Faster & cleaner transporter assays with cPgp/cBcrp double knockout, and “humanized” MDCKII cell lines stably expressing human ABCB1 (MDR1) gene encoding P-glycoprotein and ABCG2 gene encoding BCRP

Hera BioLabs
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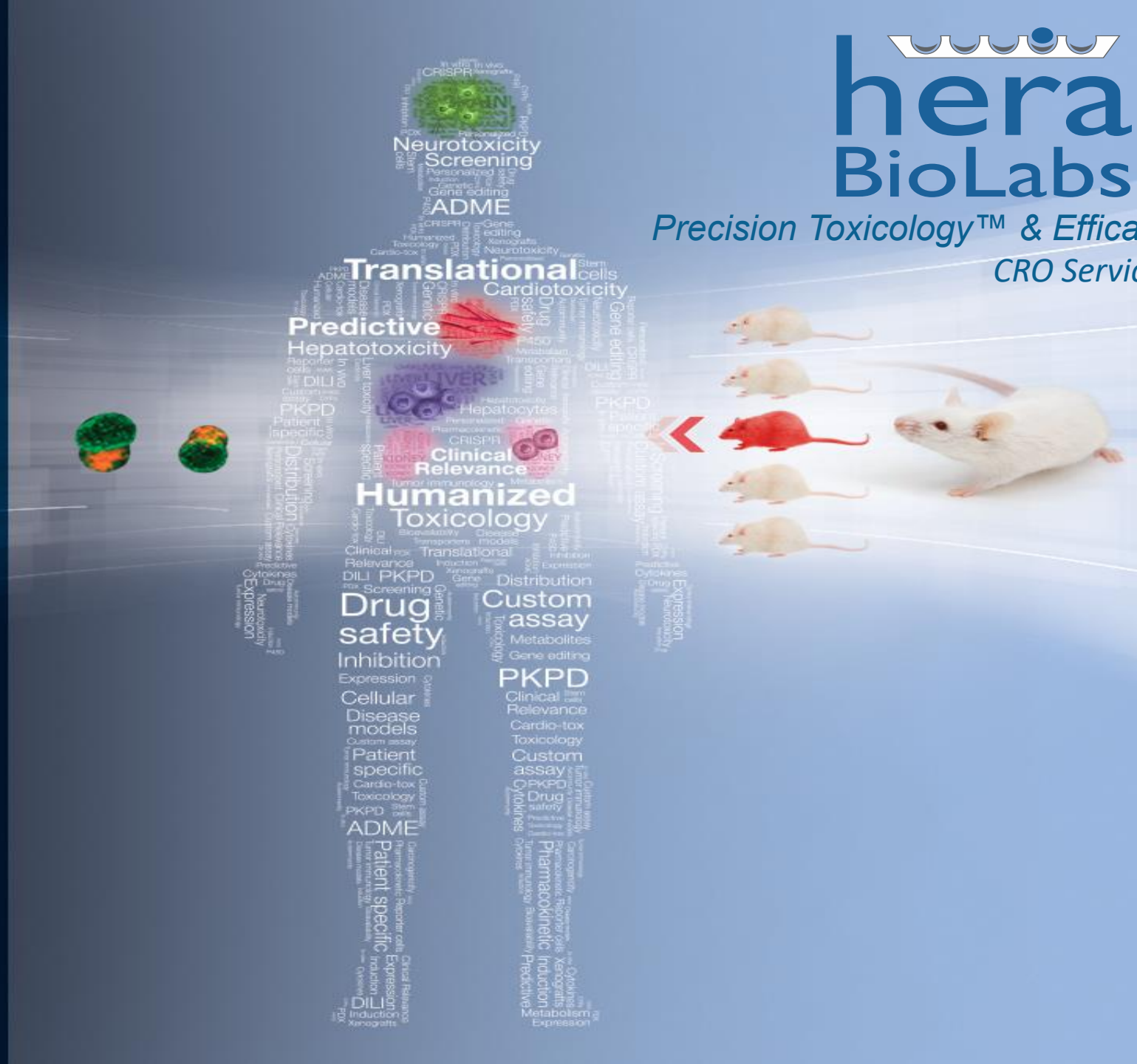


hera BioLabs

Precision Toxicology™ & Efficacy CRO Services

About Hera BioLabs

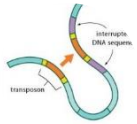
Precision Toxicology™ & Efficacy: utilizing precisely gene-edited models such as SCID rats, humanized rodents and engineered cell lines for producing more rapid, consistent and clinically-relevant data

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

Gene editing technology & capabilities

Gene Editing Tool Box

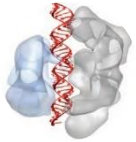
Site-Specific Nucleases



piggyBac™

Bioinformatics

TALEN



CRISPR/Cas9

Hyperactive *piggyBac™*

Excision-only *piggyBac™*

- Better project design
- Off-target analysis

- Gene knock-in & knockout

- Stable, transgenic integrations
- 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 has freedom to operate through multiple licenses to issued and pending patents

Faster & cleaner transporter assays

- Why use humanized MDR1 and BCRP-MDCKII cells?

- MDCK canine cells are desired for easy handling and a high proliferation rate. MDCKII cells form monolayers for transporter assays in 3 days *vs* Human Caco-2 cells take 21-days. However, endogenous canine transporters interfere in transfected MDCKII cells

- Hera Biolabs has generated cPgp/cBcrp double knockout MDCKII cell line (***MDCKII-BP-null™***), and "humanized" cell lines stably expressing ABCB1 (MDR1) gene encoding hPgp and ABCG2 gene encoding hBCRP on the MDCKII-BP-null cell background (***hMDR1-MDCKII-BP-null*** and ***hBCRP-MDCKII-BP-null***)

- Application: (1) MDR1 or BCRP substrate assessment; (2) MDR1 and BCRP inhibitor assessment.

Making Humanized MDCKII Cells

NextGEN CRISPR/Cas9 transfection into MDCKII
+ dual reporter plasmid with targeting sites

↓

Sort RFP^+/GFP^+ cells
(highly concentrate cells in which CRISPR/Cas9 cut properly)

↓ *Check cutting efficiency (Cell I assay)*

Single cell clones isolation

↓ *Genomic verification (PCR, sequencing)*
Functional verification (transporter assay)

MDCKII-BP-null cell line

MDCKII-BP-null cell line

↓

PB-hMDR1/PB-hBCRP + PiggyBac transposase transfection

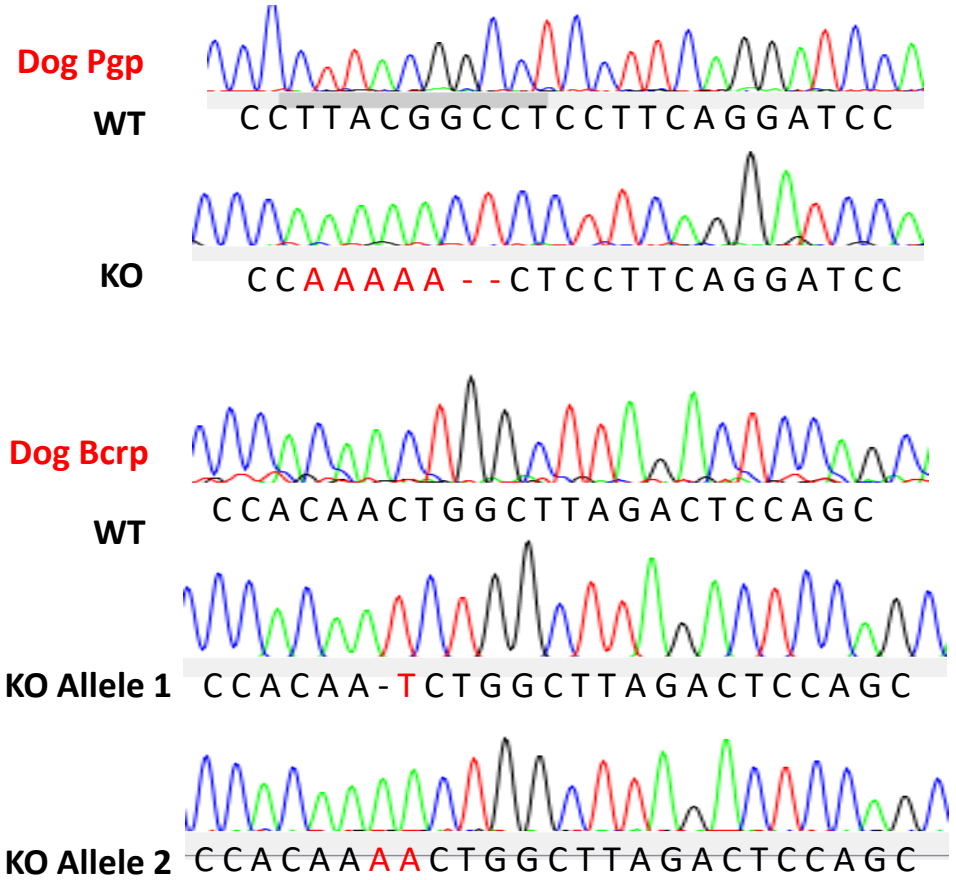
↓ *G418 selection*

Single cell clones isolation

↓ *Genomic verification (PCR, sequencing)*
Gene expression (RT-PCR, IF staining)
Functional verification (transporter assay)

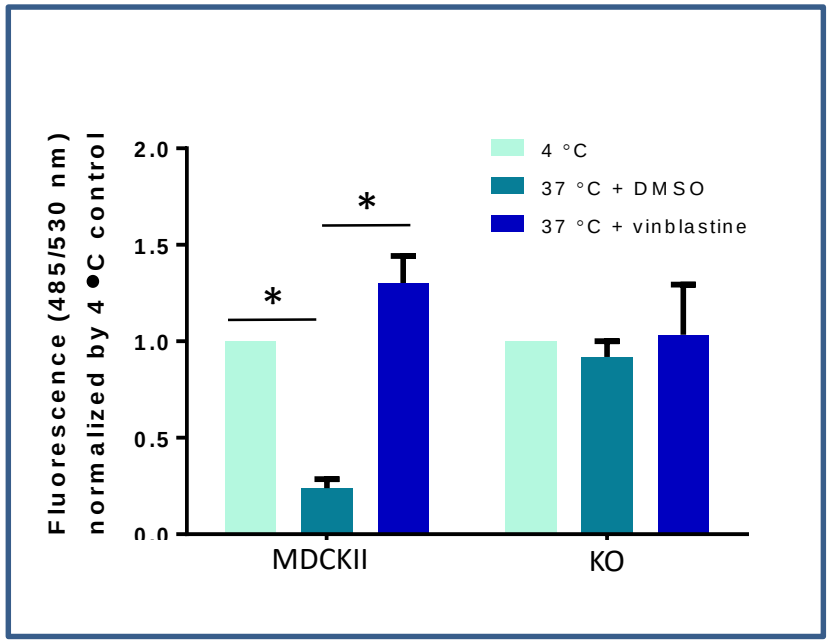
hMDR1-MDCKII-BP-null cell line
hBCRP-MDCKII-BP-null cell line

MDCKII-BP-null™ cells



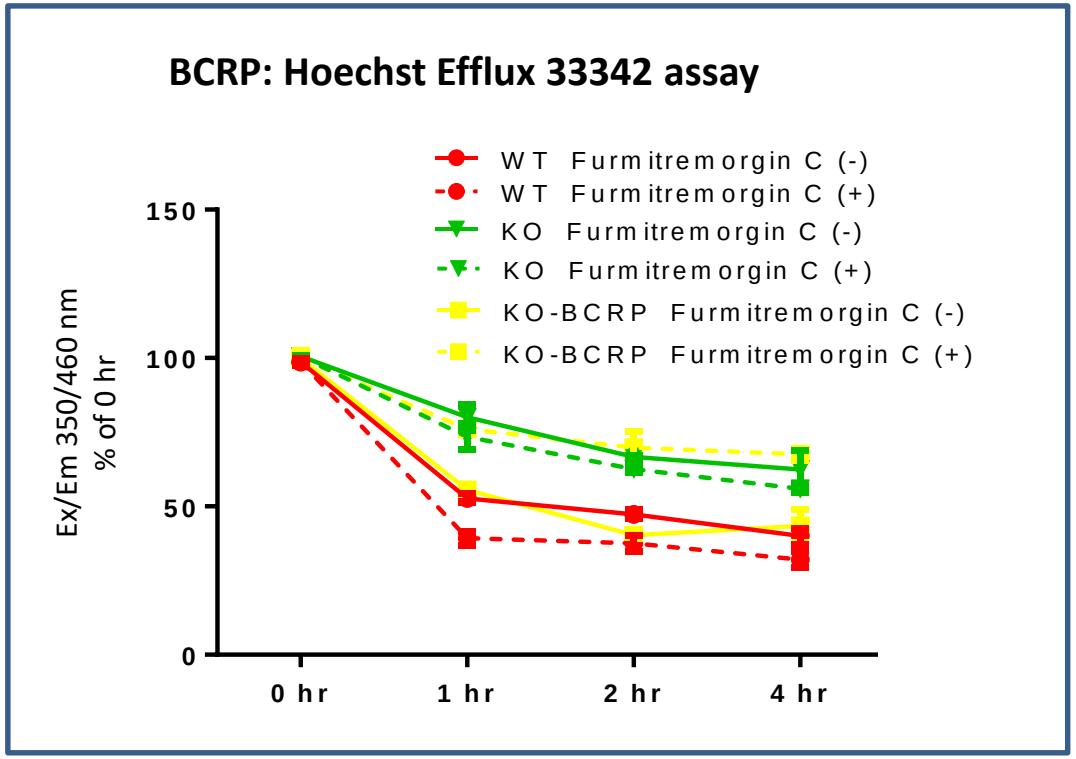
Frameshift mutation on cPgp and cBcrp gene lead to premature stop codons downstream in early exons.

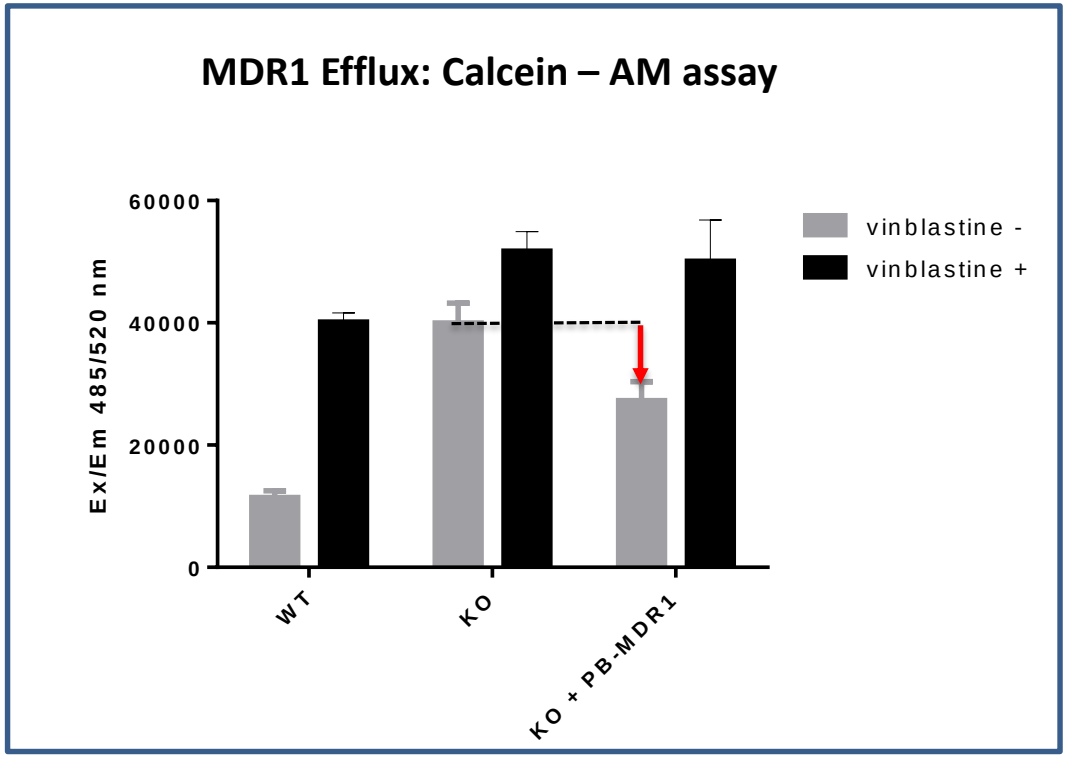
Multidrug Resistance Direct Dye Efflux Assay



Transport activity of cPgp and cBcrp was abolished in MDCKII-BP-null cells. The Pgp and Bcrp activity to transport substrate $DiOC_2(3)$ were detected by Multidrug Resistance Direct Dye Efflux Assay (ECM910, Millipore).

hMDR1/hBCRP-MDCKII-BP-null cell pool characterization

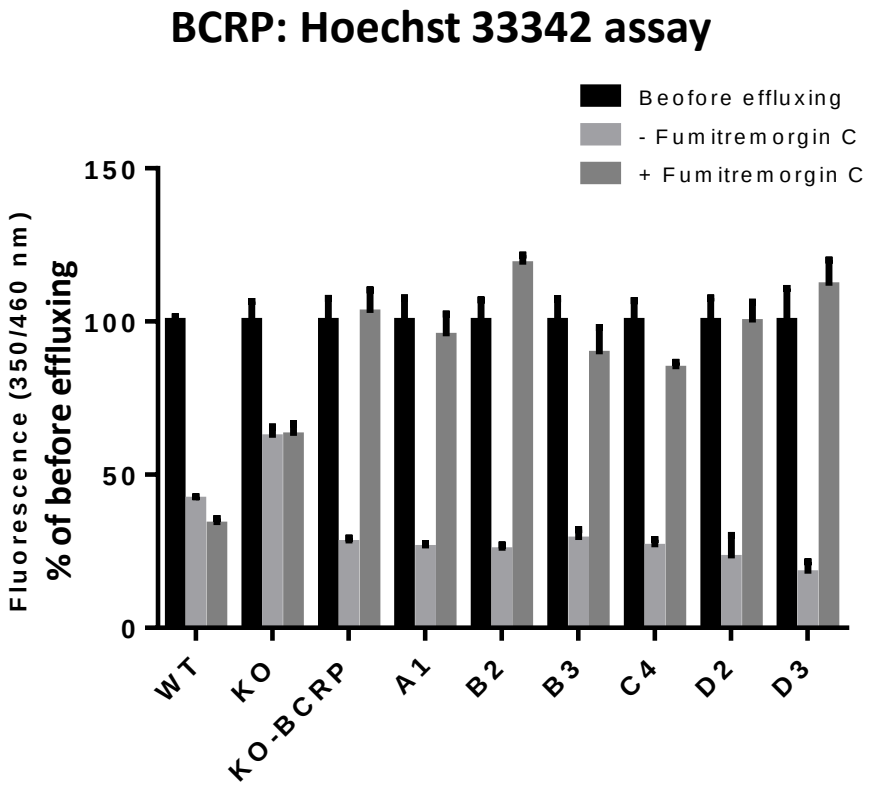


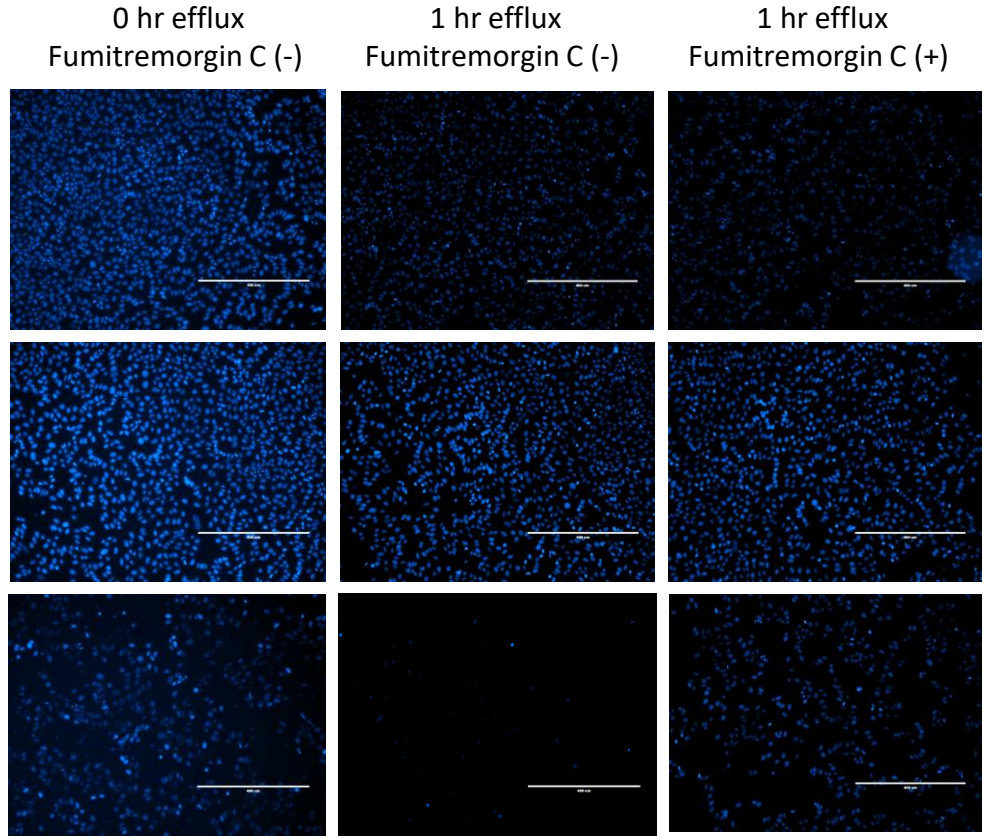


MDCKII WT, MDCKII-BP-null (KO) and hBCRP-MDCKII-BP-null (KO-BCRP) pool cells were incubated with 5 µM Hoechst for 1 hour at 37°C (before effluxing). Then cells were switched to Hoechst-free medium with/without BCRP inhibitor Fumitremorgin C for 1, 2 and 4 hours. Hoechst remaining in the cells was measured in a fluorescence spectrophotometer (Ex/Em=350nm/460nm), and was expressed as percentage of the value determined after 1 hours of incubation. *hBCRP-MDCKII-BP-null cells pool show BCRP-mediated efflux as BCRP specific inhibitor Fumitremorgin C inhibits the efflux.*

MDCKII WT, MDCKII-BP-null (KO) and hMDR1-MDCKII-BP-null (KO+ PB-MDR1 pool cells were incubated with 5 µM Calcein AM for 1 hour with/without MDR1 inhibitor vinblastine. Calcein-AM accumulating in the cells was measured in a fluorescence spectrophotometer (Ex/Em=485nm/520nm). *Compared to MDCKII-BP-null cells, hMDR1-MDCKII-BP-null cells show lower Calcein-AM accumulation, indicating possible human MDR1 activity.*

hBCRP-MDCKII-BP-null cell clonal line characterization





MDCKII WT, MDCKII-BP-null (KO), hBCRP-MDCKII-BP-null pool (KO-BCRP) and individual clones (A1, B2, B3, C4, D2, D3) cells were incubated with 5 µM Hoechst for 1 hour at 37°C (before effluxing). Then cells were switched to Hoechst-free medium with/without Bcrp inhibitor Fumitremorgin C for 1 hours. Hoechst remaining in the cells was measured in a fluorescence spectrophotometer (Ex/Em=350nm/460nm), and was expressed as percentage of the value determined after 1 hours of incubation. *Multiple MDCKII-KO-BCRP single cell colonies show BCRP-mediated efflux (because BCRP specific inhibitor Fumitremorgin C inhibits the efflux)*

Cell imagines of MDCKII cells in Hoechst efflux assay. Hoechst dye binds to the cell nucleus (blue). MDCKII WT cells (WT) efflux Hoechst dye (blue intensity decrease) through transporters other than canine Bcrp, because Bcrp inhibitor Fumitremorgin C did not inhibit the efflux. MDCKII-BP-null (KO) cells show much lower efflux activity. ***hBCRP-MDCKII-BP-null (KO-BCRP B2)*** *cells show obvious dye efflux which is inhibited by BCRP inhibitor, indicating the human BCRP contributes the efflux.*

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

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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