HepG2-CYP™ cell panel

New tools to assist in identifying human routes of metabolism, CYP phenotyping and drug/drug interactions

Hera BioLabs

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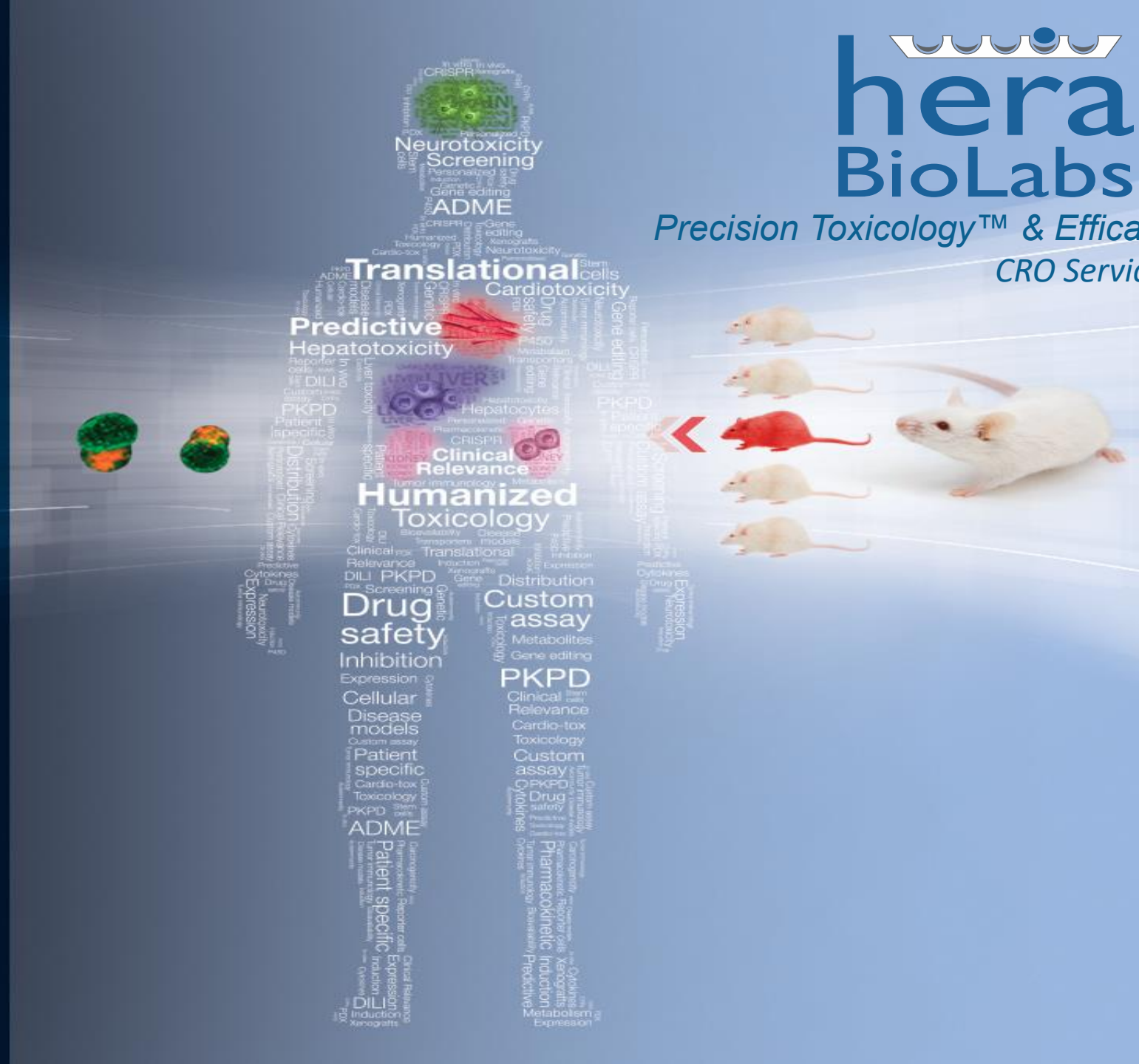


hera BioLabs

Precision Toxicology™ & Efficacy CRO Services

Why use the HepG2-CYP cell panel?

- HepG2 is one of the most commonly used cell systems for *in vitro* screening, but HepG2 cells have low expression and activity of CYP enzymes which leads to missed or underestimated cytotoxicity of test compounds and/or metabolites
- Hera Biolabs has assembled a panel of HepG2 transgenic cell lines that each stably express one of the major human CYPs
- Hera has also created a CYP reductase (POR) knockout HepG2 cell line
- Used in appropriate assay panels, these cells can address a variety of questions pertaining to CYP mediated metabolism and drug/drug interaction
  - CYP metabolism phenotyping can be accomplished directly with minimal or no use of inhibitors
  - Direct or indirect determination of CYP inhibition
  - CYP specific cytotoxicity

About the HepG2-CYP Panel

HepG2-CYP Cell Panel	Additional Cell Lines
HepG2 CYP reductase KO	HepG2-CYP1A1
HepG2-CYP1A2	HepG2-CYP2A6
HepG2-CYP2B6	HepG2-CYP2E1
HepG2-CYP2C9	HepG2-CYP2D6
HepG2-CYP3A4	HepG2-CYP2C19
	HepG2-CYP2C8

- The HepG2-CYP cell panel has been fully validated in-house at Hera and published in our SOT 2017 poster *Effect of Transfecting HepG2 with Human CYP Enzymes on Chemical Toxicity*
- Additional Cell Lines were created by & validated at Takeda Pharmaceutical Company Limited (Osaka, Japan)

- The CYP reductase (POR) knockout was created using gene editing technology at Hera
- The transgenic CYP HepG2 cell lines were created and published by Takeda Pharmaceutical Company Limited (Osaka, Japan)

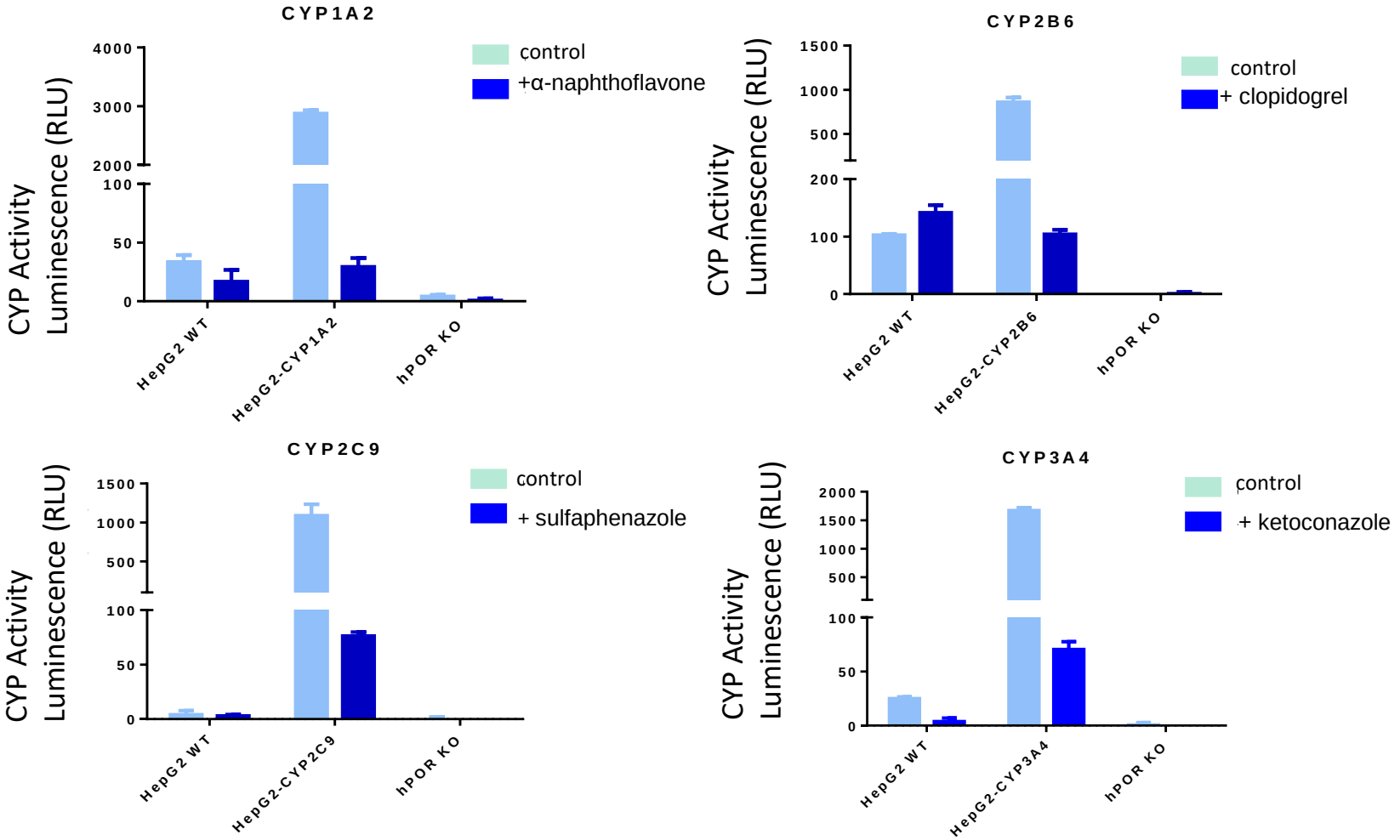
Takeda transgenic CYP publications

•*Establishment of the transformants expressing human cytochrome P450 subtypes in HepG2, and their applications on drug metabolism and toxicology* (Hashizume et al, 2010)

•*Advantages of Human Hepatocyte-Derived Transformants Expressing a Series of Human Cytochrome P450 Isoforms for Genotoxicity Examination* (Yoshitomi et al, 2001)

•*In vitro micronucleus test in HepG2 transformants expressing a series of human cytochrome P450 isoforms with chemicals requiring metabolic activation* (Hashizume et al, 2009)

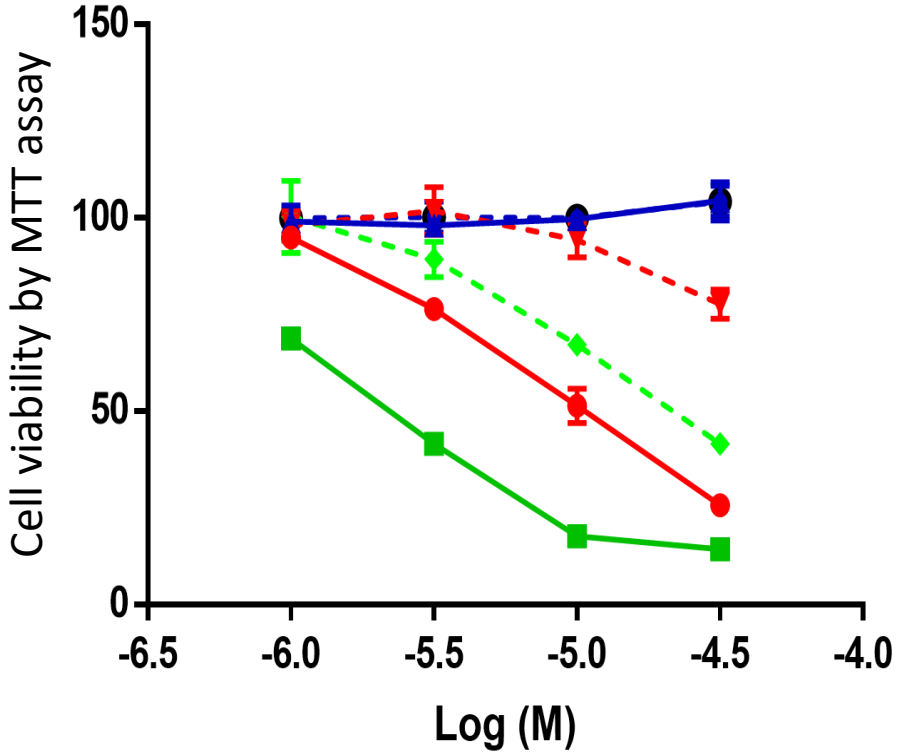
HepG2-CYP™ cell restore while reductase KO abolishes CYP activity



CYP activity in HepG2 WT, HepG2-CYP transgenic and HepG2-CYP reductase KO (POR) cells

CYP activity was measured with P450 –Glo™ assay kits. Specific CYP activity in transgenic cells was confirmed with CYP-selective competitive inhibitors, namely 5 µM α-naphthoflavone (CYP1A2), 1 µM clopidogrel (CYP2B6), 2 µM sulfaphenazole (CYP2C9), and 1 µM ketoconazole (CYP3A4).

HepG2-CYP panel correctly identifies CYP3A4 role in Aflatoxin B1 cytotoxicity



- Cytotoxicity is absent in CYP reductase (POR) KO **indicating CYP mediated toxicity**
- CYP3A4 specific cells demonstrates increased cytotoxicity **suggesting 3A4 involvement**
- CYP3A4 specific **inhibitor** partially prevents cytotoxicity in WT and CYP3A4 transgenic **confirming 3A4 involvement**

HepG2-CYP products & services

- The HepG2-CYP cell lines are available as a panel or can be purchased individually

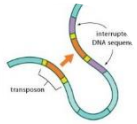
- Hera provides *in vitro* screening services using the HepG2-CYP panel as well other cell lines including: primary hepatocytes, HepaRG, Upcyte hepatocytes, Corning HepatoCells
  - Services include:
    - Cytotoxicity - cell viability (MTT & ATP)
    - Metabolic stability & metabolite profiling
    - CYP phenotyping and drug-drug interaction studies

- Custom gene editing services (knockout, knock-in and transgenic) to improve metabolic capability of any cell line as well as further modifications to any of the cell lines from the HepG2-CYP panel

Hera's gene editing technology & capabilities

Gene Editing Tool Box

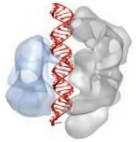
Site-Specific Nucleases



piggyBac™

Bioinformatics

TALEN



CRISPR/Cas9

Hyperactive *piggyBac™*

Excision-only *piggyBac™*

- Better project design
- Off-target analysis

- Gene knock-in & knockout (*CYP reductase KO*)

- Stable, transgenic integrations (*HepG2-CYPs*)
- Genetic screens

- Footprint-free™ gene editing
- Gene editing enhancements

- Hera's gene editing tool box for product development
- Custom gene editing, phenotyping and screening services in cells, rats & mice available

Hera's products & services

Cancer Xenografts

- Xenograft/PDX Efficacy studies
- Off-the-shelf SCID rats models

In Vivo & In Vitro Lead Optimization, Toxicity and Metabolism

- HepG2-CYP™ metabolism and toxicity cell panel
- hu-MDCK™ humanized transporter cells
- Humanized liver rodent models
- *In vivo* early discovery services

Disease Modeling

- *In vivo* liver gene delivery for disease model creation and gene therapy efficacy
- Custom genome engineering in rat and mouse
- Colony management and phenotyping

Links for specific product and service information above

About Hera BioLabs

Precision Toxicology™ & Efficacy: utilizing precisely gene-edited models such as SCID rats, humanized rodents and engineered cell lines for producing more rapid, consistent and clinically-relevant data

2015	2016	2017	2017/18 plan
Hera spun-out of Transposagen & licenses IP for gene editing technology; development of SCID rats begins; awarded phase II SBIR grant	Completion of a 10,000 ft^2 facility; Scientific team assembled with *in vitro* & *in vivo* efficacy & toxicity capabilities	Introduction of SDR™ & SRG™ SCID rats and efficacy services; Engineered HepG2 and MDCK cells; *in vivo* toxicity studies and humanized liver mice; custom gene editing, breeding and screening services in mouse and rat	Humanization of the liver & immune system of SRG™ rats for toxicity and immuno-oncology services

Hera BioLabs Leadership

Jack Crawford, M.S.

CEO

Formerly directed the Sales, Marketing, and Business Development Divisions at Transposagen. Experience in product development, licensing, technology and patent evaluation, and fundraising.

Tseten Yeshi, Ph.D.

VP, R & D

Former Director of R&D at Transposagen. An expert in genome editing with well-developed scientific program management skills and experience.

Chris Chengelis, Ph.D., DABT

Senior Scientific Advisor

Former CSO at WIL Research. 35 years+ experience in the preclinical toxicology industry, facility design, study design and execution

Fallon Noto, Ph.D.

Senior Scientist

10+ years working with mice and rats, expertise in rodent humanization, cell and tissue transplantation, microsurgery, and ethical animal care.

Kamesh Ravi, Ph.D.

Senior Scientist

10+ years of experience in preclinical oncology, cancer xenograft models, tumor efficacy studies and onco-nephrology.

Goutham Narla, M.D., Ph.D.

SAB Member & Consultant

The Pardee Gerstacker Professor of Cancer Research and a Medical geneticist at Case Western Reserve University. CSO and Scientific Founder of Dual Therapeutics, Inc. Expertise in cancer genetics and xenograft and transgenic models of cancer with over 58 publications in the field.

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