Gastrointestinal Toxicity in Model Animal Species using Organ Tissue Equivalents

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Trust Your Gut Workshop
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DTRA “Noah’s ARK” Project

Problem:

In drug development, achieving FDA approval is dependent upon using an animal model most representative of human response. Therefore, knowing which species to use prior to initiating pivotal studies can greatly improve the chance of MCM approvals using the Animal Rule

Solution:

Construct functional Organ Tissue Equivalents (OTEs) from multiple species and organs and compare their responses to drugs with human-derived OTEs

DTRA “Noah’s ARK” Project

Procedure:

After characterization, OTEs are screened with known drugs, biological responses are recorded, and differences are evaluated

Utility:

In addition to aiding in MCM development when using the Animal Rule, long term, the technology could replace the use of animals in drug development

Species Selected for DTRA Program

Human

Mouse

Rat

Dog

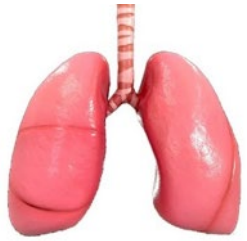
Primate

Organ Tissue Equivalents (OTEs) Constructed from each Species

LIVER

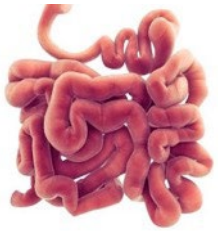
Hepatocytes
Stellate cells
Kupffer
Endothelial
Cholangiocytes

LUNG



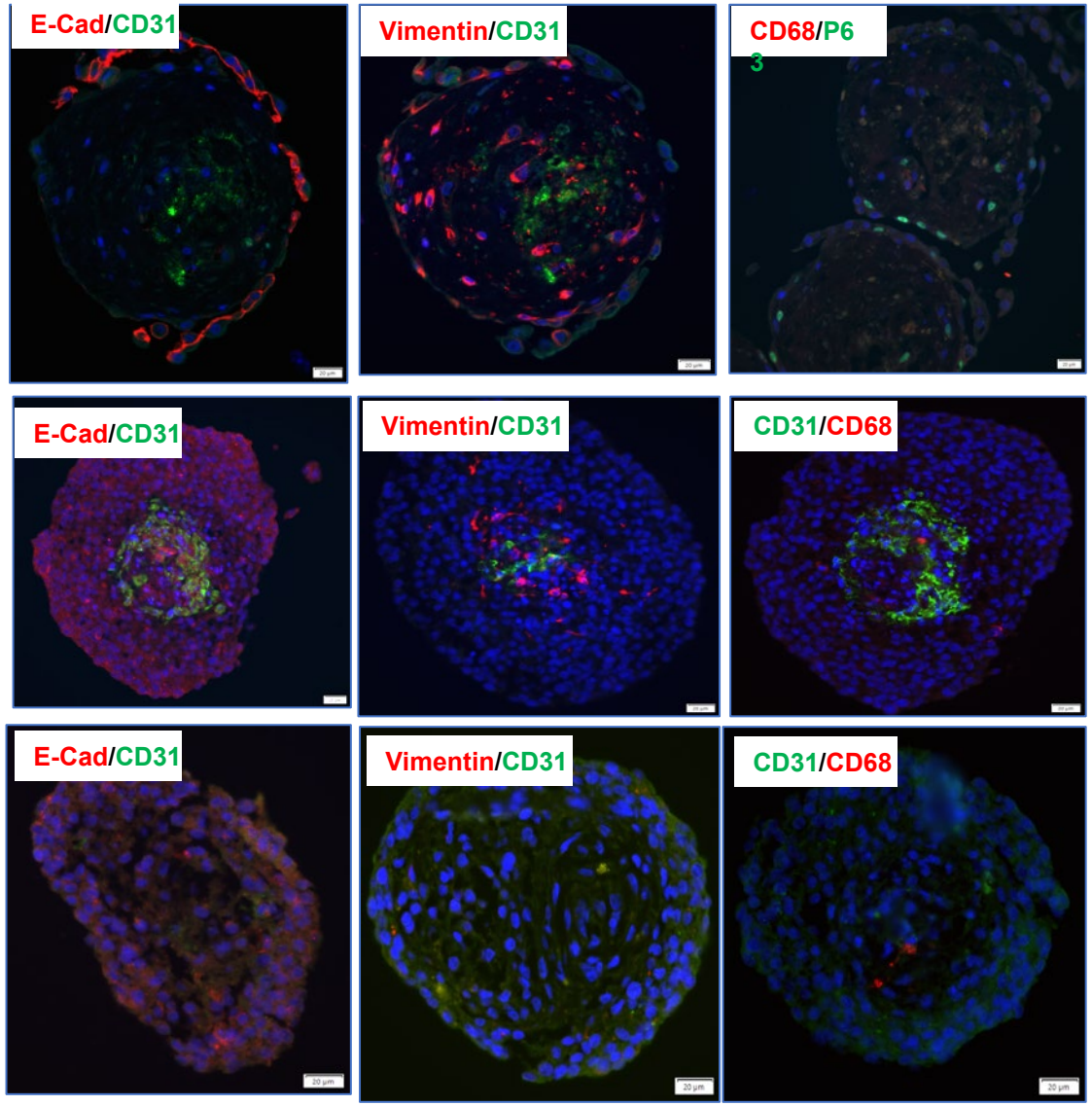
Bronchial
Epithelial cells
lung fibroblasts
microvascular
endothelial
macrophages

GUT



Enterocytes
Enteroendocrine cell
Goblet Cells
Paneth cells
Stem cells

Human, Mouse and Rat lung OTE's



HUMAN

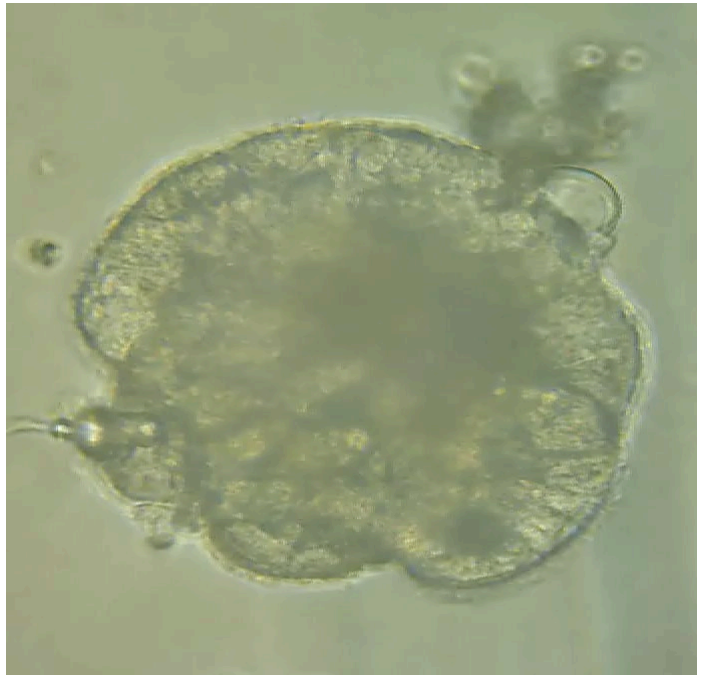
MOUSE

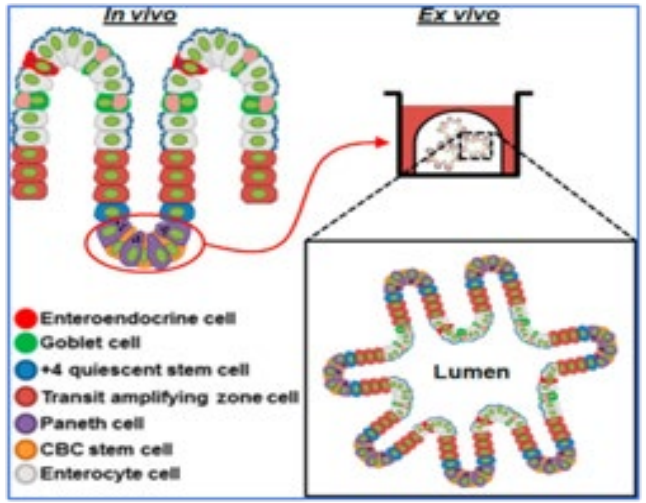
RAT

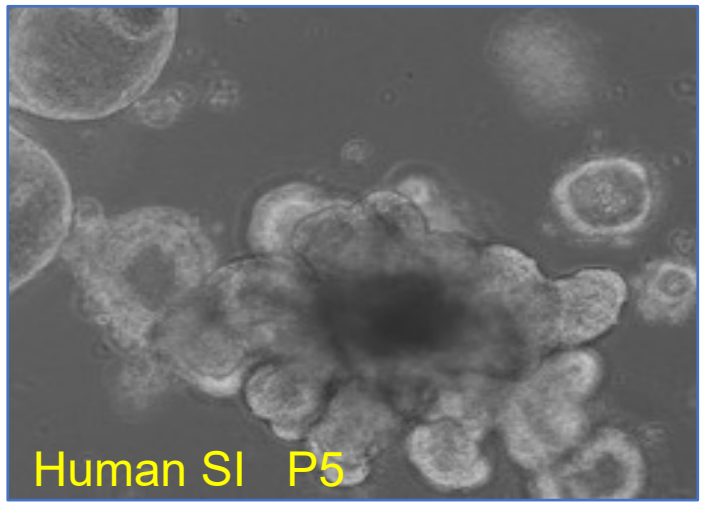
E. Cadherin	Epithelial tight junctions
Vimentin	Fibroblasts
CD31	Endothelial cells
CD68	Macrophages
P63	lung basal/stem cells

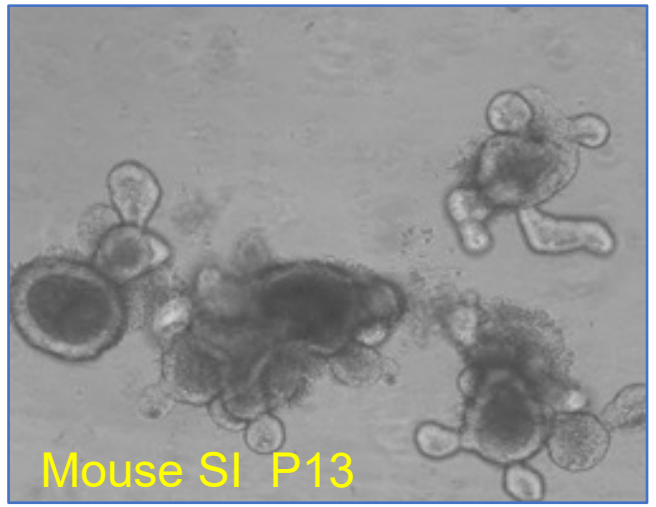
Human Lung OTE Functional Characterization

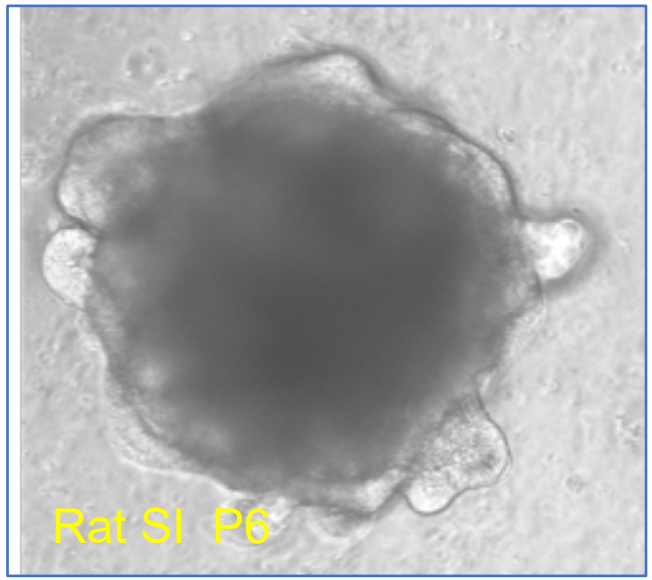
- Mucus production and cilia beat frequency are two major functions of lung tissue relevant for drug/tox studies
- Lung spheroids form an outer cilated epithelial cell layer with presence of mucus producing goblet cells.
- Cilia beating is clear with video microscopy of individual spheroids (right)

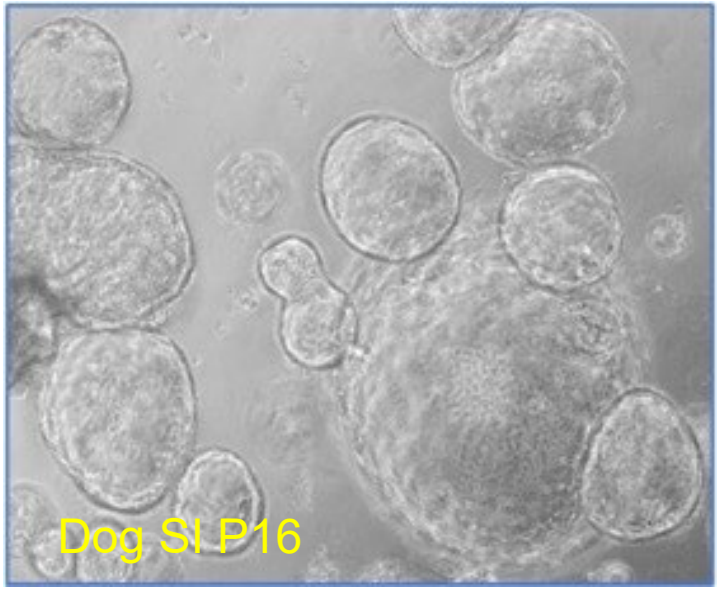



In vivo
Ex vivo
Lumen
Enteroendocrine cell
Goblet cell
+4 quiescent stem cell
Transit amplifying zone cell
Paneth cell
CBC stem cell
Enterocyte cell


Human SI P5


Mouse SI P13

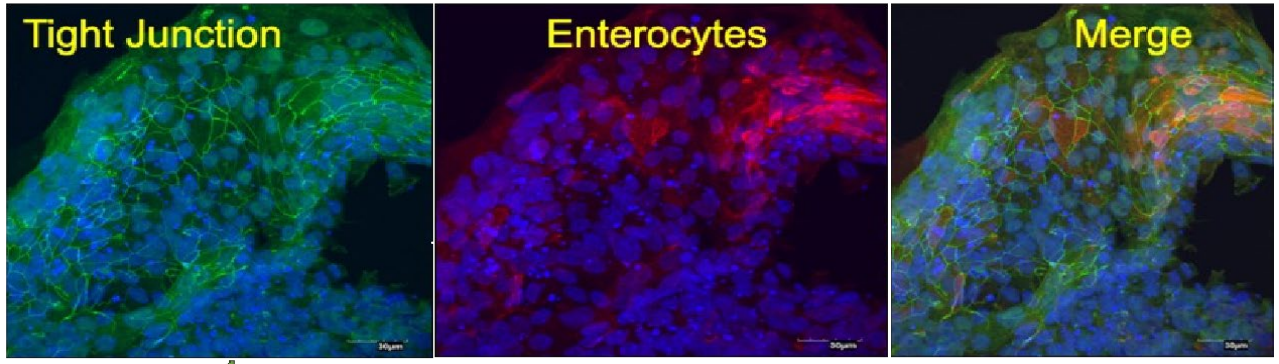

Rat SI P6


Dog SI P16

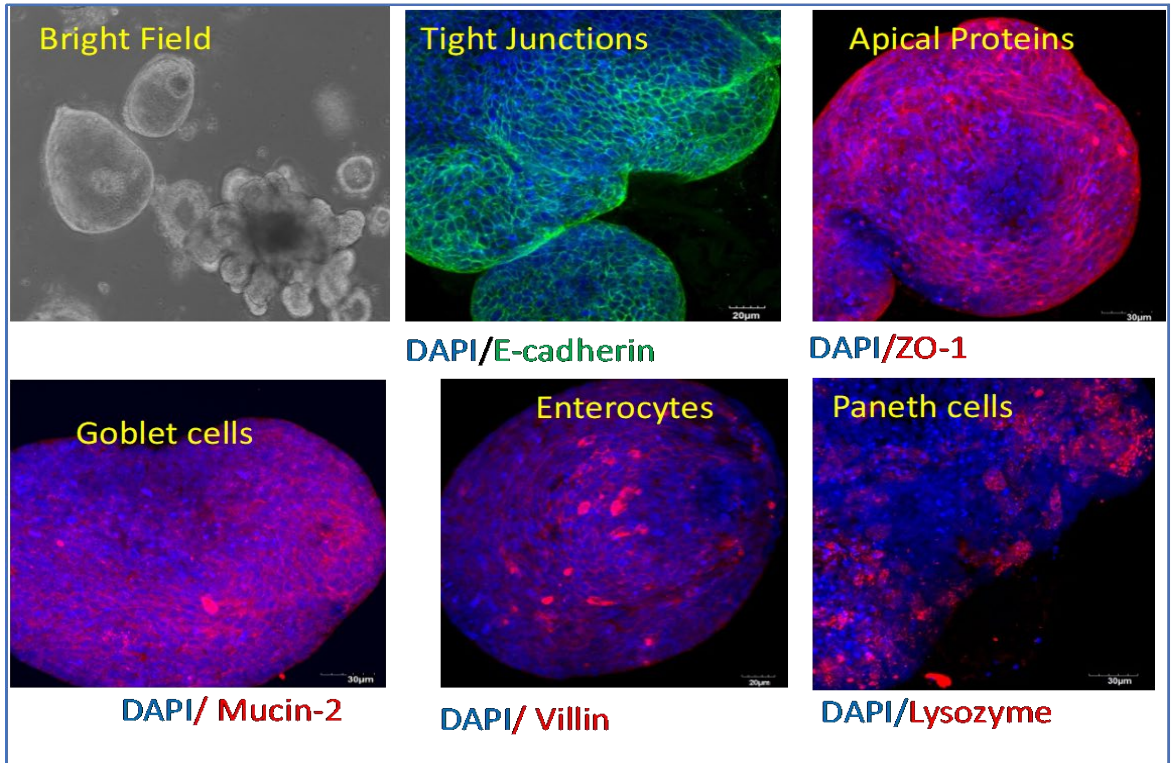

Baboon SI P8

Characterization of Colon OTEs derived from isolated Crypts

Human

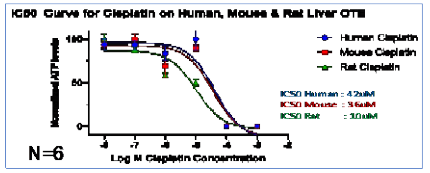


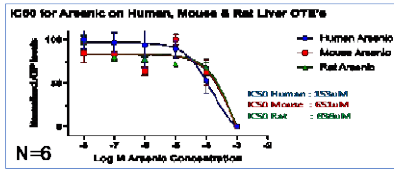
Baboon

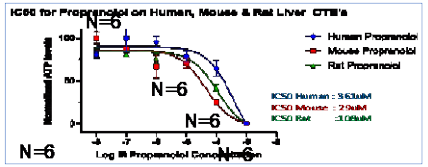


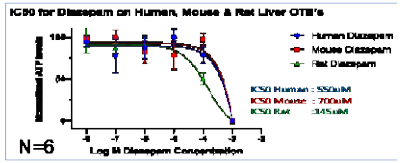
Drug Screening of OTES derived from Human Mouse and Rat

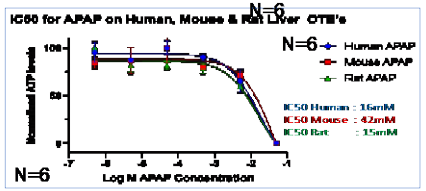
Universal	CISPLATIN
	ARSENIC(III) OXIDE
Metabolites	DIAZEPAM
	PROPRANOLOL
Hepatotoxins	APAP
	AFLOTOXINB1

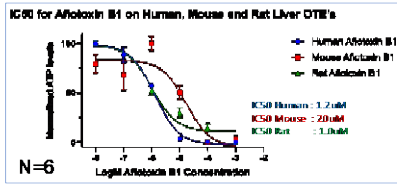


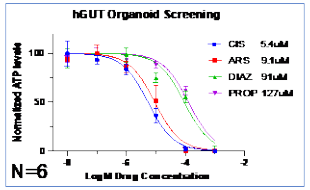


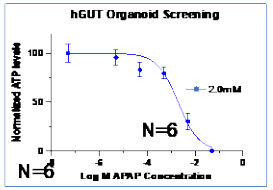


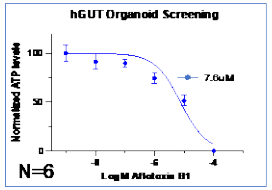


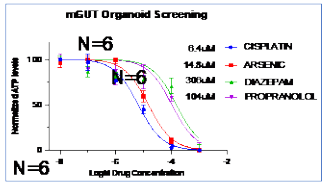


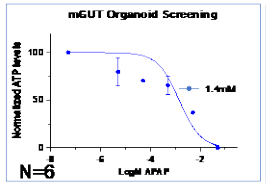


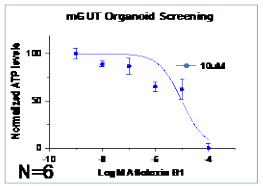


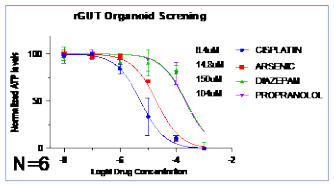


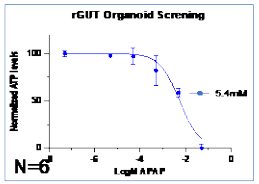


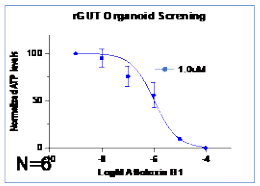


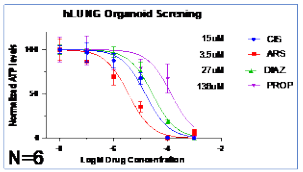


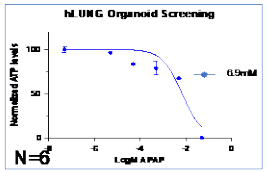


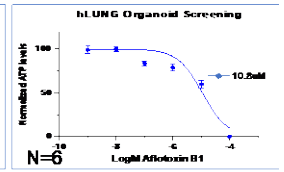


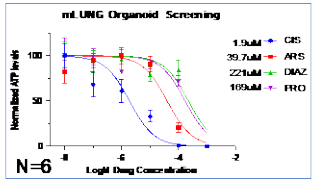


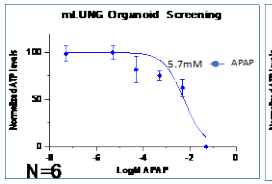


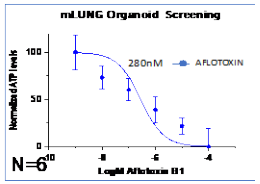


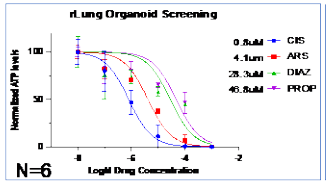


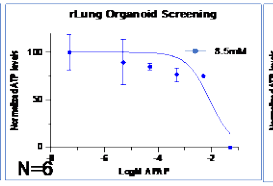


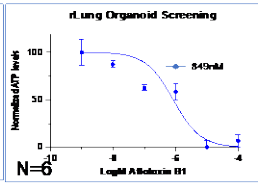












Summary of IC50 Values obtained from Screening Human, Mouse and Rat OTEs

OTE	CISPLATIN	ARSENIC	DIAZEPAM	PROPRANOLOL	APAP	AF. B1
hLIVER	42uM	153uM	550uM	361uM	16mM	1.2uM
mLIVER	36uM	651uM	700uM	29uM	42mM	20uM
rLIVER	10uM	838uM	145uM	108uM	15mM	1uM
hGUT	5.4uM	9.1uM	91uM	127uM	1.9mM	7.6uM
mGUT	6.8uM	14.8uM	306uM	104uM	1.4mM	10uM
rGUT	6.4uM	14.8	150uM	104uM	5.4uM	1.0uM
hLUNG	15uM	3.5uM	27uM	136.5uM	6.9mM	10.8uM
mLUNG	1.9uM	39.7uM	221uM	169uM	5.7mM	0.28uM
rLUNG	0.8uM	4.1uM	28.3uM	46.8uM	8.5mM	0.85uM

Screening System; Future Directions

1. Key to this work is to produce a panel of OTEs than can identify species differences in drug toxicity

2. Increase screening panel from 6 to 12 drugs including those with known species differences in liver toxicities.

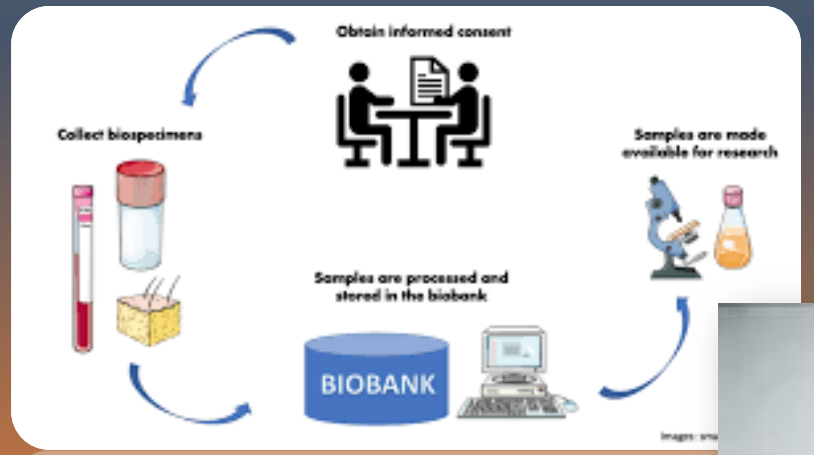
Universal	CISPLATIN
	ARSENIC(III) OXIDE
Metabolites	DIAZEPAM
	PROPRANOLOL (HCL)
Hepatotoxin	APAP
	AFLOTOXINB1

1	**Universal**	**METAARSENITE (Na)**	
		Known Liver Tox	
2	**Metabolites**	**PROPRANOLOL (HCL)**	
3	**Hepatotoxin**	**APAP**	
4		**AFLATOXINB1**	**Hum/rat Toxic >Mse**
5	**Interspecies**	**BOSENTAN**	**Rat Toxic > Hum/Mse**
6		**COUMARIN**	**Rat Toxic > Hum/Mse**
7		**FIALURIDINE (FIAU)**	**Hum Toxic > Mse/rat**
8	**Mixed**	**ETOPOSIDE**	
9		**IPRONIAZID**	
10		**TROGLITAZONE**	
11		**VPA**	
		PHENOBARB	

Development of a living biobank of intestinal organoids

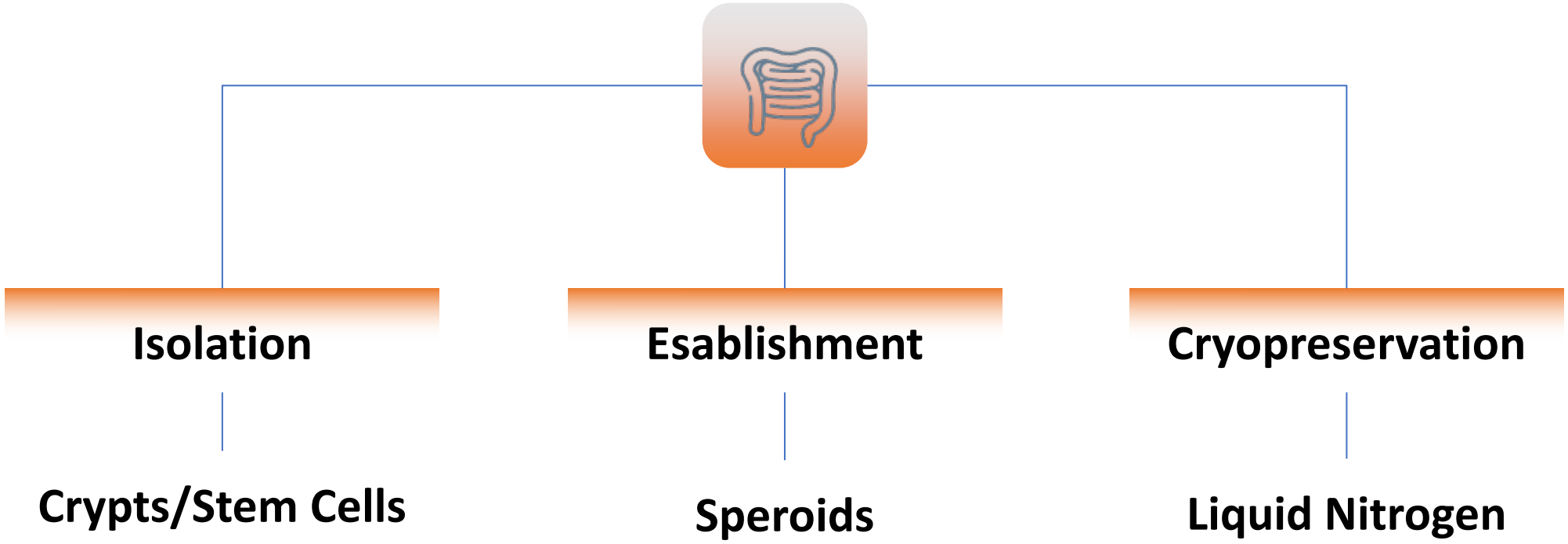
"Biobanks bridge the gap between basic and translational research"

- Viable Organoids
- Recapitulate Cell-Cell Interactions organoids
- Unique Aspects of Human Pathophysiology
- Clinical Translation



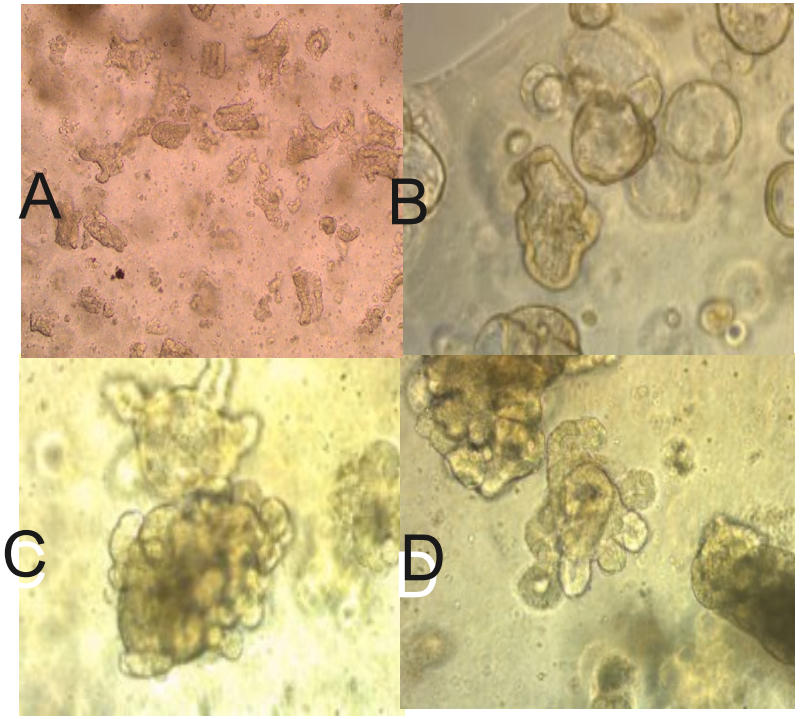
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8492003/

Gut Organoids phases

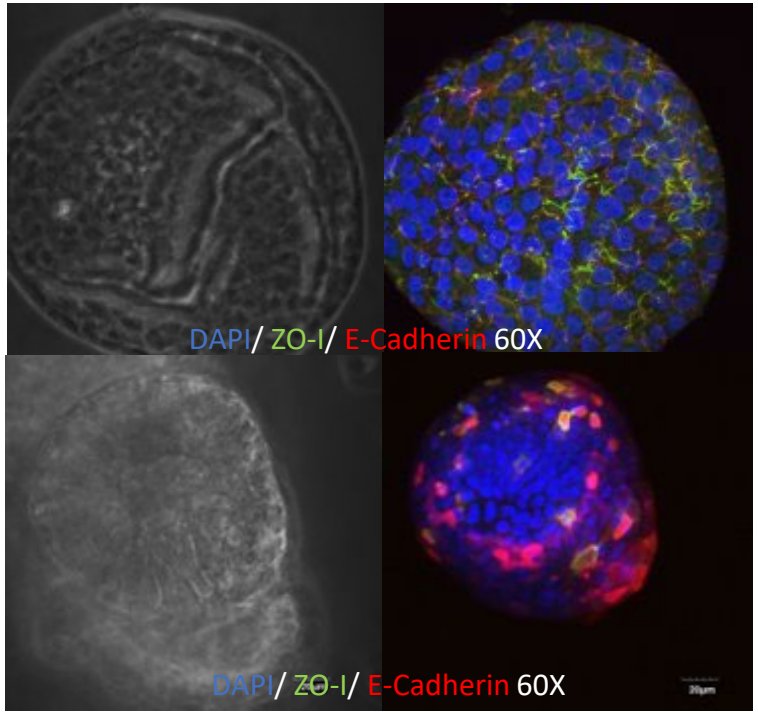


Organoid Culture

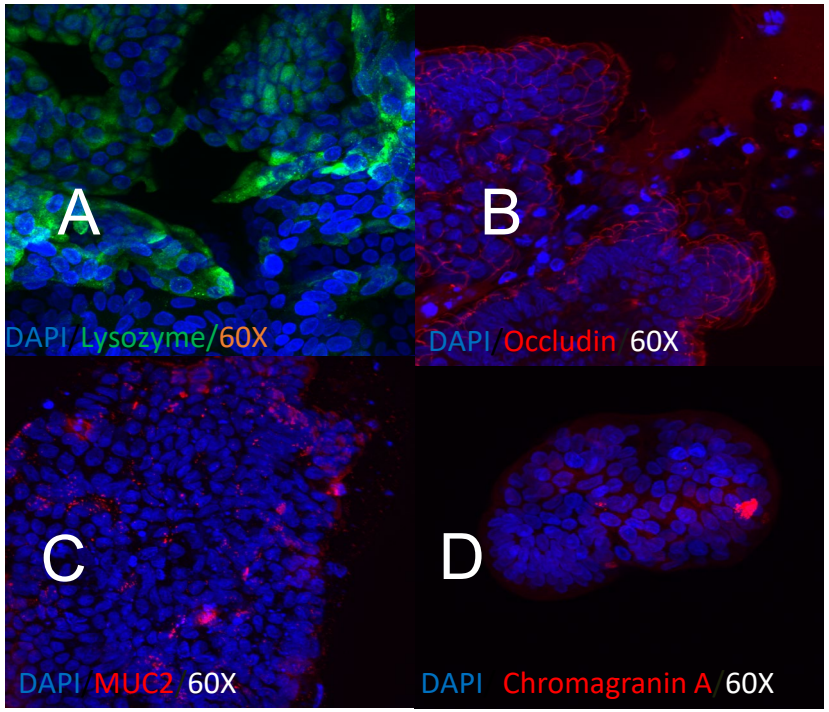
Isolation, Characterization, and Differentiation



A. Intestinal crypt B. spheroids and
C/D. Full mature organoids

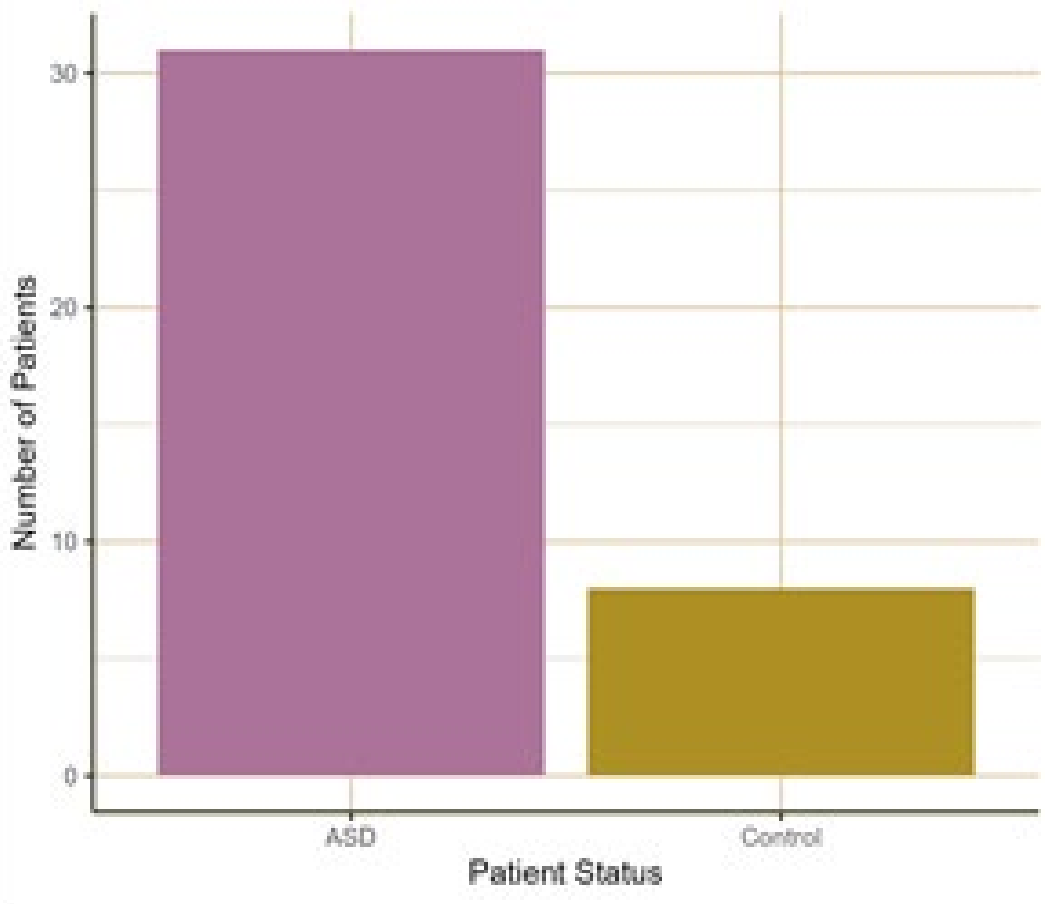


Representative organoids with basolateral-out orientation.



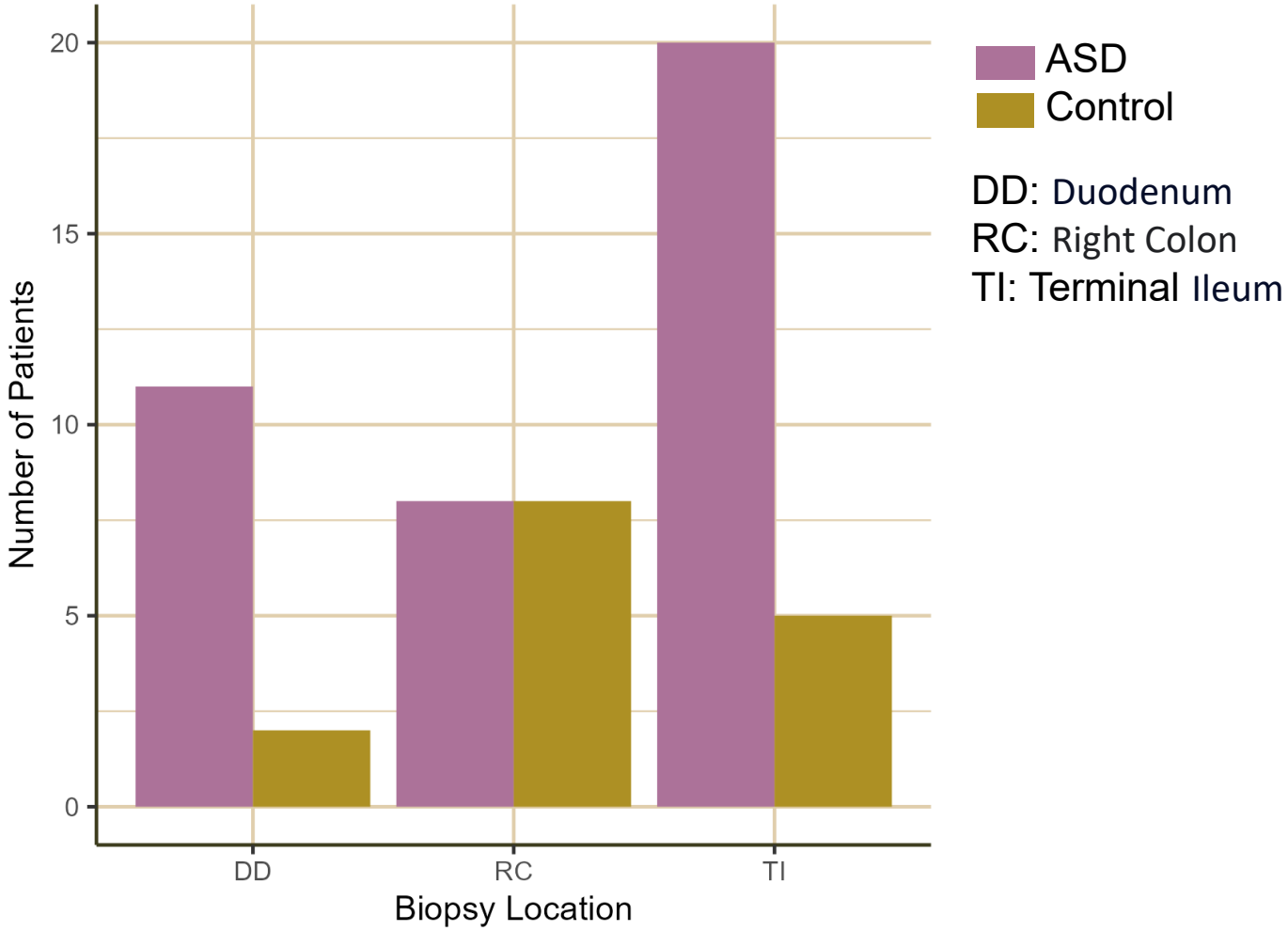
Representative organoids with apical-out orientation.

Organoid Culture / August



- **Total**: 39 viable samples / Unique sample ID
- 31 Viable ASD samples
- 8 viable Control Samples

Organoid Inventory / August 2023



ASD samples
11 from the duodenum
8 from the right colon
20 from the terminal ileum

Control samples
2 from the duodenum
8 from the right colon
5 from the terminal ileum

SUMMARY

1. **Produced functional OTEs from human, mouse, rat dog and primate.**
    - **b. Shown they are functional long term**
    - **c. Shown they show basic immune response via cytokine release after LPS insult**

2. **Screened all OTE's from 3 species with 6 drugs and obtained IC50 values for each OTE and species**
    - **a. Identified clear species differences in toxicities**

3. **Development of a living biobank of intestinal organoids from GI-symptomatic children with ASD and controls**