

What is Precision Toxicology™?

Generating high-confidence, clinically-relevant toxicology profiles with greater predictability by utilizing precisely gene-edited models such as humanized rodents and engineered cell lines.

Why Precision Toxicology™?

The pharmaceutical and chemical industry, as well as regulatory bodies such as the FDA and EPA which are parties to Tox21, are looking to modernize toxicology testing by encouraging the development of alternative models that are mechanism based, higher through-put and of greater clinical relevance. Our mission is to use our genetic engineering expertise to provide innovative, more predictive toxicology and discovery models and services:

In vitro – Reporter and disease model cell lines focused on neurotoxicity, hepatotoxicity and off-target hormonal effects with greater sensitivity and accuracy in ultra high-throughput assays.

In vivo – ‘Humanized’ rodent models (i.e. humanized liver, immune system) with improved predictability in hepatotoxicity, cancer xenograft (PDX models), DDI, and immunogenicity testing.

Common hepatic (HepaRG®, HepG2), neuronal (SH-SY5Y), epithelial (MDCK) and pluripotent (iPS and ES) cell lines used in toxicology, are now amenable to genome engineering (1, 2, 3). For in vivo studies, the rat, a favored platform for toxicology, is being engineered to model human conditions more closely (4).

Whether creating animal models or cellular assays, it is important to have the correct research tool. Below is an overview of some of the types of Precision Toxicology™ models one can make and when these models are typically used.

Reporter cell lines – contain reporter genes (e.g. luciferase) under the control of an endogenous regulatory sequence (promoter) or targeted within the endogenous gene of interest. Different regulatory sequences or targeted genes are chosen to detect genetic expression involved in the process or pathway of interest (i.e. toxicity). Reporter cell lines can be used to screen for toxicity by “tagging” a gene that is known to be activated during a toxicity event with a reporter gene such as luciferase. A Precision Toxicology™ reporter cell line created in SH-SY5Y cells was developed using a proprietary neurotoxicity gene as described in Figure 2.

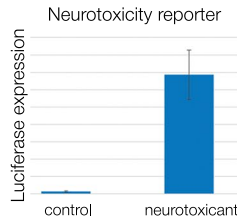


Figure 2: Creation and validation of neurotoxicity reporter cell lines. In searching for improvements to the current screening methodologies for neurotoxicant compounds, 96 genes expressed in neurons or their progenitors, were screened to identify genes most consistently upregulated after exposure to a number of toxicants. We have utilized gene editing to insert two different reporters under the control of neurotoxicant-responsive endogenous sequences. Following model creation we have validated the potential of this system for a cost-effective, high-throughput assay for neurotoxicity screening.

Disease or patient specific models – these cells can be derived from patient populations, in which case correcting the mutation associated with the disease or phenotype via gene editing is required to confirm the mutation being studied is responsible for the phenotype, and not a background mutation. On the other hand, one could use a well characterized cell line and create the mutation associated with the disease in this cell line. Either way the research model and its isogenic companion are required for accurate discovery and toxicology assays. Cellular assays using patient specific cells can be used for toxicity and efficacy screens, and can even help screen patients for enriched clinical trials (5).

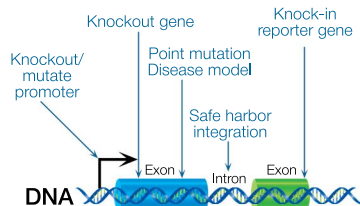
Choose your:

1. System

- ☞ Disease models
- ☞ Reporter cell lines
- ☞ Humanized rodents
- ☞ Cancer xenografts



2. Precision gene edit



3. Assay

- ☞ CYP induction/disruption
- ☞ Phase II metabolism enzymes
- ☞ Nuclear receptors
- ☞ Neurotoxicity
- ☞ Drug transport & DDI

Figure 1: Precision Toxicology™ in three simple steps from off the shelf services to custom solutions: 1-choose the desired in vitro or in vivo system. 2- select or develop an appropriate model system using precise gene editing. 3- run your assay and generate more clinically-relevant toxicology profiles. Some examples are depicted in the figure but the opportunities are limitless.

Humanized rodents – There are two ways an animal model can be ‘humanized’ 1 – by replacing the endogenous rodent gene with a human gene and 2 – ablating endogenous cells or tissues (i.e. hepatocytes) and replacing them with engrafted human counterparts. Both methods require genome engineering. In the later, an immunocompromised rodent background is required so that the animal will not reject the exogenous human cells. By creating the humanized rodent, the physiology becomes more relevant to human studies; examples include how the animal responds to drug toxicity and metabolism.

Humanized liver mice have demonstrated proper human specific hepatotoxicity when traditional animal models did not (6). Humanized immune system rodents provide an in vivo model of human disease for tumor immunology, infectious disease, and autoimmunity, that promises to overcome the limitations of traditional animal models and boost the translation rate from target identification to the clinic (7). Humanized rodent models may also be ideal for studying diabetes due to the inaccessibility of the human pancreas and the inability to analyze the interaction of immune cells with islets in vivo (8). Xenografts in immunodeficient rats and mice of patient derived cells or primary tumor tissues (PDX, PDTT, or PDTX) provide a better model that overcomes the limitations of traditional in vivo models and more sufficiently represent human cancer characteristics especially with regard to metastasis and drug sensitivity. Patient derived xenografts are being used to provide results with better translational potential that have eluded researchers using traditional rodent models (9).

While the use of humanized mice has transformed pre-clinical approaches to screen for human specific toxicity, toxicity studies often require multiple dosing by routes other than oral (e.g., intravenous administration once daily for 28 days), and collection of multiple blood samples at different time points, which is extremely challenging in mice. Rats are easier to surgically manipulate, and their larger tissue/greater blood volume allows for more analyses with each collected sample. Thus, rat size is appropriate for multiple dosing, particularly by routes other than oral, and for collection of multiple blood samples, which will enable investigators to assess systemic toxicity in a single animal over time.

Knockout models – contain mutations that make one or more genes inoperative or null. Knockout rodents and in vitro assays are sometimes used for functional toxicology as well as synthetic lethality screening. Functional toxicology is a mechanistically-based high throughput method used to assess genetic requirements for chemical tolerance and provides a direct link between genes and cellular toxicant response (10).

Synthetic lethality is when a combination of mutations in two or more genes leads to cell death, whereas a mutation in only one of these genes does not, and by itself is said to be viable. In a synthetic lethal genetic screen, it is necessary to begin with a mutation that does not kill the cell, although may confer a phenotype (for example, slow growth), and then systematically test other mutations at additional loci to determine which confer lethality. Synthetic lethality indicates functional relationships between genes (11).

In summary, modern genetic engineering can be used to create more relevant and precise models for toxicological assessment. Hera BioLabs is the first company to focus exclusively on the development and application of in vivo and in vitro Precision Toxicology™ models born of gene editing. Our goal is to develop and run the precise assay you need to meet your projects specific goals.

- Huang et al. (2015) Development of an optimized cytotoxicity assay system for CYP3A4-mediated metabolic activation via modified piggyBac transposition. *Toxicology In Vitro*.
- Milosch et al. (2014) Holo-APP and G-protein-mediated signaling are required for sAPP-induced activation of the Akt survival pathway. *Cell Death Dis*.
- Xie et al (2014) Seamless gene correction of-thalassemia mutations in patient-specific iPSCs using CRISPR/Cas9 and piggyBac. *Genome Res*.
- Van Boxtel et al (2011) Homozygous and Heterozygous p53 Knockout Rats Develop Metastasizing Sarcomas with High Frequency. *Am J Pathol*.
- Anson et al. (2011) Opportunities for Human iPSCs in Predictive Toxicology. *Clin Pharmacol Ther*.
- Xu et al. (2014) Fialuridine Induces Acute Liver Failure in Chimeric TKNOG Mice: A Model for Detecting Hepatic Drug Toxicity Prior to Human Testing. *Plos Med*.
- Zhao et al (2015) Humanized Mice Reveal Differential Immunogenicity of Cells Derived from Autologous Induced Pluripotent Stem Cells. *Cell Stem Cell*.
- Greiner et al (2011) Humanized mice for the study of type 1 and type 2 diabetes. *Ann AC NY Sci*.
- Nofiele, et al. (2014) Establishment of a Lung Metastatic Breast Tumor Xenograft Model in Nude Rats. *PLoS ONE*.
- Gaytan et al (2014) Functional toxicology: tools to advance the future of toxicity testing. *Frontiers in Genetics*.
- Tucker et al. (Nov 2003). Lethal combinations. *Nat Genet*.

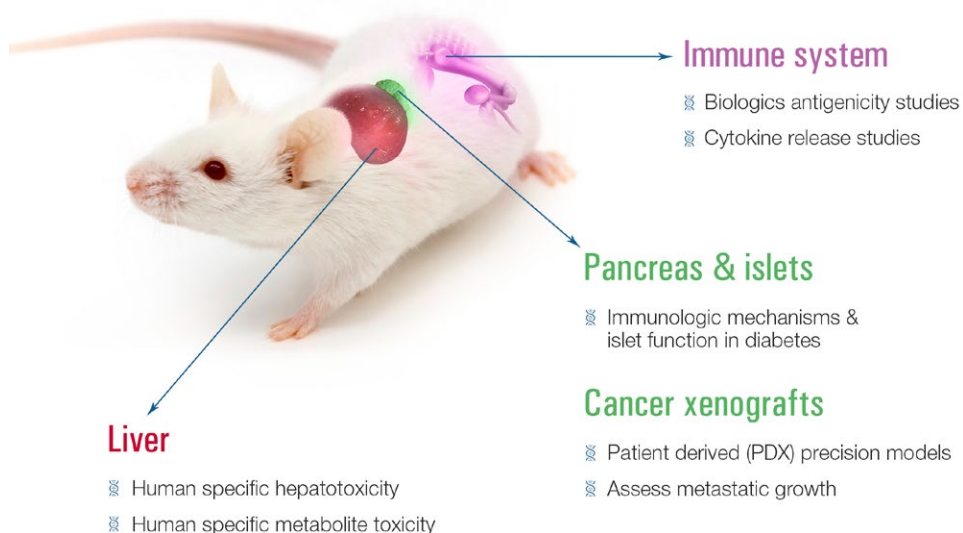


Figure 3. Overview of the humanization possibilities and applications on a fully immunocompromised rodent model.