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INVIVOCUE

Welala

ABOUT US

Set up in 2018, Invivocue is a bioscience company that aims to support medical and pharmaceutical research through its humanized rodent models. Invivocue was formed as a joint venture between Invitrocue, an innovative life sciences company providing *in vitro* liver models for drug toxicology testing as well as 3D patient-derived organoid technology for personalized oncology, and Dr Chen Qing Feng, a Principal Investigator at the Institute of Molecular and Cell Biology, whose research expertise focuses on humanized mouse models for various disease applications.

Invivocue's technologies are built upon decades of research conducted at national research institutes, organisations and industries in Singapore. Our animal models have enabled research in areas such as oncology, liver disease, auto-immune and infectious diseases. This research has been published in numerous well-regarded journals, including *Proceedings of the National Academy of Sciences*, *Gut* and *Blood*.

Our vision is to develop and provide a simpler, better and more affordable humanized mice model for drug efficacy and safety assessment.

INVIVOCUE'S PRODUCTS

Immunotherapy has been recognised as a promising tool for treatment of cancer, immune disorders and various infectious diseases. Due to missing of human-specific immune targets and genetic complexity of the human diseases, the capability of traditional murine models in recapitulating the human immune microenvironment and responses remains as the major hindrance in immunotherapeutic drug efficacy and immunogenicity evaluation.

Since 2018, Invivocue has run over 20 projects and generated over 1000 humanized mice annually to address the needs for humanized mice in immunotherapeutic research. These propriety humanization technology has unleashed the huge potential in changing the way of developing and understanding the interaction of immune cells with the host disease in an in-vivo approach. Importantly, it helps to test the drugs in a clinically relevant setting, which can be ultimately lead to better success in human trials.

Types of Humanized Mice available at Invivocue

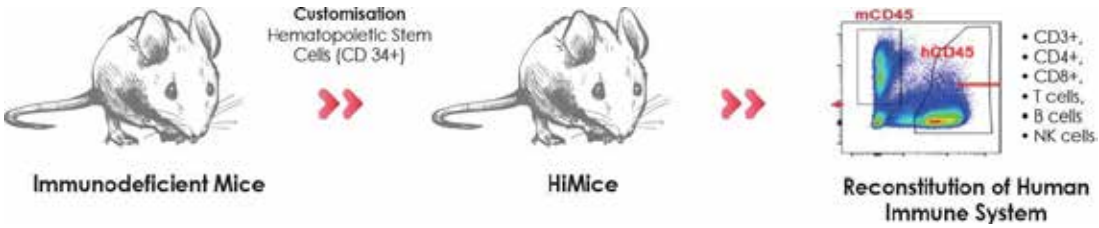
- Human Immune System Mice (HiMice)
- Human Liver Chimeric Mice (HepMice)
- Dual Humanized Mice

Types of disease models available at Invivocue

- Cancer (CDX and PDX)
- Graft vs Host Disease (GvHD)
- Acute Respiratory Distress Syndrome (ARDS)
- Inflammatory Bowel Disease (IBD)
- Non-alcoholic Steatohepatitis (NASH)
- Systemic Lupus Erythematosus (SLE)
- Immunotoxicity
- Custom research

HUMAN IMMUNE SYSTEM MICE (HIMICE)

Invivocue's proprietary HiMice are a humanized mouse model with a stably reconstituted human immune system. HiMice are constructed by engrafting human CD34+ hematopoietic stem cells derived from human cord blood into immunodeficient (NOD/SCID/IL2Rγ-/-) mice. Our HiMice stably express multiple human cell lineages in the blood circulation and organs, including the spleen, thymus, bone marrow, liver, lung and intestine after 12-16 weeks post HSC engraftment.



Advantages of Humanized Mice available at Invivocue

01 Quality
Invivocue guarantees that every single animal model is rich with human CD45+ cell lineages in peripheral blood and organs.

02 Known HLA typing
Leveraging strategic partnerships with leading cord blood banks in the region, Invivocue has built up an extensive biobank with known HLA-typing information. This allows researchers to validate their drug candidates on a sufficiently diverse pool of HLA types for donor-matched personalised therapy.

03 Consistency
Due to our unique stem cell manipulation techniques, Invivocue is able to produce over 100 mice from a single cord blood donor. This ensures that research can be repeated numerous times on HiMice with the same HLA type.

04 Customisation
HiMice can be further modified through cytokine injections in order to present specific features (See Customisation options on Page 6 for more details).

05 Customer Service
Invivocue assists customers to design and optimise research/ drug testing plans on specific disease models to meet specific requirements based on over 10 years of experience working on human disease model development.

CELL LINEAGE RECONSTITUTION

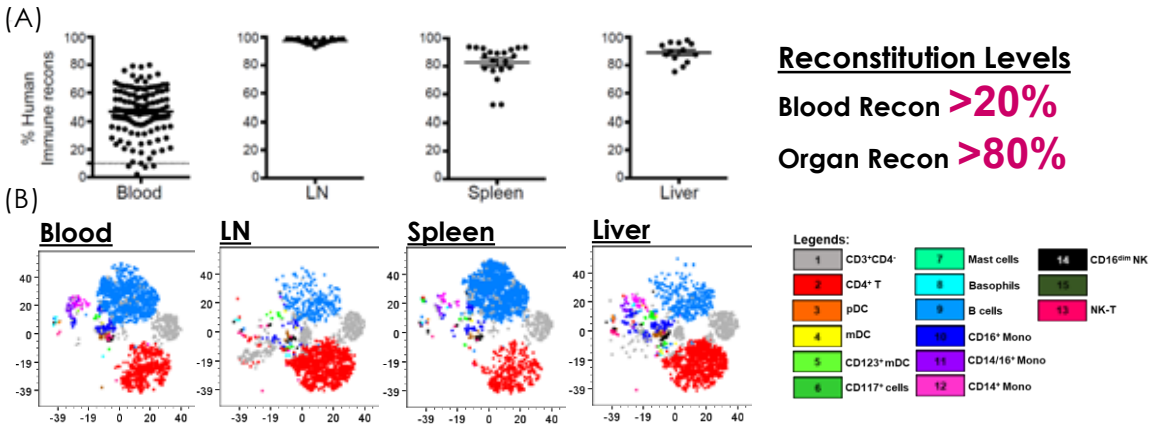
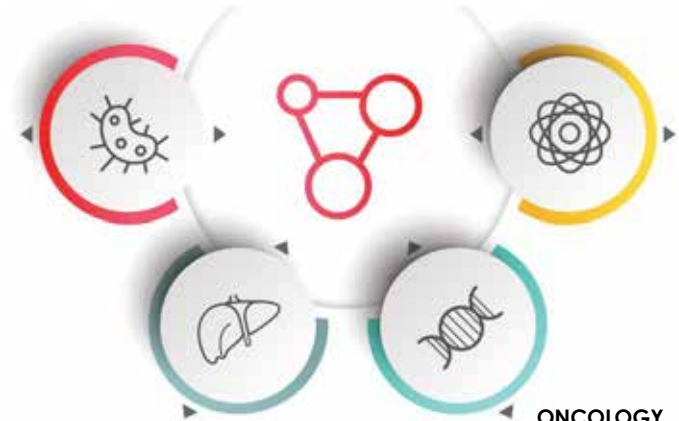


Figure 1. (A) Flow cytometry analysis of human cell lineages of HiMice. Blood samples were taken from 12 weeks old HiMice and stained with mouse CD45, human CD45 (lymphocyte common antigen), human CD56 (NK cells), human CD3 (T cells), human CD4 (Th cells), human CD8 (Tc cells), human CD19 (B cells), human CD14 (monocytes MNC) and many other cell types. (B) Human organ residential Immune cells in HiMice. Source: Merry et al. 2017. Scientific reports vol. 7,1 16642.

APPLICATIONS OF HIMICE

As HiMice express the human immune system, they are an ideal in vivo animal model for research in human immunology, oncology, inflammation and infectious diseases. Leveraging upon its research and technology expertise, Invivocue also operates as a niche CRO providing services in four key areas:



INFECTIOUS DISEASES
Dengue, Malaria, EBV, EV71, SARS-COV-2, Influenza virus

AUTOIMMUNE DISEASE & IMMUNOTOXICITY
Systemic Lupus Erythematosus (SLE)

LIVER DISEASES
Hepatitis B & C
NAFLD & NASH

ONCOLOGY
Cell line derived Xenograft (CDX), Patient derived Xenograft (PDX), Immunotherapy Efficacy Evaluation

PUBLICATIONS

1. Chen et al. **PNAS** 2009; 106 (51): 21783-21788.
2. Zhao et al. **Blood** 2017; 130(18):1995-2005.
3. Li et al. **J. Immunol.** 2013; 191(6):3192-9.
4. Zhao et al. **Gut** 2018; 67(10):1845-1854.
5. Gunawan et al. **Scientific Reports** 2017; 1644:1-11.
6. Chen et al. **J. Immunol.** 2012;189(11):5223-9.

HYDRODYNAMIC INJECTION OF CYTOKINE

Reconstitution of some human immune cells is low due to absence of **important human cytokines** which are essential for **immune cells differentiation**. Invivocue's co-founder, Dr Chen Qing Feng, invented a safe, effective and stable method of improving the human cytokine expression and reconstituting human immune cells in HiMice, known as the Hydrodynamic injection of human cytokines through the tail vein. The types of cytokines and their resulting functions are shown below :

CYTOKINES AND FUNCTIONS

- **IL-15** — T- and NK cell differentiation and proliferation
- **GM-CSF** — Granulocyte, monocyte/ macrophage and eosinophil differentiation
- **IL-3** — Supporting the proliferation of a broad range of hematopoietic cell types

ADVANTAGES OF HYDRODYNAMIC INJECTION

- **Adaptable** — Dose, cytokine combinations and timing of injection can be customized.
- **Physiological** — The expression of cytokines is transient. Cytokine DNA delivers at adult stage upon confirmation of humanization when all the key immune cells have been developed.
- **Normal Lifespan** — Do not compromise the health and lifespan of HiMice.

GM-CSF/IL-3 Supplementation

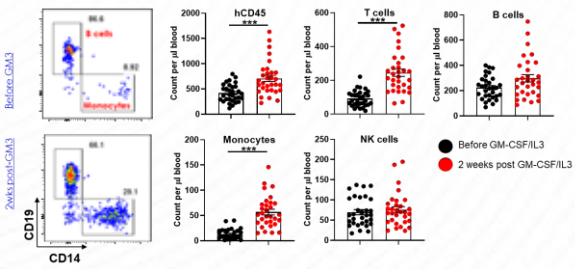


Figure 2. GM-CSF/IL-3 supplementation significantly Improves the reconstitution of immune cells including T-cells and monocytes in adult HiMice. Data is presented as mean absolute number per microliters of blood from 47 mice generated by same donor.

IL-15 Supplementation

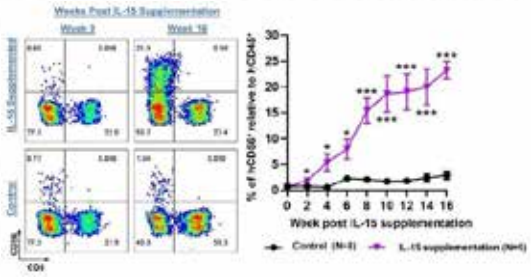
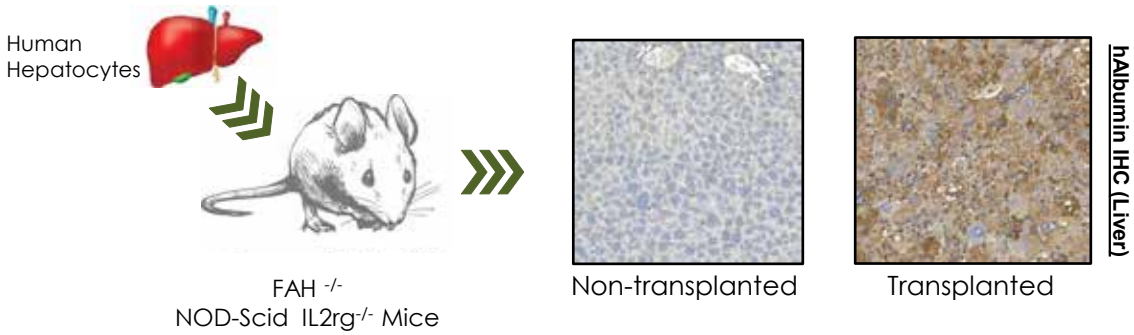


Figure 3. IL-15 supplementation starts to increase the percentage of CD56⁺ NK significantly 2 weeks after IL-15 supplementation and last more than 16 weeks.

HUMAN LIVER CHIMERIC MICE (HEPMICE)

Human liver chimeric mice (HepMice) is a mouse model with resident human hepatocytes within mice liver organ . This mice are designed by engrafting human adult hepatocytes from certified donors into immunodeficient FAH knock-out mice (Fah$^{-/-}$ NOD-SCID-IL2rg$^{-/-}$). Repetitive cycles of liver injury and repair eventually leads to replacement of mouse hepatocytes with human hepatocytes. High level of human hepatocytes repopulations were reported in the liver of HepMice. This model has huge potential in drug metabolism studies and efficacy studies for fatty liver, Hepatitis viral infection, malaria and for hepatotoxicity studies.



Model Validation

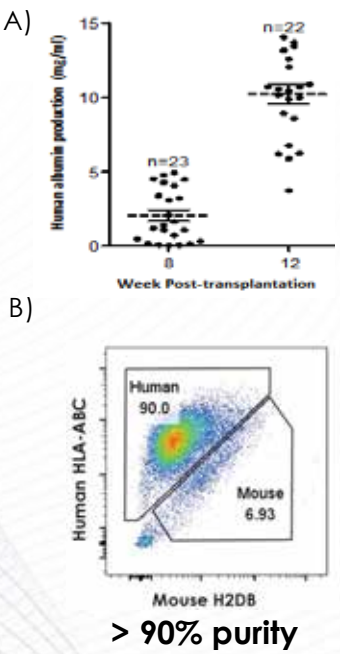
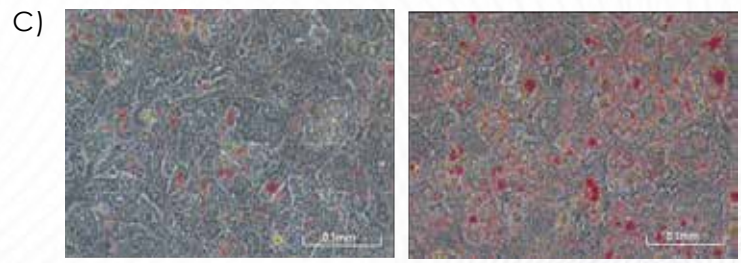
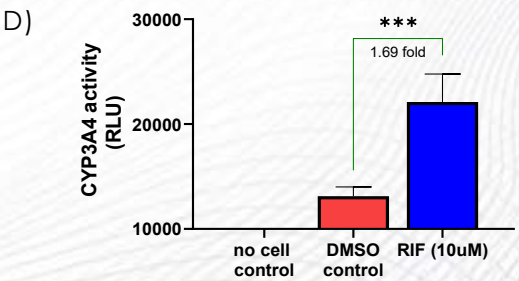


Figure 4. A) Human albumin detected in serum after 8-12 weeks of hepatocyte transplantation. B) Purity of human hepatocytes isolated from HepMice. C) Intracellular lipid accumulation (Oil Red O stained in red) in humanized hepatocytes after 24hours of Oleic Acid treatment. D) CYP3A4 induction in humanized hepatocytes after Rifampicin stimulation





DUAL HUMANIZED MICE

Many pathogenesis of human liver diseases involve both human liver and liver-resident immune system, such as hepatitis B, C viral infection, Non-Alcoholic Steatohepatitis (NASH) and cirrhosis. At Invivocue, we understand the need for a more robust rodent model with double humanization possessing stably engrafted human immune system and hepatocytes for preclinical research in animals.

In this model, human CD34+ hematopoietic stem cells and primary human hepatocytes were transplanted consecutively into an immunodeficient FAH knock-out mice with NOD-Scid IL2rg$^{-/-}$ background. After 18-20 weeks of reconstitution, dual humanized mice are found to possess high levels of human liver repopulation and hCD45+ lymphocytes.

Model Validation

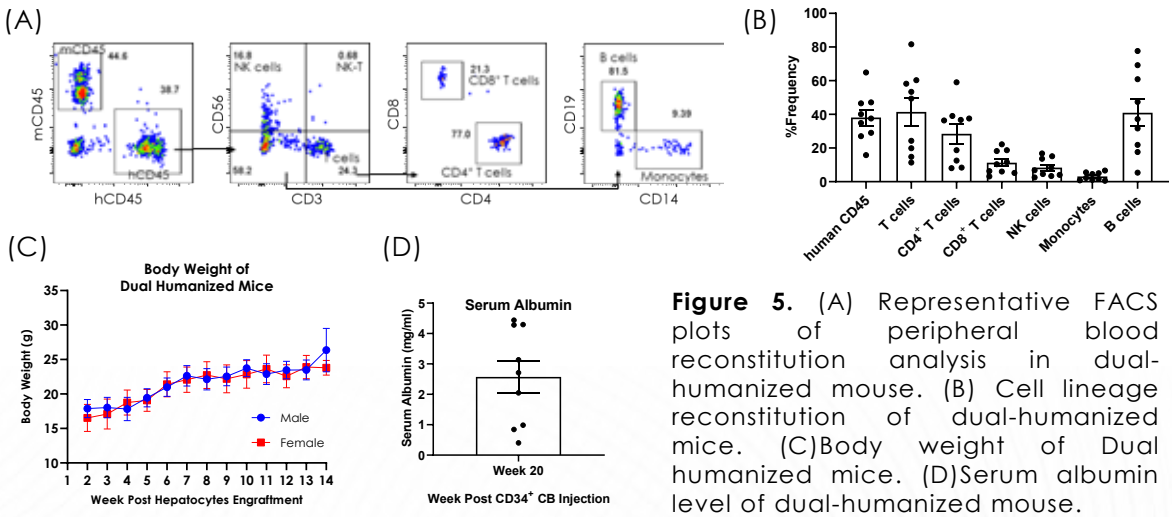


Figure 5. (A) Representative FACS plots of peripheral blood reconstitution analysis in dual-humanized mouse. (B) Cell lineage reconstitution of dual-humanized mice. (C)Body weight of Dual humanized mice. (D)Serum albumin level of dual-humanized mouse.

Key Features of Dual Humanized Mice

01 Functionality

Allows hepatitis viral infection, liver disease modelling and drug efficacy evaluation

02 Quality

Reconstitution of hCD45+ cell lineages and human hepatocytes repopulation in individual mice are quality assured.

03 Scalability

100 mice can be generated from single batch of CD34+ HSC and hepatocytes

04 Flexibility

HLA and gender selection are allowed to match the study requirements

05 Ready to use

No additional charge for use in profitable research and services

Potential Applications of Humanized Mice

IMMUNO-ONCOLOGY

Recapitulation of human immune-tumour microenvironment in HiMice model serves as the most advanced and promising platform for immunotherapeutic drug efficacy and safety assessment. In Invivocue, we establish the tumor models using **Patient-derived (PDX or PDO-X)** or **Cell Line- derived Xenograft (CDX).** It has been scientifically proven that human tumor growth in HiMice evolves significantly in the presence of human immune system and provides notable expression of immune checkpoints compared to conventional nude, immunodeficient and syngeneic mouse model. Importantly, the research outcomes provide a better representation of the clinical conditions.

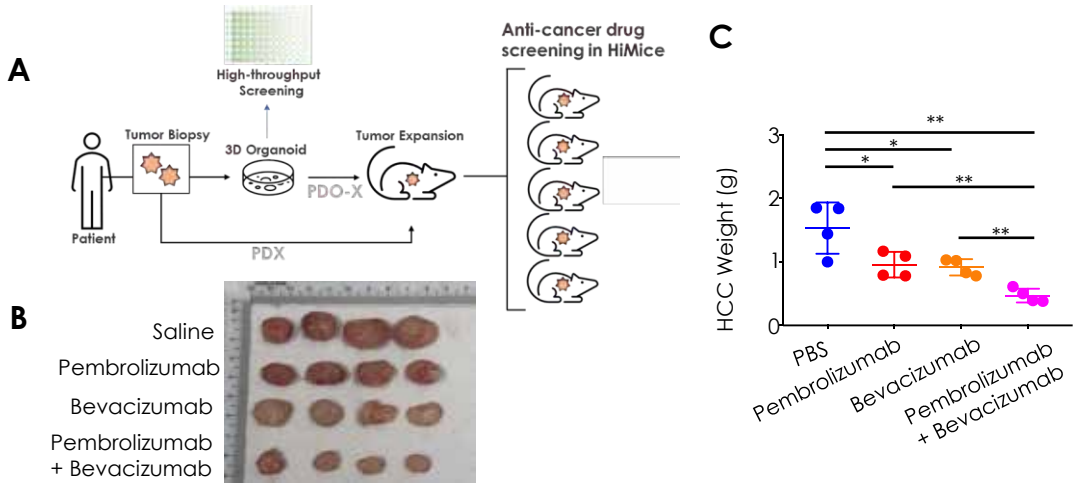
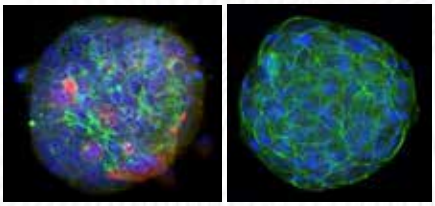


Figure 6.(A) Establishment of PDX using Patient tumor biopsy or patient derived Organoid (PDO) in HiMice. (B) PDX from HiMice showed positive response to the anti-cancer drug treatment such as Pembrolizumab (PD-1 inhibitor), Bevacizumab (anti-VEGF antibody) and combinational therapy. (C) Tumor weight reduced significantly after anti-cancer drug treatment (adapted from Zhao et al. Hepatology 2021; Vol 74, No 3: 1395-1410).

IN VITRO CO-CULTURE OF TIF AND TUMOR CELLS

Monoculture of tumor cell is poor in recapitulating the tumor microenvironment in vitro. From CDX, PDX or PDO-X, Tumor Infiltrating Lymphocyte (TIL) which contribute mainly to anti-tumor immune response are isolated. These cells are then used in an in-vitro high-throughput co- culture system with tumor cells (either cancer cell lines or PDO's). Such co-culture system established significantly improves in-vitro preliminary testing capability for infiltration, activation and function of immune cells toward human tumors.



Colorectal Cancer PDO | Breast Cancer PDO

Images credit: Invitrocue Pte Ltd

SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

SLE is a chronic autoimmune disorder, characterized by numerous anti-nuclear autoantibodies, series of systemic and organs inflammation and lymphopenia as a consequence of impaired immune system. At invivocue, we have successfully recapitulated most of the hallmark of clinical and immunological features of SLE by single pristane induction in **HiMice**. Cytokines, autoantibodies and T-B cells responses in this SLE model are human-specific. Hence, it provides an exclusive in-vivo platform for the study of disease pathogenesis, as well as immunotherapeutic target identification and drug evaluation.

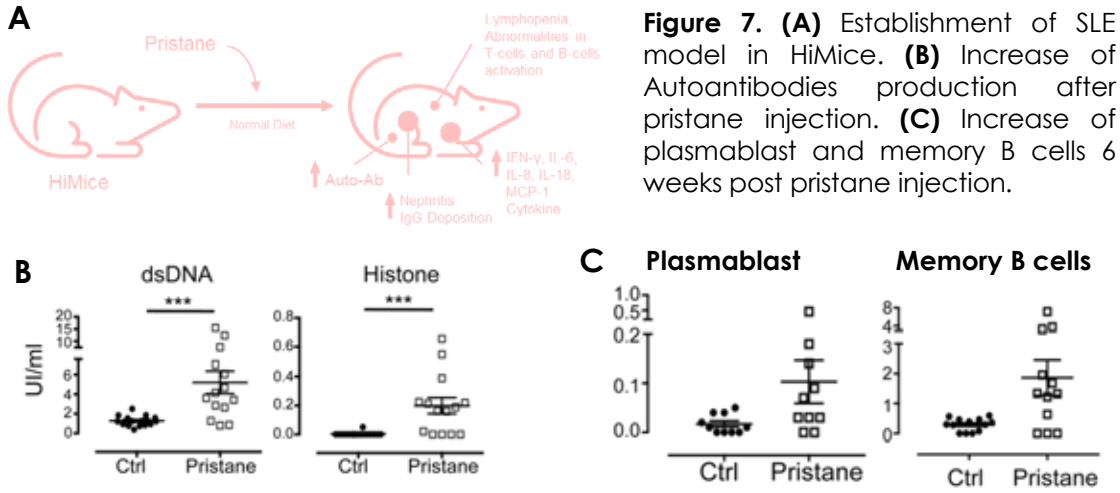


Figure 7. (A) Establishment of SLE model in HiMice. **(B)** Increase of Autoantibodies production after pristane injection. **(C)** Increase of plasmablast and memory B cells 6 weeks post pristane injection.

NAFLD AND NASH

Non-alcoholic Fatty Liver Disease (NAFLD) is one of the common metabolic disorders with excessive lipid accumulation in hepatocytes. It may progress into severe liver fibrosis and cancerous stage as a result of persistent inflammation, called Non-alcoholic Steatohepatitis (NASH). Traditional NAFLD/NASH models are usually genetically modified or chemically induced and lack relevant human liver or immune correlation. In contrast, we have successfully recapitulated the NAFLD features using only High-Fat High-Carbohydrate (HFHC) diet in **HiMice or HepMice**. Interaction between immune cells and hepatocytes during the NASH progression could be studied intensively using **Dual Humanized Mice.** This dual chimeric model has advantages over others in new immunotherapeutic target identification and anti-fibrotic drug evaluation.

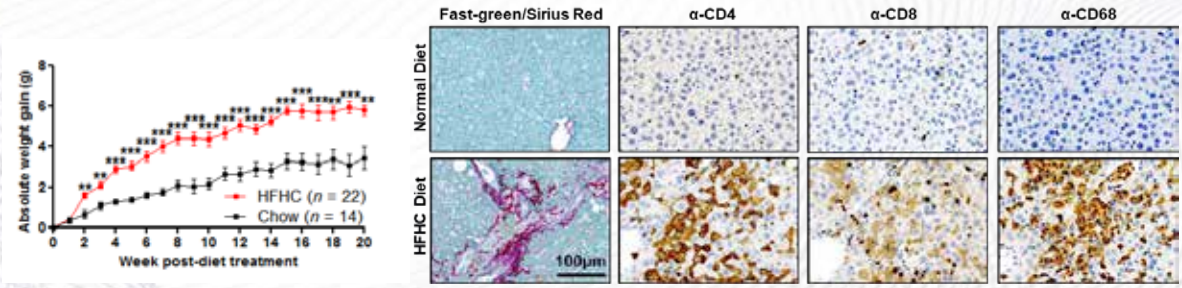


Figure 8. HiMice increased body weight substantially post 20 weeks of HFHC diet. Immunohistochemistry staining on the HiMice with HFHC diet also showed Increased intrahepatic infiltration of CD4+ T-, CD8+ T cells and CD68 monocytes into fibrotic region of liver. (Adapted from Her et al. 2020. Front. Immunol. 11:580968)

HEPATITIS VIRAL INFECTION

HepMice has been demonstrated as a promising alternative in-vivo model to transgenic mice, and are highly susceptible to patient-derived hepatitis B and C viral infection due to the stable engraftment of primary human hepatocytes in the mouse liver. This model has provided a valuable insight to the viremia study. However, the immune response induced by hepatitis virus infection is still lacking. To circumvent this, we have combined the human hepatocytes and immune system in a dual chimeric approach. This **dual humanized mice** model not only allows the persistent viral infection, but also acts as a unique platform for studying the immunopathogenesis of Hepatitis viral infection such as liver fibrosis, cirrhosis, and investigating the potential immunotherapies for viral clearance.

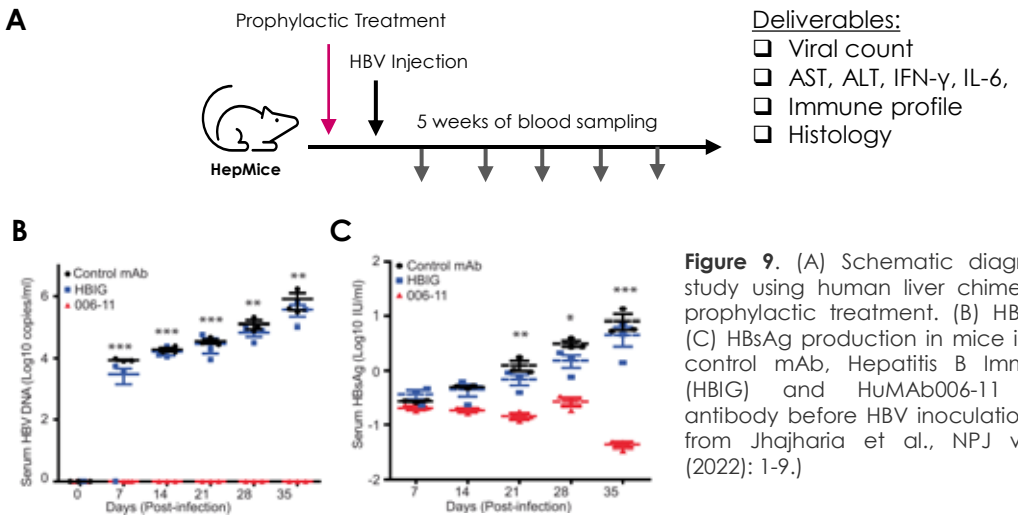


Figure 9. (A) Schematic diagram of HBV study using human liver chimeric mice for prophylactic treatment. (B) HBV DNA and (C) HBsAg production in mice injected with control mAb, Hepatitis B Immunoglobulin (HBIG) and HuMAb006-11 neutralizing antibody before HBV inoculation. (adapted from Jhajharia et al., NPJ vaccines 7.1 (2022): 1-9.)

DMPK AND HEPATOXICITY

Liver is known to be the primary organ responsible for xenobiotic metabolism, pharmacokinetics and prodrug activation. Whether it's **HepMice** or isolated human hepatocytes from HepMice, it has become an attractive and capable translational tool to predict the clinical xenobiotic profile for preclinical validation of a new drug. Our recent study showed that HepMice demonstrated high levels of human chimeric (>70% human hepatocyte), human albumin and human-specific cytochrome P450 (CYP) expression which were comparable to donor liver profile. Exposure of isolated hepatocytes to Rifampicin (a CYP3A4 inducer) resulted in significant increase of CYP3A4 enzymatic activity, thereby proving that hepatocytes or liver microsomes are applicable in drug-drug interaction, drug metabolism and drug-induced liver injury (DILI) assays for new drug investigation.

To know more about our animal models and services, please visit our website www.invivocue.com or contact our technical experts at contact@invivocue.com

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