Profiling Drug Activity of Human and Ortholog Xenobiotic-Sensing Receptors
PXR, CAR, AhR and **FXR**

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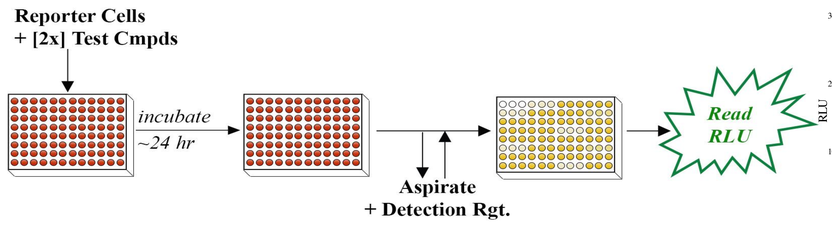
Introduction

Pre-clinical studies utilize animals as human surrogates to assess a drug's pharmcokinetic and toxicologic profiles. These studies are often confounded by the fact that the ligand preferences and response dynamics of the clinically important xenobiotic-sensing receptors (PXR, CAR, AhR and FXR) can vary dramatically between the human receptor and the corresponding ortholog receptors. A drug with cross-activity to one or more of the xenobiotic-receptors can effect expression of a variety of drug transporters, CYP's, and other phase I & II drug metabolism enzymes, fundamentally altering the ADMET profile of that drug and its potential to induce adverse drug-drug interactions (DDIs). Accordingly, selecting an animal model that provides the most representative human-surrogate must involve an assessment of the drug-induced activity profiles of the human *vs.* ortholog forms of these xenobiotic-receptors. Here we utilize cell-based mouse, rat, monkey and dog PXR assays, mouse CAR and FXR assays, and rat AhR assay as tools to assess drug-specific differences in the activities of these ortholog receptors relative to their corresponding human receptors. A variety of reference ligands are used to treat reporter cells engineered to express the human form and various ortholog forms of PXR, CAR, AhR and FXR. The majority of tested ligands showed dramatic differences in the relative activity profiles (potency and/or efficacy) that were measured between the human and ortholog receptors. These findings underscore the importance of characterizing the species dependent drug-induced activation profiles of the xenobiotic nuclear receptors to better understand human relevance of pre-clinical animal model systems.

Methods

Figure 1. Cell-based assays for Xenobiotic-Sensing Receptors. INDIGO Biosciences reporter cells express either full-length receptors (AhR) or Gal4-DBD hybrid receptors (PXR, CAR, FXR). For each assay, the reporter gene is firefly luc functionally linked to the appropriate upstream regulatory element. Changes in luciferase activity provide a direct correlation to the receptor's transcriptional activity. Relative Light Units (RLU) are quantified using a plate-reading luminometer.



Results

Reference drugs were used to determine comparative activity profiles of human and select orthologs of the xenobiotic-sensing receptors PXR, CAR, AhR and FXR.

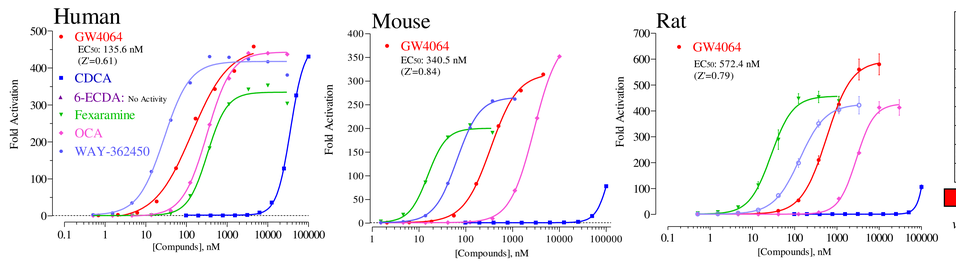
Figure 2 presents both dose-response plots and corresponding tables summarizing drug activity between the respective human and ortholog receptors. In particular, PXR and CAR profiles reveal stark difference in ligand preferences, potencies, and efficacy.

The CAR receptor presents added complexity in terms of splice variants. Whereas the rat and mouse express one predominate active form of CAR, humans express three variants. These isoforms, designated 1, 2 & 3, are expressed from mRNA splice variants that derive from a single gene, and each isoform differs by only 5 aa acids. Interestingly, the human isoform 1 and rat CAR receptors are constitutively active. Therefore, ligand interactions with these receptors will produce either an agonist activity response or inverse-agonist activity response, as exemplified in the drug response plots for human CAR-1 (Figure 2).

Profiling the Activities of Human, Mouse, Rat, Monkey and Dog Xenobiotic-Sensing Receptors

Figure 2. Activity Profiling of Xenobiotic Receptors. Dose-response plots and the corresponding tables summarize the potency and efficacy of reference drugs in human *vs.* ortholog PXR, CAR, FXR and AhR reporter cells. **Tables** present color-coded EC_{50} (nM) values of respective drugs. **Blue** = drug potency in the ortholog receptor is similar to that of the human receptor; **Red** = potency is higher than the human receptor; **Green** = potency is lower than the human receptor. Drugs often show no significant activity (*nsa*), or very low activity (*vla*) that precluded EC_{50} determinations, in respective human and ortholog receptor assays.

FXR

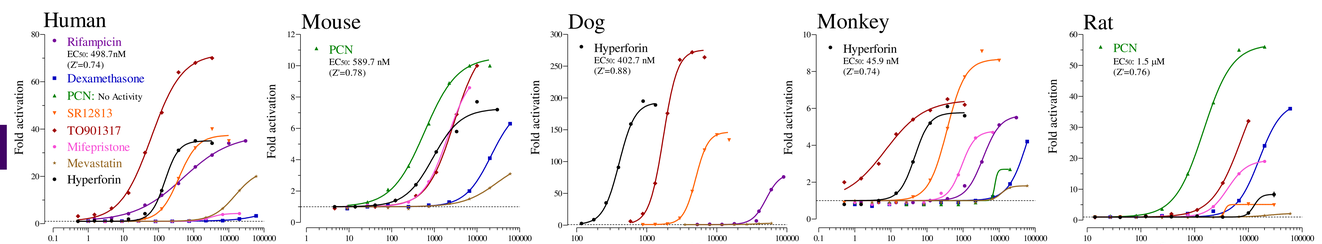


Drug Potencies (EC_{50}, nM): Human FXR *vs.* Ortholog FXR			
Drug	**Human**	**Mouse**	**Rat**
GW4064	136	341	572
WAY-362450	28.1	64.5	132
OCA	320	2,870	2,890
Fexaramine	328	15.4	27.7
CDCA	36,100	*vlp*	*vlp*
6-ECDA	*nsa*	*nsa*	*nsa*

Higher potency vs. Human; Similar Potency to Human; Lower potency vs. Human

vlp: very low potency *nsa*: no significant activity

PXR

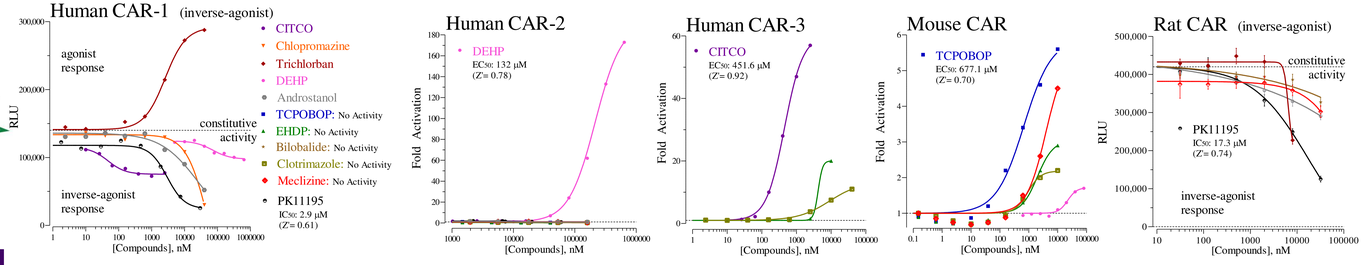


Drug Potencies (EC_{50}, nM): Human PXR *vs.* Ortholog PXR					
Drug	**Human**	**Monkey**	**Mouse**	**Rat**	**Dog**
Rifampicin	499	3,560	*nsa*	*nsa*	*vlp*
TO90137	60.0	6.30	2,820	*vlp*	1,730
Hyperforin	152	44.0	937	12,700	402
SR12813	376	360	*nsa*	3,620	5,000
Mifepristone	3,000	912	2,040	4,120	*nsa*
Mevastatin	15,000	*vlp*	*vlp*	24,300	*vlp*
Dexamethasone	*vlp*	*vlp*	*vlp*	15,800	*nsa*
PCN	*nsa*	*vlp*	540	1,460	*nsa*

Higher potency vs. Human; Similar Potency to Human; Lower potency vs. Human

vlp: very low potency *nsa*: no significant activity

CAR

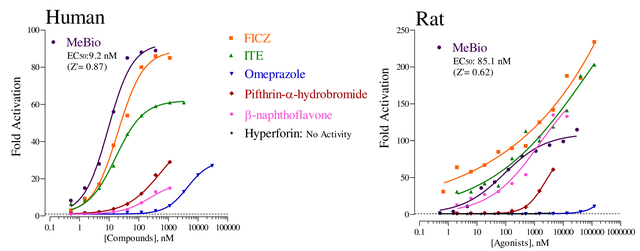


Drug Potencies (EC_{50}, nM): Human CAR *vs.* Ortholog CAR					
Drug	**hCAR1**	**hCAR2**	**hCAR3**	**Mouse**	**Rat**
PK11195	2,980	*nsa*	*nsa*	*nsa*	*17,300*
Trichlorban	2,571	*nsa*	*nsa*	*nsa*	*vlp*
Androstanol	*26,100*	*nsa*	*nsa*	*nsa*	*vlp*
Chlopromazine	51,100	*nsa*	8,930	*nsa*	*nsa*
DEHP	71,300	214,000	*nsa*	*vlp*	*nt*
CITCO	*vlp*	*nsa*	452	*nsa*	*nt*
TCPOBOP	*nsa*	*nsa*	*nsa*	677	*nsa*
EHDP	*nsa*	*nsa*	4,230	1,980	*nt*
Bilobalide	*nsa*	*nsa*	*nsa*	*nsa*	*vlp*
Clotrimazole	*nsa*	*nsa*	*nsa*	935	*nt*
Meclizine	*nsa*	*nsa*	*nsa*	3,890	*vlp*

Higher potency vs. Human; Similar Potency to Human; Lower potency vs. Human

vlp: very low potency *nsa*: no significant activity *nt*: not tested

AhR



Drug Potencies (EC_{50}, nM): Human AhR *vs.* Ortholog AhR		
Drug	**Human**	**Rat**
MeBIO	9.2	85.1
FICZ	17.6	*vlp*
ITE	16.5	*vlp*
Omeprazole	4,390	*vlp*
Pifthrin-a-hydrobromide	543	2,320
β-Naphthoflavone	233	1,540
Hyperforin	*nsa*	*nsa*

Similar Potency to Human; Lower potency vs. Human

vlp: very low potency *nsa*: no significant activity

Discussion

The assessment of a drug candidate's cross-activity with human xenobiotic-sensing receptors provides important early indications of that drug's potential for downstream DDI liabilities. The next stage in the drug development process is ADMET studies using animal models. However, these data demonstrate that corresponding ortholog receptors often have very different activity profiles relative to the human receptor. Indeed, the receptors of even closely related species, such as mouse and rat, sometimes exhibit dramatically different ligand preferences and responses. This creates a challenge when selecting the most human-relevant animal model for pre-clinical studies. The cell-based assays described herein provide researchers with an *in vitro* method to rapidly assess a drug candidate's activity profile comparing human and ortholog xenobiotic receptors, thus allowing a more informed selection of the most suitable animal model for ADMET studies.