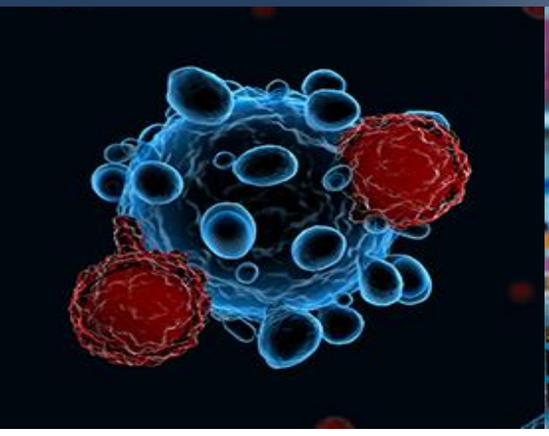
INVITROCUE®
Transforming bioanalytics

Preclinical Pharmacology Services



www.invitrocue.com | Unit 612, Biotech Center 2, 11W Science Park West Avenue, Shatin, N. T., Hong Kong | contact.hk@invitrocue.com

INVITROCUE®
Transforming bioanalytics

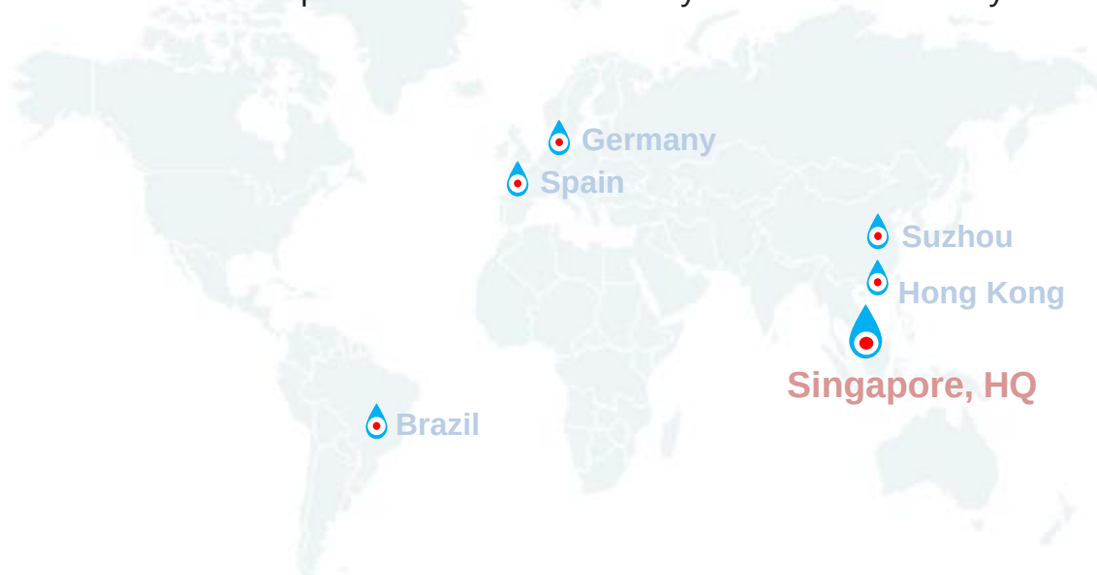
Our Vision is to develop predictive and comprehensive translational in vitro and in vivo models to healthcare and pharmaceutical industry to create better, faster and more affordable disease treatments for a healthier society.

Our Story

Founded in 2012, Invitrocue is Singapore-based innovative life sciences company focusing on commercializing a series of cell-based technologies developed from Singapore's Agency for Science, Technology and Research (A*STAR) Institute, Singapore. These technologies and know-how have since been successfully developed into multiple commercial services in different research areas such oncology, respiratory, liver and immunology.

To further strengthen our pipeline in oncology, Invitrocue has co-developed a clinical services called Onco-PDO® test in 2016 with Genome Institute of Singapore (GIS) for personalized treatment decision making. In 2018, Invitrocue formed Invivocue to commercialize an advanced in vivo humanized mice model called HiMice for various disease applications.

Invitrocue strives to be the best-in-class resource center for biomedical researchers and industry partners with the superior technical platform, and established a good reputation within the biomedical industry powered by our dedicated and talented scientific team. We proudly work together with researchers from world-renowned academic institute across the world as well as pharmaceutical or nutraceutical companies both domestically and internationally.



INVITROCUE®
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PRECLINICAL PRODUCTS AND SERVICES

AT INVITROCUE, we develop in vitro and in vivo models that improve the efficacy and safety evaluation for new drugs for biotechnology, pharmaceutical and consumer healthcare companies. Our expertise in developing predictive preclinical models helps to reduce the time and cost of product development and minimize the risks of downstream testing.

01 Immunity

- Phagocytosis
- T and B Cell Proliferation
- NK Cytotoxicity
- ROS Inhibition
- Cytokine Release

02 Cell Metabolism

- Fat Uptake
- Cellular Energy
- Glucose Uptake
- Fatty acid, Glucose & Glutamine Oxidation

03 3D Cell Models

- 3D Airway Model
- 3D Hepatocyte and Co-culture Model
- Skin Model
- Cardiovascular

04 Anti-cancer

- Patient-derived Organoid (PDO)
  - Breast, Colorectal, Lung, Pancreatic, Ovarian

05 Neuroscience

- Brain Signaling Pathway Analysis
- Reporter Assay for GPCR Binding analysis
- Neurological Disease Models

06 Customized

- GLP - DMPK/Toxicity
- Anti-aging
- Immune and Cytokine Profiling
- Histology – H&E and IHC

CONTACT US
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IN VITRO IMMUNOLOGY ASSAYS

Human immune defence system is made up of 2 major arms: Innate Immunity and adaptive immunity. In vitro and In vivo Immunology studies are commonly conducted for investigating the immunomodulatory effects of test article in promoting body immunity and assessing the undesirable immunotoxicity. We offer a wide selection of validated immunology assays for therapeutic drugs and natural products screening.

- **Macrophage Differentiation**
- **Macrophage Phagocytosis**
- **ROS/superoxide Inhibition**
- **Immune-hepatocyte Co-culture Model**
- **T- and B-cell Proliferation and Activation**
- **Cytokine/Chemokine Release**
- **Antibody (IgG/IgM) Production**
- **Natural Killer (NK) Cytotoxic Activity**

In innate Immunity, chemical defends such as **Interferons** and **Interleukin-1** and cellular defends such as **Natural Killer Cells**, **Mast Cells** and **Phagocytes** are plays important role as first line of immune defence in order to eliminate and prevent the spread of pathogens.

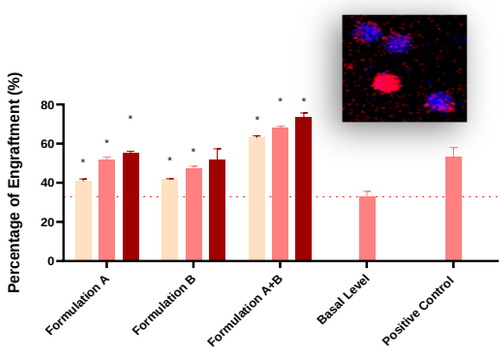


Fig 1: Phagocytic activity of Macrophage

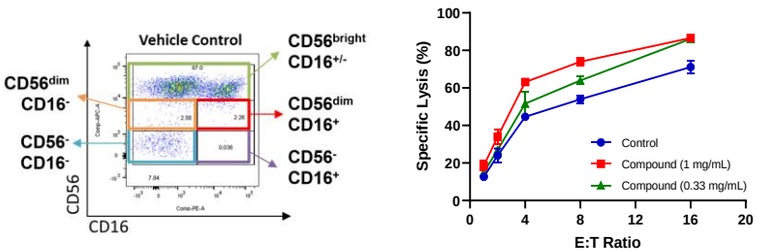


Fig 2: Proliferation of NK cells subsets and specific killing of NK cells.

If innate immunity fails to defend the threats, adaptive immunity will be activated via cell-mediated immune response which is mainly driven by mature **T-cells** and secretion of **cytokine**, and the humoral immune response which produces antigen-specific antibodies and is primarily controlled by **B-cells**.

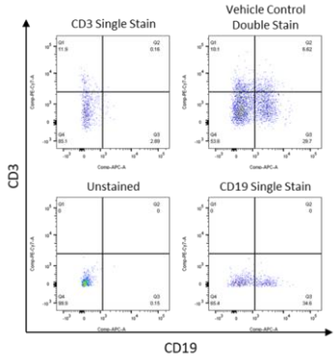


Fig 3: Flow cytometry analysis of CD3+ T-cells and CB19+ B-cells in Human PBMCs.

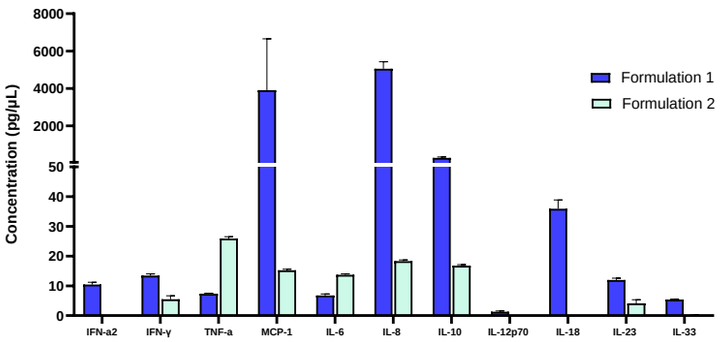


Fig 4: Proinflammatory cytokine release after 6 hours of treatment with different drug formulations.

IN VITRO CELL METABOLISM

We utilize comprehensive cellular metabolic assays to carry out detailed analysis of cellular energy demand, mitochondrial metabolic status and cellular capability to metabolize various energy substrate in response to different stimuli. This is essential when trying to understand the impact of drugs, nutraceutical products and healthy foods on cell metabolism.

Cell Based Immunoassays

- **Cellular Energy Demand**
- **Mitochondrial Metabolism**
- **Fat/Glucose/Glutamine Oxidation**
- **Fat Uptake Assay**
- **Adipocyte Differentiation**
- **Glucose Uptake Assay**

Mitochondrial function, metabolic activity and effects of test articles in energy substance oxidation can be analyzed in real time by measuring the Oxygen Consumption Rate (OCR) in cellular level.

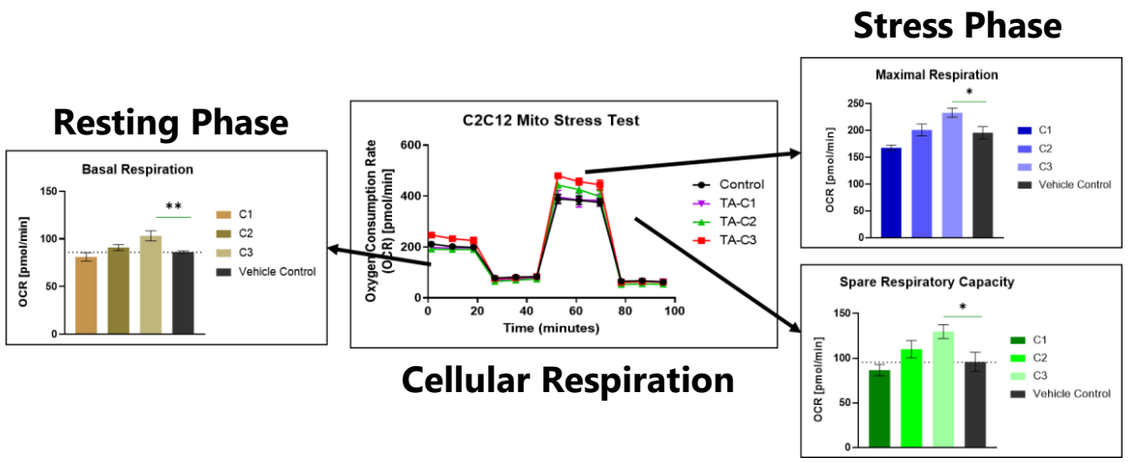


Fig 5: Mitochondrial metabolic profile (In resting and stress condition) of C2C12 muscle cells after treated with various test articles (C1, C2 and C3) using Seahorse XF Cell Mito Stress Test.

In diabetes and weight management studies, glucose uptake and adipocyte (fat) formation are one of the key parameters for product efficacy assessment.

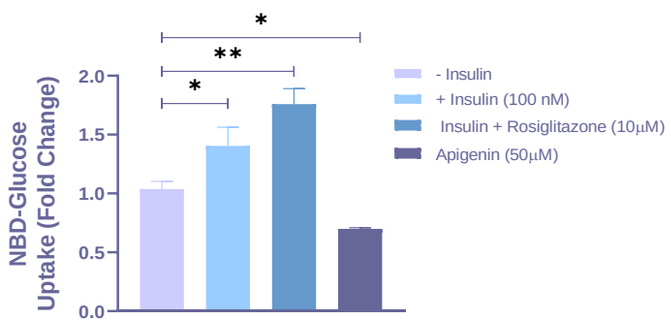


Fig 6: Effect of Rosiglitazone and Apigenin on glucose uptake by C2C12 muscle cells in presence of insulin

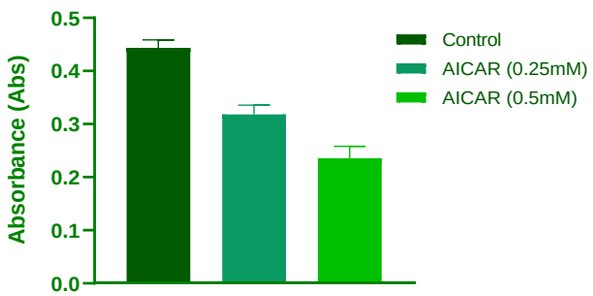
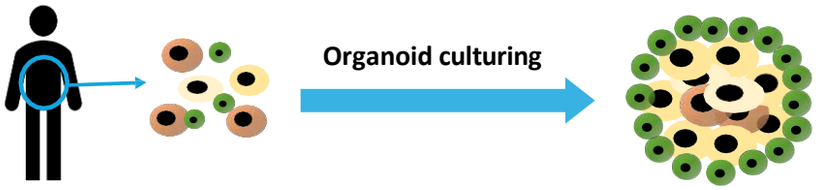


Fig 7: AICAR inhibition 3T3-L1 adipocyte differentiation after 3 days treatment. Intracellular lipid was stained using Oil Red O and quantified spectrophotometrically at 510nm.

3D CELL MODELS

Invitrocue's expertise in 3D culture allows us to successfully recapitulate *in vitro* the normal and disease conditions of humans. Our 3D cell culture techniques are more cost-effective and physiologically relevant compared to conventional 2D monolayer cultures.



Patient-derived cells | Realistic micro-anatomy

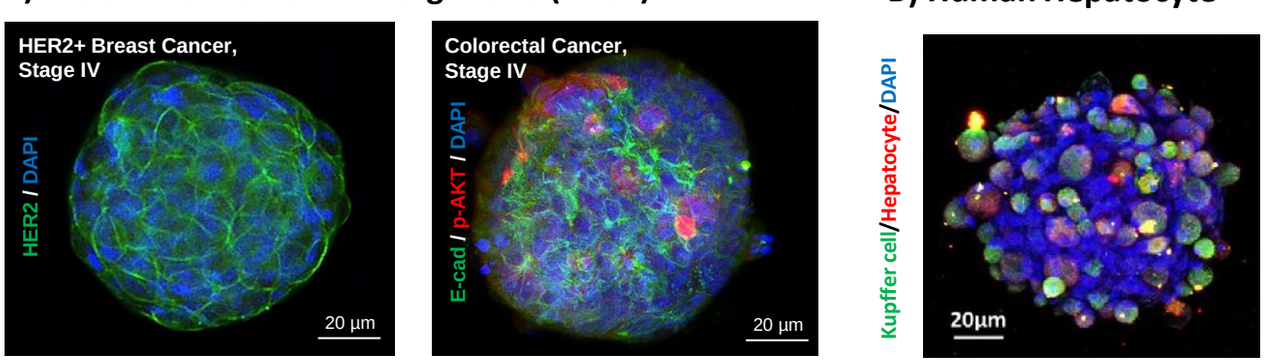
Why Use 3D Cell Culture?

2D cell culture	3D cell culture
Lack of cell-cell and cell-ECM interaction	Recapitulates cell-cell and cell-ECM interaction
No gradients present	Drugs, oxygen, nutrients diffusion in gradient
Co-culture unable to establish a proper microenvironment	Co-culture of multiple cell types mimic in vivo microenvironment
Poor clinical correlation	More reliable and better estimate of in vivo responses

Our custom 3D cell culture Services allows you to speeds up your drug discovery using established 3D models in 3 key areas below.

A) Patient-derived Cancer Organoids (PDOs)

B) Human Hepatocyte



C) Human Nasal Epithelial

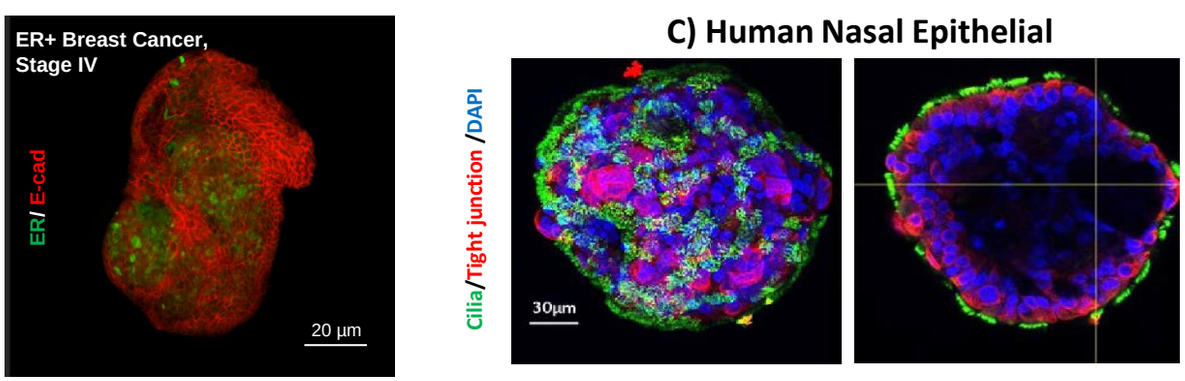


Fig 8: Confocal Images of patient derived organoids, human hepatocyte and human airway.

APPLICATIONS OF 3D CELL MODELS

3D HEPATOCYTES & IMMUNE CELLS CO-CULTURE

Kupffer cells (KCs) are innate immune cells found in the liver that play a role in immune defense against pathogens. They may also be involved in drug-induced liver injury (DILI) and liver diseases by triggering inflammatory responses. Our unique techniques allow us to establish 3D spheroid co-culture model of hepatocytes and Kupffer cells to mimic physiological condition. The co-culture model exhibits far better responses to hepatotoxicity tests compared to single culture system and are far more suitable for disease model development.

Applications

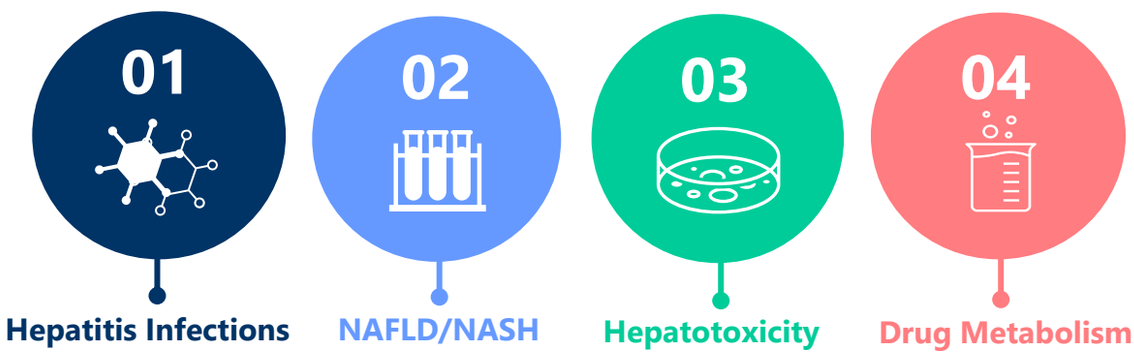
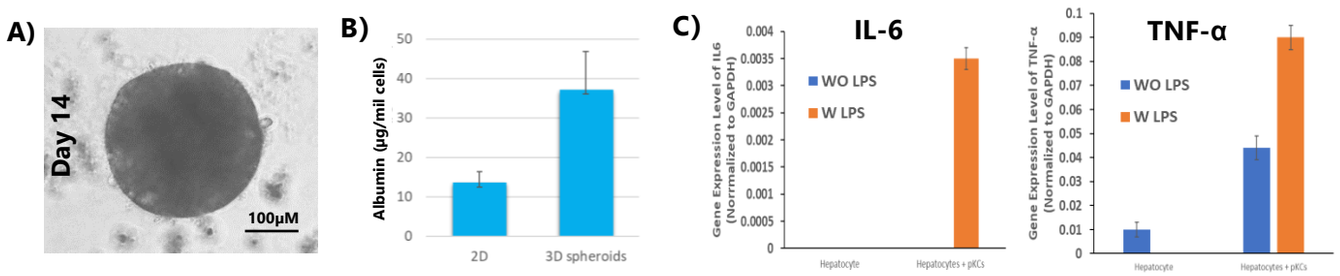


Fig 9. (A) Primary Kupffer cells form compact 3D spheroid after 14 days of co-culture with hepatocytes. B) Human primary hepatocytes showed a better hepatic function in albumin secretion in 3D compared to 2D culture. C) Kupffer cells response to Lipopolysaccharide (LPS) treatment by stimulating the pro-inflammatory IL-6 and TNF-α mRNA expression in supernatant.



Immune-Induced Hepatotoxicity Study

Compared to monoculture of hepatocyte, co-culture of primary Kupffer cell with hepatocyte (Hep:KC) allows the investigation of direct hepatotoxicity and Immune-related hepatotoxicity in single model, which is more sensitive to representative to clinical outcomes.

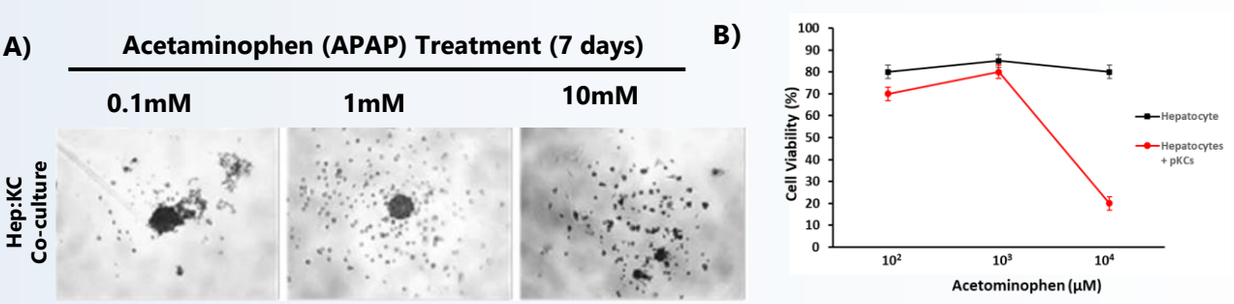


Fig 10. Cell viability of Human Hepatocyte and KCs was measured using CTG assay after exposure to Acetaminophen (APAP) for 7 days. Morphology (A) and Cell viability (B) was compared to monoculture of hepatocytes in the presence of LPS. Data demonstrated that Hep:KC co-culture able to recapitulate the immune-induced liver injury by APAP.

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APPLICATIONS OF 3D CELL MODELS

3D AIRWAY EPITHELIAL MODEL

Our airway epithelium is a ready-to-use, 3D mucociliary tissue model consisting of human-derived nasal or bronchial epithelial cells, ciliated cells and goblet cells. Our unique technique of purifying airway epithelial progenitors can help to maintain differentiation capacity and allow our model to recapitulate the in vivo phenotypes of barrier function, mucociliary responses, infection, and toxicity responses.

Applications

01 Viral & Bacterial Infections
- HRV , HSV, Influenza virus, Coronavirus

02 Airway inflammation / Rhinitis
- microorganism, pollen, allergen

03 Inhalation Toxicity
- Toxin, inhalation drug testing

Human rhinoviruses (HRV) Infection

HRV is the most common respiratory pathogen that causes upper respiratory tract infection in Human.

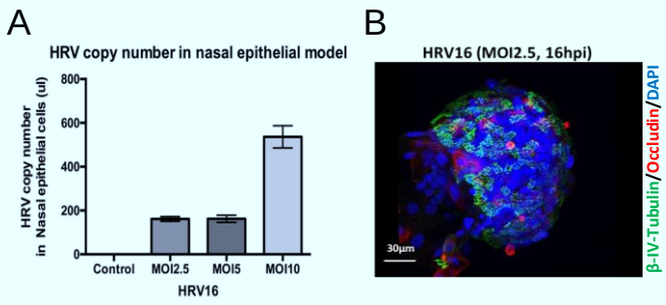
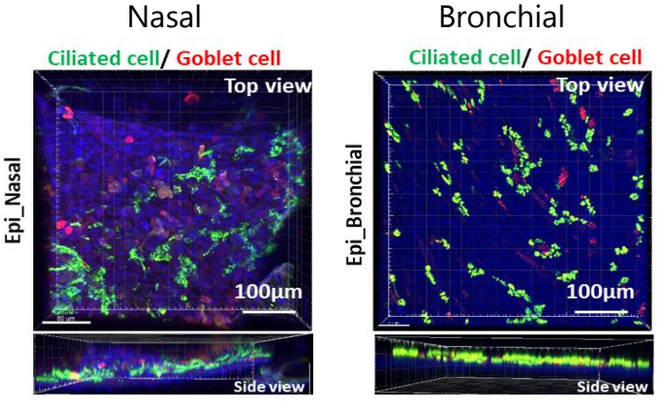


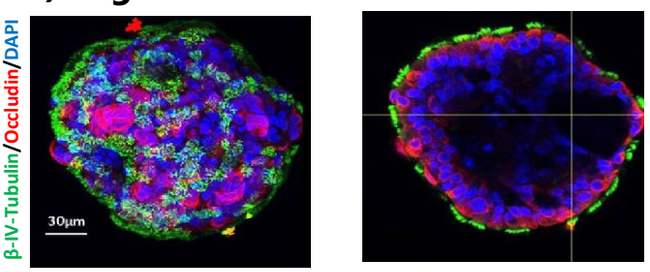
Fig 11. A) Nasal epithelial model allows the infection of HRV and characterized with increase of Goblet cells and decrease of ciliated cells. B) HRV infection disrupted the tight junction and membrane integrity of the nasal epithelial organoid.

Type of Airway Models

a) Air-Liquid Interface Culture



b) Organoids



Features:

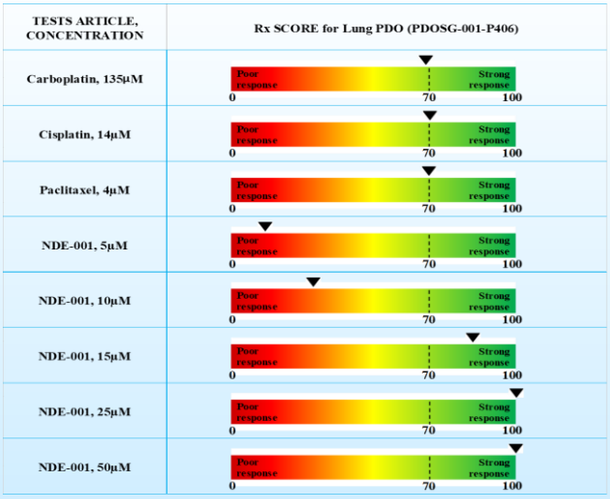
- Formed by Human Nasal Epithelial Progenitor cells (hNEPCs).
- Able to differentiated into multilayer structure with ciliated columnar cells and goblet cells, resembling the in vivo mucociliary airway epithelium.
- Allows study of mucociliary clearance and mucus hypersecretion.
- Nasal (upper airway) or Bronchial (lowe airway) Organoids are suitable for high-throughout drug screening.
- Allows long-term study up to Day 35

APPLICATIONS OF 3D CELL MODELS

PATIENT DERIVED ORGANOID (PDO)

Invitrocue's Patient-derived tumor organoids (PDOs) are multicellular in vitro tissue construct derived from patients diagnosed with metastatic cancer. PDOs recapitulates the genetic, morphological features and heterogeneity of the native tumor. At Invitrocue, we regenerate the 3D tumour microenvironment using our propriety 3D cell culture technology. This provides a more clinically relevant model for preclinical drug efficacy and toxicity assessment.

Case Study Report for New Drug Entity (NDE) screening on Lung and Colorectal Cancer PDO as a comparison with Standard of Care (SOC) drugs.



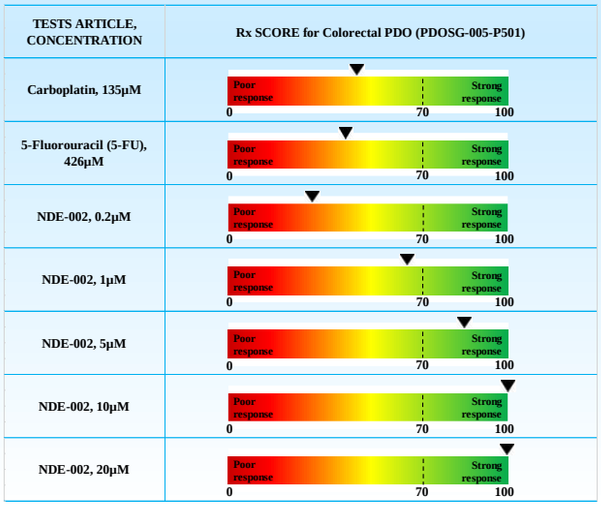


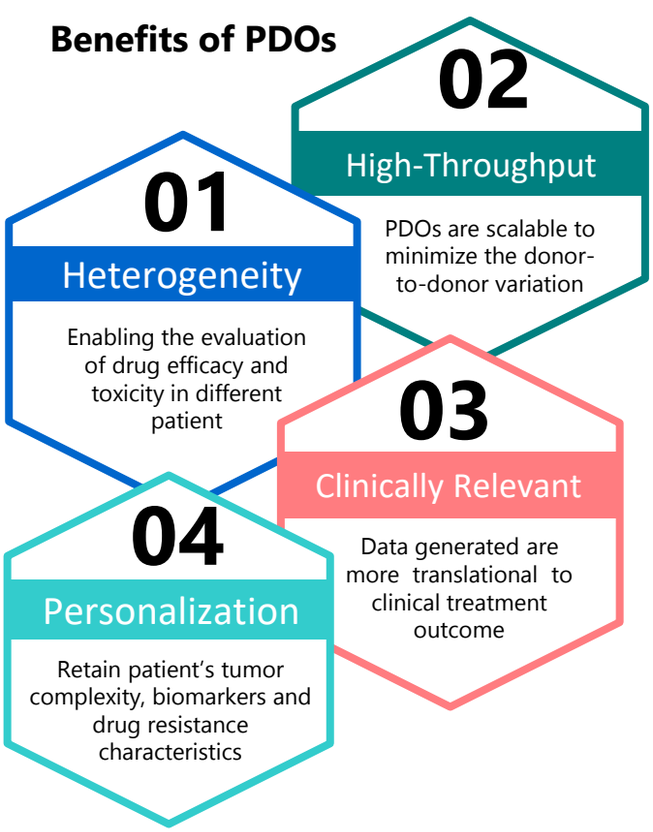
Figure 12. Rx Score of NCE and SOC Drugs on (A) Lung and (B) Colorectal Cancer PDO at different concentrations. Rx score indicates the percentage of cell death caused by test article treatments at 72 hours.

Type of Cancer PDO available for Research

- ❖ Breast Carcinoma, Stage IV
- ❖ Colon Adenocarcinoma, Stage IV
- ❖ Colorectal Adenocarcinoma, Stage IV
- ❖ Lung Adenocarcinoma, Stage IV
- ❖ Ovarian Adenocarcinoma, Stage IV
- ❖ Pancreatic Adenocarcinoma, Stage IV

* Tissue samples were collected with approval of IRB and patient informed consent.

Benefits of PDOs



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NEUROSCIENCE

Pathway-Centric Drug Discovery

Attempts to discover new drugs for genetically complex brain disorders have proven extremely difficult. Insufficient knowledge of the underlying biological mechanisms represents a critical challenge impacting the drug discovery process.

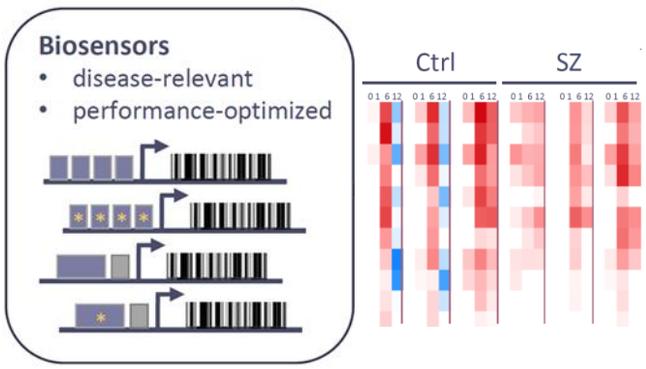
Through our collaboration with Systasy, we address this need by delivering unmatched mode-of-action insights through highly multiplexed phenogenomic pathway profiling technologies for target selection, lead discovery, and safety profiling.

Our capabilities in Neuroscience Research:

cisPROFILER

High Content Pathway Analysis

Measure >100 distinct biosensors
Cover 20 different pathways.

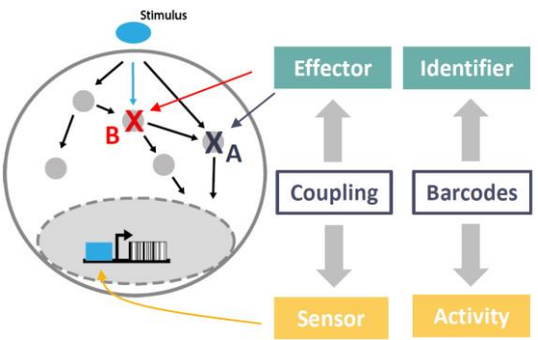


a. Cell Fate
b. Cellular Stress
c. Synaptic Activity
d. Calcium Signalling
e. Stem Cell Pluripotency
f. Immune Response
g. Metabolism
h. Immediate Early Genes (IEG) response

pathSCREENER

Gene Decoding and Target Identification

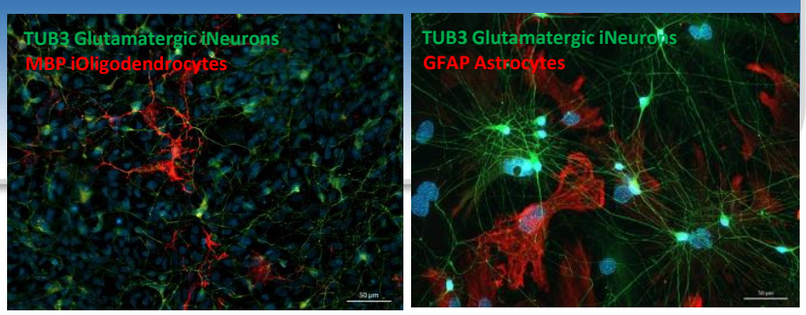
Sensor linked sgRNA libraries for >7000 genes
Capture disease relevant pathway activities
Customized Neuron cells (Mouse and Human) using CRISPR Gene Editing Technology



Human Neurological Disease Modelling

Drug Screening with full characterized IPSC-derived Neuron cells for **Schizophrenia** and **Neuropathy model.**

- PBMCs available for reprogramming
  - **Bipolar Disorders, Depression, Alzheimer Disease**



Images by Systasy Bioscience

Empower by

systasy BIOSCIENCE

For more information, please visit our website at https://extassay.com/

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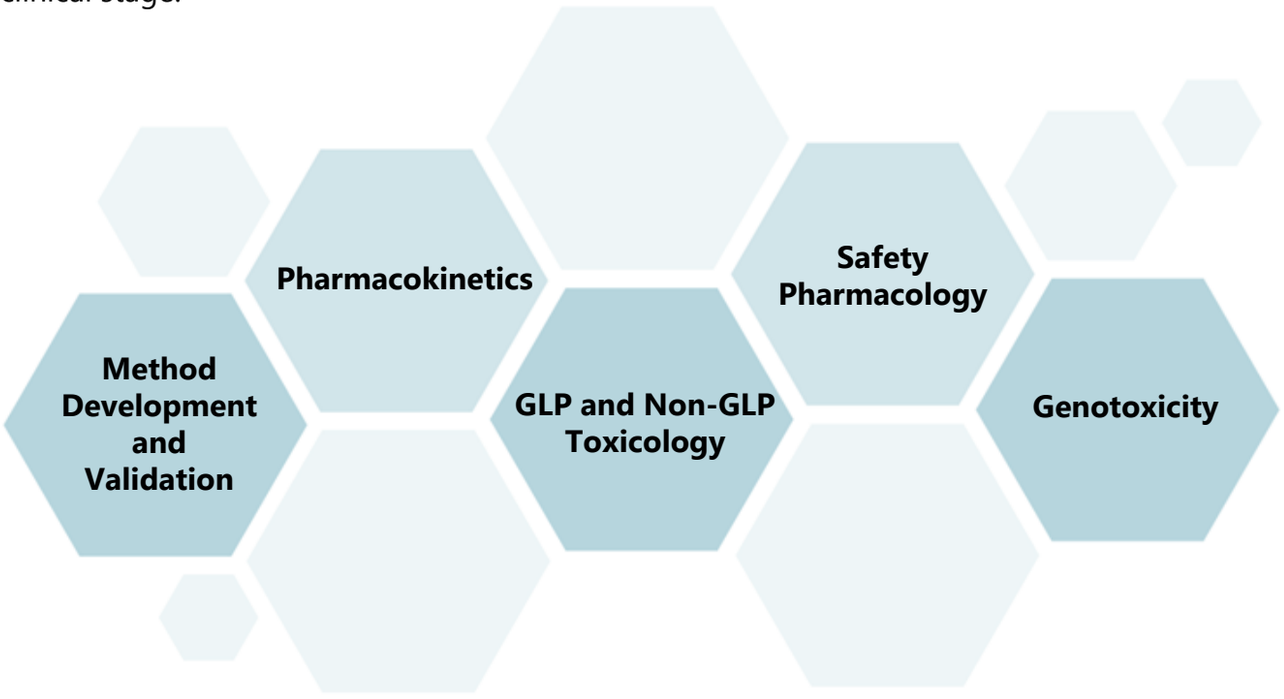
SAFETY ASSESSMENT

Through collaborations with local and international GLP laboratories, Invitrocue provides services to help clients understand the role that physicochemical properties, drug metabolism, pharmacokinetics and drug-drug interactions play in the safety and efficacy of the drug discovery and development process. We offer both in vitro and in vivo models for lead candidate selection and optimization in the preclinical stage.



Our Capabilities in Preclinical Development:

1. **Method Development and Validation**
   Dose Formulation
   Bioanalytical

2. **Pharmacokinetics**
   Single Dose Bioavailability PK Study
   Single Dose Escalation PK Study

3. **GLP and Non-GLP Toxicology**
   7 Days Tolerability Study
   14 Days Dose Range Finding Study
   28/90 Days Repeated Dose Study

4. **Safety Pharmacology**
   hERG Assay, CNS, Respiratory and Cardiovascular System

5. **Genotoxicity**
   Bacterial AMES Test
   In vitro Chromosomal Aberration Test
   In vivo Micronucleus Test (MNT)

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TO LEARN MORE

INVITROCUE®
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In partnership with :
INVIVOCUE

THE GLOBAL PARTNER ALONGSIDE YOUR BREAKTHROUGH

PRECLINICAL CAPABILITIES FOR MEDICAL ADVANCEMENT

Products

- Rodent Models
  - **HiMice** (Humanized Mice)
  - **HepMice** (Human Liver Chimeric Mouse)
  - **Dual Humanized Mice**
- Humanized Hepatocytes

Connect with us!

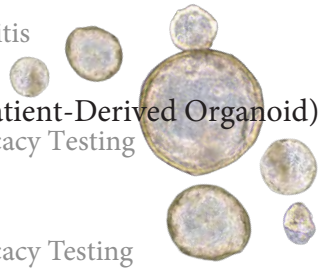
Services

SAFETY ASSESSMENT

- ADME Properties
- DMPK Studies
- Cytotoxicity Study
- Bioavailability Study
- hERG Assay
- AMES Test
- Micronucleus Test
- In Vivo 7, 14, 28, 90-days Toxicity
- OECD GLP Certified Testing Services
- Method Validation and Dose Formulation

IN VITRO SERVICES

- Cellular Metabolic Assays
  - Cellular Energy Demand
  - Mitochondrial Metabolism
  - Diabetes and Weight Management Studies
- 3D Liver Model
  - Hepatotoxicity
  - Hepatitis Infections
- 3D Airway Model (Organoid /ALI)
  - Viral and Bacterial Infection
  - Airway Inflammation / Rhinitis
  - Inhalation Toxicity
- Anti-Cancer Model (Patient-Derived Organoid)
  - High-Throughput Drug Efficacy Testing
  - Toxicity Assessment
- Anti-Viral CPE Model
  - High-Throughput Drug Efficacy Testing
  - Inhibition Assay
- Neuroscience
  - Brain Signaling Pathway Analysis
  - Reporter Assay for GPCR Binding Analysis
  - Schizophrenia and Neuropathy Disease Models



IN VIVO SERVICES

- In Vivo Model
  - Immunodeficient Mice (Nod-scid IL2rg$^{-/-}$)
  - CD34^{+} Humanized Mice (HiMice)
  - Enhanced Humanized Mice (Myeloid or NK Cell)
  - ACE2 Humanized Mice (hACE2-HiMice)
  - PBMC Humanized Mice (Mature T-cells)
  - Human Liver Chimeric Mouse (HepMice)
  - Dual Humanized Mice (Human Immune + Liver System)
- Disease Models
  - Oncology Model (CDX, PDX, PDOX, Syngeneic Mice)
  - Immunotherapy Model (CAR-T induced CRS)
  - Inflammation Model (GvHD, Acute Lung Inflammation)
  - Autoimmune Model (SLE, Inflammatory Bowel Disease)
  - Liver Model (NAFLD, NASH)
  - Infection Model (SARS-CoV-2, ARDS, Hep B, Hep C, H1N1)
  - Safety Assessment (Immunogenicity and Immunotoxicity)

BIOANALYTICAL SERVICES

- ELISA | ELISPOT | Flow Cytometry | Histology
- HLA Typing | Imaging | Quantitative RT-PCR

CUSTOMIZED ASSAYS & DISEASE MODELLING

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Ver. No.: 04 | Date: 01 Sep 2023

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To know more about our products and services, please contact our technical experts

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