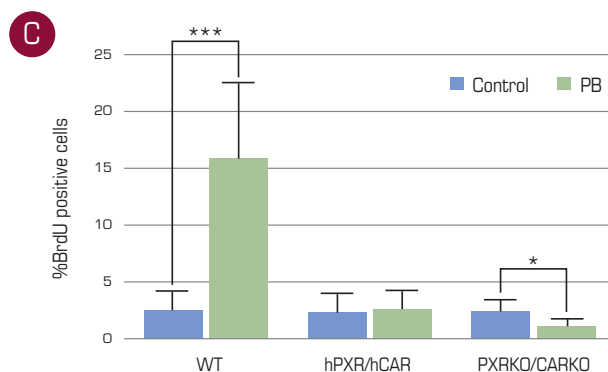
Many marketed drugs are non-genotoxic carcinogens in rats and/or mice (e.g. benzodiazepines, barbiturates, phenytoin). Their proliferative effects are believed to be driven through murine nuclear receptors such as PXR and CAR, but do not appear to be supported by human PXR or CAR—an outcome replicated in the Humanized PXR-CAR Mouse. For example:

- In wild-type mice, phenobarbital and chlordane treatment increased hepatocellular proliferation labelling index (S-phase) by approximately 7-fold and 11-fold, respectively.
- However, no change in S-phase was detected in either the Humanized PXR-CAR Mouse model or the Pxr-Car Knockout Mouse model following chlordane or phenobarbital treatment.

Improved Cytochrome P450 (CYP) induction screening.

CYP induction is an important cause of drug-drug interactions. Current screens for CYP induction have marked limitations. Human hepatocytes show high inter-individual variability, and therefore low reproducibility. Some CYP induction (especially through CAR) requires intact metabolic systems, and therefore induction events may be missed *in vitro*. Although *in vitro* PXR transactivation assays are available, accurate CAR transactivation assays are not.

CYP induction can be studied in animals, but profound species differences in PXR and CAR response limit relevance to man. Therefore it is not unusual for human induction events to go undetected until a compound enters the clinic. **The solution: humanized receptor mice demonstrate human-like CYP induction accurately and reproducibly.**



▲ Figures C & D: WT = C57BL/6; hPXR/hCAR = Humanized PXR-CAR Mouse; PXRKO/CARKO = Pxr-Car Knockout Mouse.

Using these novel models, we have shown that it is unlikely that exposure to chlordane and phenobarbital poses a hepatocarcinogenic hazard to humans. These observations support the utility of these humanized models in demonstrating lack of relevance to man.

FUNCTIONALLY HUMANIZED MODELS FOR PXR AND CAR

	PXR Humanized	CAR Humanized																																																																		
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transADMET	KEY MODELS		APPLICATIONS	
CYTOCHROME P450 MOUSE PANEL	Humanized CYP3A4/3A7 Mouse Cyp3a (7-gene) Knockout Mouse Humanized Liver & Gut CYP3A4 Mouse Humanized Liver CYP3A4 Mouse Humanized Gut CYP3A4 Mouse Cyp2d Knockout Mouse <i>More models coming soon</i>	In vivo effects of human metabolites • Toxicity, efficacy and/or PK effects of human-specific metabolites in an <i>in vivo</i> system	Impact of gut vs. liver metabolism on bioavailability • Effects of first pass intestinal vs. liver CYP3A4 on bioavailability	In vivo effects of CYP inhibition • Effects on PK, metabolism and tissue distribution
NUCLEAR RECEPTOR MOUSE PANEL	Humanized PXR-CAR Mouse Humanized PXR Mouse Humanized CAR Mouse Pxr-Car Knockout Mouse Pxr Knockout Mouse Car Knockout Mouse <i>More models coming soon</i>	Improved CYP induction screening • Increased accuracy, reproducibility vs. hepatocytes • KOs to dissect cause	In vivo effects of CYP induction • <i>In vivo</i> implications of induction seen <i>in vitro</i> • Effects on PK and metabolism	Non-genotoxic carcinogenicity • Relevance to man of rodent non genotoxic liver carcinogenicity
DRUG TRANSPORTER MOUSE PANEL	Humanized MRP2 Mouse Humanized PXR-CAR-MRP2 Mouse Humanized MDR1 Mouse <i>More models coming soon</i>	Tissue Distribution • Investigate transporter effect on distribution <i>in vivo</i>		

Commercial Availability

The first nuclear receptor models and cytochrome P450 models are available now, with additional models to follow.

Contract services: CXR are co-exclusive suppliers of contract research services using transADMET™ mice. We also offer consultancy and advice to our customers.

Collaborative research: special pricing terms are available to companies who enter into collaborative agreements with CXR and Taconic.

Off the shelf mice: Mice may be purchased directly from Taconic. To purchase transADMET™ mice, please visit: www.taconic.com/transADMET

- **Pxr-Car Knockout Mouse**
(Taconic model 8222)
- **Humanized PXR-CAR Mouse**
(Taconic model 8223)
- **Humanized CYP3A4/3A7 Mouse**
(Taconic model 8842)
- **Cyp3a (7-gene) Knockout Mouse**
(Taconic model 8841)
- **Humanized Liver & Gut CYP3A4 Mouse**
(Taconic model 9049)
- **Humanized Liver CYP3A4 Mouse**
(Taconic model 9048)
- **Humanized Gut CYP3A4 Mouse**
(Taconic model 9047)
- **Cyp3a (8-gene) Knockout Mouse**
(Taconic model 9011, available as a Taconic Transgenic Model™)

For further details regarding contract services utilizing the transADMET™ mice, please contact:



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www.cxrbiosciences.com
www.taconic.com/transadmet