CORNING

Innovation Showcase: Advanced 3D Cell Culture Tools and Revolutionary Models

Welcome and Introduction

Tools to Accelerate 3D Cell Culture

Austin Mogen, Ph.D., Senior Field Applications Scientist, Corning Life Sciences

Patient-derived Organoids: A Revolutionary New Model to Advance Precision Medicine

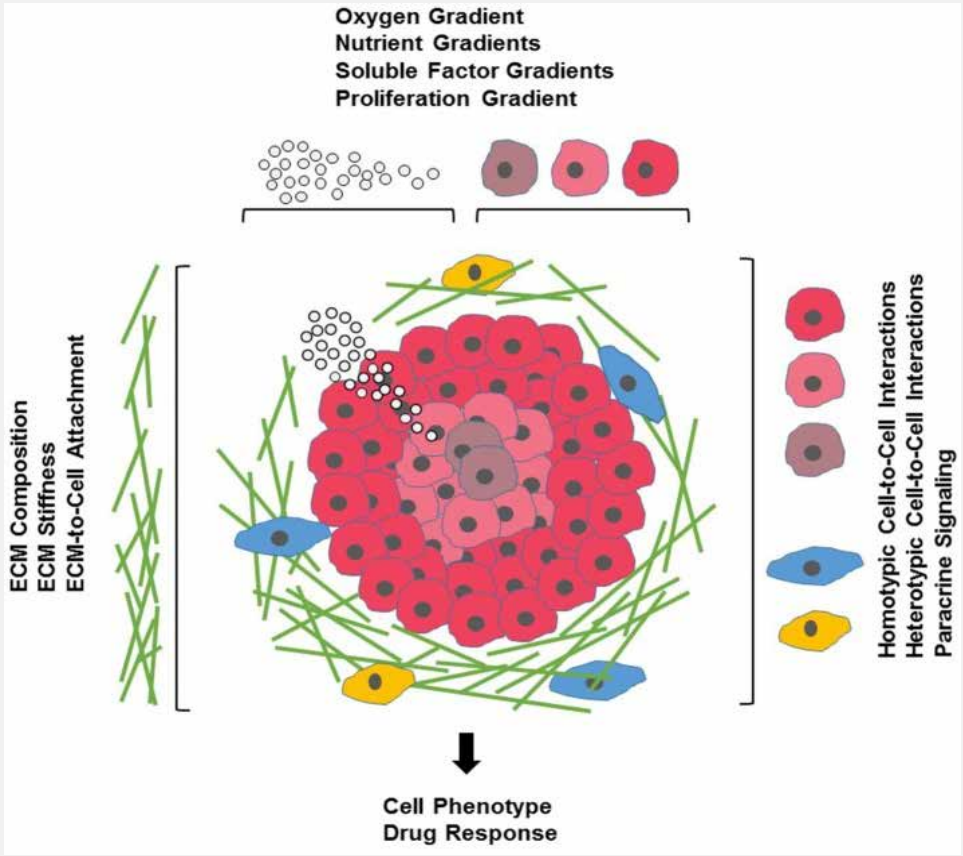
Sylvia Boj, Ph.D., Chief Scientific Officer, HUB Organoids

3D Cell Culture: Introduction

Why 3D? Biological Factors Captured by 3D Cell Culture

Extracellular Matrix Composition

Substrate Stiffness



Concentration Gradients

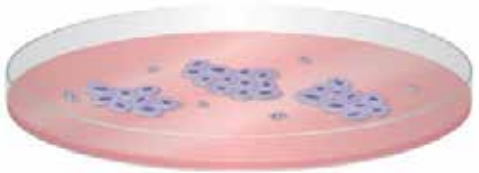
Multiple Cell Types

"An ideal 3D cell culture model would include cell-to-cell and cell-to-ECM interactions, tissue-specific stiffness, oxygen, nutrient and metabolic waste gradients, and a combination of tissue-specific scaffolding cells"

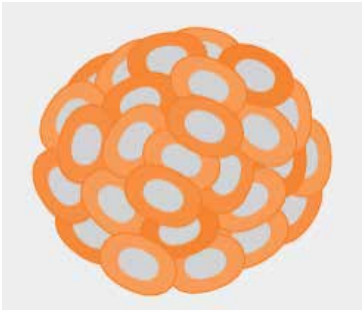
Langhans, 2018. Three-Dimensional in Vitro Cell Culture Models in Drug Discovery and Drug Repositioning.

Preclinical Models Comparison

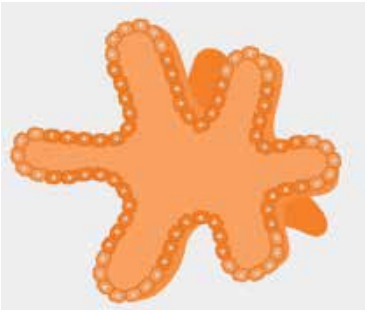
2D



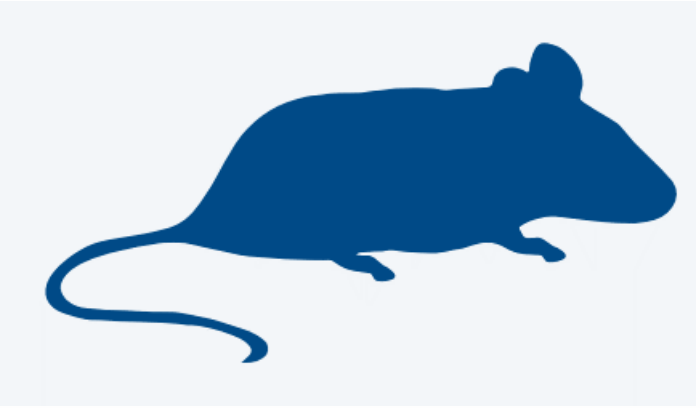
Spheroids



Organoids



Mouse



Ease of use

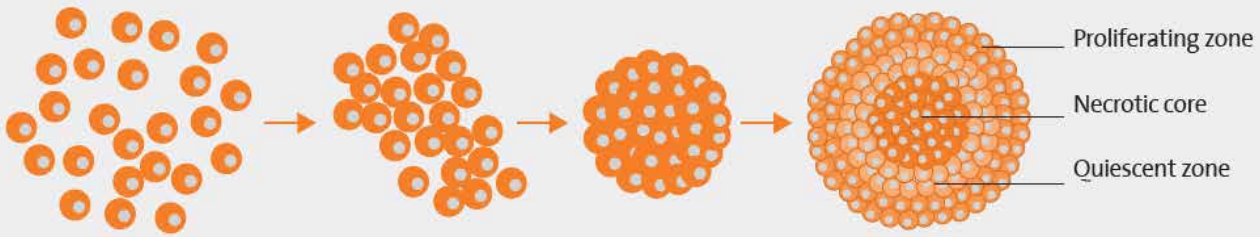
Biological Complexity

Throughput

CORNING | FALCON® AXYGEN® PYREX®

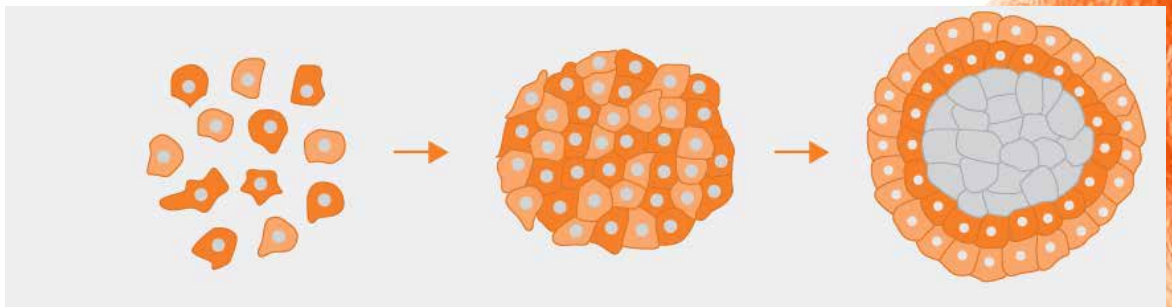
Spheroids vs. Organoids

Spheroids



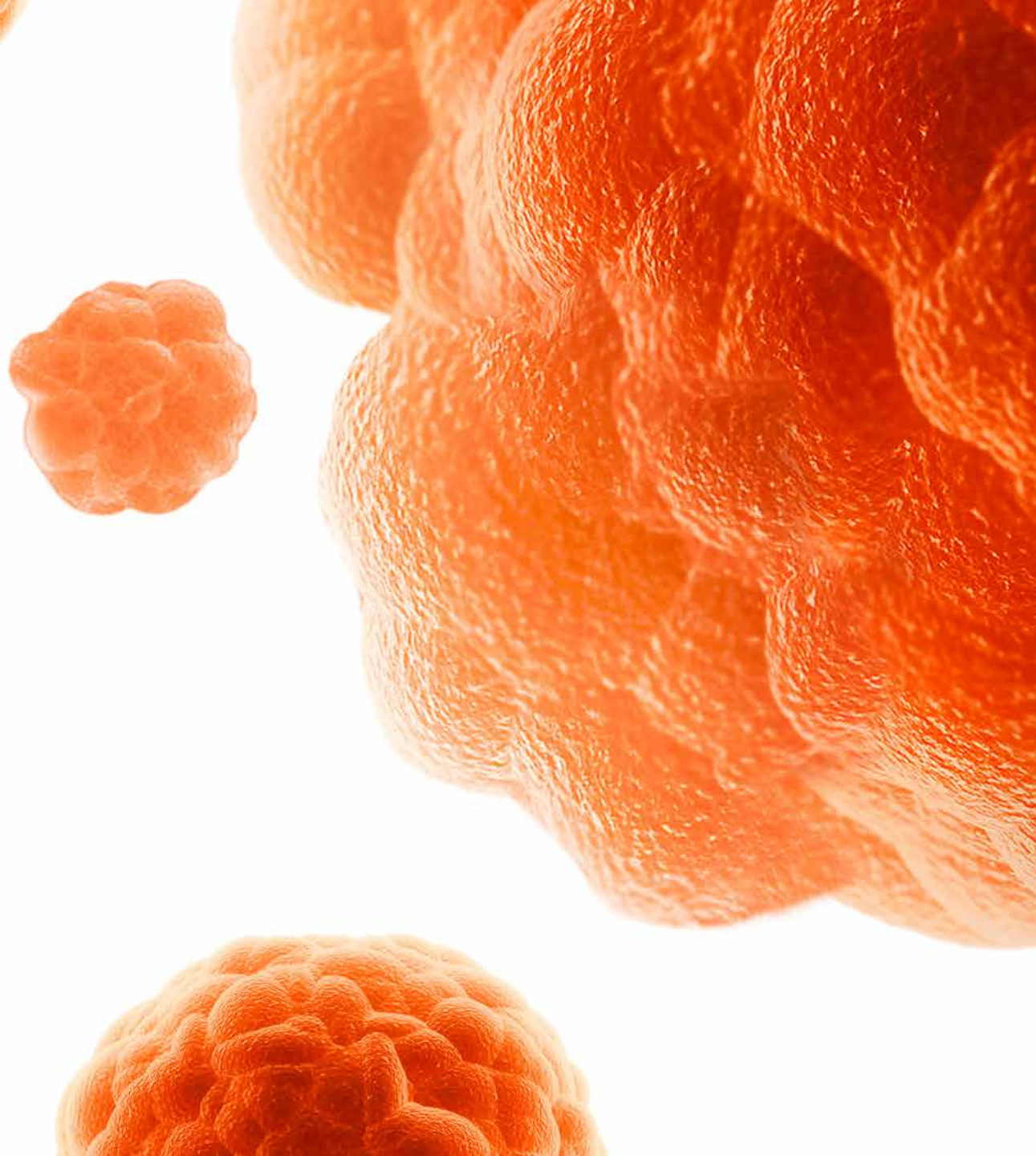
- Cell lines or primary cells
- One or multiple cell types
- Cell and concentration gradients
- Limited self-organization and polarity
- Do not require ECM

Organoids



- Generated from stem or progenitor cells
- Multiple organ-specific cell types
- Self organizes and has polarity
- Require ECM
- Recapitulates organ function

Tools for Generating Spheroids and Organoids



Corning 3D Cell Culture: A History of Innovation

Corning® Matrigel® Matrix

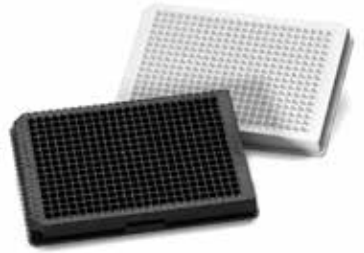
Transwell® Permeable Supports

Corning Spheroid Microplates

Corning Elplasia® Plates

Corning Matrigel for Organoids

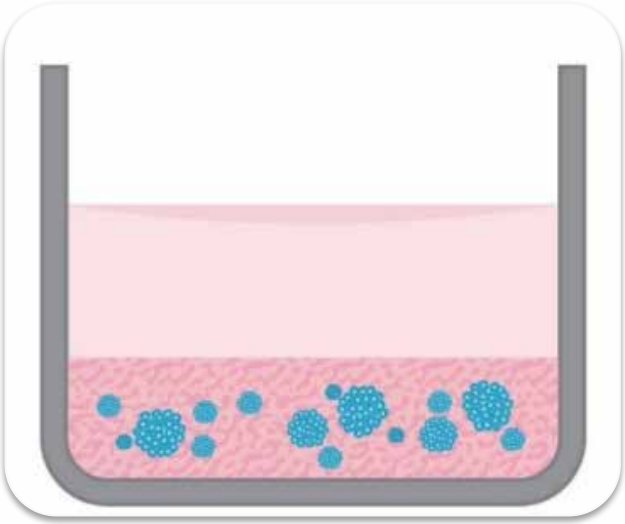
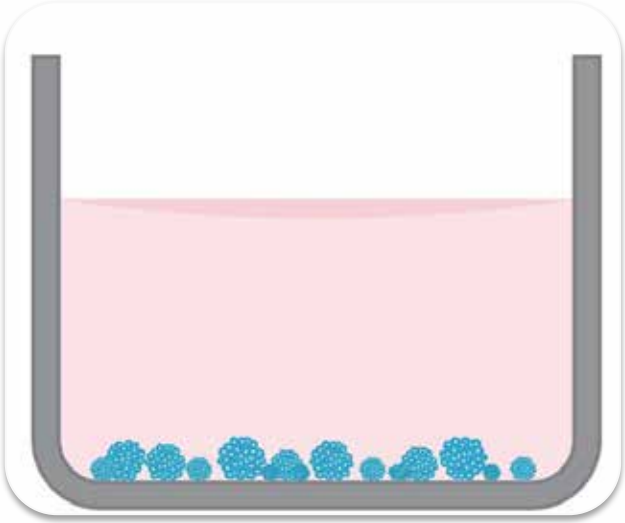
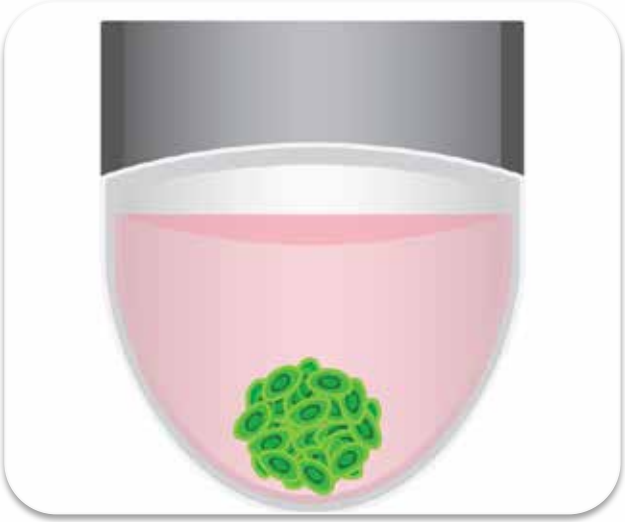
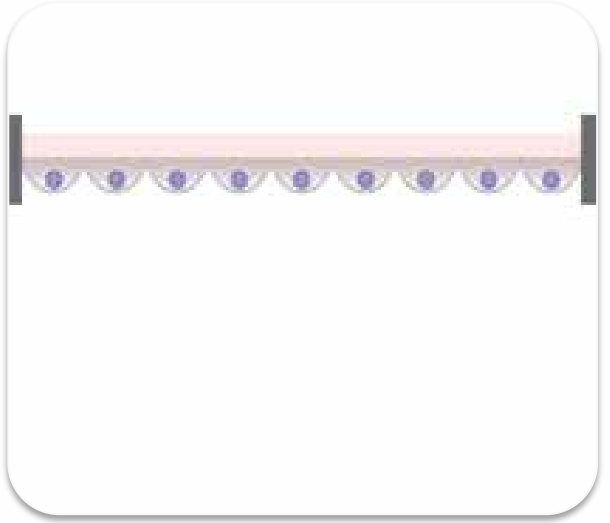
Corning Matrigel Matrix-3D Plates



Corning Matribot® Bioprinter

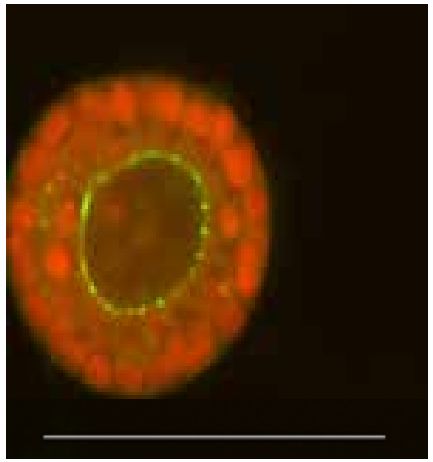
Corning, Falcon®, Axygen®, and PYREX® brands.

Methods to Form Spheroids and Organoids

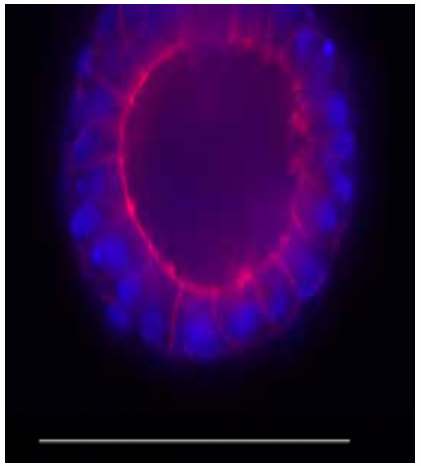
Scaffold	Scaffold-free		
Gel-based	**Multiple Non-uniform**	**Singular Uniform**	**Multiple Uniform**
			
• Corning® Matrigel® Matrix • Corning Matrigel Matrix-3D Plate • Other ECM hydrogels (Collagen)	Ultra-Low Attachment Surface Products	Corning Spheroid Microplate	Corning Elplasia® Plate

3D Technologies: Biological Hydrogels

Example: Corning® Matrigel® matrix, Collagen, Fibronectin, BME, Laminin.

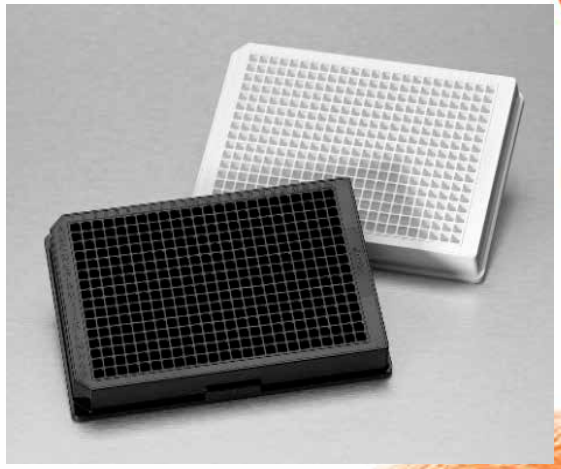


Polarized MDCK Cysts (40X)



Corning Matrigel Matrix

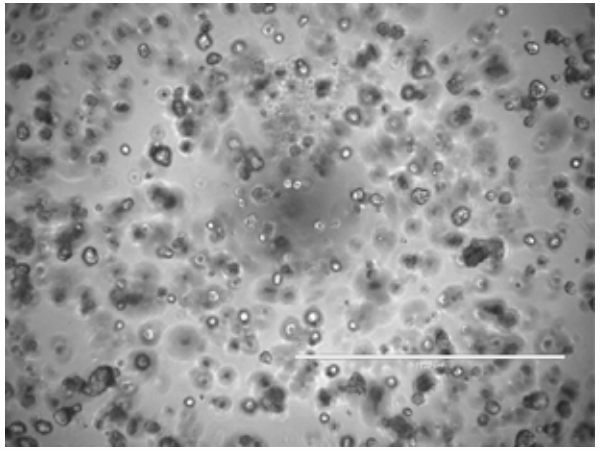
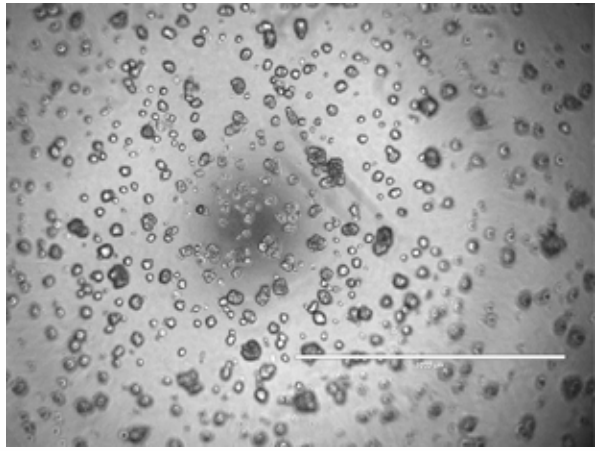
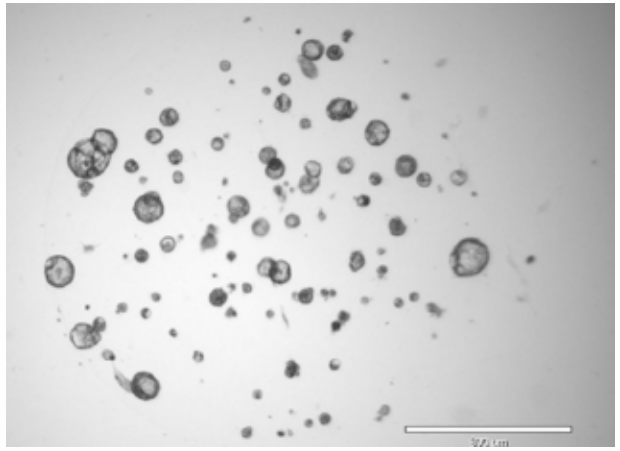
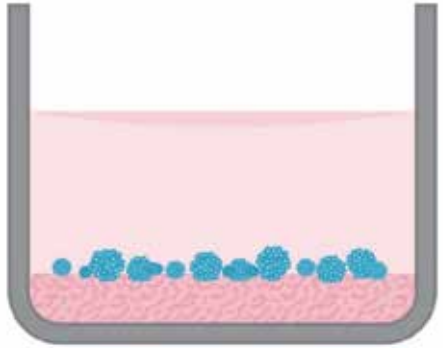
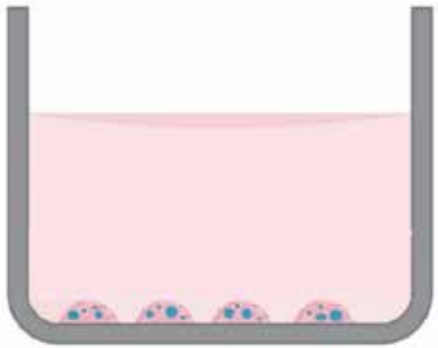
Corning Matrigel Matrix for Organoid Culture



Corning Matrigel Matrix-3D Plates

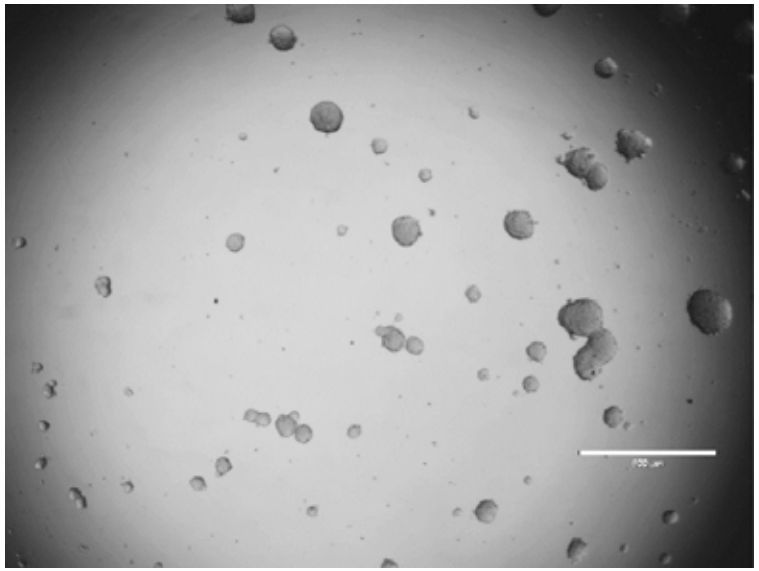
- Mimics ECM
- Allows for cell polarity
- Flexibility to embed cells, culture on top, control stiffness
- Historically difficult to work with for high throughput applications

3D Corning® Matrigel® Culture Methods

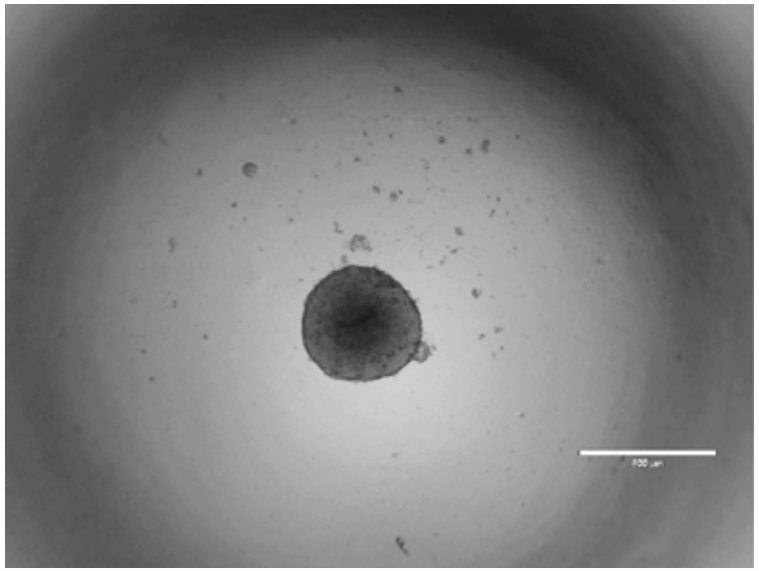
Embedded	Sandwich	Dome
		
		

Often used for organoid culture

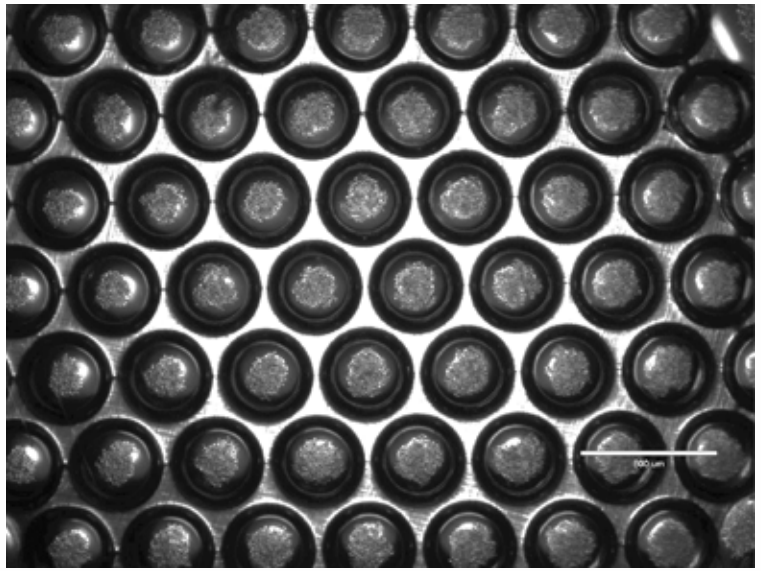
3D Technologies: Ultra-Low Attachment (ULA) Surface



Flat bottom



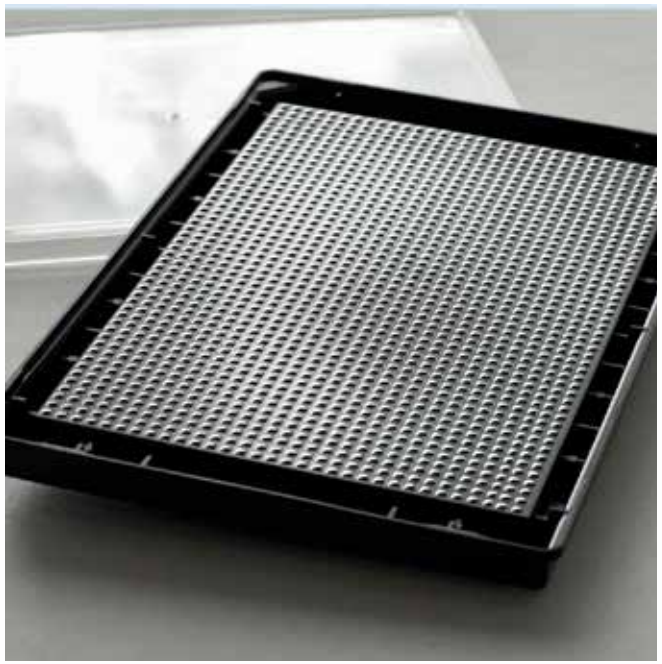
Round bottom
(spheroid microplate)

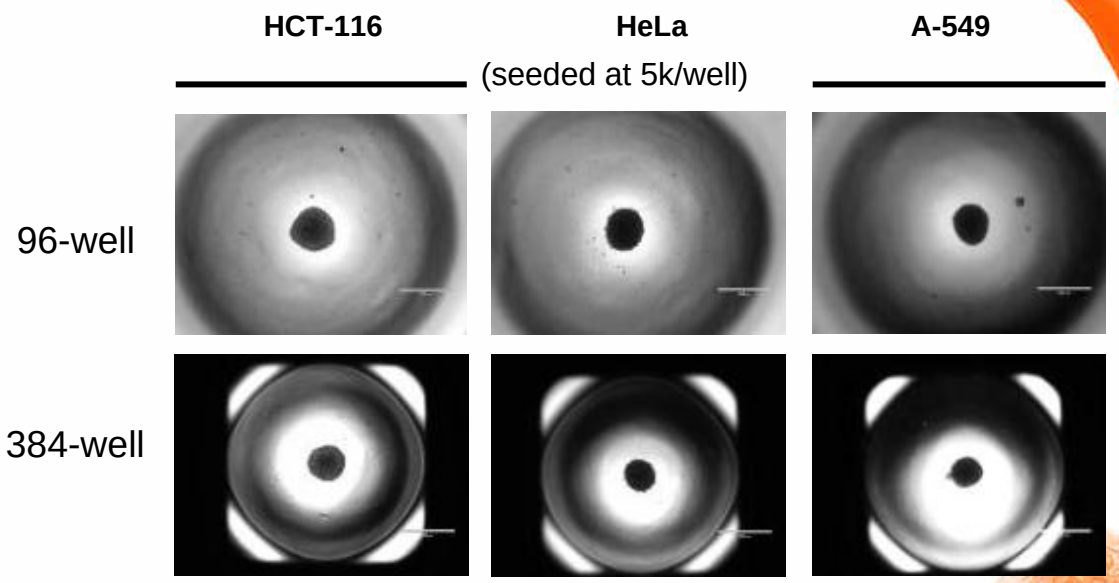


Microcavity
(Corning® Elplasia® plate)

- Hydrogel coating prevents attachment
- Promotes self-aggregation of cells
- Assays can be done directly in plate
- Easy handling and automation friendly

Corning® Spheroid Microplates

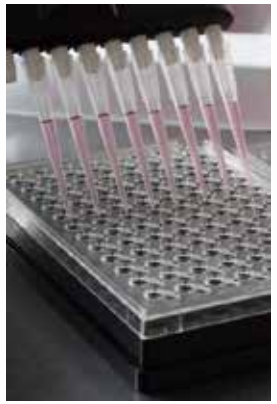




- Unique round bottom geometry
- Single, uniform sized spheroids per well
- Culture and assay in the same plate
- Scalable formats: 96-, 384-,1536-well
- >40+ cell types demonstrated

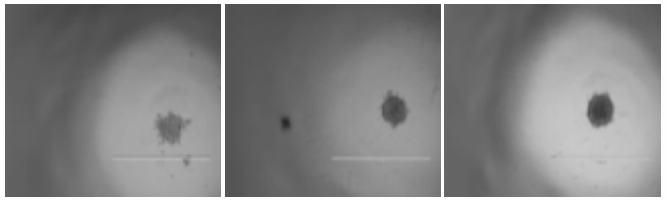
Round Bottom Design Enables “Plug and Play” Protocols:
Optimization considerations

1. Plate cells.



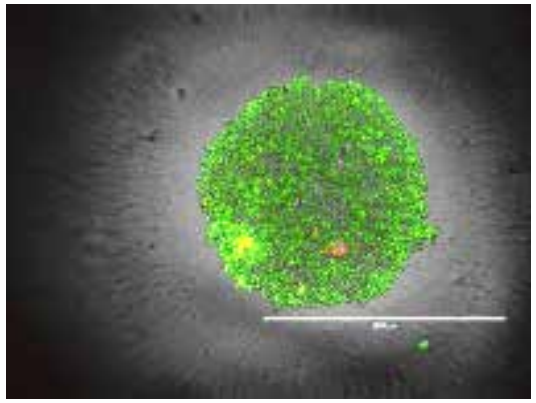
Centrifuge (optional)

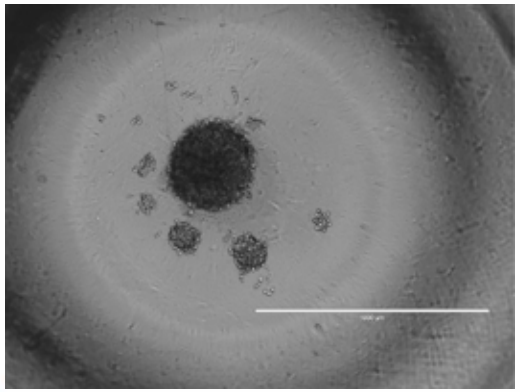
2. Generate and culture spheroids in microplate.



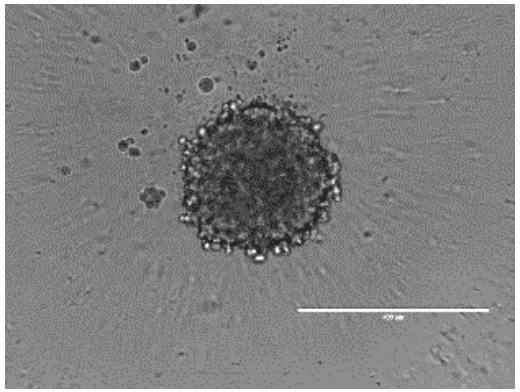
24 hr. 48 hr. 72 hr.

3. Assay spheroid directly in well.

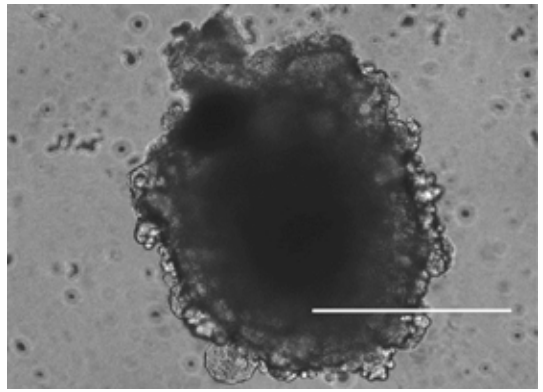




Satellites
Centrifuge and seed with a single cell suspension



Co-culture
1:1 A549 and fibroblast

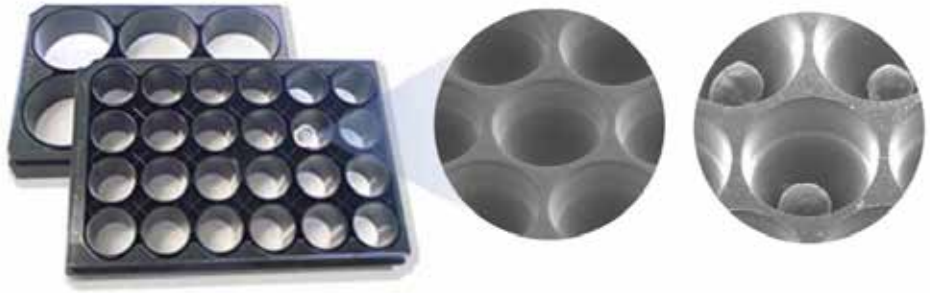


Overlay
2.2 mg/mL Corning Matrigel matrix

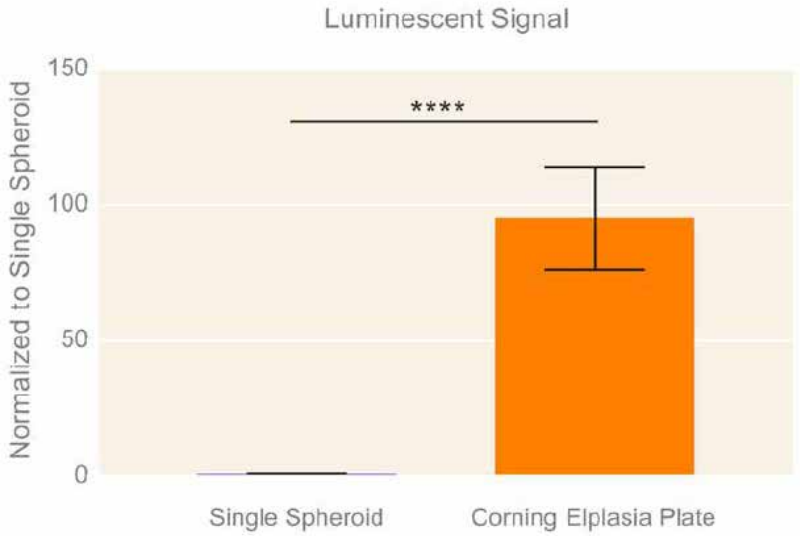
Corning® Elplasia® Microcavity Plates

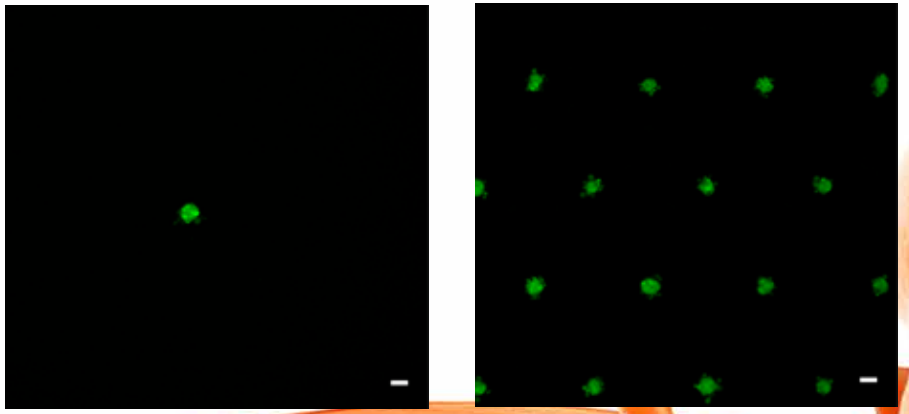
- Bulk spheroid production
- Increase fluorescent/luminescent signal
- Bulk RNA/Protein isolation in 3D
- No need to pre-wash wells before use
- From 79 to 2,885 spheroids per well

Round Bottom

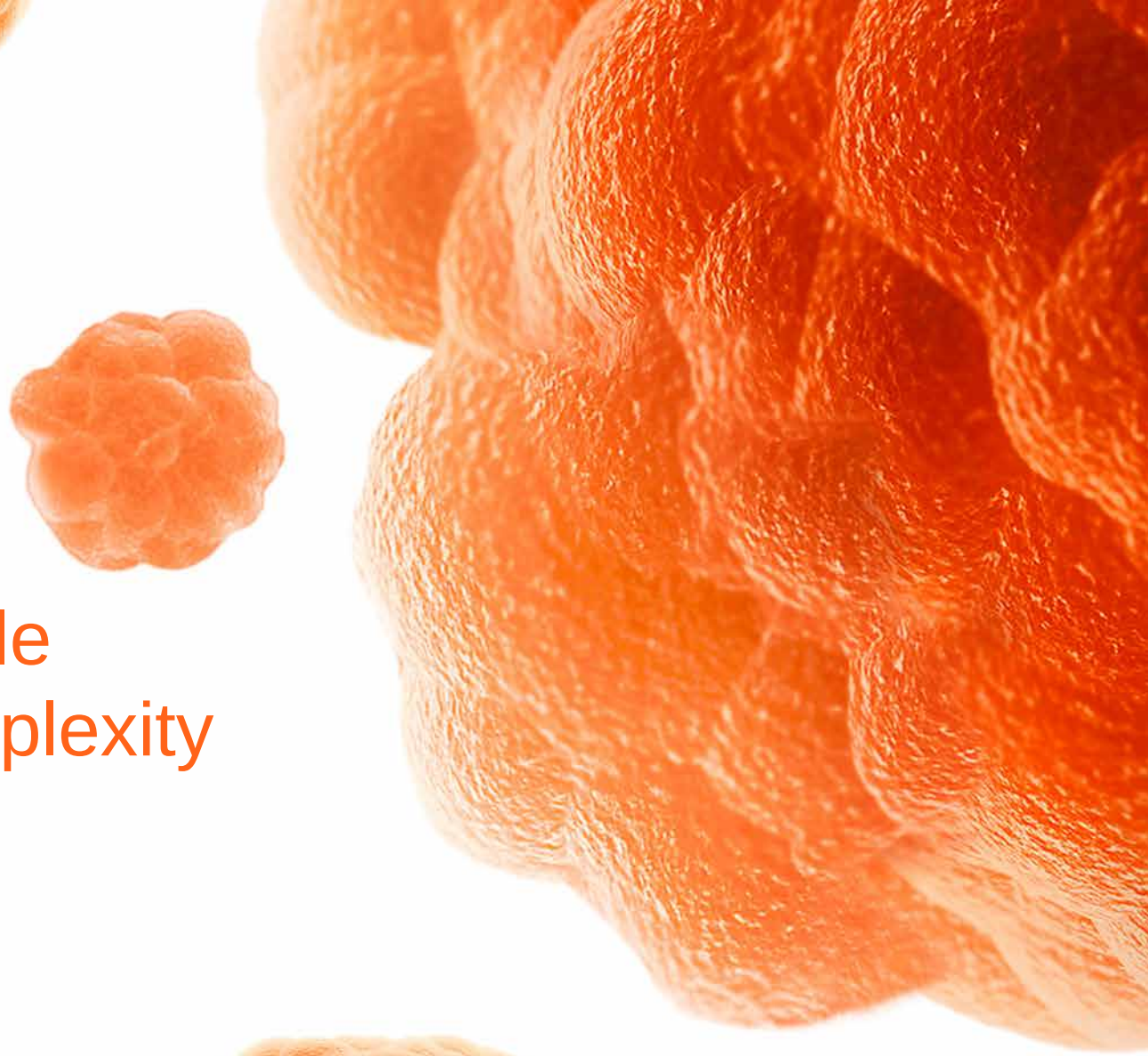


ULA surface coated – Ideal for bulk spheroid formation





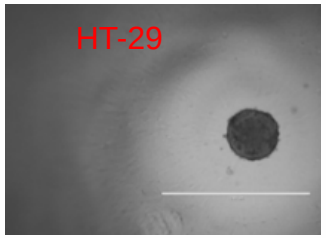
Increasing Throughput While Maintaining Biological Complexity in 3D

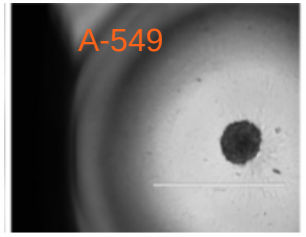


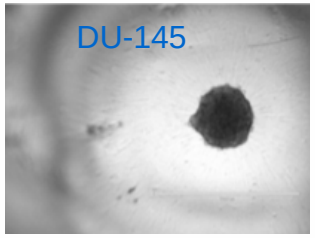
HT Growth Analysis and Drug Screening

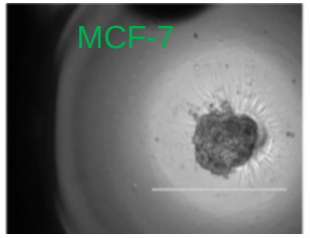
ATP viability assay using Promega CellTiter-Glo® 3D

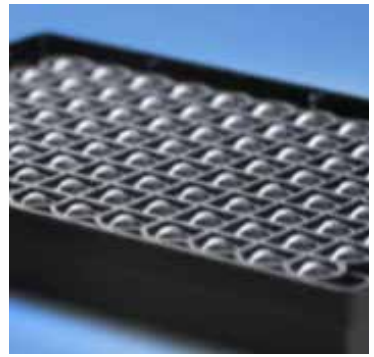
- High throughput method for tumor spheroid growth quantification
- Suitable for drug screening assays in 3D

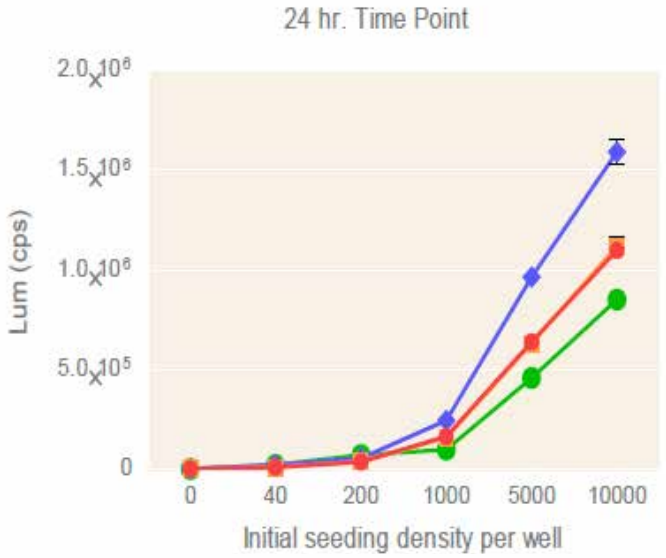


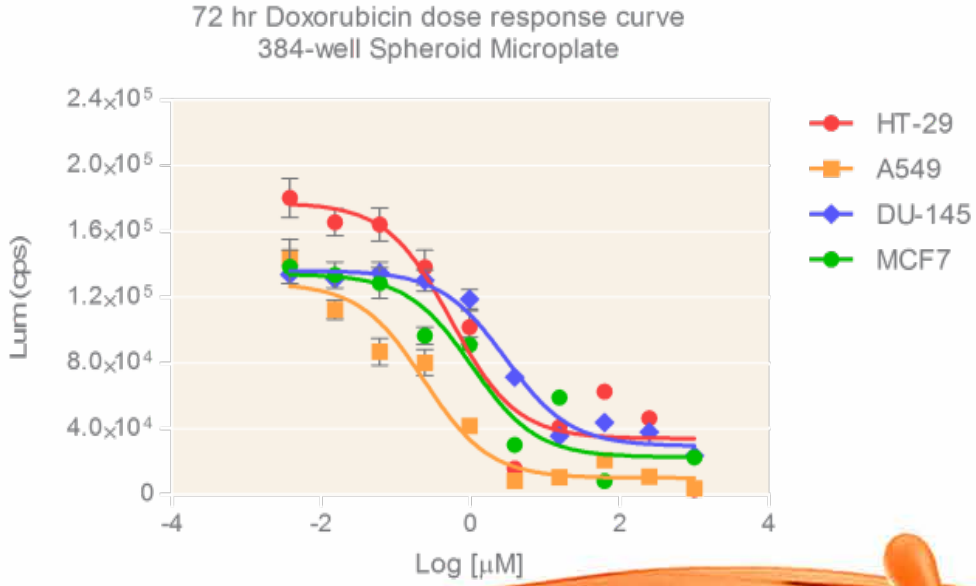








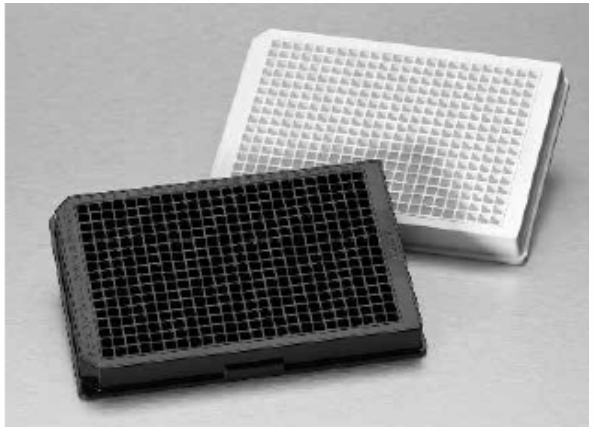


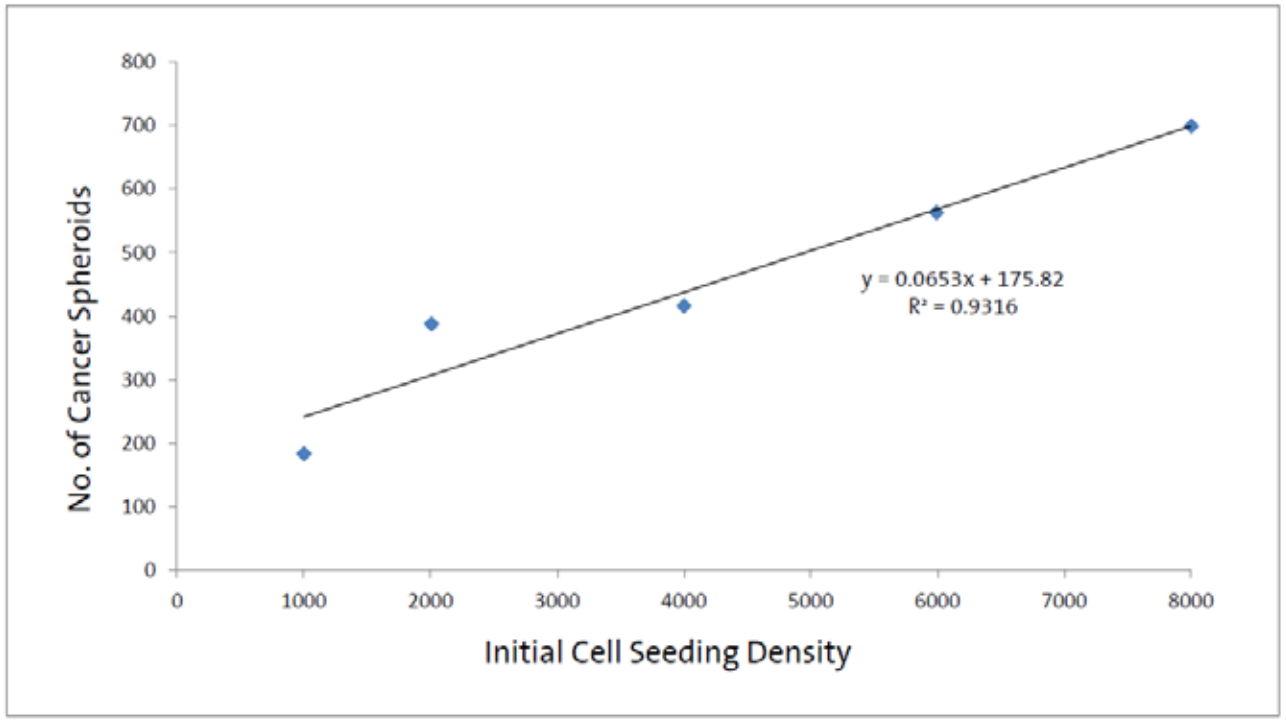


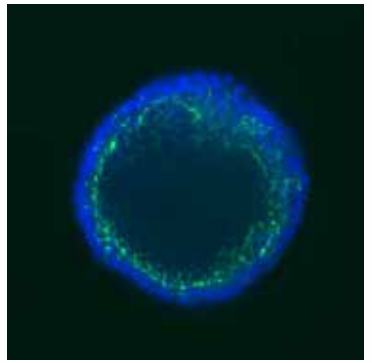
Pardo 2014. Corning Spheroid microplates and Promega CellTiter-Glo 3D cell viability assay provide a novel approach for high throughput screening of multicellular spheroids. Poster.

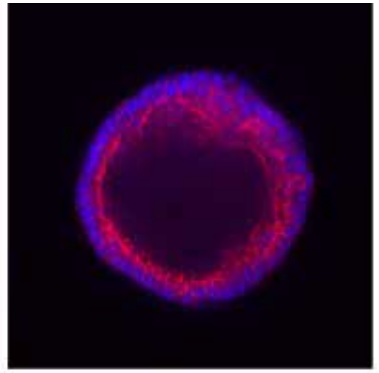
Corning® Matrigel® Matrix-3D Plates

- Convenient – no need to manually prepare Matrigel matrix plates
- Consistent – built in quality control and consistent Z' values
- Optimized – ideal thickness for 3D applications





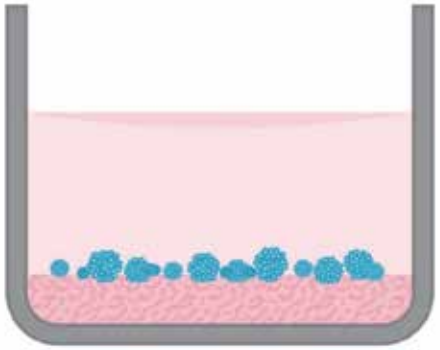




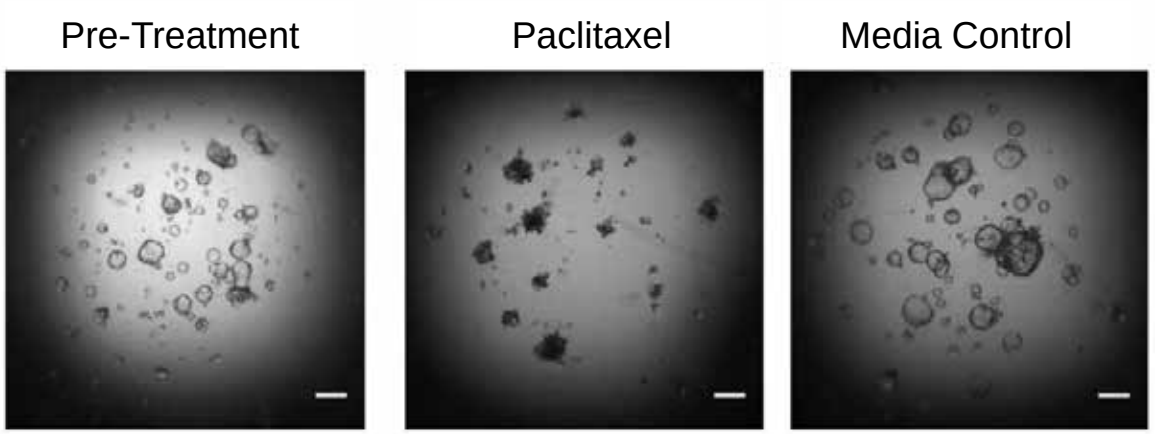
HT Generation of Pancreatic Cancer Organoids

Using Corning® Matrigel® Matrix-3D Plates

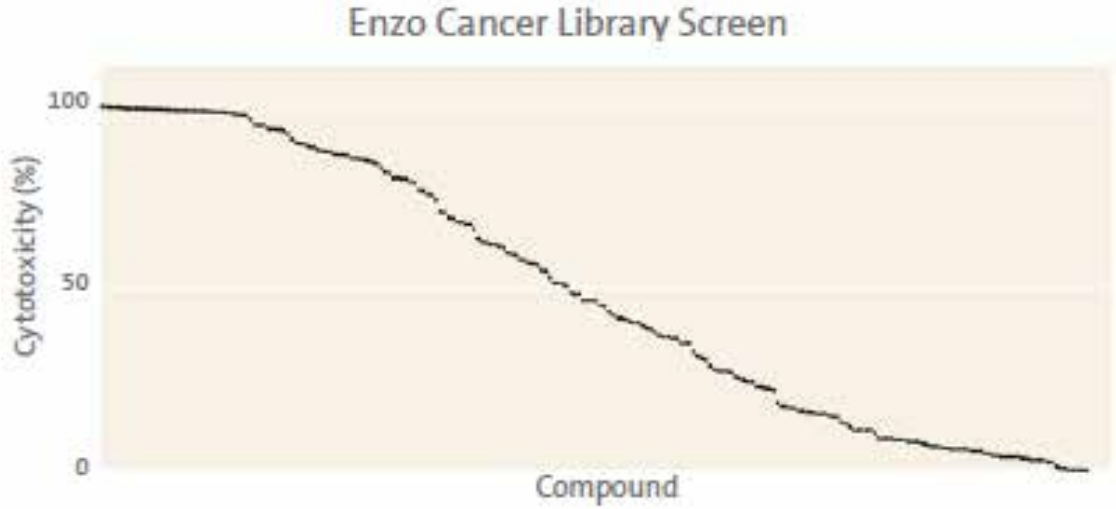
20 µL of organoid suspension added to polymerized Matrigel matrix

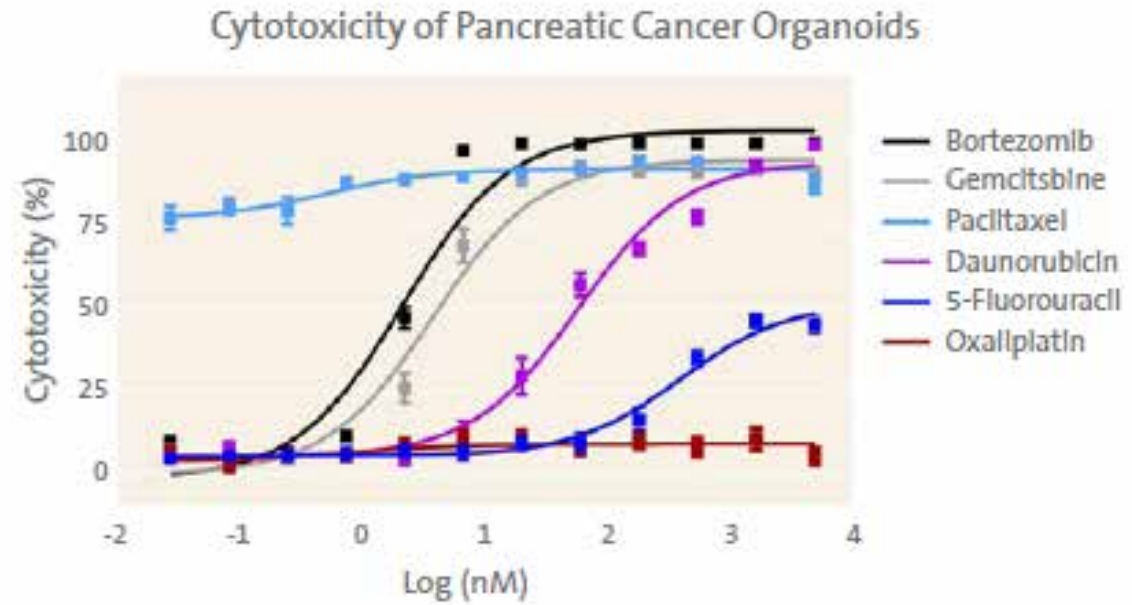


After 48 hours, add drugs and incubate additional 5 days

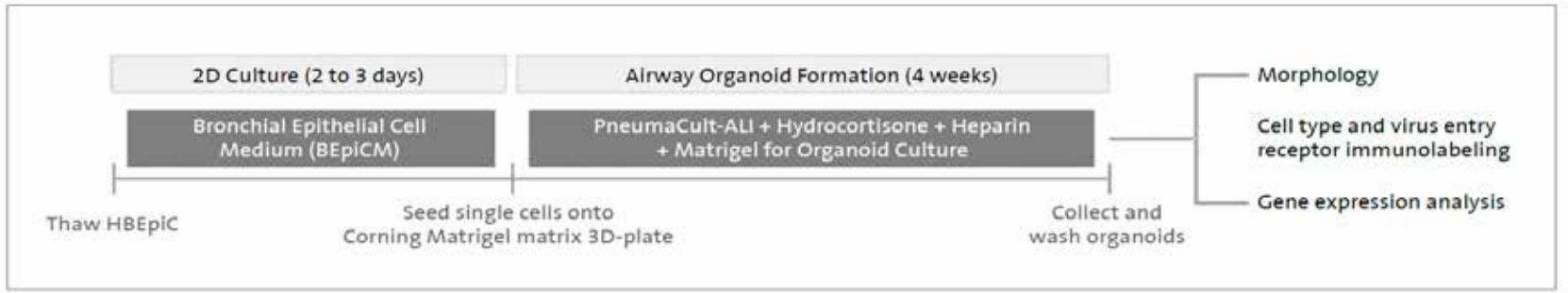


Viability Assessed Using CellTiter-Glo® 3D

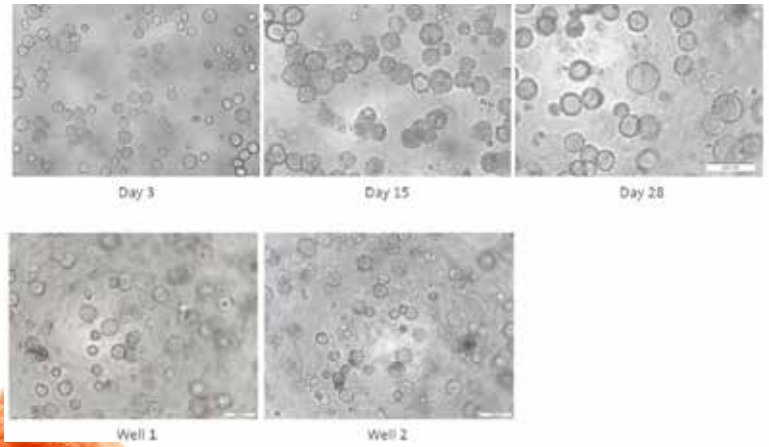




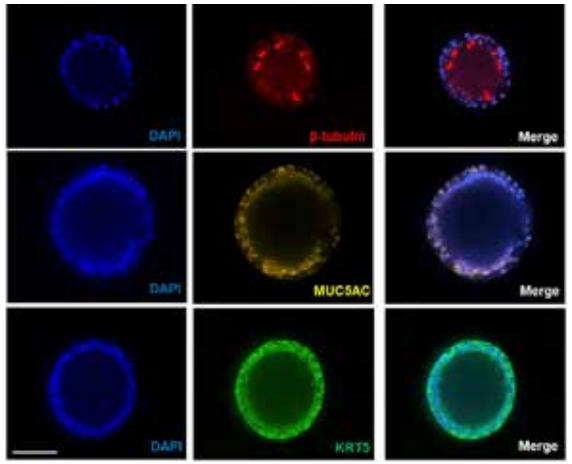
HT Airway Organoid Formation on Corning® Matrigel® Matrix-3D Plates:
Model for testing respiratory virus infectivity



Images of Airway Organoid Formation

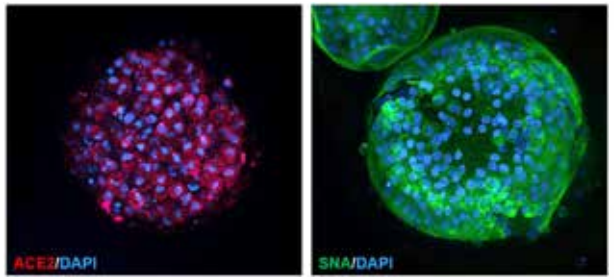


Fluorescent Labeling of Airway Cells



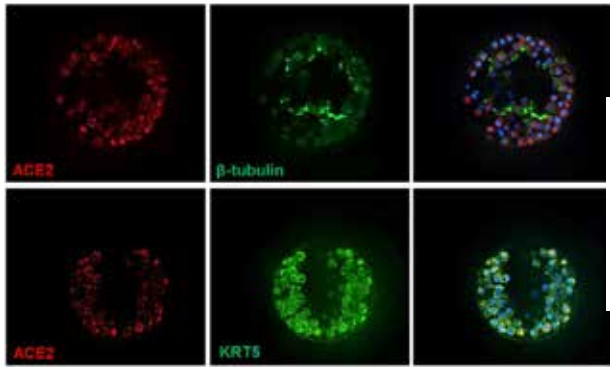
Blue: Nuclei
Red: Ciliated cells
Yellow: Goblet cells
Green: Basal cells

Virus-specific Entry Receptors



Red: ACE2
Green: α2-6-linked sialic acids
Blue: nuclei

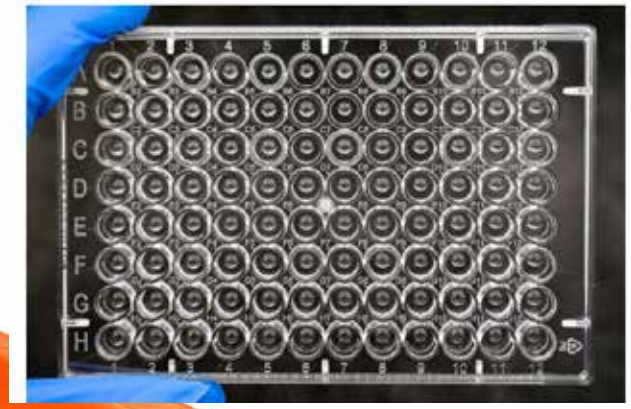
Cell-specific ACE2 Expression

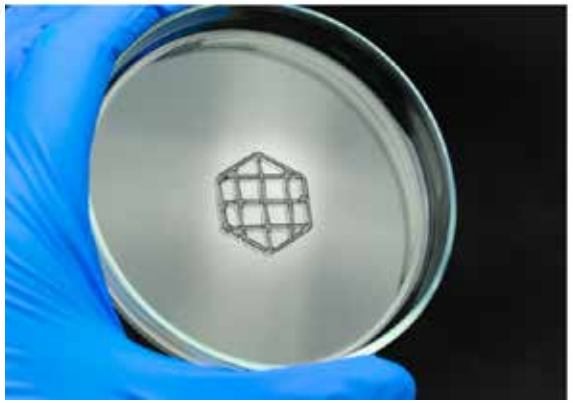


Red: ACE2
Green (top): ciliated cells
Green (bottom): basal cells

New Product Spotlight: Corning® Matribot® Bioprinter

- Accurately dispense hydrogels and print tissues
- Automate 3D workflows
- Ideal for:
  - Dispensing Corning Matrigel® matrix and organoid domes
  - Entry level bioprinting applications





Corning® Matribot® Bioprinter Features

Small footprint
(14.6 x 12.8 x 15 in.)
Benefit: *Benchtop unit that fits easily into hood/BSC*

Cooled syringe printhead (0°C-RT)
Benefit: *Use of Corning Matrigel® matrix/Collagen and other RT hydrogels*

Thermal nozzle insulators
Benefit: *Reduces clogging of at nozzle for temperature-sensitive hydrogels*

Curing System
UV 405 nm LED and Temp. of printbed (RT-60°C)
Benefit: *Multiple modes to crosslink hydrogels and maintain 3D structure*

Calibration
Automatic and Manual
Benefit: *Better Consistency*

Receiving Vessels
Microplates, multiwell plates, Petri dishes
Benefit*: Compatible with various vessel formats*

Controls
LCD screen with knob or software-mediated access. The software supports both Windows® or macOS operating systems.
Benefit: *Flexible operations*

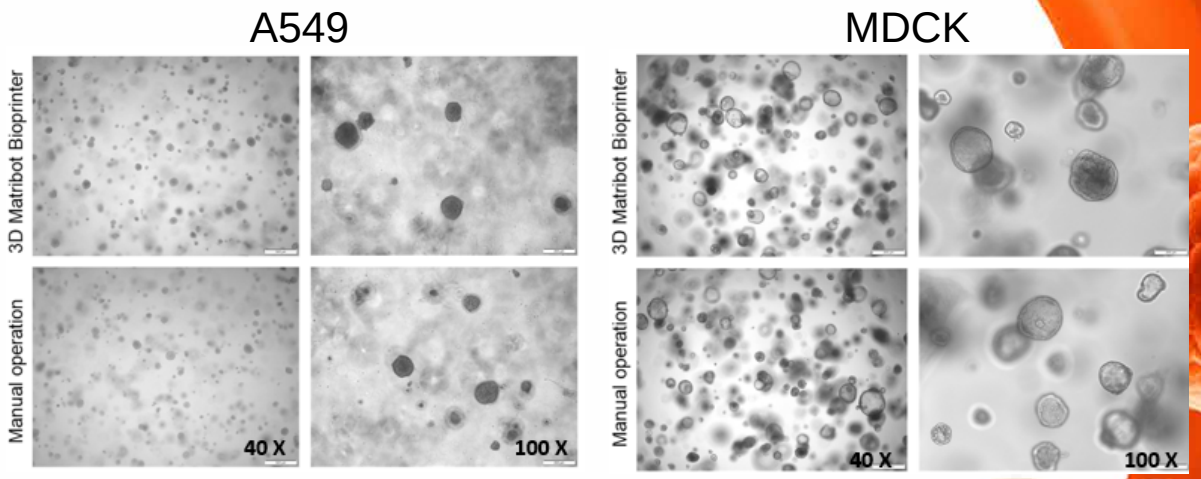
Materials
Bioinks/Hydrogels (with or without cells)
Corning Matrigel matrix and Collagen
Alginate-based bioinks
Benefit: *Supports temperature-sensitive and ambient-temperature hydrogels*

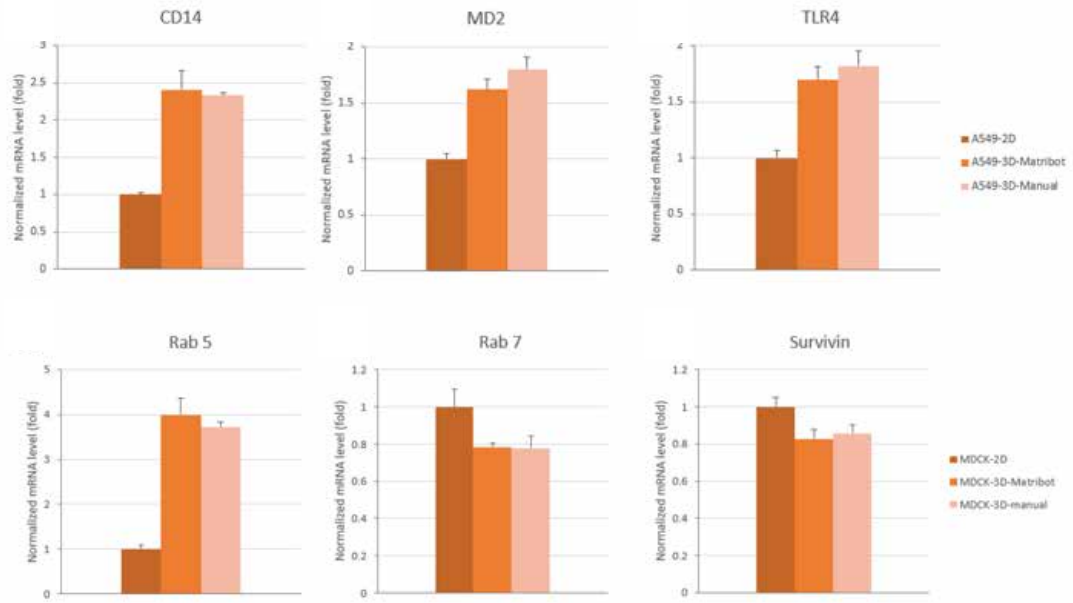
Corning® Matribot® Bioprinter Application

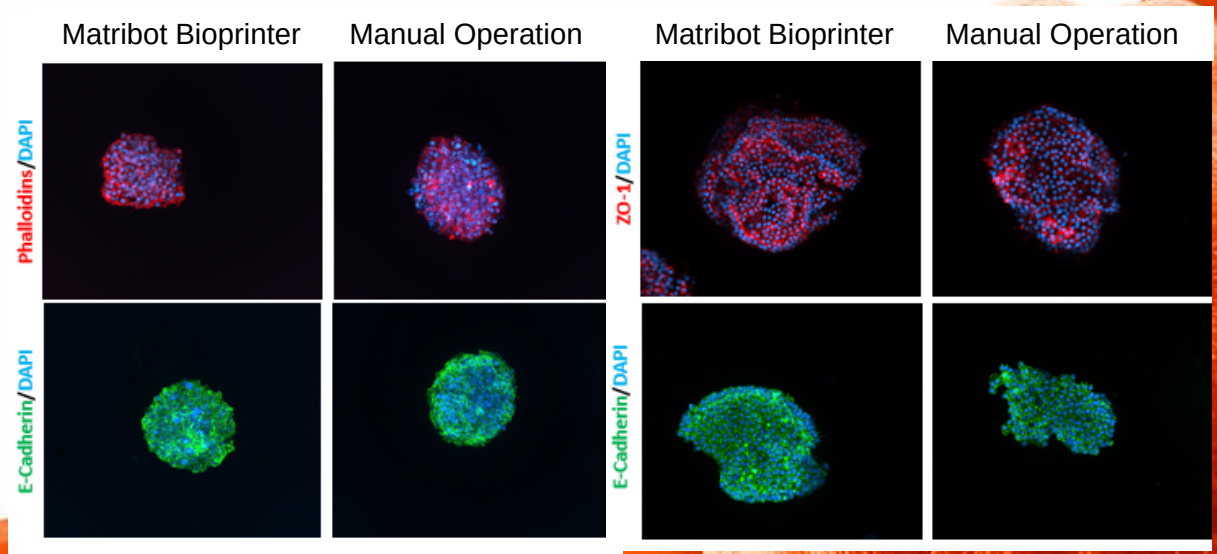
Automating spheroid droplet dispensing

50 µL droplet per well of 24-well microplate

10 and 7 days in culture



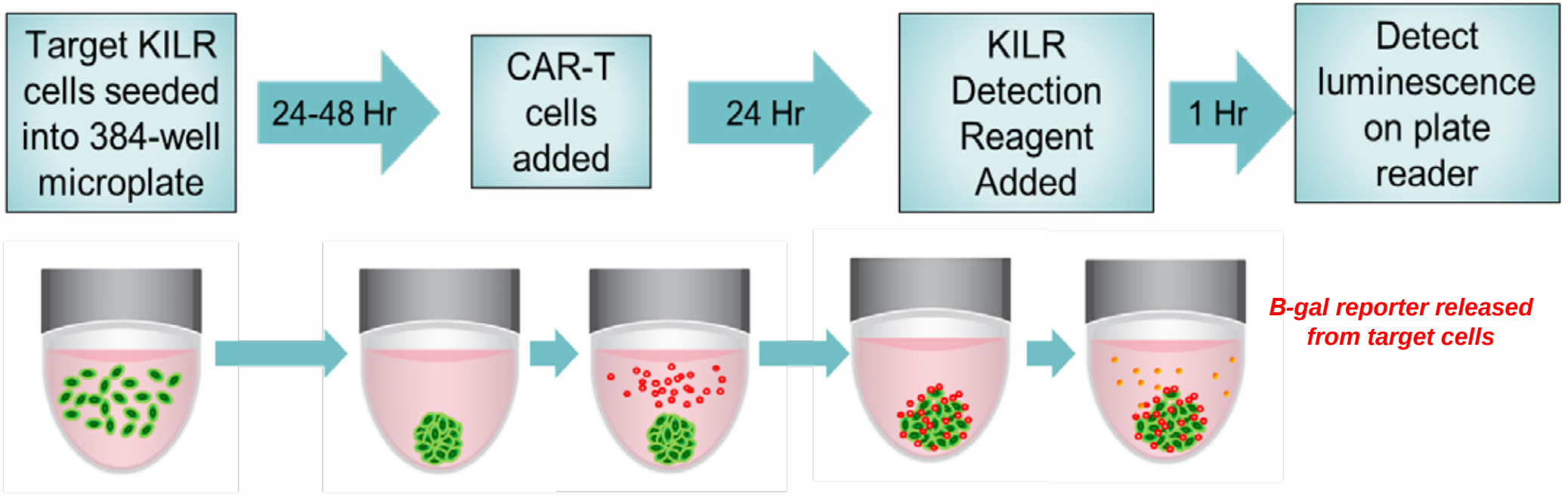




Immuno-oncology Application

3D Cytotoxicity Assay: CAR-T Cell Screening of Tumor Cytotoxicity

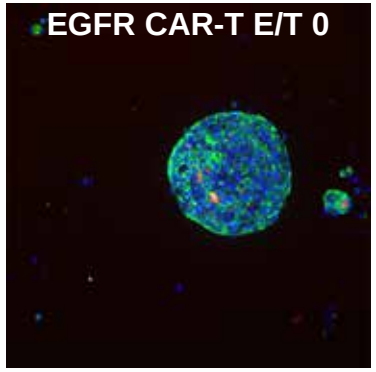
- Corning® Spheroid Microplates – Tumor spheroid formation
- DiscoveRx KILR® Cytotoxicity Assay – Quantification of tumor cytotoxicity

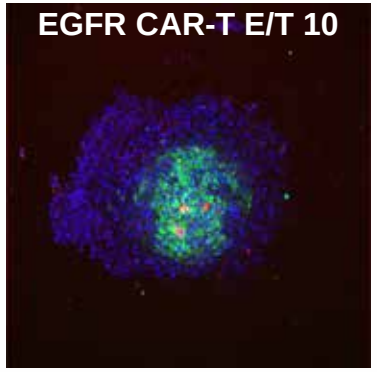


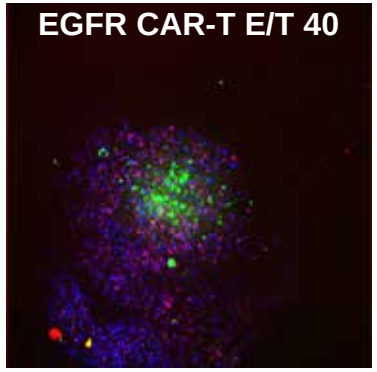
CLS-AN-447

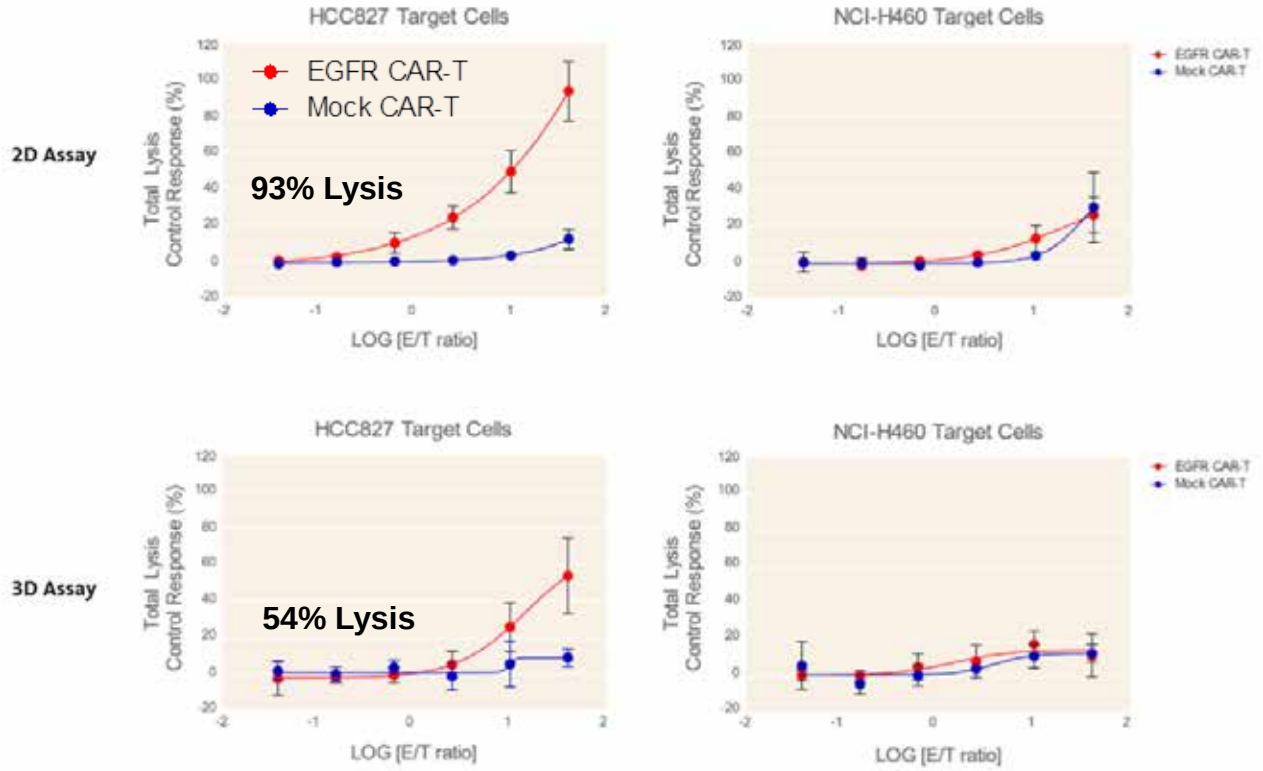
CAR-T Cells are More Effective in Lysing 2D vs. 3D Tumor Cells

- Effector: CAR-T cells
- Target: lung adenocarcinoma cell line (HCC827)









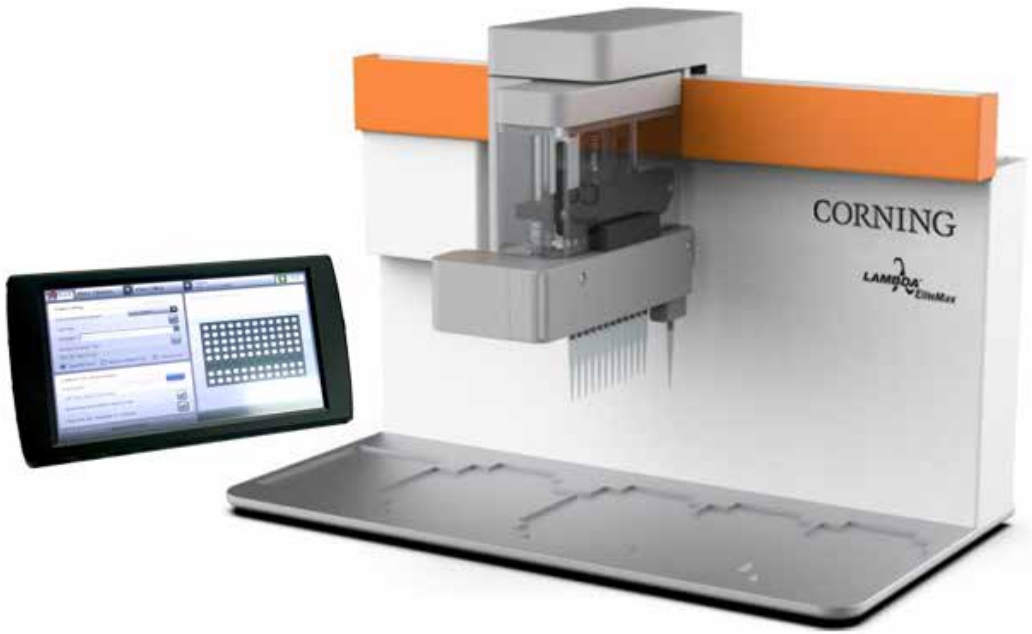
Corning® X-LAB® and X-WASH® Platforms:
Semi-automated MNC Isolation and Buffer Exchange

Disposable Cartridge
- Starting material volume: 40-240 mL
- Sterile, single-use, easy to use
- Harvest volume: 3-40 mL

Control Module
- Opens and closes cartridge valves
- Runs protocol in centrifuge
- Records speed, time, states

Docking Station
- Charges control module
- Uploads protocols to control module
- Downloads processing data

Corning® Lambda™ EliteMax Semi-automated Benchtop Pipettor



High throughput automation with a small footprint

- Easy-to-use touch screen user-interface controller
- Dual single-channel and multi-channel head configurations
- 5 deck seating positions with both landscape and portrait orientation options
- USB flash drive port supports data exchange and backup files

Corning Elplasia Microcavity Flask

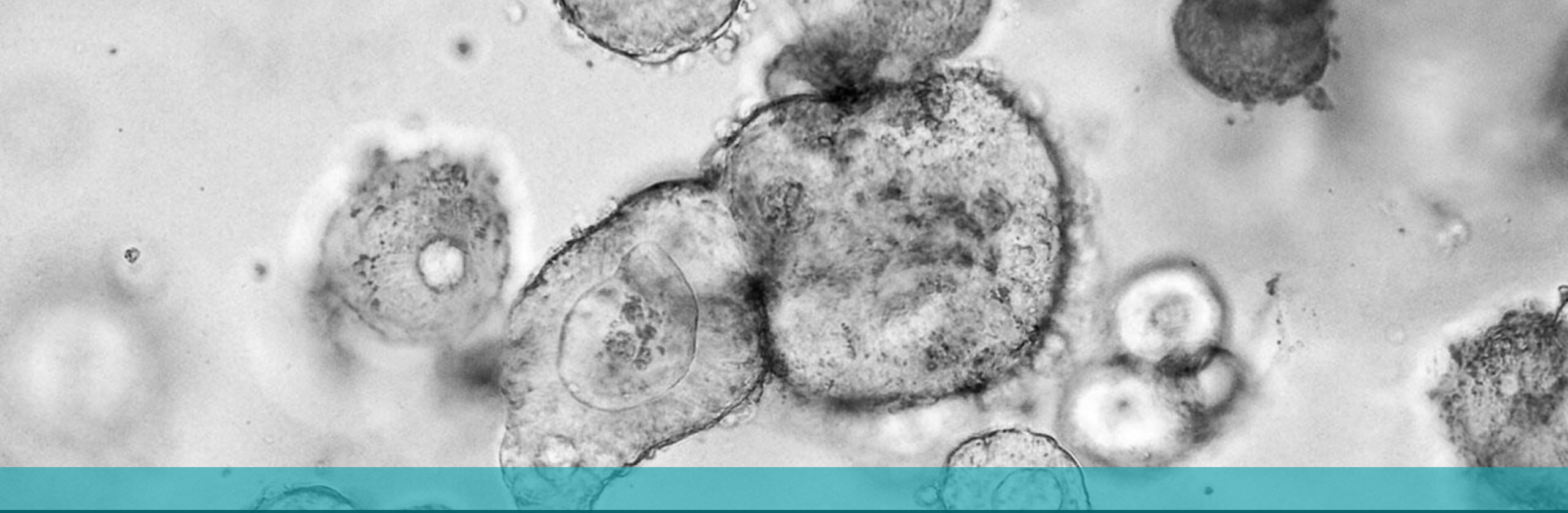
~12,000 spheroids per flask

New Product Features

Corning® Matribot® Bioprinter

Corning Cell Counter
for Organoids and Spheroids

Corning Lambda™ EliteMax
Semi-automated Benchtop Pipettor



Patient-derived Organoids: a revolutionary new model to advanced precision medicine

HUB ORGANOIDS

ISSCR
San Francisco, USA
16th June 2022

Drug Development – Inefficient, Unpredictable, Expensive

Pre-Clinical Development

Clinical Phase I-III

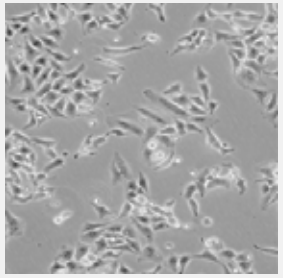
Post Registration

90% clinical failures of drug development

Small Molecules
Antibodies
IO Drugs

Highly Innovative Drugs

Average investment:
10-15 years and $1-2 billion



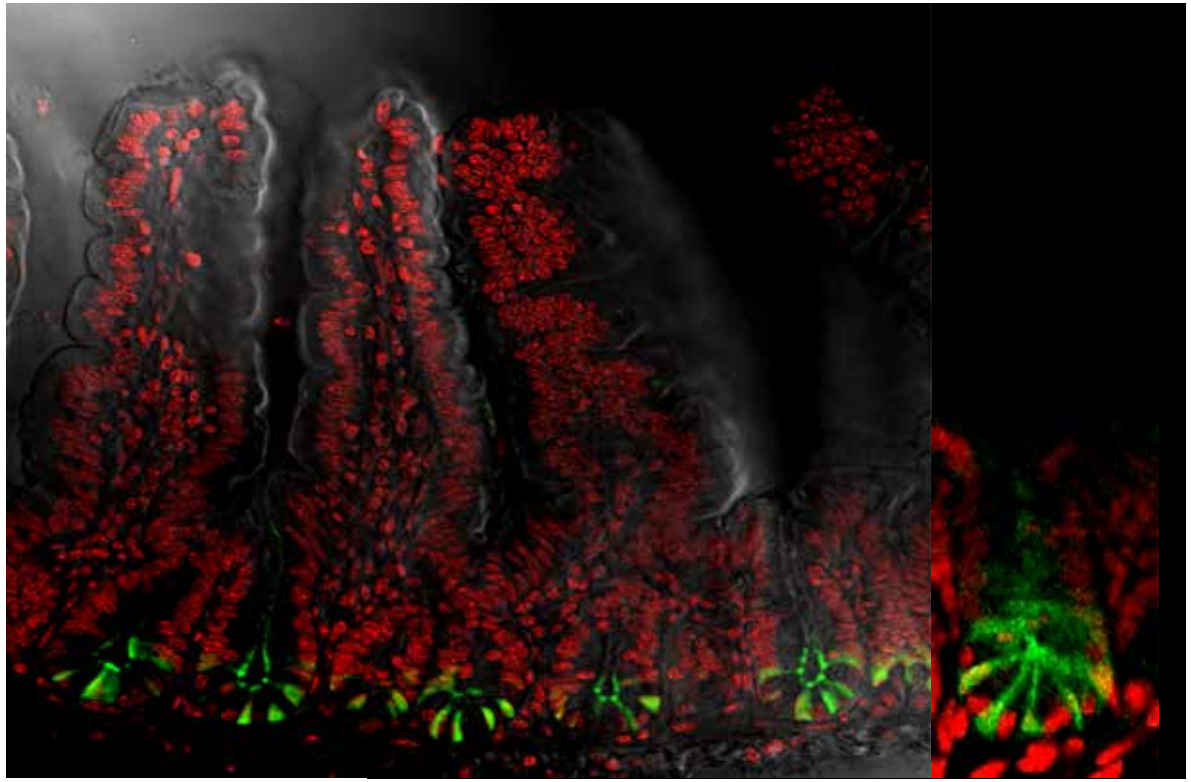
Bridge the Gap Between Lab & Clinic

Patient in the Lab

HUB ORGANOIDS

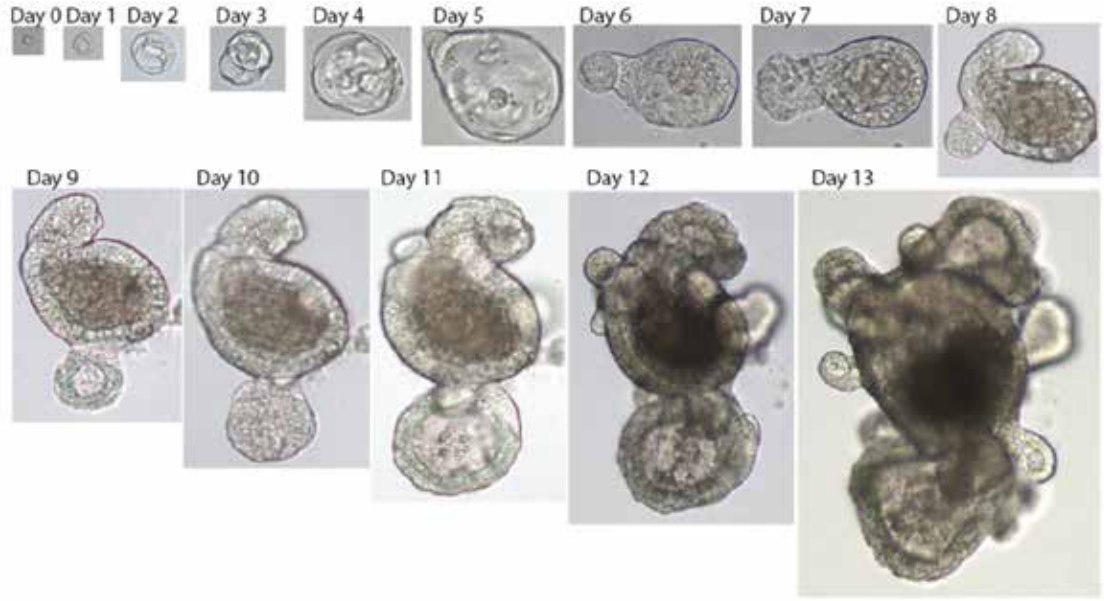
Key Scientific Breakthroughs that Led to ASC-Organoid Technology

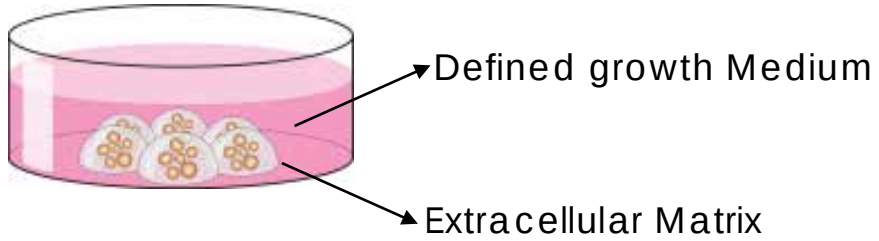
- Identification of LGR5 as adult stem cell marker of the intestinal mouse epithelium



Barker N. *et al.* Nature 2007

- Identification of LGR5 as adult stem cell marker of the intestinal mouse epithelium





Sato T. *et al.* Nature 2009

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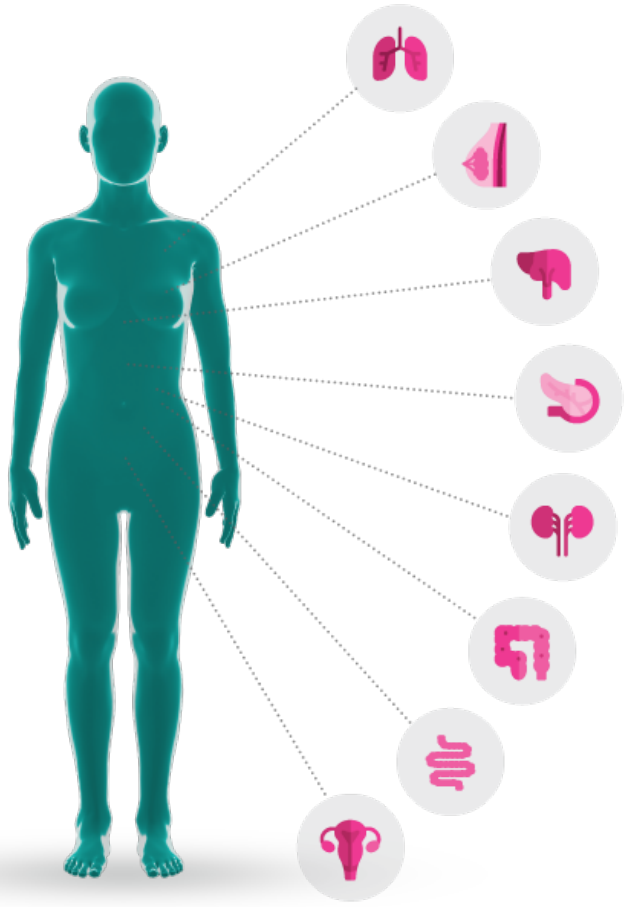
Patient-derived Organoid Biobanks at HUB

- ASC-Organoid Technology allows the generation of Patient-derived Organoids models from different tissues to build “living” biobanks representing different diseases with a relevant level of characterization



DNA-seq

RNA-seq

In vitro Drug Testing

Clinical Data

HUB ORGANOIDS

Key HUB Organoids Features

HUB ORGANOIDS

Applications of HUB Organoid Technology for Drug Discovery and Development

HUB ORGANOIDS

Oncology

colon, pancreas, breast, lung, ovarian, H&N organoids

Cystic fibrosis

colon and lung organoids

Inflammatory diseases

colon and lung organoids

Infectious diseases

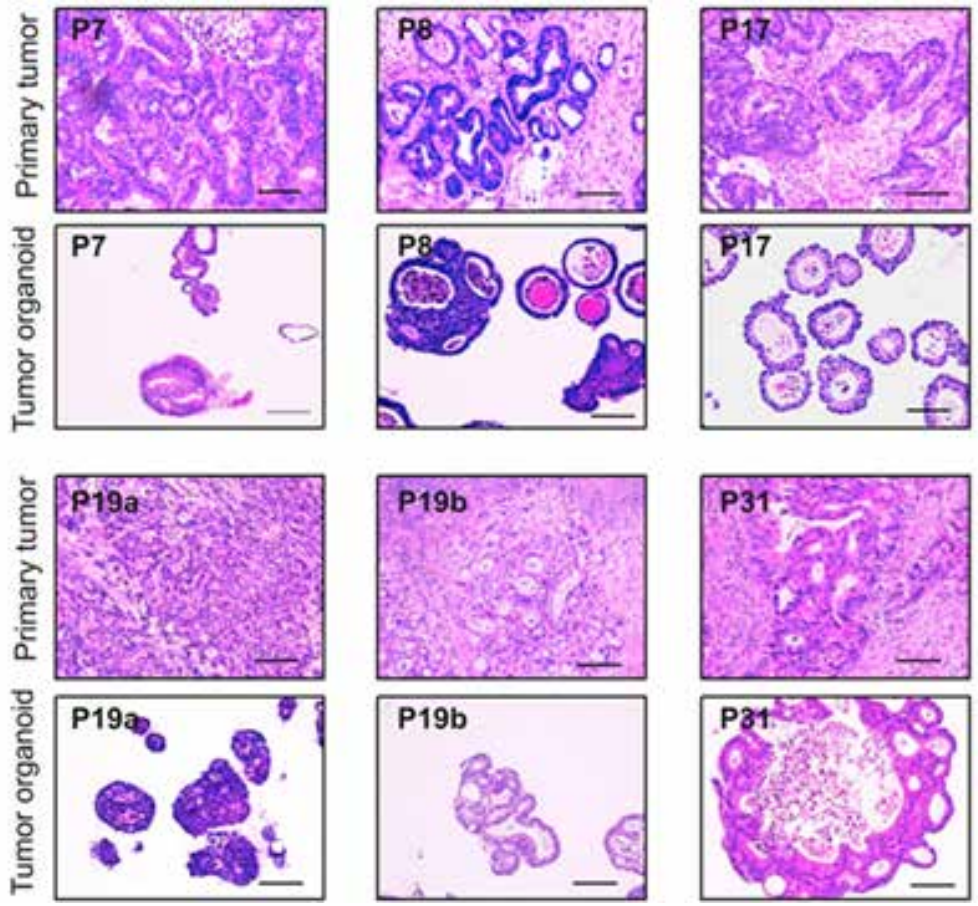
liver, lung and intestinal tract organoids

Toxicology

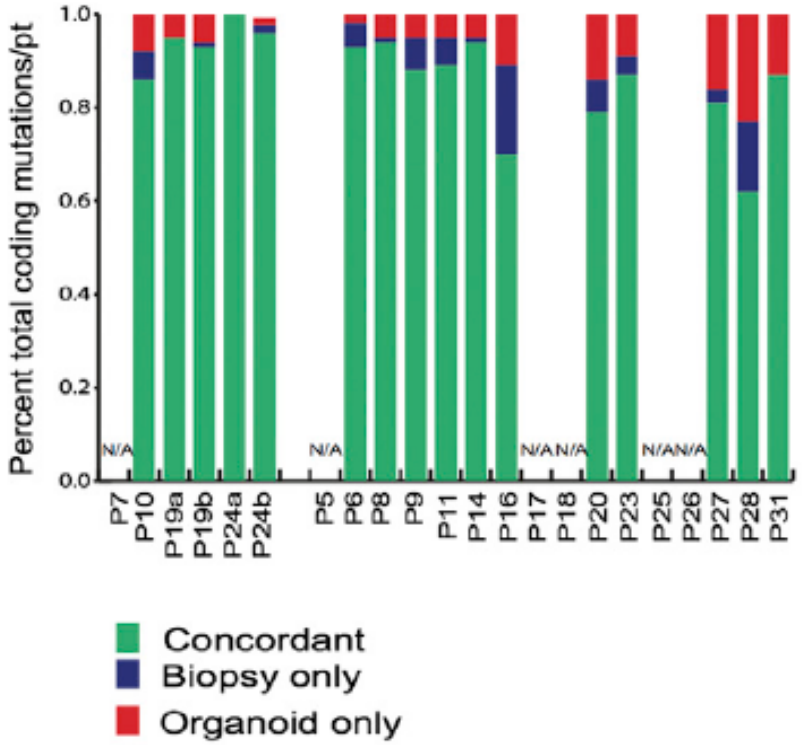
liver and intestinal tract organoids

Colorectal Organoids Resembles Primary Tumour

- CRC PDOs show comparable histological characteristics to original tumor tissue



- High concordance of mutations identified in both original tumor tissue and patient-derived organoid

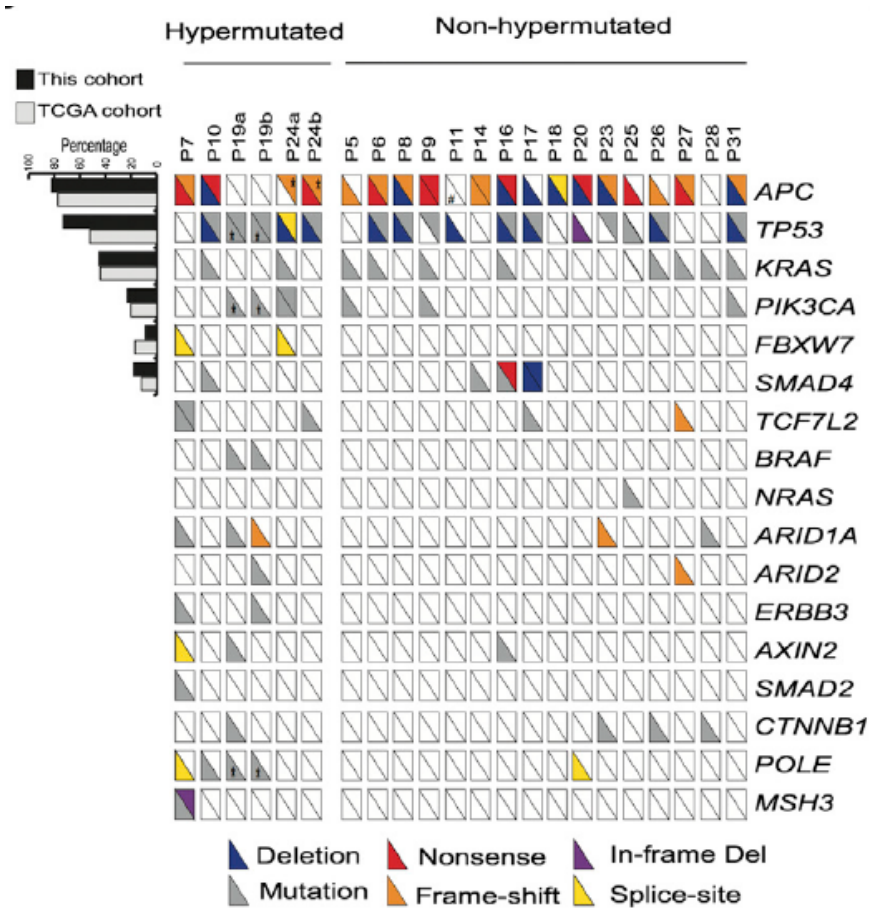


van de Wetering *et al.*, Cell 2015

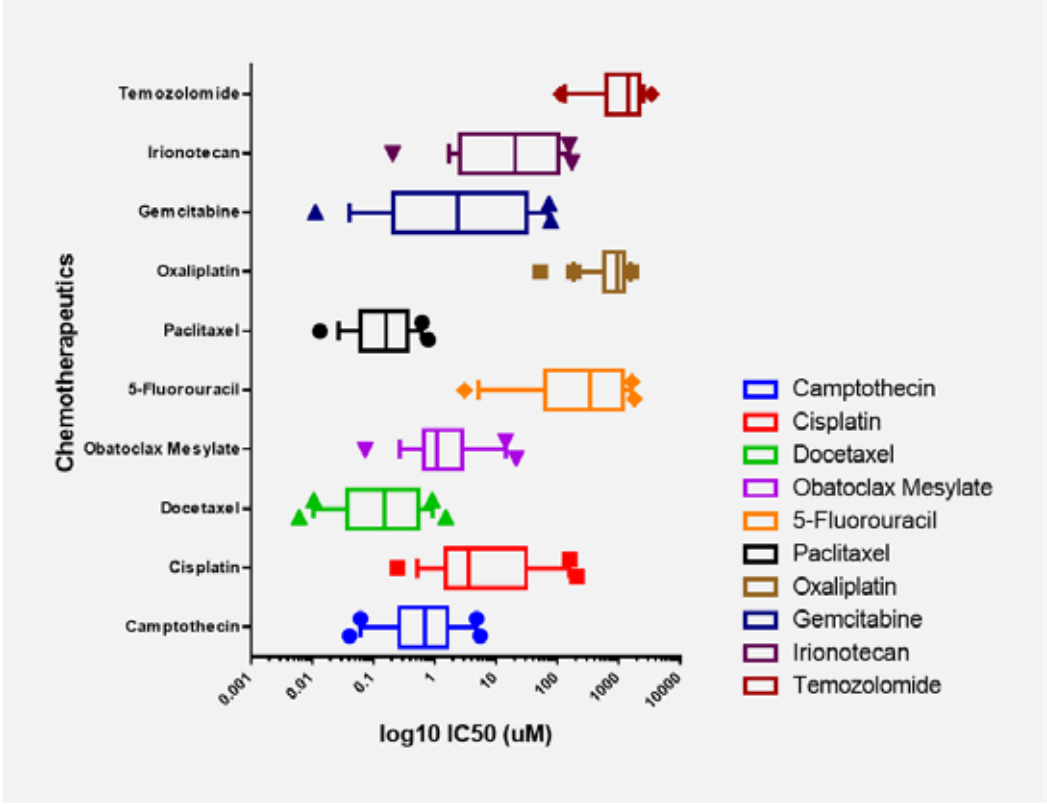
HUB ORGANOIDS

Genomic Alterations and Drug Response in Colon Cancer Patient-derived Organoids

- CRC PDOs represent inter-tumor heterogeneity observed in CRC patient populations



- CRC PDOs show an heterogenous drug response to standard of care compounds as observed in CRC patient populations



van de Wetering *et al.* Cell 2015

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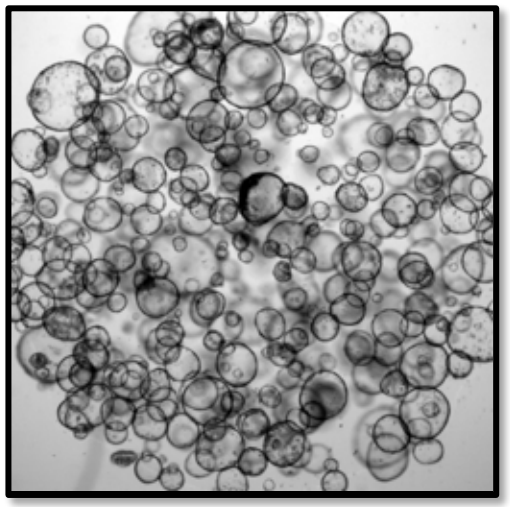
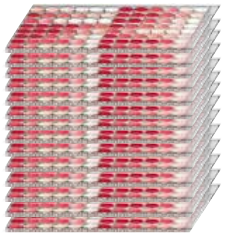
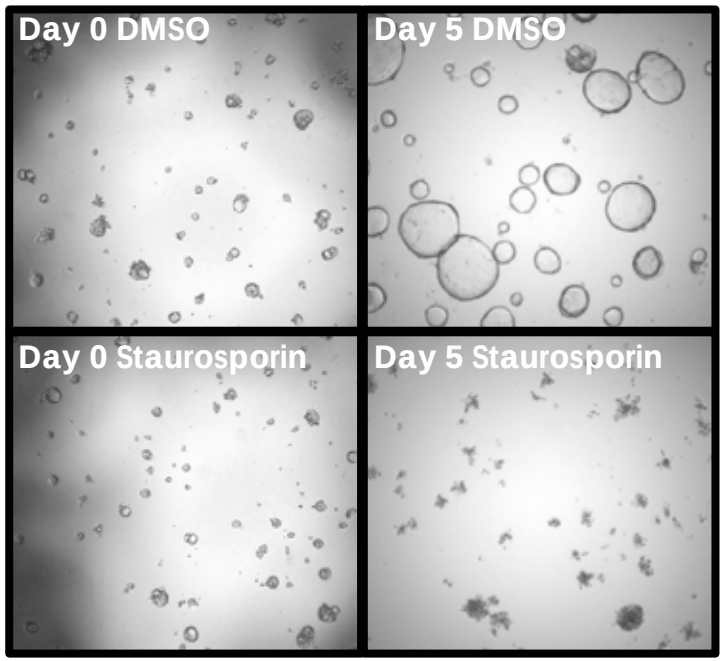
Middle-throughput Drug Screening with Patient-derived Organoids

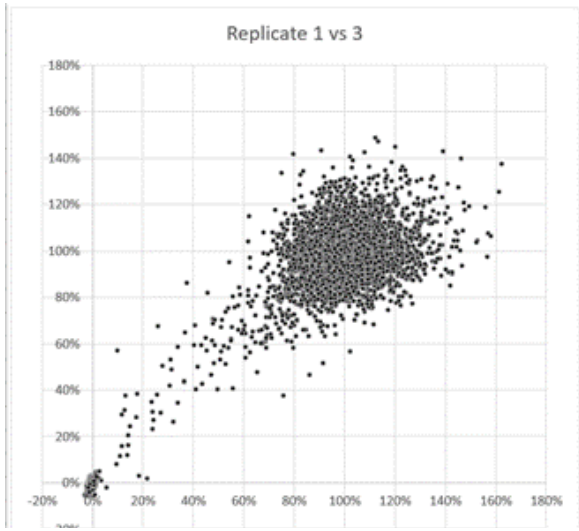
Organoids are expanded (passaged every week)

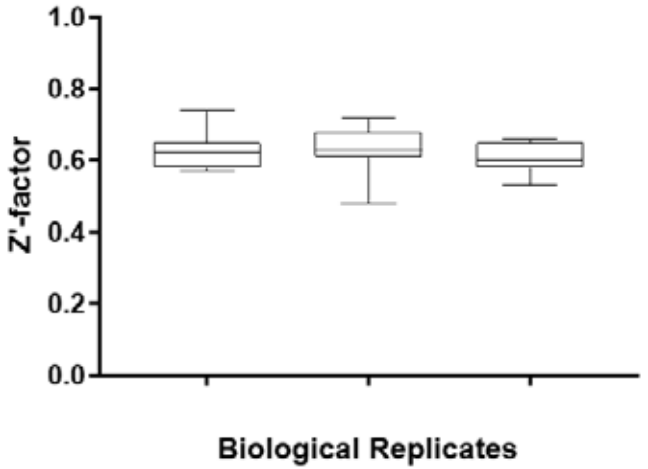




> 12.000 screening wells







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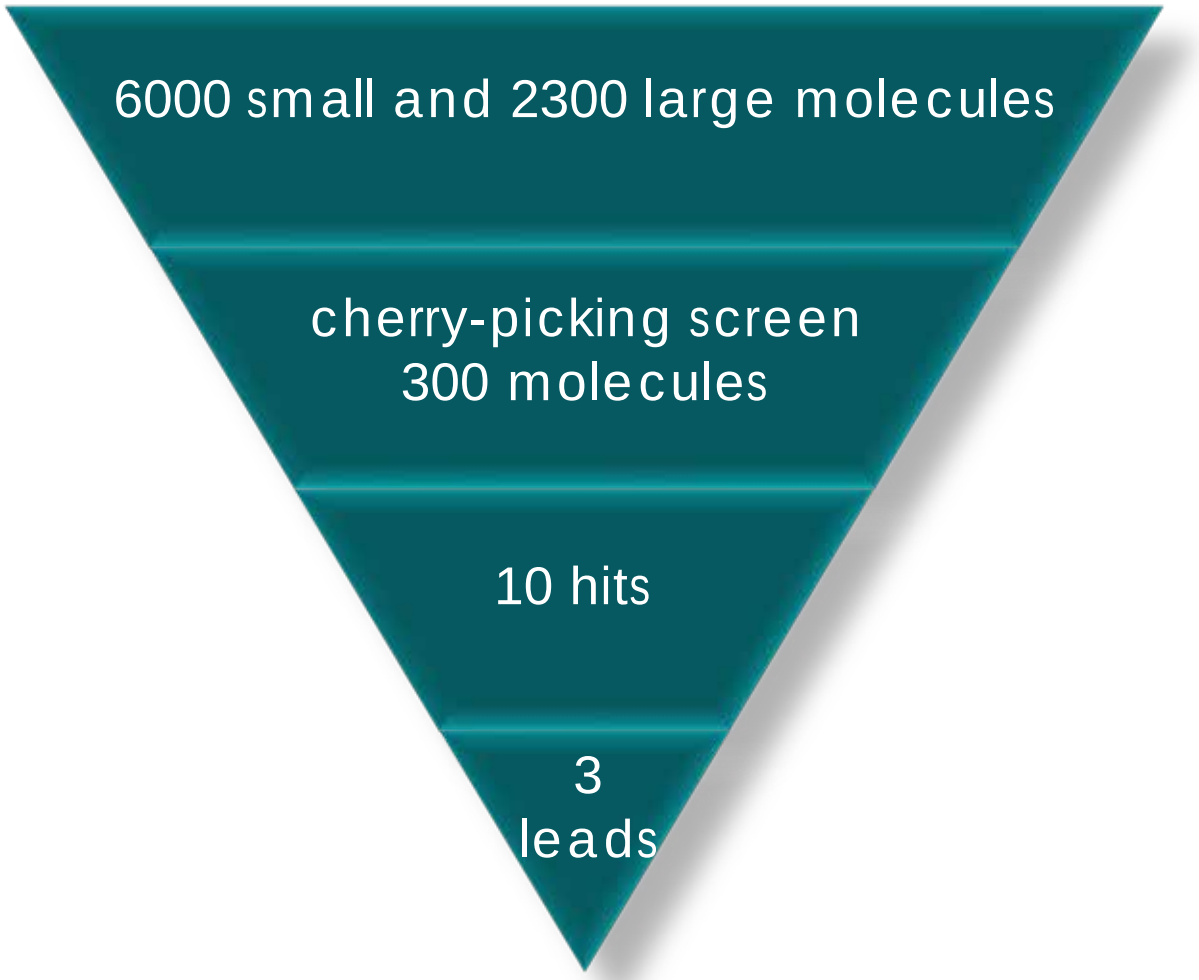
Drug Discovery Platform using CRC PDOs



Increasing Number of CRC PDOS used along the screening pipeline

Mechanism and Lead development

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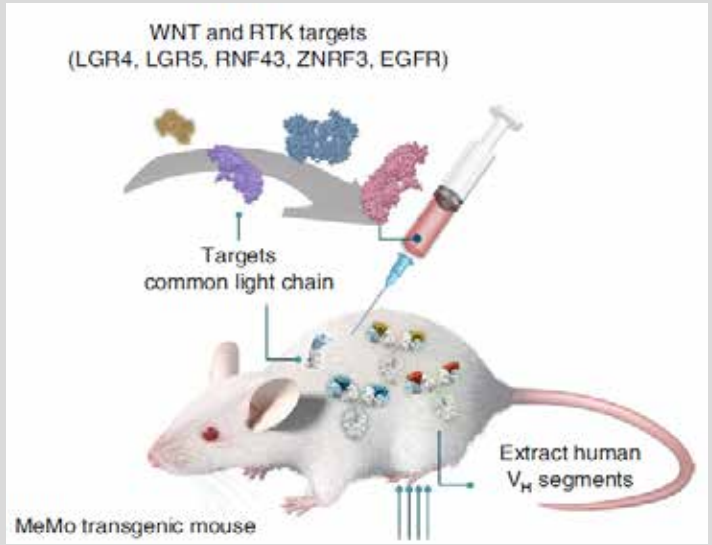
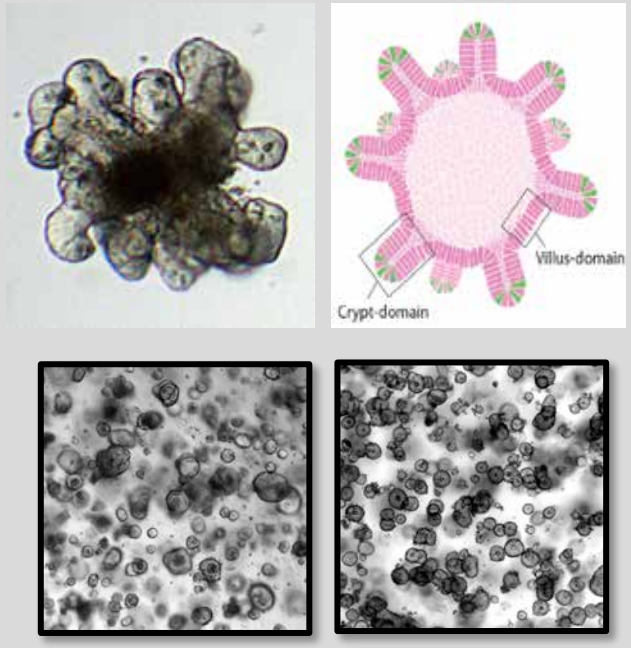
First Organoid Based Drug in Clinical Phase I

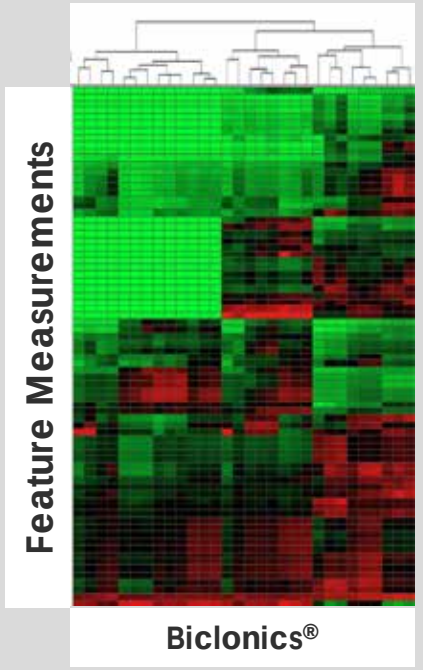
Biclonics® targeting CRC



Organoids – *ex vivo* patient-derived tumor tissue



High content screening on tumor and normal organoids



Merus

HUB ORGANOIDS

Ocello
Discover the full potential

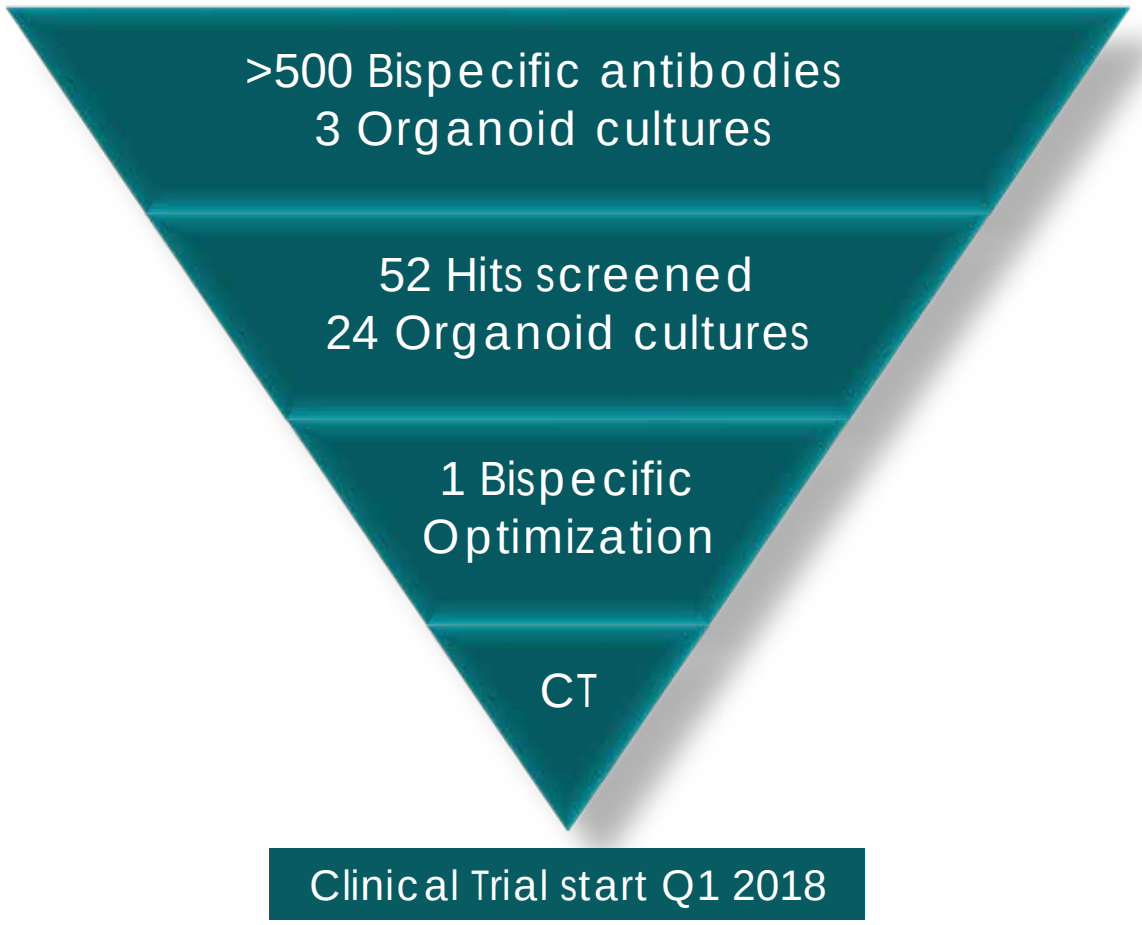
CROWN BIOSCIENCE

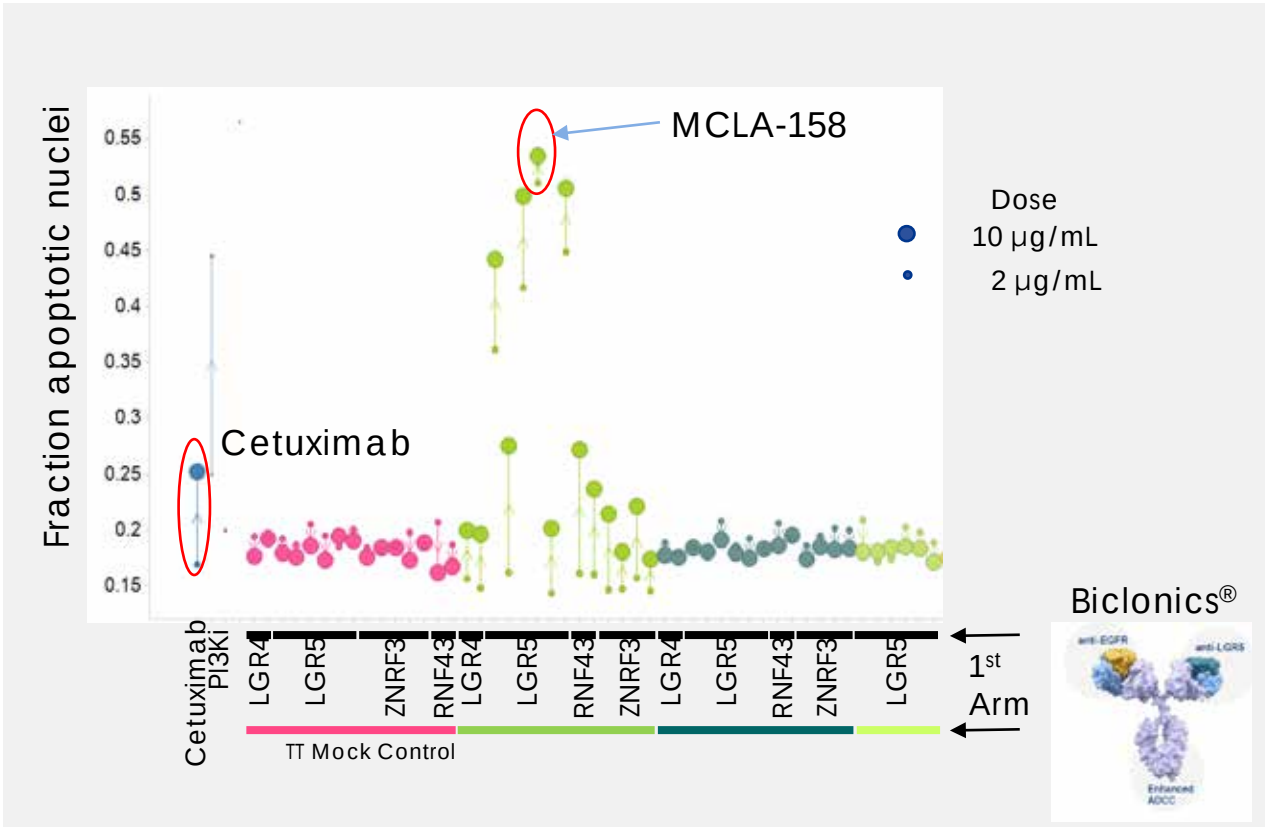
IRB BARCELONA

wellcome trust sanger institute

HUB ORGANOIDS

First Organoid Based Drug in Clinical Phase I



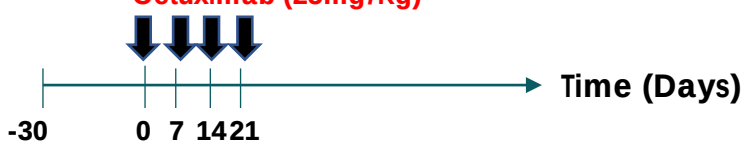


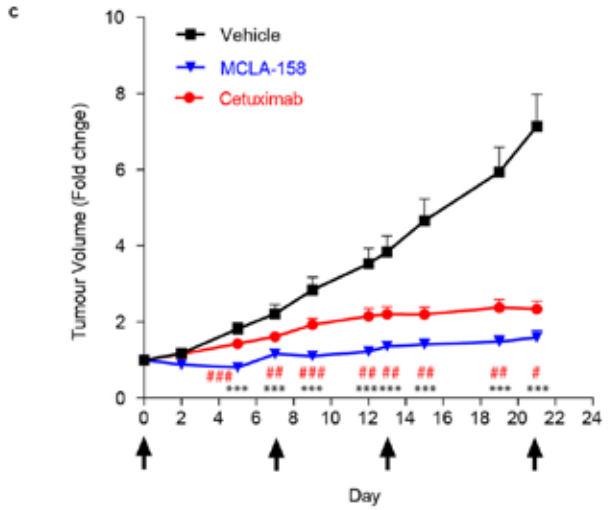
Herpers B. *et al.* Nature Cancer 2022

HUB ORGANOIDS

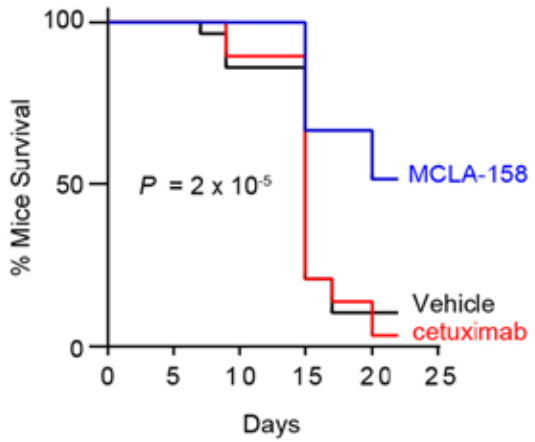
Effective Translation Into PDX Model

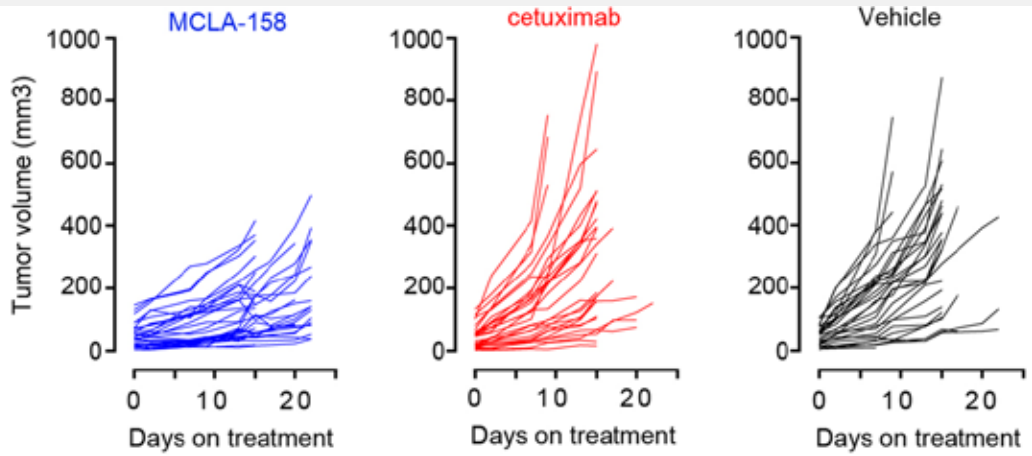
Vehicle
MCLA-158 (25mg/Kg)
Cetuximab (25mg/Kg)





	P values	vs	MCLA-158
	Cetuximab		Vehicle
d2	0.005		0.005
d5	### 0.0001	***	0.0001
d7	## 0.006	***	0.006
d9	### 0.0005	***	0.0005
d12	## 0.001	***	0.001
d13	## 0.004	***	0.004
d15	## 0.006	***	0.006
d19	## 0.006	***	0.006
d21	# 0.01	***	0.01





Herpers B. *et al.* Nature Cancer 2022

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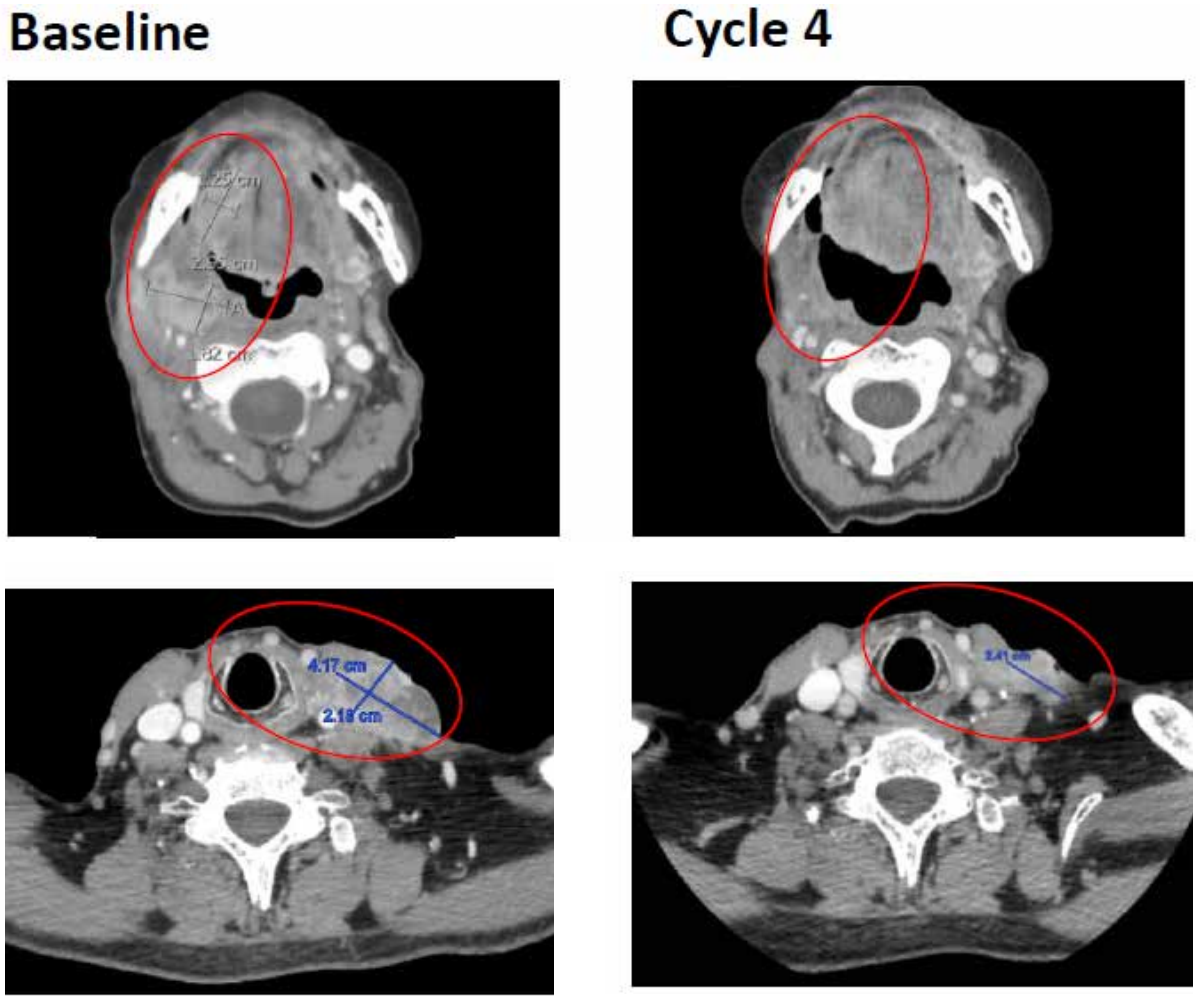
Validation of MCLA-158 in Clinical Trials



67-year-old male

Lesion location: larynx

MCLA-158 cycles: 6+

Best response: PRc (-41%)

Prior treatment: platinum + paclitaxel + durvalumab

59-year-old female

Lesion location: tongue

MCLA-158 cycles: 4+

Best response: PR (-88%; CR reported after data cut-off)

Prior treatment: RT → pembrolizumab + platinum + 5-FU

- **Substantial and promising antitumor activity** was observed in the first 7 patients with HNSCC.
- Well tolerated with a **manageable safety profile.**
- Exploration of MCLA 158 in HNSCC is continuing and is planned in **other tumour indications.**

Poster at AACR-NCI-EORTC Virtual Conference on Molecular Targets and Cancer Therapeutics (October 7-10, 2021)

Oncology

colon, pancreas, breast, lung, ovarian, H&N organoids

Cystic fibrosis

colon and lung organoids

Inflammatory diseases

colon and lung organoids

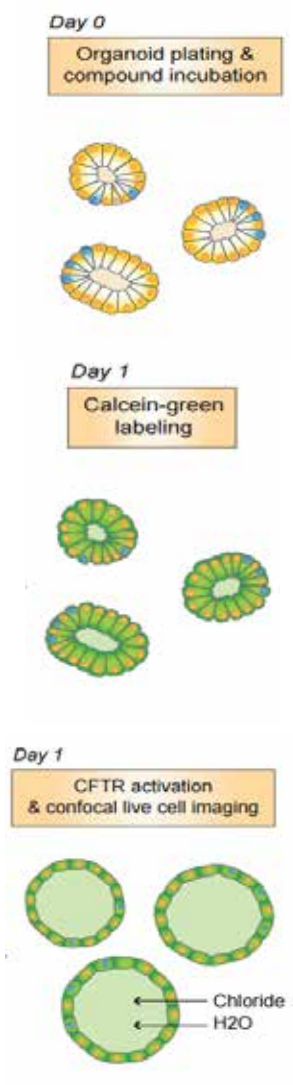
Infectious diseases

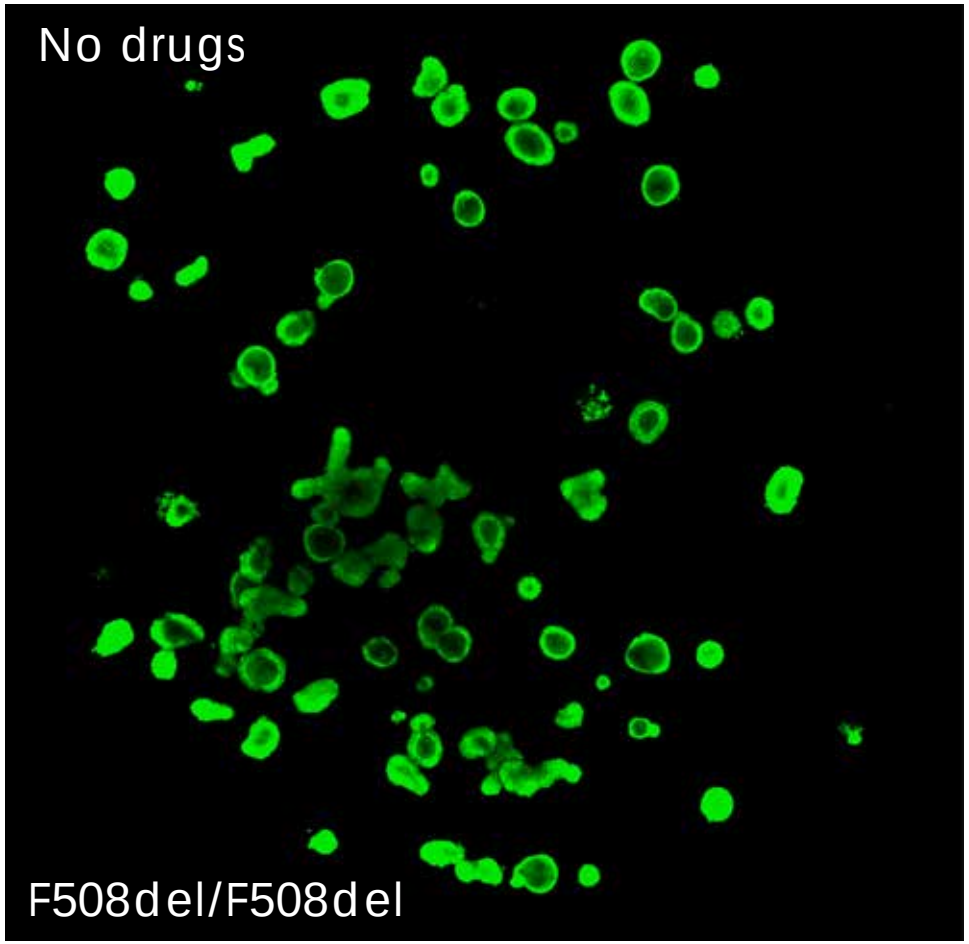
liver, lung and intestinal tract organoids

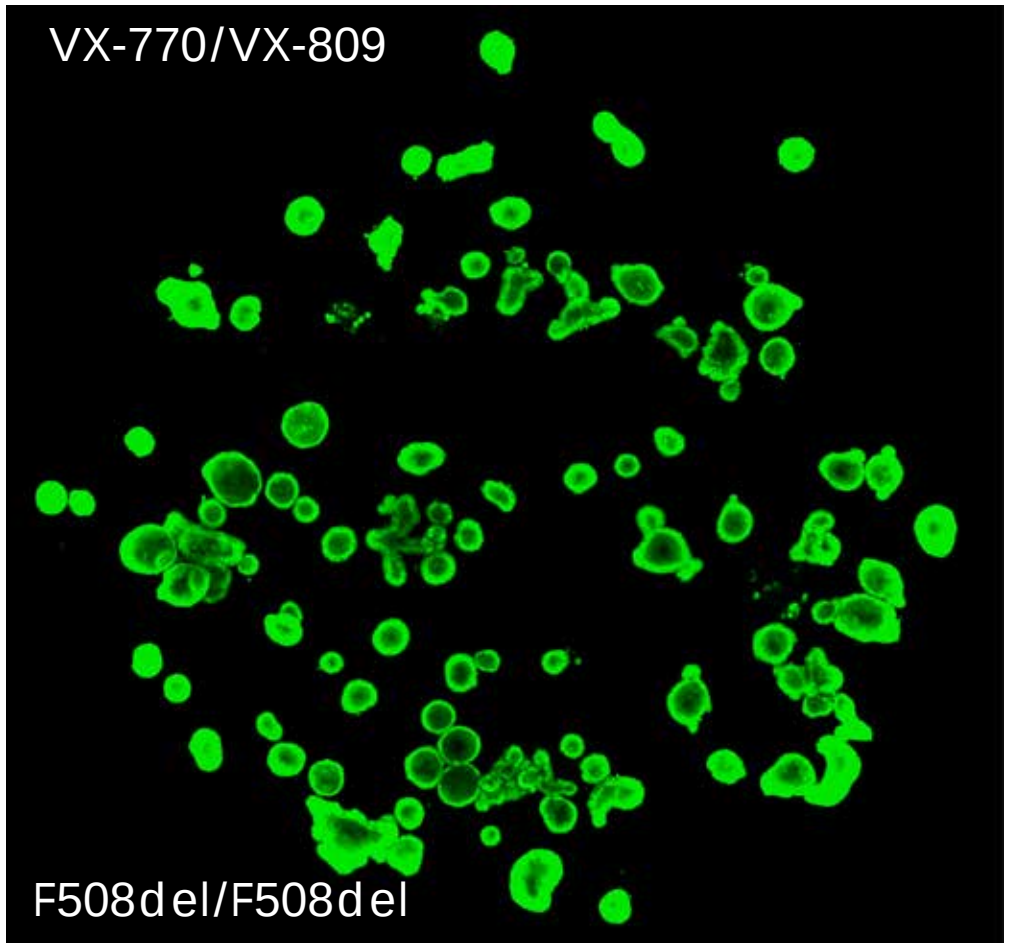
Toxicology

liver and intestinal tract organoids

Forskolin-induced swelling (FIS) assay







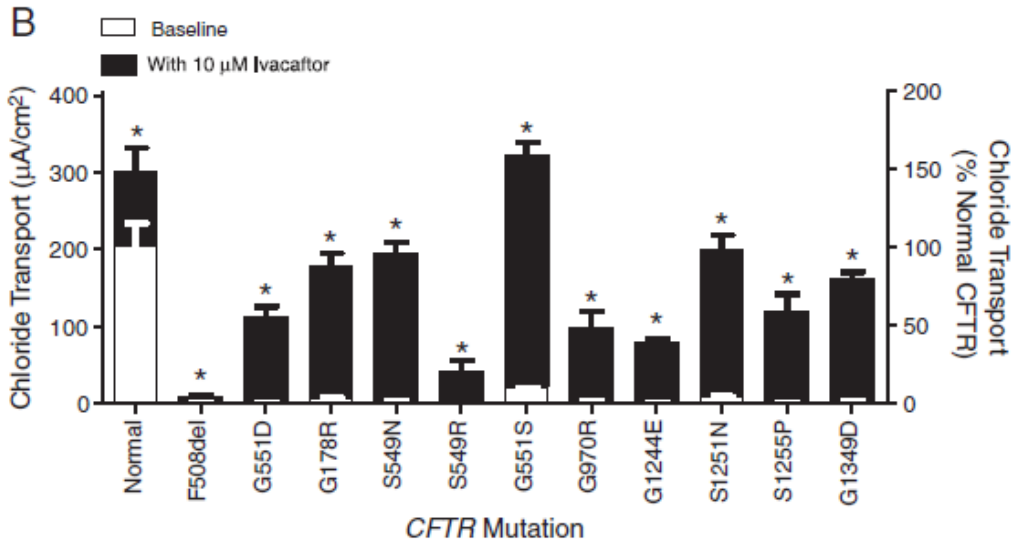
Adaptation from **Dekkers and Beekman (WKZ,** Utrecht, the Netherlands**)**

HUB ORGANOIDS

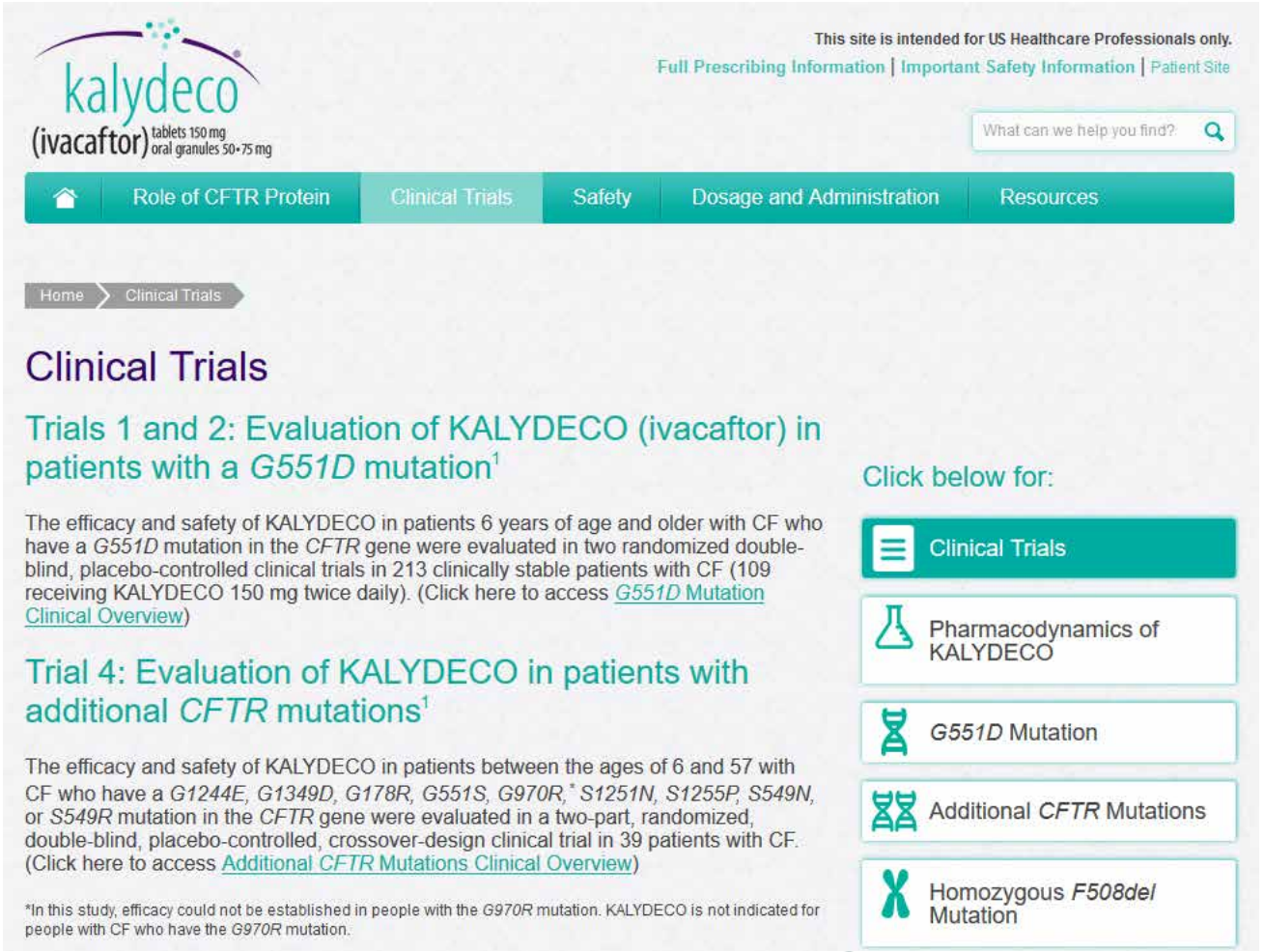
Rectal Organoids as Predictive Tool for Patient Response

Ivacaftor potentiation of multiple CFTR channels with gating mutations

Haihui Yu, Bill Burton, Chien-Jung Huang, Jennings Worley, Dong Cao, James P. Johnson Jr., Art Urrutia, John Joubran, Sheila Seepersaud, Katherine Sussky, Beth J. Hoffman, Fredrick Van Goor *

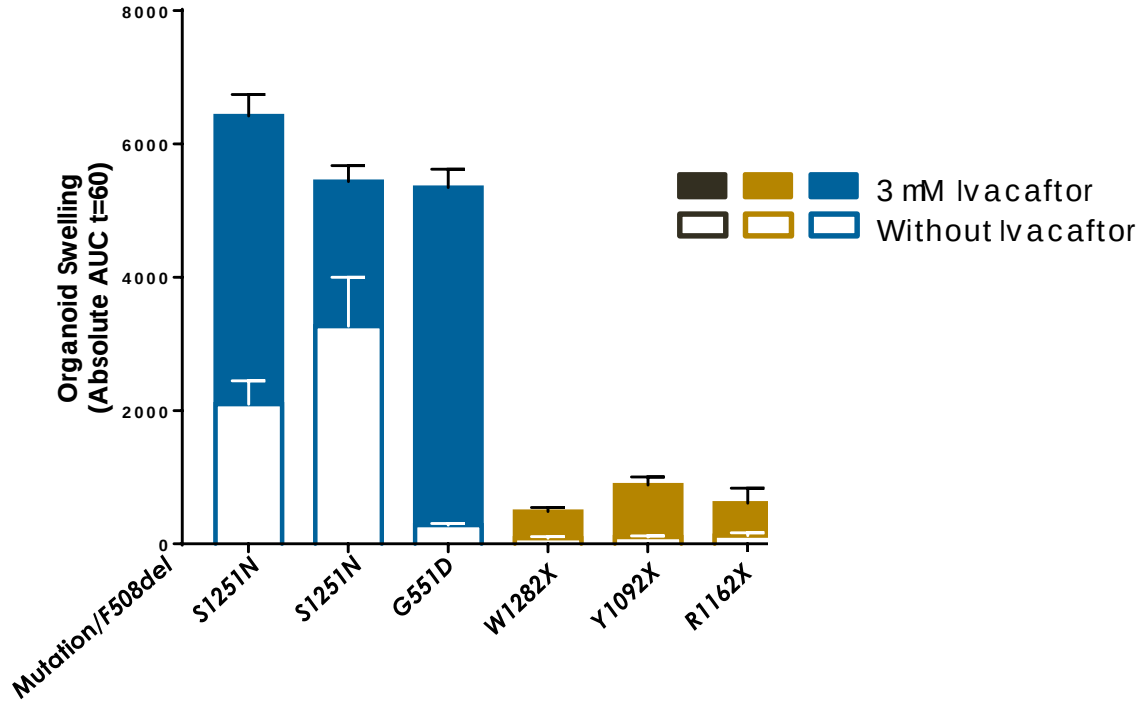


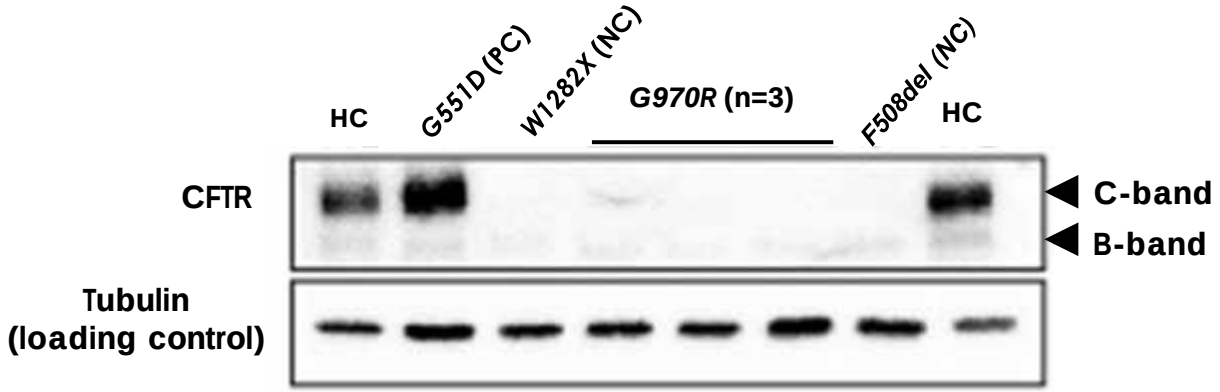
Yu *et al. J Cyst Fibros.* 2012



HUB ORGANOIDS

Rectal Organoids as Predictive Tool for Patient Response





Fidler M., et al. Presented at the 40th European Cystic Fibrosis Conference 7-10 June 2017, Seville, Spain. Abstract WS18.5.

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Clinical Predictive Value for Patient-derived Organoids

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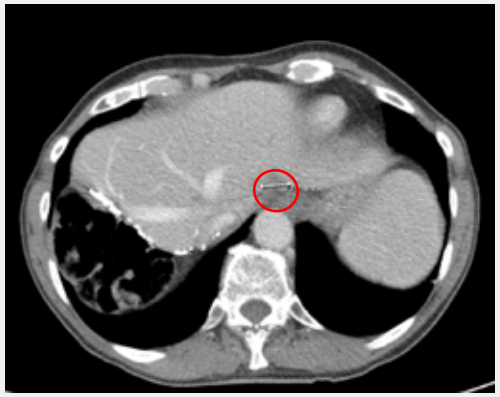
Patient-derived Organoids Mimic Patient Treatment Response

Clinical Response

Patient A (responder)

- Capecitabine:
  - *Progression-free survival:* 34.6 month,
  - *Best RECIST response:* partial response

Liver metastasis (30 mm)

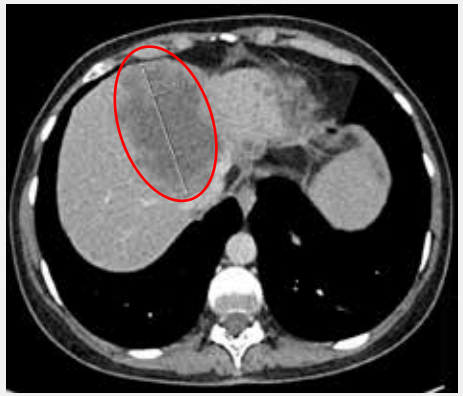


Liver metastasis (18 mm)

Patient B (non-responder)

- 5FU + Oxiliplatin (FOLFOX):
  - *Progression-free survival:* 1.8 month,
  - *Best RECIST response:* progressive disease

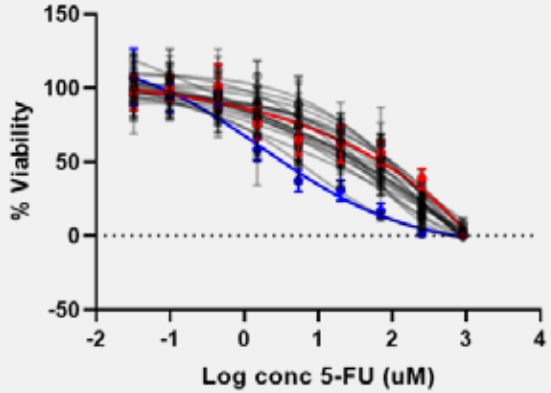
Liver metastasis (57 mm)



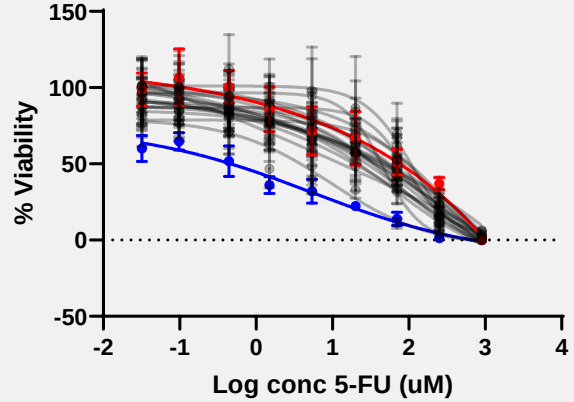
Liver metastasis (80 mm)

Organoid Response

5FU



5FU + 5,3µM Oxaliplatin



Clinical Data provided by **Wensink** and **Roodhart**

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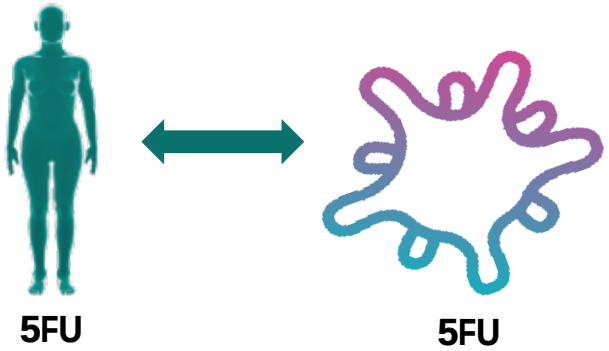
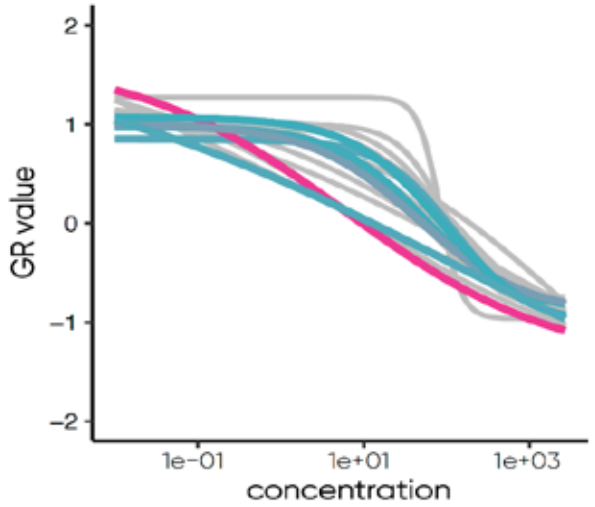
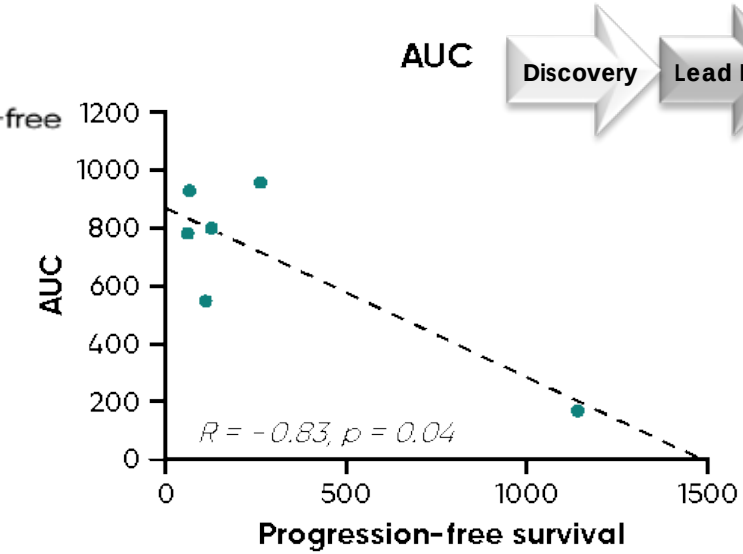
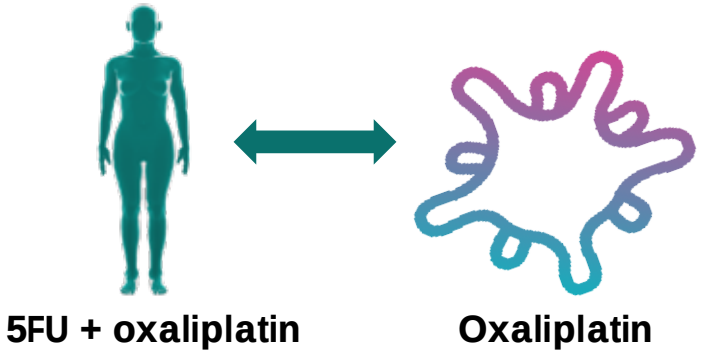
CRC Organoids Mimic Patient Clinical Responses

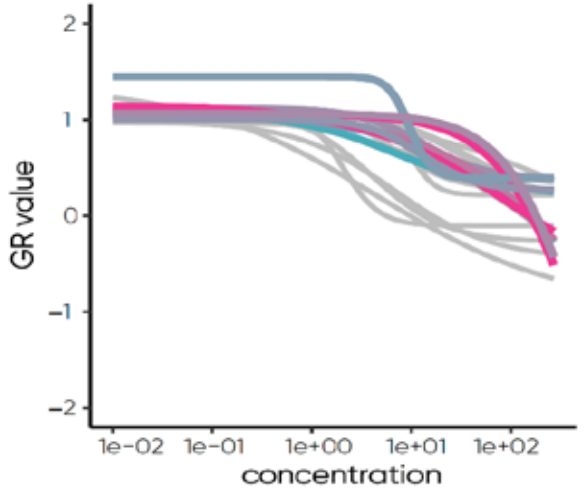


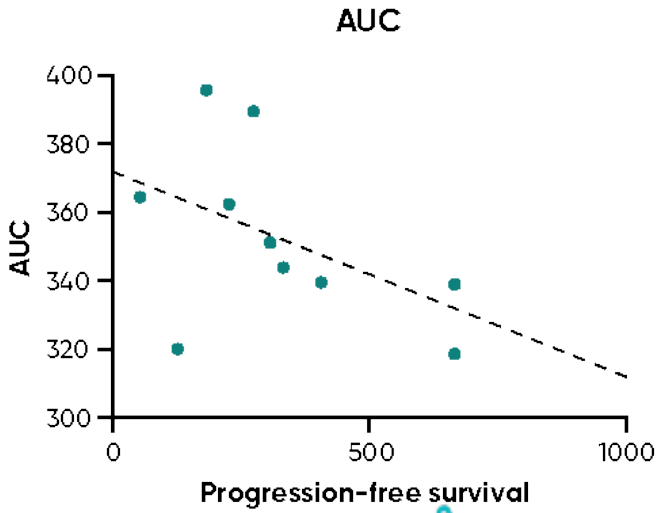




Discovery → Lead Dev → Clinical







UMC Utrecht

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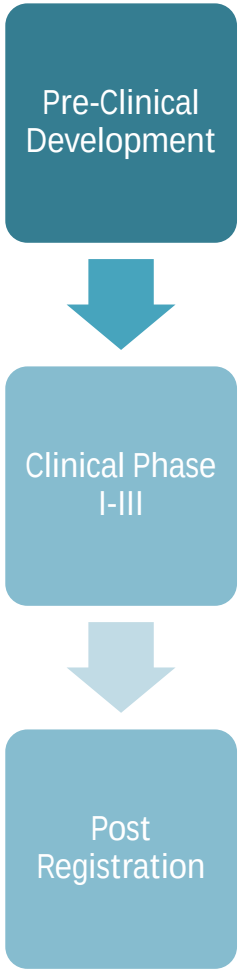
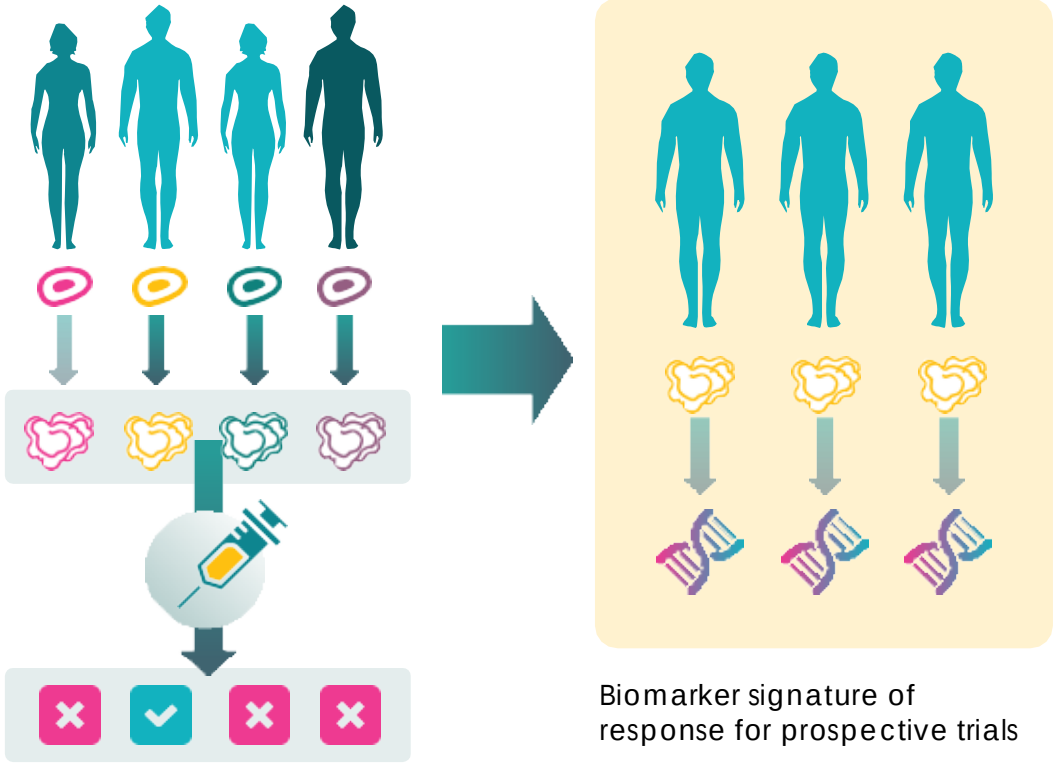
Clinical Trials on a Dish

"Clinical Trials on A Dish or Avatar Clinical Trials"



Clinical trial in a dish
Patient-stratification based on PDO response



Biomarker signature of response for prospective trials

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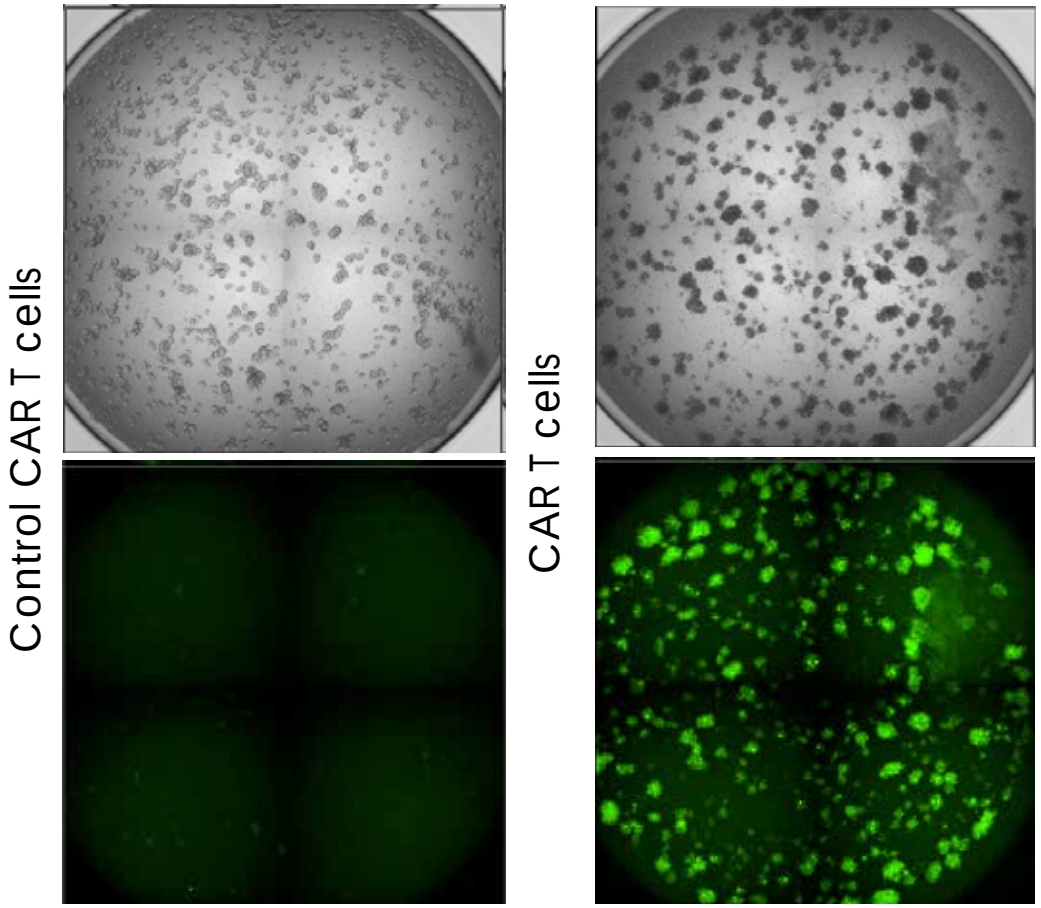
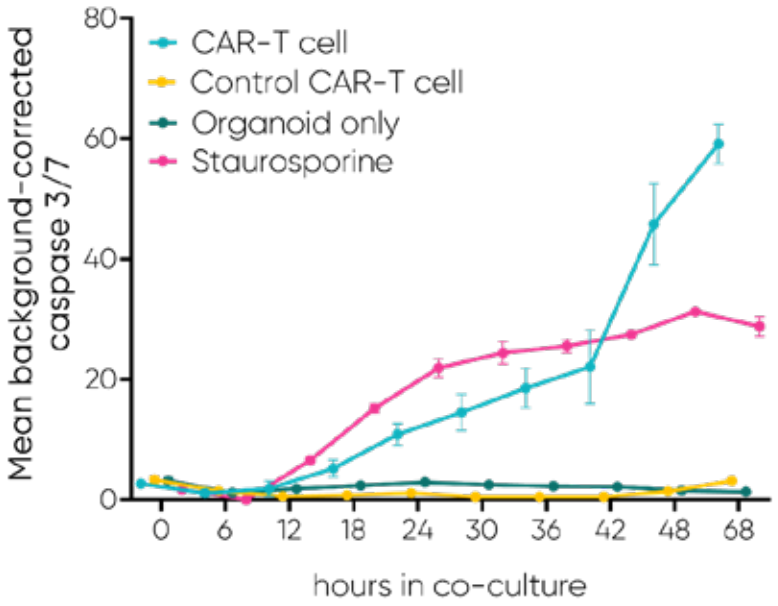
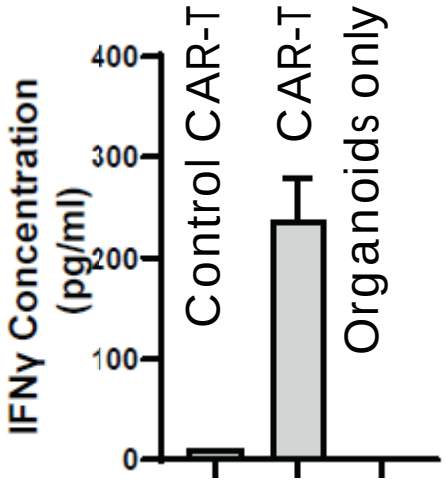
Development of Co-Culture Assays with Imaging-based Readouts

- Caspase3/7 signal can be used to evaluate tumor organoid killing by T-cells in a time course.



CART-CRC co-culture, 72h





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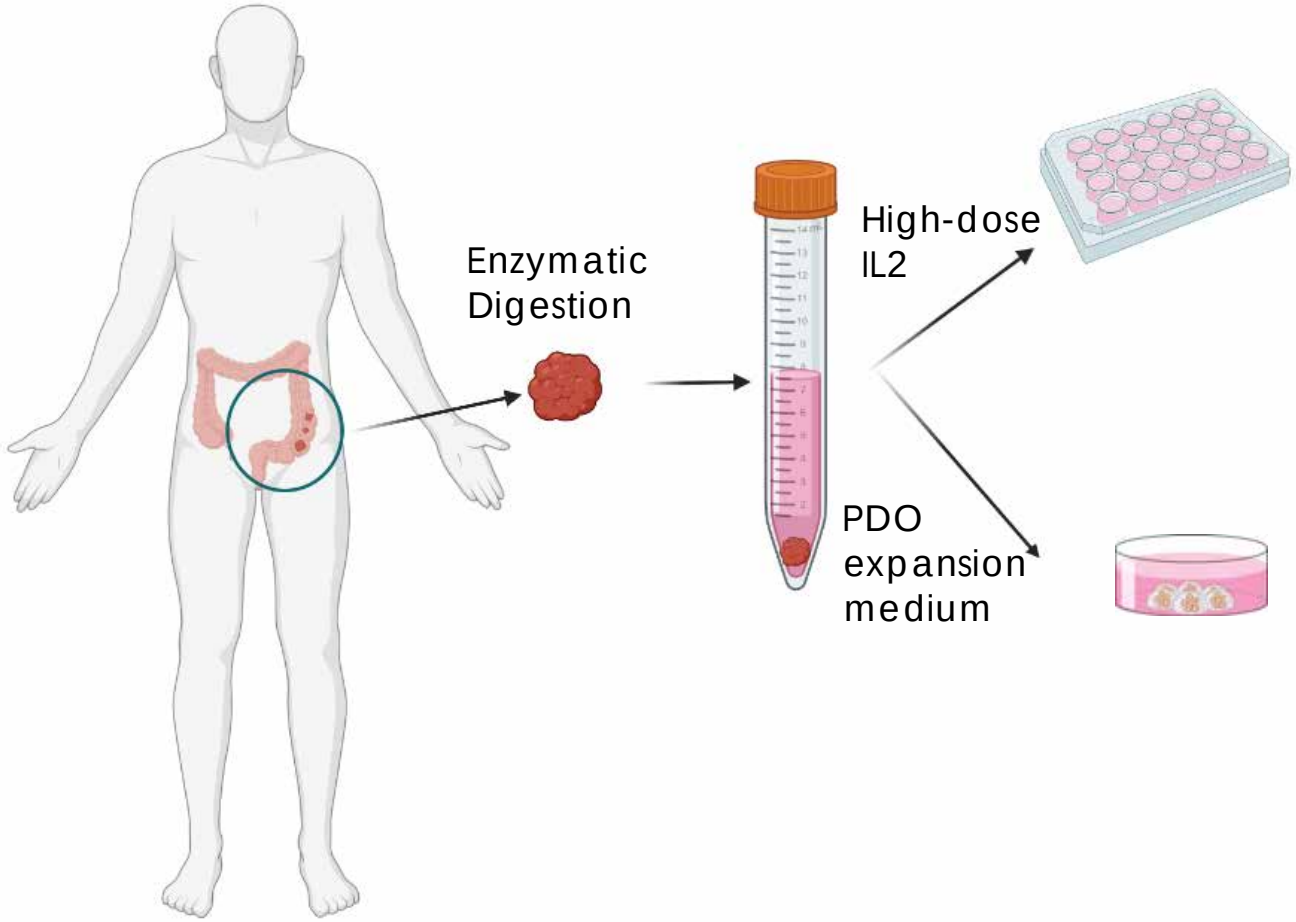
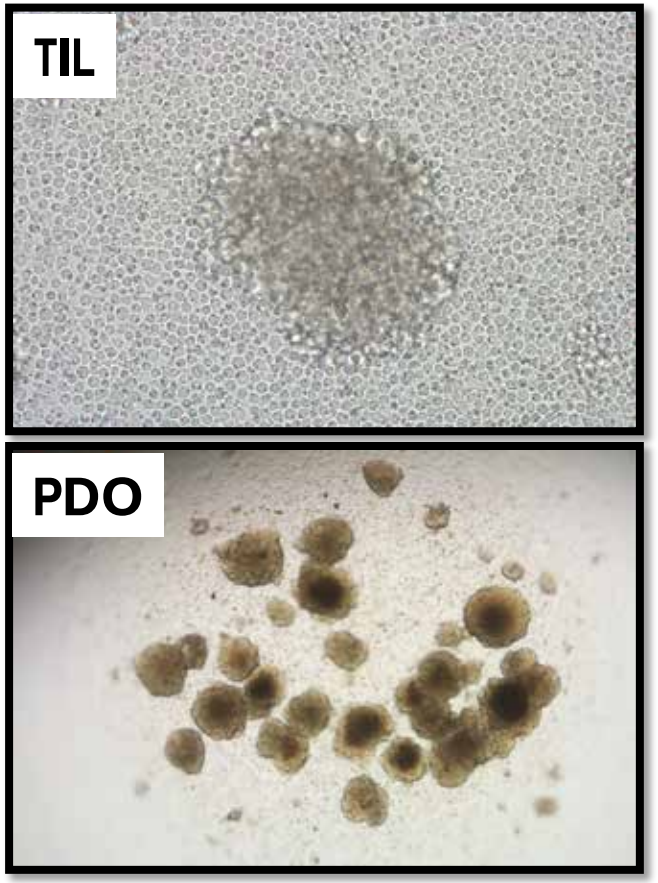
Establishment of autologous TIL - PDO Biobanks

- Generation of IO Biobanks by isolating and establishing biobank of tumor PDOs and associated Tumor Infiltrated lymphocytes (TILS)





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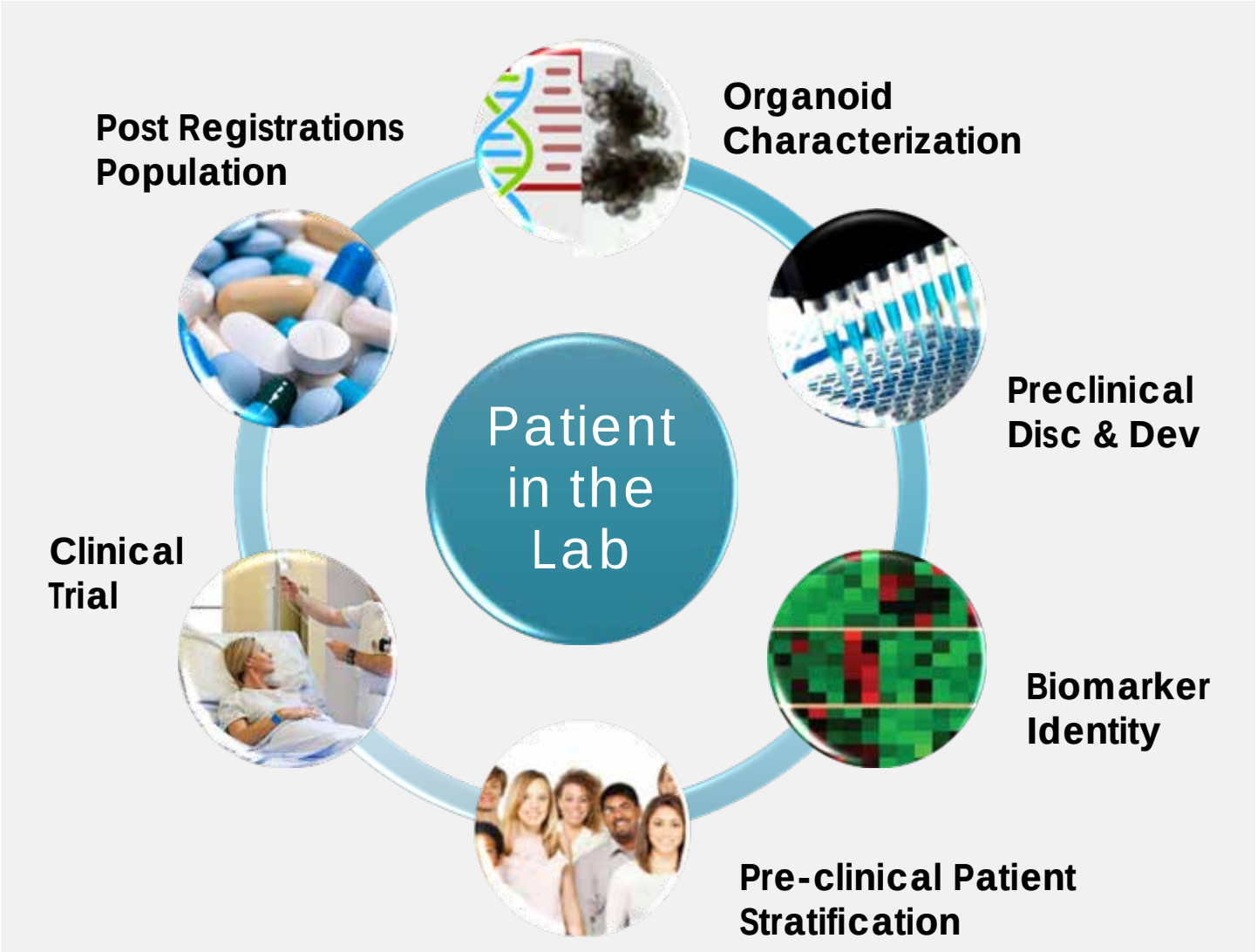
HUB Organoids – From Drug Discovery to Patient Application

Pre-Clinical Development

Clinical Phase I-III

Post Registration

From Traditional to Patient Centric



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ABOUT **HUB**

Hubrecht Organoid Technology (HUB) was founded by the Hubrecht Institute, the University Medical Center Utrecht, and the Royal Netherlands Academy of Arts and Sciences (KNAW).

HUB's technology constitutes a paradigm-shifting platform for drug discovery and development, (pre)clinical patient stratification, predictive diagnostics, personalized medicine, clinical trials, regenerative medicine, and companion diagnostics.

As the global leader in the field of Organoid Technology, HUB offers licenses to its proprietary technology, provides services and access to its living organoid biobanks.

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