Humanized Liver Mice

Toxicology & PK/PD

Gene Therapy Efficacy & Infectious Disease

services@herabiolabs.com
859-414-0648
### 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

<table>
<thead>
<tr>
<th>2015</th>
<th>2016</th>
<th>2017</th>
<th>2017/18 plan</th>
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<tbody>
<tr>
<td>Hera spun-out of Transposagen &amp; licenses IP for gene editing technology; development of SCID rats begins; awarded phase II SBIR grant</td>
<td>Completion of a 10,000 ft² facility; Scientific team assembled with <em>in vitro</em> &amp; <em>in vivo</em> efficacy &amp; toxicity capabilities</td>
<td>Introduction of SDR™ &amp; SRG™ SCID rats and efficacy services; Engineered HepG2 and MDCK cells; <em>in vivo</em> toxicity studies and humanized liver mice; custom gene editing, breeding and screening services in mouse and rat</td>
<td>Humanization of the liver &amp; immune system of SRG™ rats for toxicity and immuno-oncology services</td>
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Gene editing technology & capabilities

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
Making humanized liver mice

1) TK-NOG mice are injected with 15mg/kg ganciclovir IP

2) Human hepatocytes transplanted via the spleen

3) Human albumin detected in the serum; histology to visualize human cells
Human albumin is detected in the serum of TK-NOG liver-humanized mice. Blood was collected every 2 weeks starting at 2 weeks post-transplant of primary human hepatocytes in TK-NOG mice. Each line represents a single animal. 1-4, males. 5-6, females. Human albumin levels correlate with level of human chimerism (Hasegawa et al., 2011).
Humanized liver mice

Detection of human hepatocytes within the chimeric TK-NOG liver. **Left:** H&E staining of liver sections confirms the presence of human cells, which are less eosinophilic than mouse hepatocytes and appear pale in comparison (arrow). **Right:** immunohistochemistry for human albumin (brown staining).
Example *in vivo* hepatotoxicity data (non-humanized rats): clinical pathology

**Clinical pathology in animals treated with known hepatotoxins - CCl4 and ANIT**

Alpha-naphthyl isothiocyanate/ANIT study (right): 75mg/kg dosed IP on days 0, 1, 2 (3 doses, analysis 48 hours post-dose). Blood collection via cardiac puncture for clinical pathology on day 3. Liver extracted, fixed, and processed for histopathology on day 3.

CCl4 study (left): 5ml/kg dosed PO on days 0, 1 (2 doses, analysis 48 hours post-dose). Blood collection via cardiac puncture for clinical pathology on day 3. Liver extracted, fixed, and processed for histopathology on day 3.
Example *in vivo* hepatotoxicity data (non-humanized rats): histopathology

**CCl₄-treated**

- Cytoplasmic vacuolation, centrilobular fibrosis, mononuclear cell infiltration

**ANIT-treated**

- Portal changes: bile duct epithelial hypertrophy and hyperplasia; inflammatory cell infiltration; increased mitotic activity
Additional technical skills for toxicity studies

- Blood collection from live animals:
  - Tail vein
  - Jugular vein
  - Saphenous vein
  - Facial vein

- Urine collection

- Traditional serum chemistry panels for clinical pathology

- Full necropsy of all organs, including images if desired

- Fixation or flash freezing of organs

- Histopathology, immunohistochemistry
Transgenes and virus can be delivered to humanized liver or wild type mice & rats

- Disease model creation
- *In vivo* gene delivery efficacy testing
- Therapeutic gene efficacy screening
- Gene therapy toxicology screening
Humanized rodent models in the pipeline

- Humanized liver models allow for the detection of human specific liver toxicity and metabolism studies
- Humanized liver mouse models have been successfully generated at Hera and services are available
- Humanized liver rat models are under development and expected in late 2017

- Humanized immune system models are utilized for immuno-oncology studies & where the test article interacts with the immune system
- Humanized immune system mice are commercially available for studies at Hera
- Humanized immune system rats are under development and expected in 2018
Hera’s facility and scientific team

- Dosing by multiple routes including i.p., oral and i.v.
- ACLAM and AALAS certified staff
- Digital data acquisition via StudyLog® systems software

- Vivarium featuring all 100% HEPA filtered, disposable IVC caging specifically designed for immunocompromised animals
- Molecular and cell culture facility
Hera has the freedom to operate through multiple licenses to issued and pending patents for CRISPR, piggyBac and TALEN gene editing technologies.

Hera’s SCID rats are covered under issued patents claiming knockout rat phenotypes for immune system disorders, SCID and cancer. US Patent Numbers: 8,558,055; 9,314,005; 8,722,964.
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.

Contact Us: services@herabiolabs.com 859-414-0648
2277 Thunderstick Dr. #500 Lexington, KY 40505