

Humanised Metabolism Models for *in vitro* Screening

The next step

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About ITI Scotland

Established in 2003, ITI Scotland has three divisions, ITI Life Sciences, ITI Energy and ITI Techmedia. Our role is to identify global market opportunities, create research programmes based on these opportunities and then commercially exploit the resulting Intellectual Property for Scotland's economic benefit.

“Humanised mice can provide new insights into the drug development process and potentially lead to earlier and better prediction of human response”

Species differences in drug metabolism and responsiveness between mice and humans mean that it is difficult to extrapolate results obtained in animal trials to humans. Therefore humanised mice for the key drug metabolising enzymes, transporters and the transcription factors that regulate them can provide new insights into the drug development process.

ITI Life Sciences' Transgenic Screening & Safety Models Programme has developed an impressive range of humanised and knockout (KO) models that are all available from a single source on the same genetic background. All humanisations are done using a targeted integration strategy to ensure physiological expression levels and tissue distribution.

This world-leading programme represents a significant advance on current safety and toxicology models through improved prediction of the human drug response. Humanised models offer the ability to flag up potential drug-drug interaction and metabolite issues earlier in the drug development pathway.

The models are grouped into four key panels covering each element of the drug metabolism pathway – nuclear receptors, cytochrome (CYP) P450 enzymes, transporters and a reporter panel.

The development of *in vitro* systems could be used as a fast and efficient screen to determine which *in vivo* model is best suited to answer the particular safety or toxicology issue.



Models currently available for *in vitro* development

Receptor Panel	hPXR
	hCAR
	PXR KO
	CAR KO
	hPXR/hCAR
	hPXR/CAR KO
	PXR KO/hCAR
	PXR KO/CAR KO

Receptor Panel

- The nuclear receptors, PXR and CAR, play a key role in the regulation of genes involved in metabolism. Hence, understanding their interactions is both important and complex, particularly due to the cross-talk between receptors.
- The Receptor Panel offers two key advances in the study of PXR and CAR that will further the dissection of their involvement in the study of drug metabolism.
- Firstly, the fact that each nuclear receptor has been humanised means these models more closely resemble the situation in man than wild type mice.
- Secondly, the range of models available, including single and double humanised plus KO models for both receptors, means it is now easier to isolate which receptor is involved in which step of metabolism.

CYP Panel	Cyp3a11 KO
	Cyp3a cluster KO
	hCYP3A4
	hCYP3A4/hCYP3A7
	Cyp2c cluster KO
	hCYP2C9
	Cyp2D cluster KO
	hCYP2D6

CYP Panel

- The CYP Panel contains KO and humanised models for the major Phase I CYP genes that are involved in drug metabolism.
- Within the CYP3A family (which metabolise >50% of marketed drugs) there is a KO of the entire murine Cyp3a locus and models humanised for CYP3A4 and CYP3A7. Within the CYP2C family (which metabolise >20% of marketed drugs) there is a Cyp2c KO and a model humanised for CYP2C9. Finally within the CYP2D family there is a Cyp2d KO and a model humanised for CYP2D6.
- Many of these models are available on a human PXR or CAR background
- These models can be used alongside existing CYP *in vitro* assays to determine human-specific induction or inhibition and identify human specific metabolites

Transporter Panel	Mdr1a KO
	Mdr1b KO
	Mdr1a/b KO
	hMDR1
	hMRP2
	hMRP2/hPXR/hCAR

Transporter Panel

- Among the ABC trans-membrane proteins the MDR1 & MRP2 genes are a major confounding factor for drug development and are believed to play a key role in drug resistance.
- MDR1 encodes for P-glycoprotein (P-gp) and is known to export a large number of drugs including cytotoxic agents and immunosuppressants. MRP2 is important in mediating the transport of anionic drugs and metabolites.
- The Transporter Panel includes both KO and humanised forms of both mouse Mdr1 genes – Mdr1a & b and also a double KO and humanisation of Mdr1a/b. It also contains a humanised MRP2 mouse.

Reporter Panel	rCYP2B6_LacZ
	rCYP2B6_LacZ/hCAR
	rCYP2B6_LacZ/hCAR/hPXR
	rCYP2D6_Zs Yellow/hPXR
	rCYP2D6_Zs Yellow/hPXR/hCAR
	rCYP2B6_LacZ/rCYP2D6_Zs Yellow/hPXR/hCAR
	rCyp3a11_luc
	rCYP3A4_Zs Green_hCG
	rMDR1_luc

Reporter Panel

- The Reporter Panel enables visualisation of the induction of key metabolism genes via luciferase and lacZ activation.
- These models provide direct *in vivo* information of the ability of drugs to induce or repress specific CYP's, transporter genes and the transcription factors that regulate them.
- Key genes tagged with a reporter include: CYP2D6, CYP2B6, MDR1, Cyp3a11 and CYP3A4. Moreover, many of these reporter genes are on a PXR/CAR background.
- Additional benefits of the reporter panel include the assessment of organ-specific enzyme induction – e.g. liver vs. intestine and the easy read out of the *in vivo* interaction of a compound with PXR and CAR.

References

These models have been developed by ITI Life Sciences together with its Research Providers – CXR Biosciences and TaconicArtemis. More information about the Nuclear Receptor Panel can be found in the following publication: "A novel panel of mouse models to evaluate the role of human pregnane X receptor and constitutive androstane receptor in drug response". Scheer, N., Ross, J., Rode, A., Zevnik, B., Niehaves, S., Faust, N. and Wolf, C.R. (2008) *J. Clin. Invest.* 118:3228-3239.

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