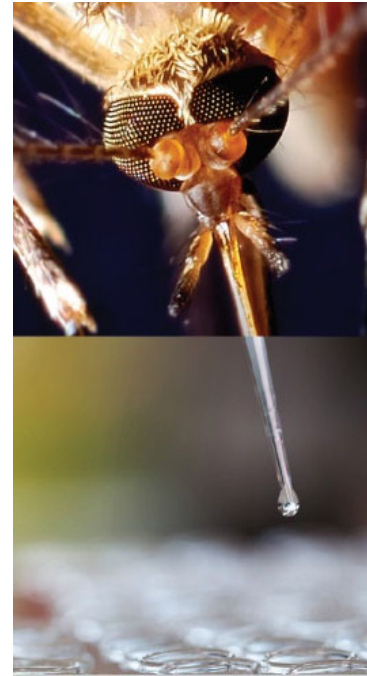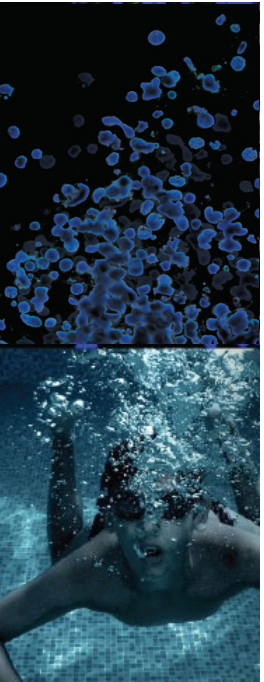# Novel 3-D In Vitro Models for Studying Pancreatic Cancer Drug Response and Resistance

Naomi Walsh, MPH, PhD
Associate Professor, Life Sciences Institute,
School of Biotechnology, Dublin City University

Credible Leads to Incredible™

About ATCC

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD

- World's largest, most diverse biological materials and information resource for cell culture – the "*gold standard*"

- Innovative R&D company featuring gene editing, differentiated stem cells, advanced models

- Partner with government, industry, and academia

- Leading global supplier of authenticated cell lines, viral and microbial standards

- Sales and distribution in 150 countries, 19 international distributors

- Talented team of 550+ employees, over one-third with advanced degrees

ATCC®

Utilization of novel 3D *in vitro* models for studying pancreatic cancer drug response and resistance

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School of Biotechnology,
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Ireland

DCU
Ollscoil Chathair
Bhaile Átha Cliath
Dublin City University

Objectives/Key Points

Describe the role of preclinical systems focusing on organoids in modelling pancreatic cancer

Present a method for the establishment of isogenic primary cell lines from patient derived organoids (PDOs) and the recapitulation to 3D cell line organoids (CLOs)
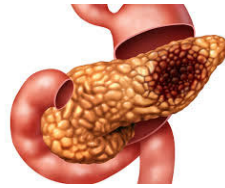
Demonstrate that 3D CLO culture method can be used as an expandable, easy scale-up, affordable, and less time-consuming research model

Highlight a methodology for the development and characterization of drug resistance using pancreatic cancer organoids

Pancreatic cancer

- Pancreatic ductal adenocarcinoma (PDAC) is the most common type

- Aggressive, poor prognosis with 5-year survival rate 13%, distant metastases 3%*.

- Diagnosed at late state
  - Silent progression
  - Nonspecific symptoms
  - More than half of patients diagnosed at an advanced stage

- Treatment
  - Surgery offers curative intent however, approximately 20% patients are operable
  - Chemotherapy and/or radiotherapy as the standard of care for most patients.
  - Inherent or acquired resistance

- 7th leading cause of cancer mortality in the World
  - Increasing trends in incidence and mortality of PDAC across the World

easing Survival
Pancreatic
ncer Patients
unding and Research Breakthroughs

ted 5-Year Survival Rate

5%

6%

2010
FOLFIRINOX
Improves Survival
Combination chemotherapy
becomes standard of care
for stage IV patients.

2013
Recalcitrant
Research Ac
The landmark R
Research Act si
to develop a pla

ABRAXANE®
Combo treatme
standard of care

2011
PanCAN Advocates Secure
New Federal Funding Source
Pancreatic cancer is included as a
disease eligible for funding in the
Department of Defense's Peer
Reviewed Cancer Research
Program for the first time ever,
creating a second avenue of federal
funding in addition to the NCI.

2003
PanCAN's
Research Grants
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d

2005
Critical Help for
Patients
PanCAN Patient Services
hits a milestone: 10,000
patients and families
served since the program's
founding in 2002.

* SEER = Surveillance, Epidemiology, and End Results (2013-2019)

Greatest challenge in cancer treatment

- Predict response because cancer and its treatment are patient-specific

- Personalised medicine, individual prevention, and treatment strategies based on unique patient-specific variables
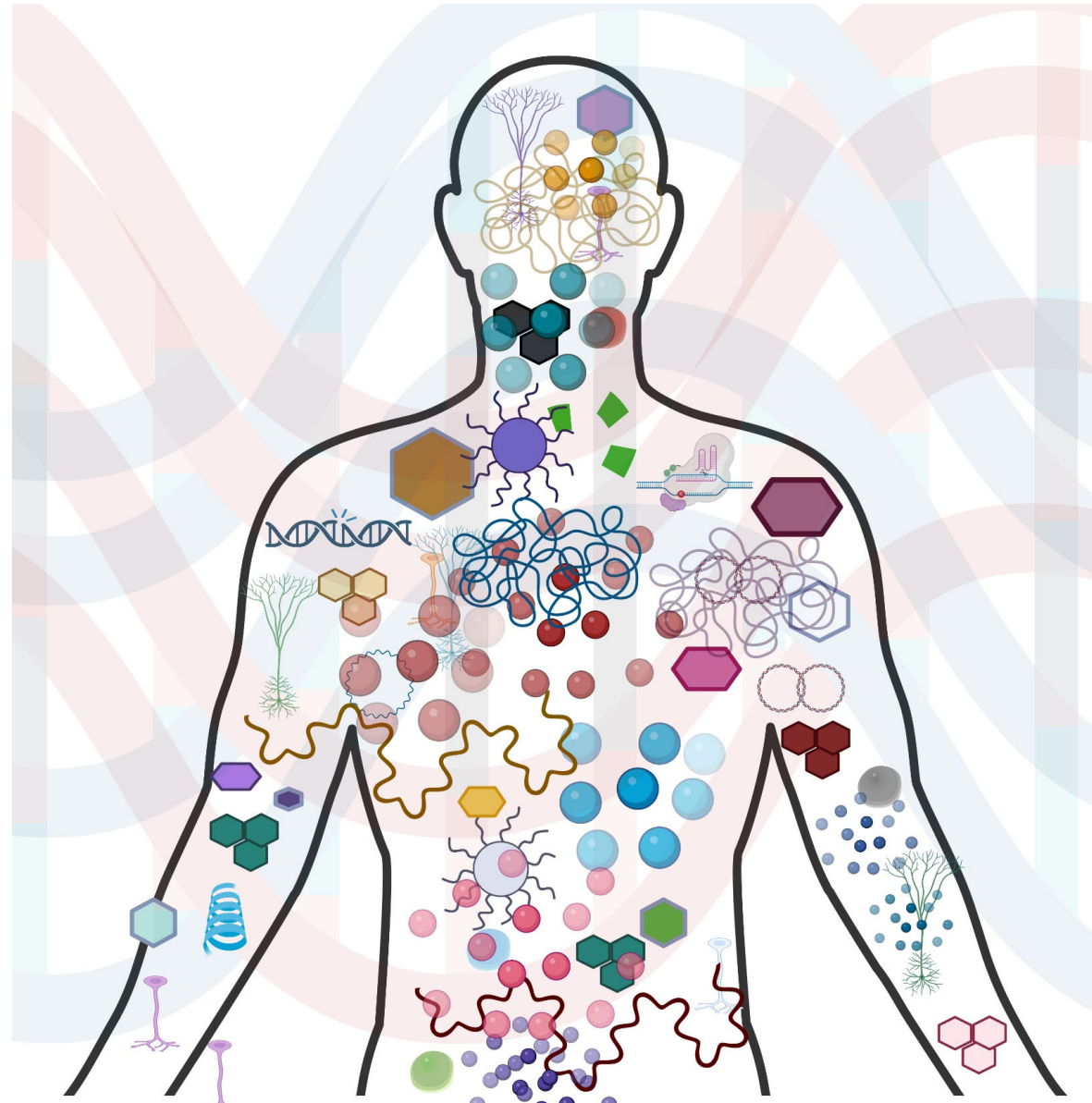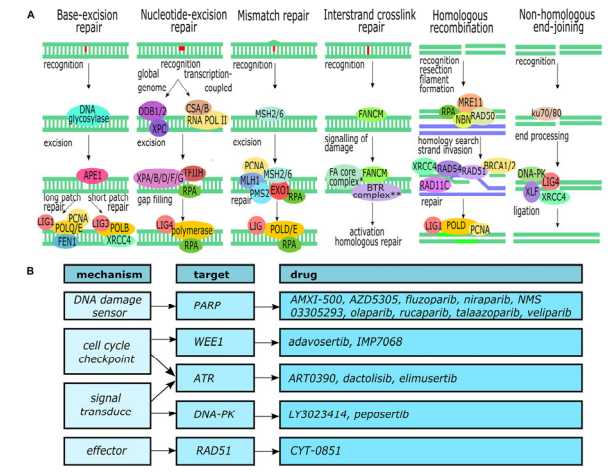
Landscape of PDAC

- Genomic and transcriptomic subtypes can enrich for therapeutic vulnerabilities
  - > 90% cases present with KRAS mutation
  - *TP53*, *SMAD4* and *CDKN2A* inactivated in > 50%
 - Limited success in targeted therapy strategies
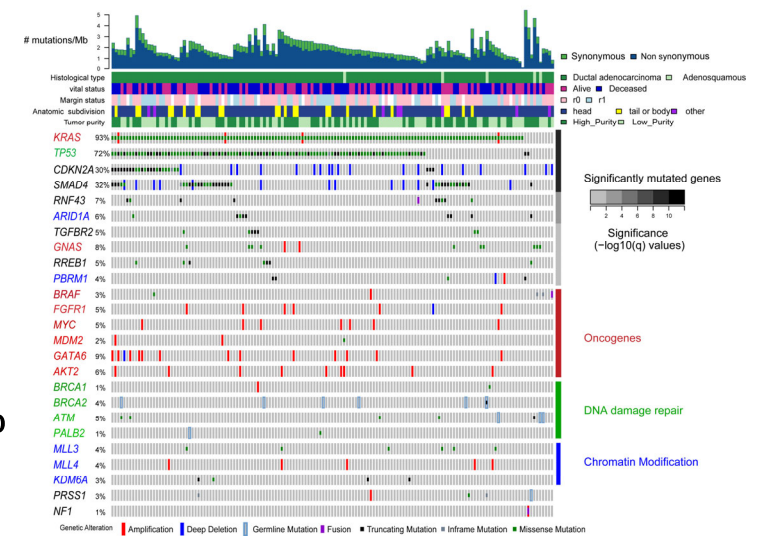
- Familial predisposition
  - Genetic syndromes account for 5-10% of PDAC
  - Germline mutations in DNA damage repair pathway (*BRCA1/BRCA2* etc)

Cancer Genome Atlas Research Network. *Cancer Cell*. 2017;32(2):185-203.e13.
Stoof et al. *Frontiers in cell and developmental biology*. 2021;*9*, 749490.

Preclinical models of pancreatic cancer
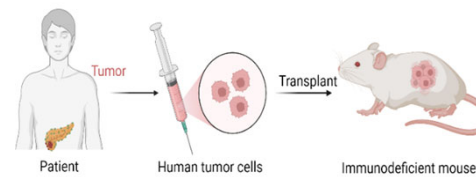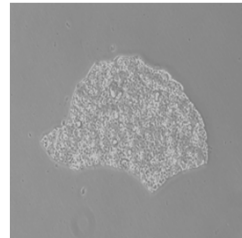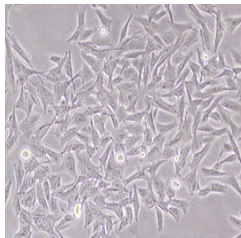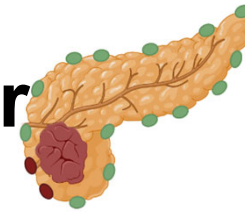
Established cell lines

👍
- Widely available
- Represent common genetic features
- Cost effective
- Easy to manipulate
- High throughput screening

👎
- Homogenous
- Genomic drift
- Low in vivo relevance

Primary cell lines

- Mirrors heterogeneity and tumor characteristics
- Personalized drug screening

- Difficult to establish
- Limited doubling
- Stromal cell contamination

Patient-derived xenografts

- Mirrors heterogeneity and tumor characteristics
- Response reflects clinical results
- Unlimited resource of tumor – generations

- Time consuming
- Lacks functional immune system
- Expensive
- Animals

GEMM

- De novo tumor development
- Retain the histopathological and molecular features of human tumors

- Expensive, time consuming and complex
- Mouse biology
- Animals

Cancer organoids as models of disease

- **Miniaturized** and **simplified** 3D structures
- Patient specific as derived from **patient tissue**
- **Self-organizing, resembling the original tumor architecture**
- Show **heterogeneity** of tumors
- **Long-term growth** potential - biobanking
- **Alternative to animal models**
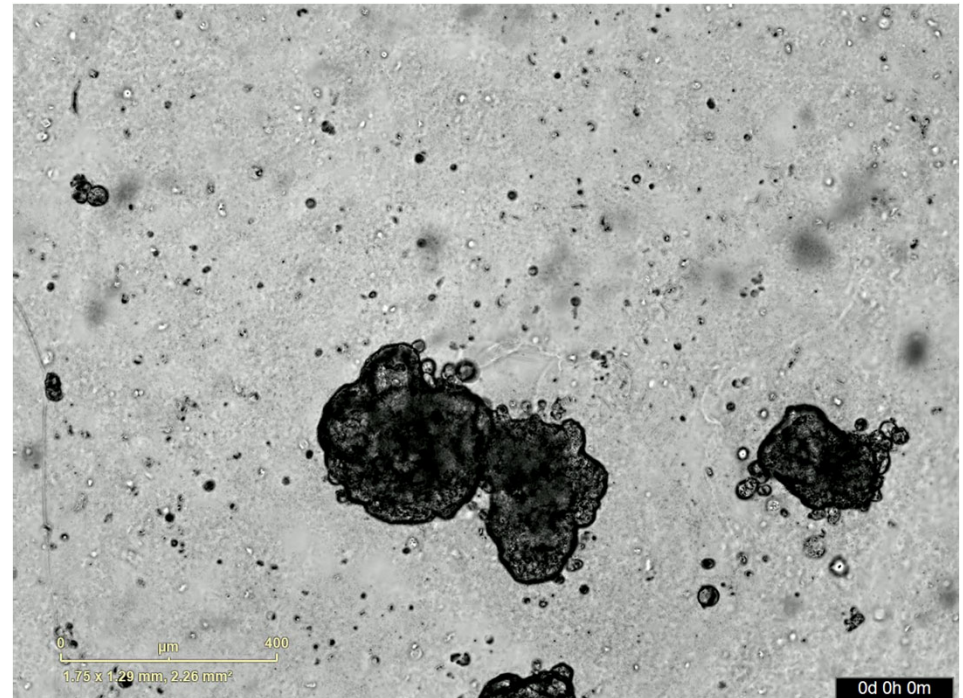
PDOs in personalized medicine

Personalized therapy development – providing accurate and reliable **drug screening systems**

Uncover **underlying mechanisms driving cancer progression** – genetic mutations, signalling pathways etc.

Can be developed into **more complex models to mimic tumor microenvironment**

Platform to study early and late stages of tumor development

Developing 3D organoid systems to model pancreatic cancer

Development of 3D organoid models

xenograft

Removal of infiltrating mouse cells
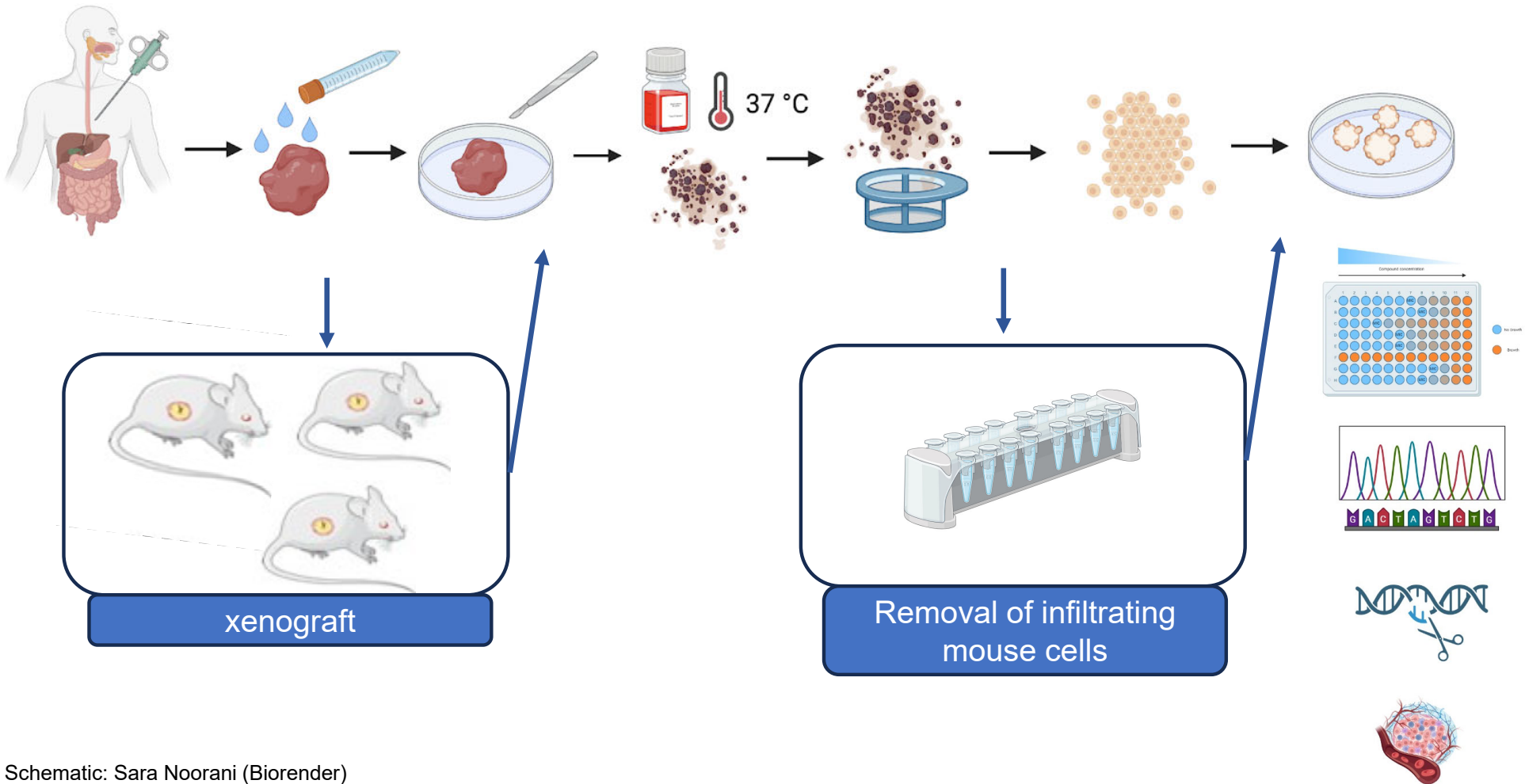
Schematic: Sara Noorani (Biorender)
Nelson et al., *Scientific reports*, 2020;*10*(1), 2778

Created with BioRender.co

Development of primary 2D cell lines from organoids
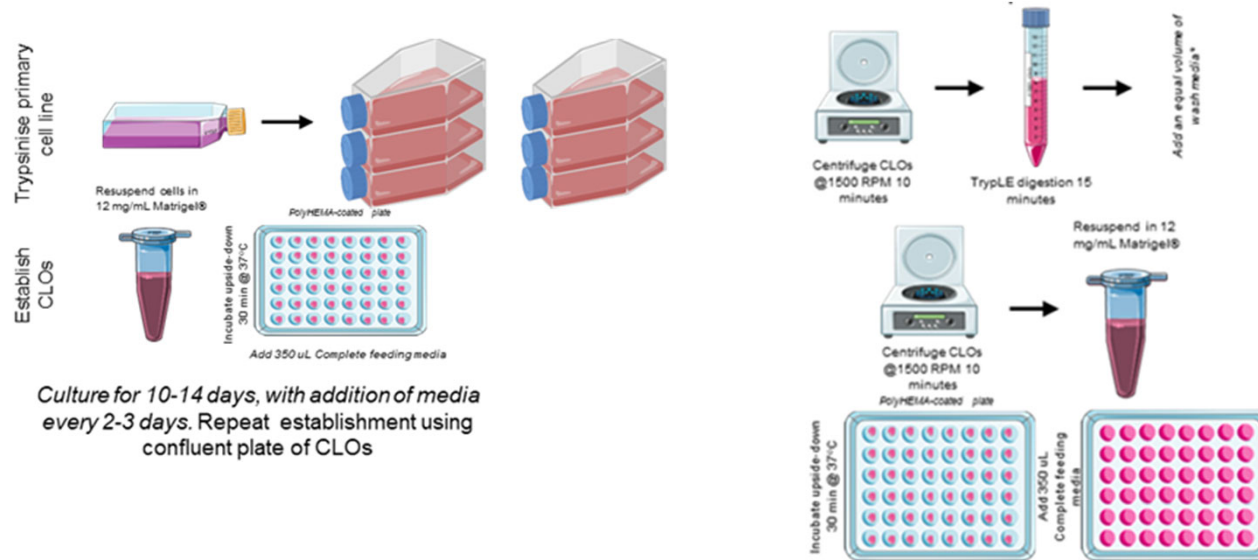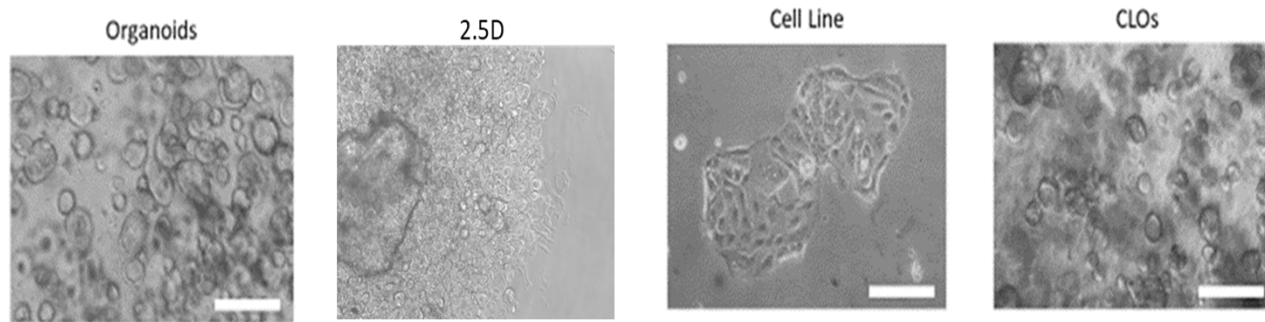
Generation of organoid-derived primary cell lines and 3D cell line organoids (CLOs)
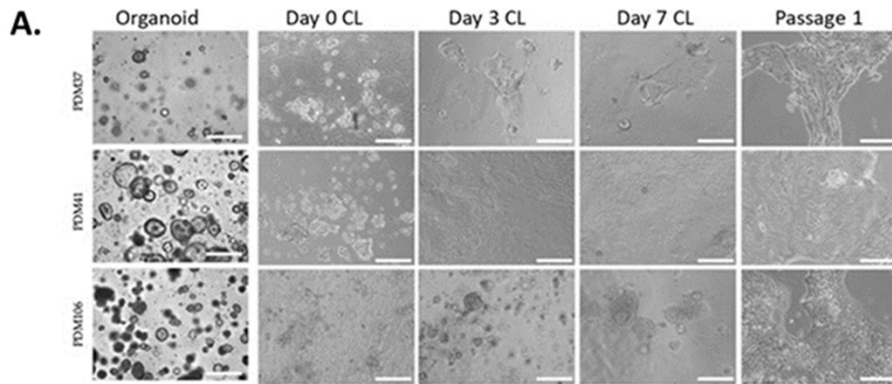
- Developed three new primary 2D cell lines derived from 3D organoids purchased from ATCC
    - HCM-CSHL-0090-C25 (ATCC® PDM-37™)
    - HCM-CSHL-0094-C25 (ATCC® PDM-41™)
    - HCM-BROD-0008-C25 (ATCC® PDM-106™)
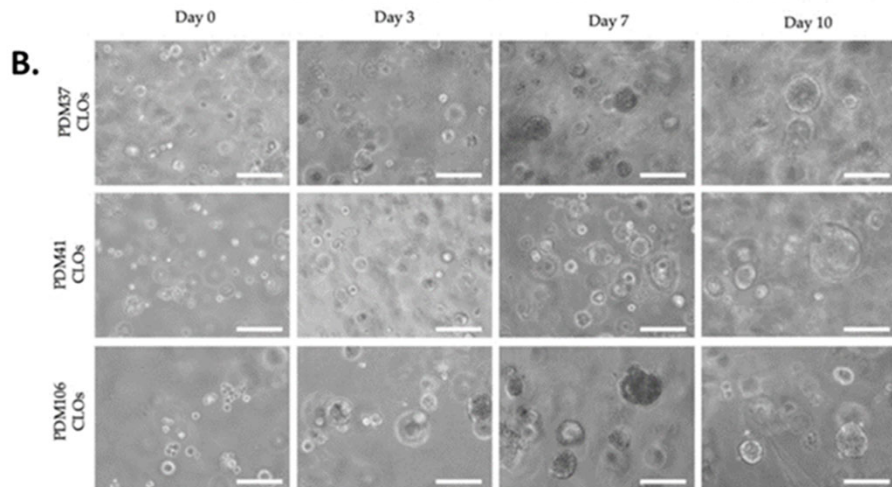
- 2D cell lines were expanded over 2 passages and recapitulated to cell line organoids (CLOs) using 3D organoid culture conditions

CLOs maintain the phenotypic and growth characteristics of similar to organoids
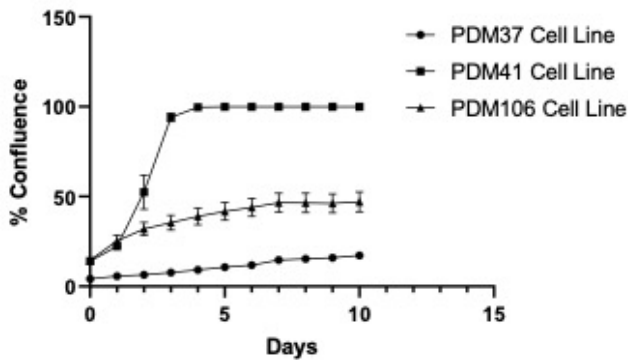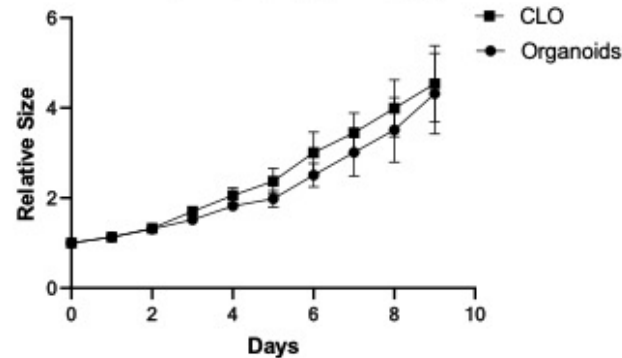
Differential proliferation rate observed between the three cell lines

Comparison of proliferation between CLOs and respective isogenic matched organoid revealed similar rates of proliferation between PDM37 and PDM106 models;
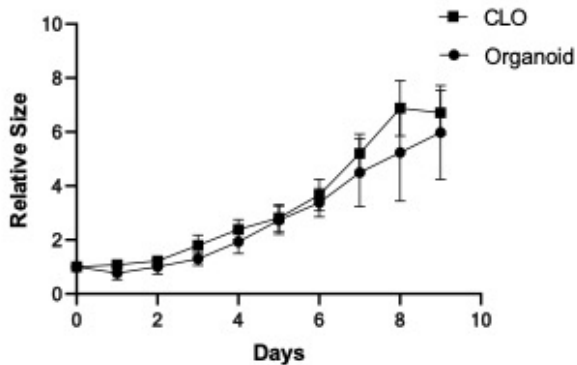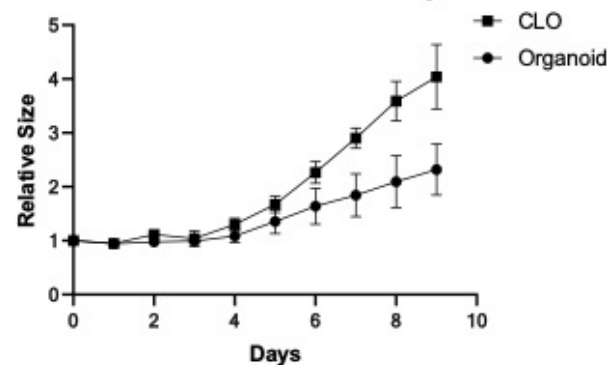
PDM41-CLO proliferated faster compared to PDM41-organoid

Noorani et al., *Organoids*. 2022; 1(2):168-183.

CLOs retain therapeutic drug response comparable to derived organoids

Noorani et al., *Organoids*. 2022; 1(2):168-183.
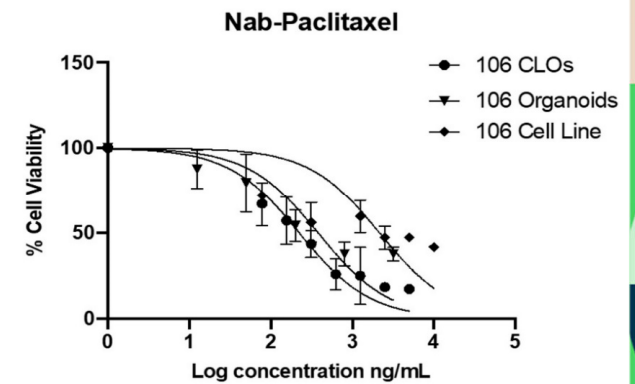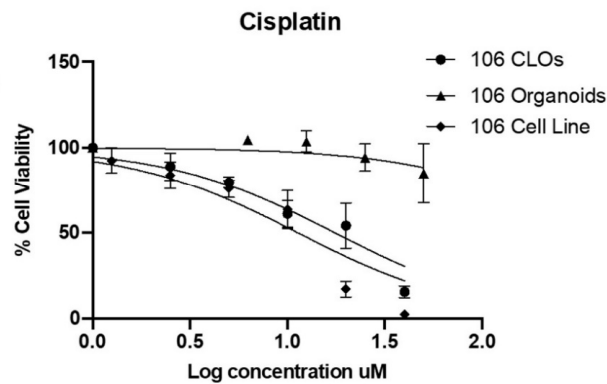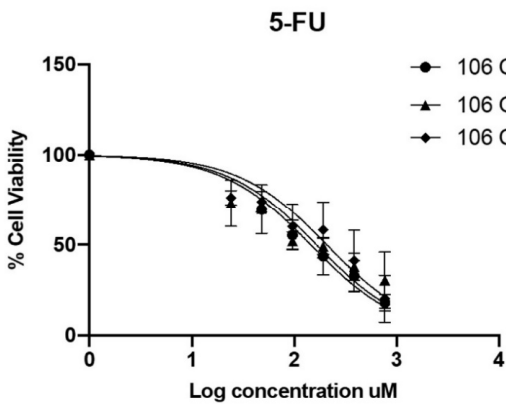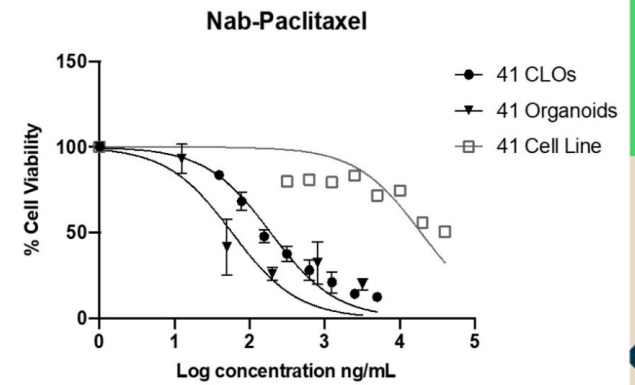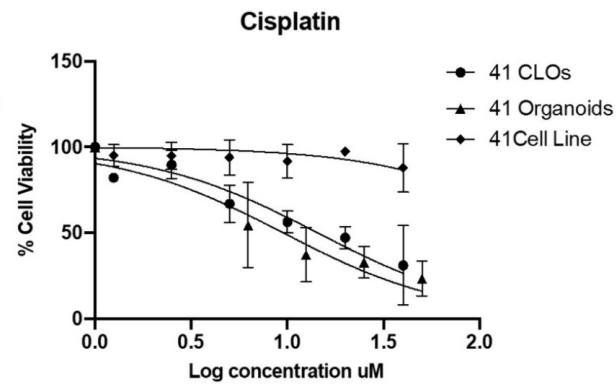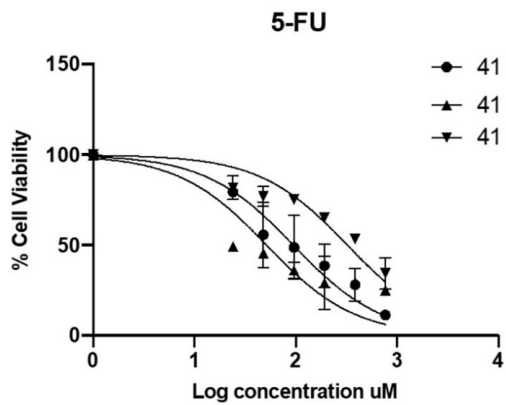
Stem cell marker expression in 2D cell line, CLOs and organoids

A. PDM37
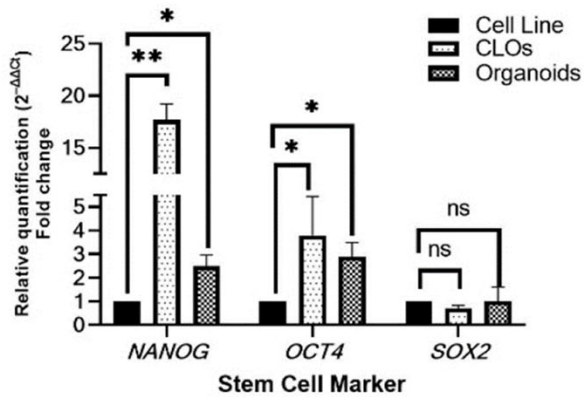
B. PDM41
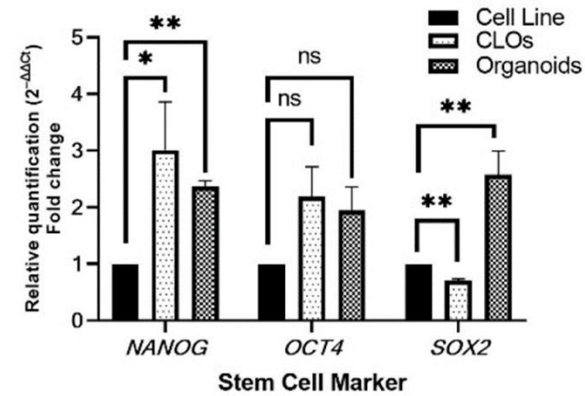
C. PDM106
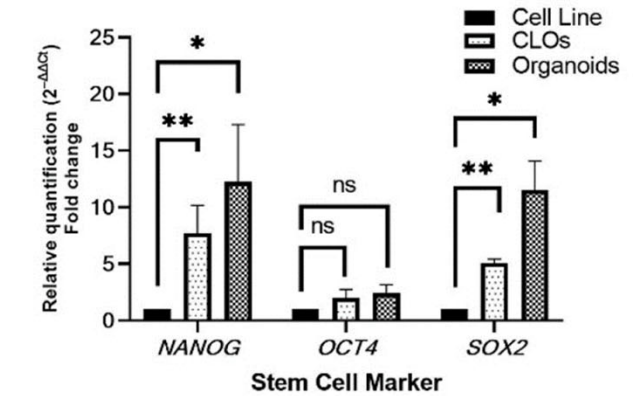
Overexpression of Cancer Stem Cell markers in 3D models compared to 2D cell lines

Noorani et al., *Organoids*. 2022; 1(2):168-183.
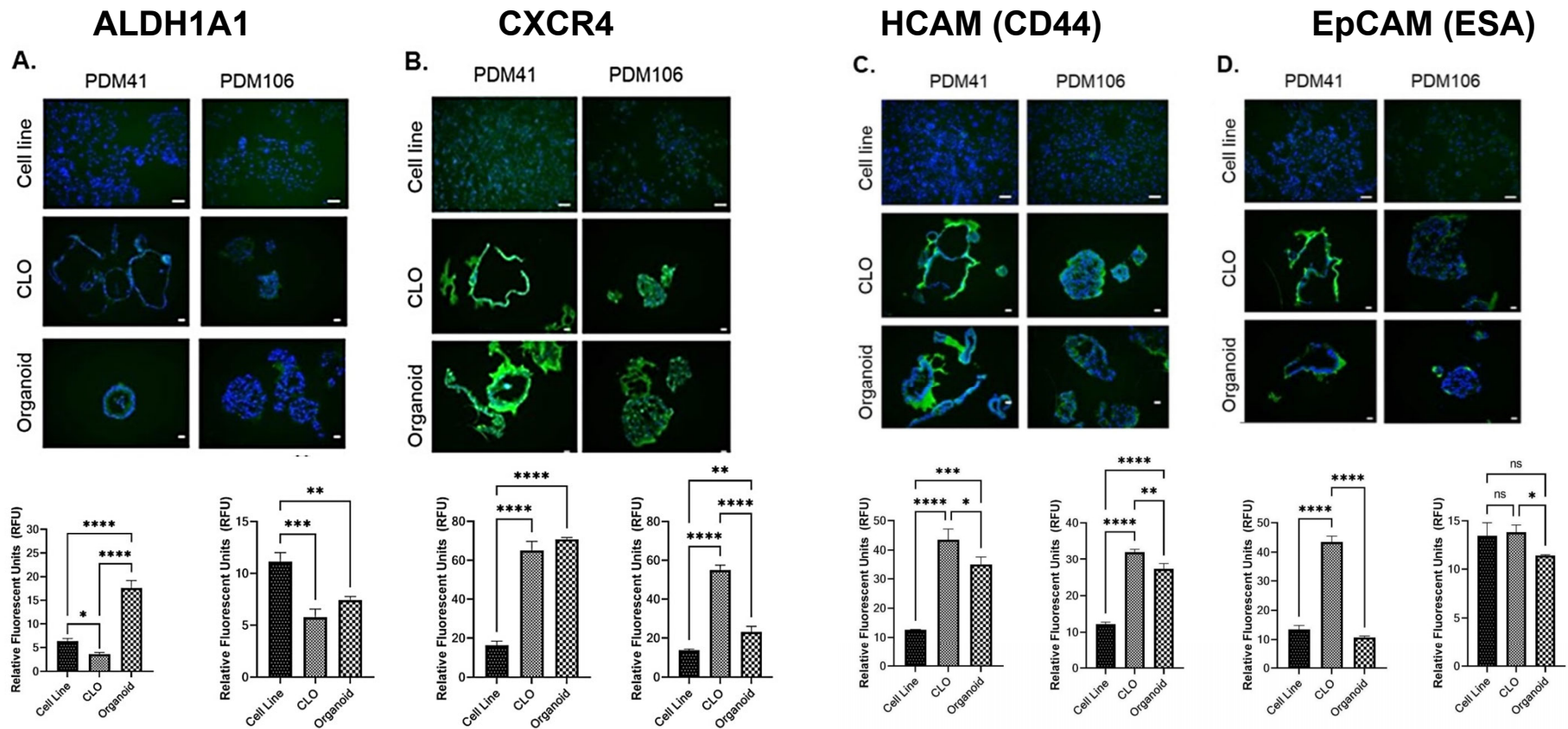
RNA-Seq transcriptomic analysis identifies similar CLO and organoid signature

Nelson et al., *Scientific reports*. 2020;*10*(1), 2778
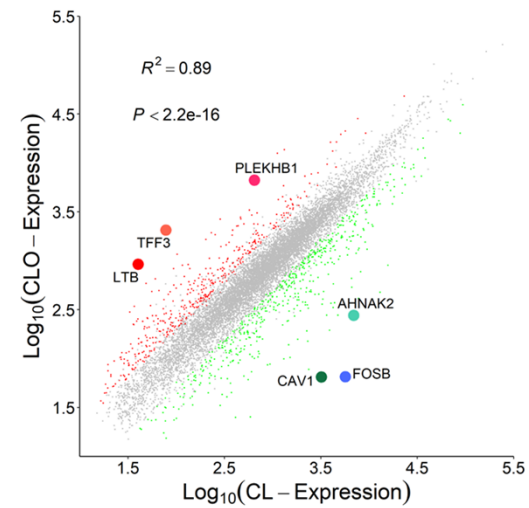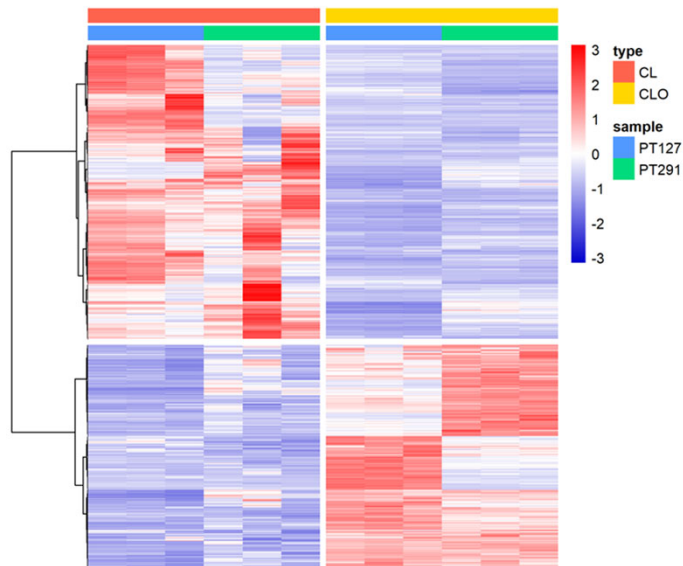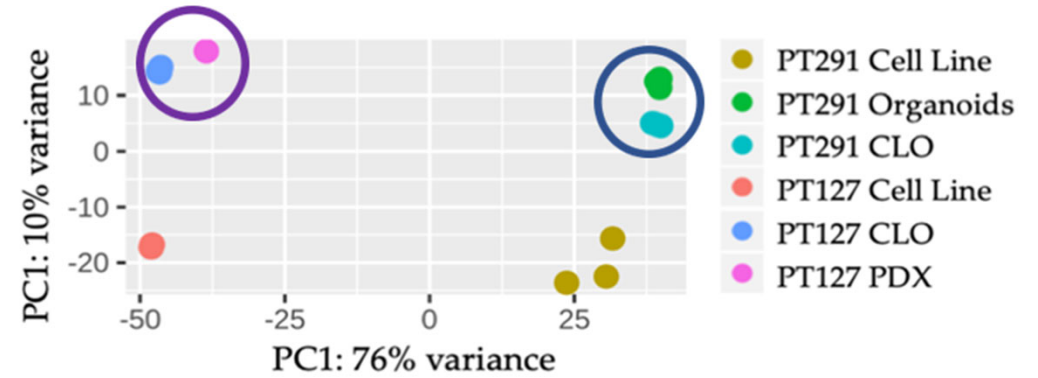
CLOs accurately reflect the cellular architecture and heterogeneity of organoids *in vivo*
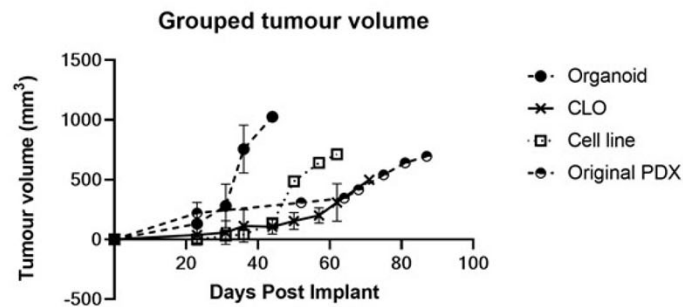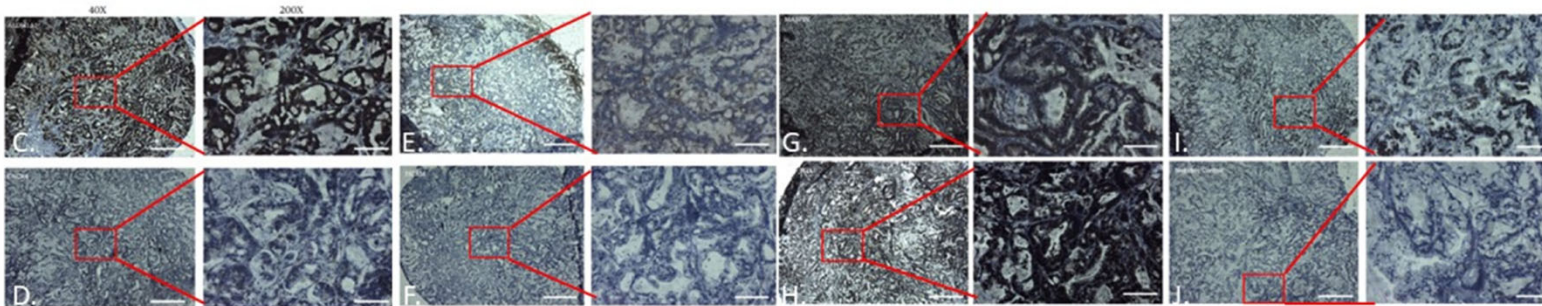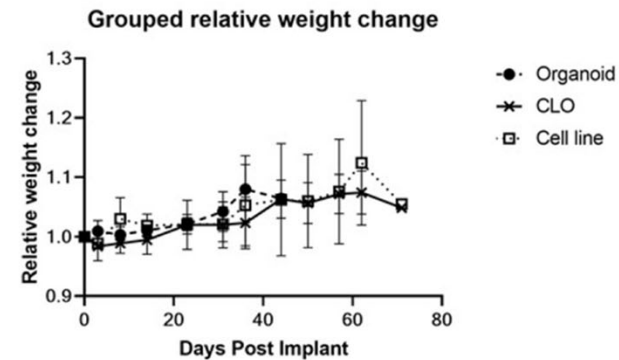
(**C**) ALDH1A1, (**D**) CXCR4, (**E**) ESA/EpCAM, (**F**) CD44, (**G**) MASPIN, (**H**) PDX1, (**I**) Ki67, and (**J**) negative control.

Noorani et al., *Organoids*. 2022; 1(2):168-183.

Cancer Stem Cell expression altered in long-term cultured 2D cell lines

PDM37 cell line
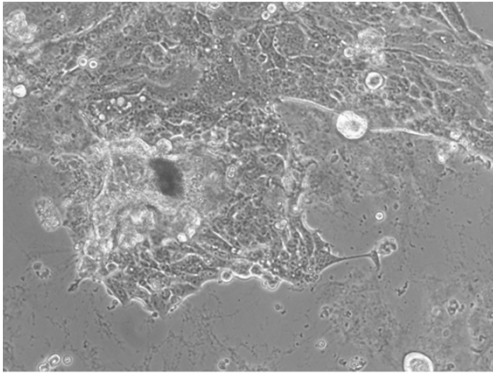
PDM41 cell line
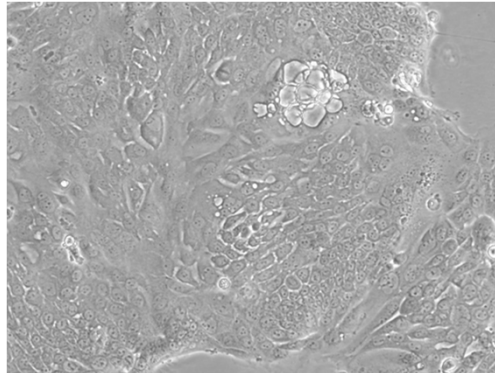
PDM106 cell line
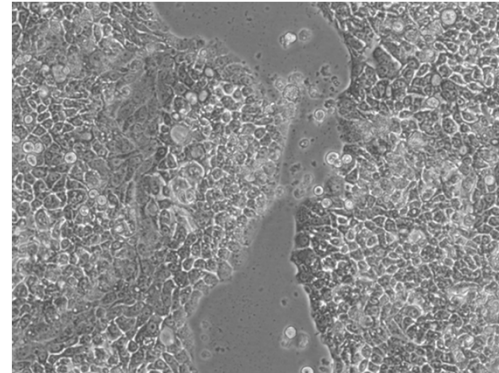
Long-term 2D culture loses Cancer Stem Cell CD133 expression

Long-term 2D culture expression of CD44/CD24/ESA is cell line dependent
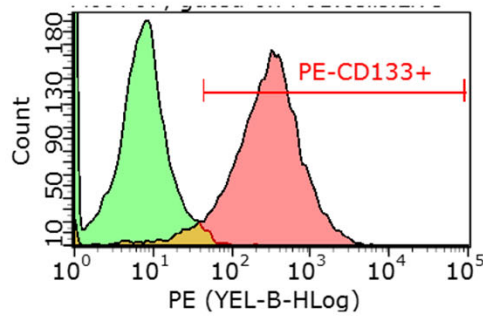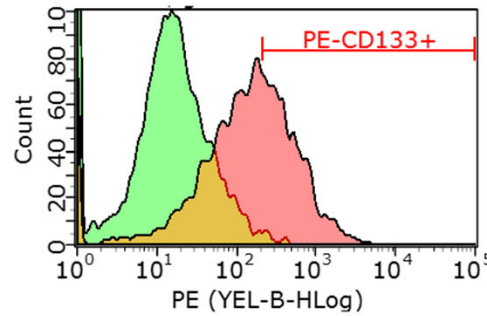
PDM41
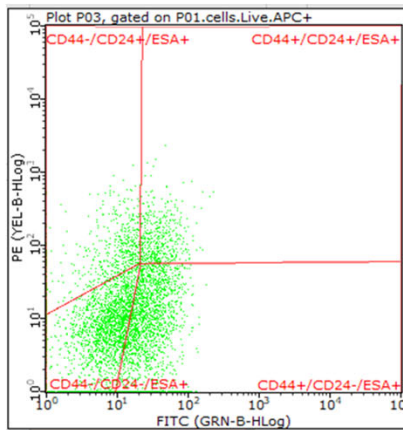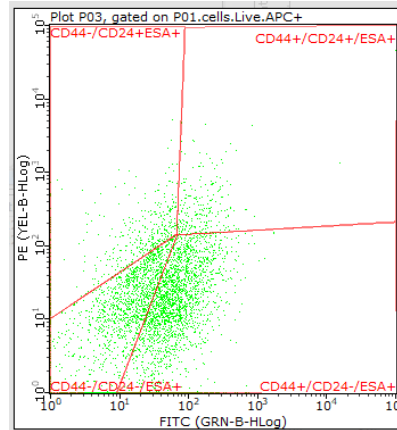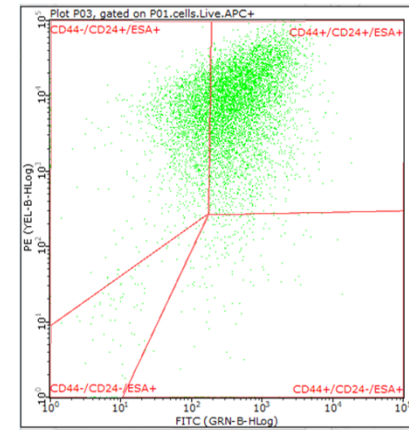
Organoid CLO Cell line

PDM106
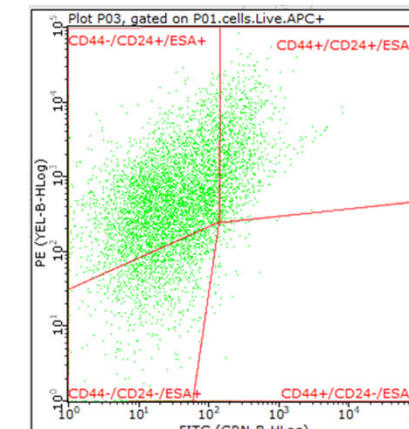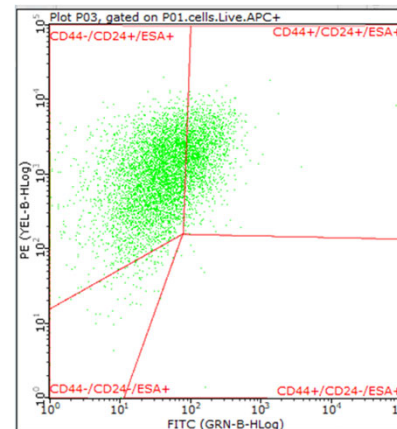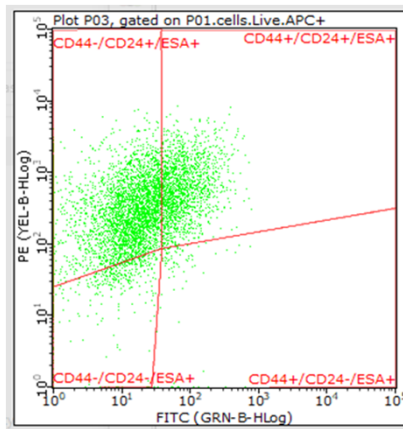
Stem cell plasticity is reconciled by culture conditions
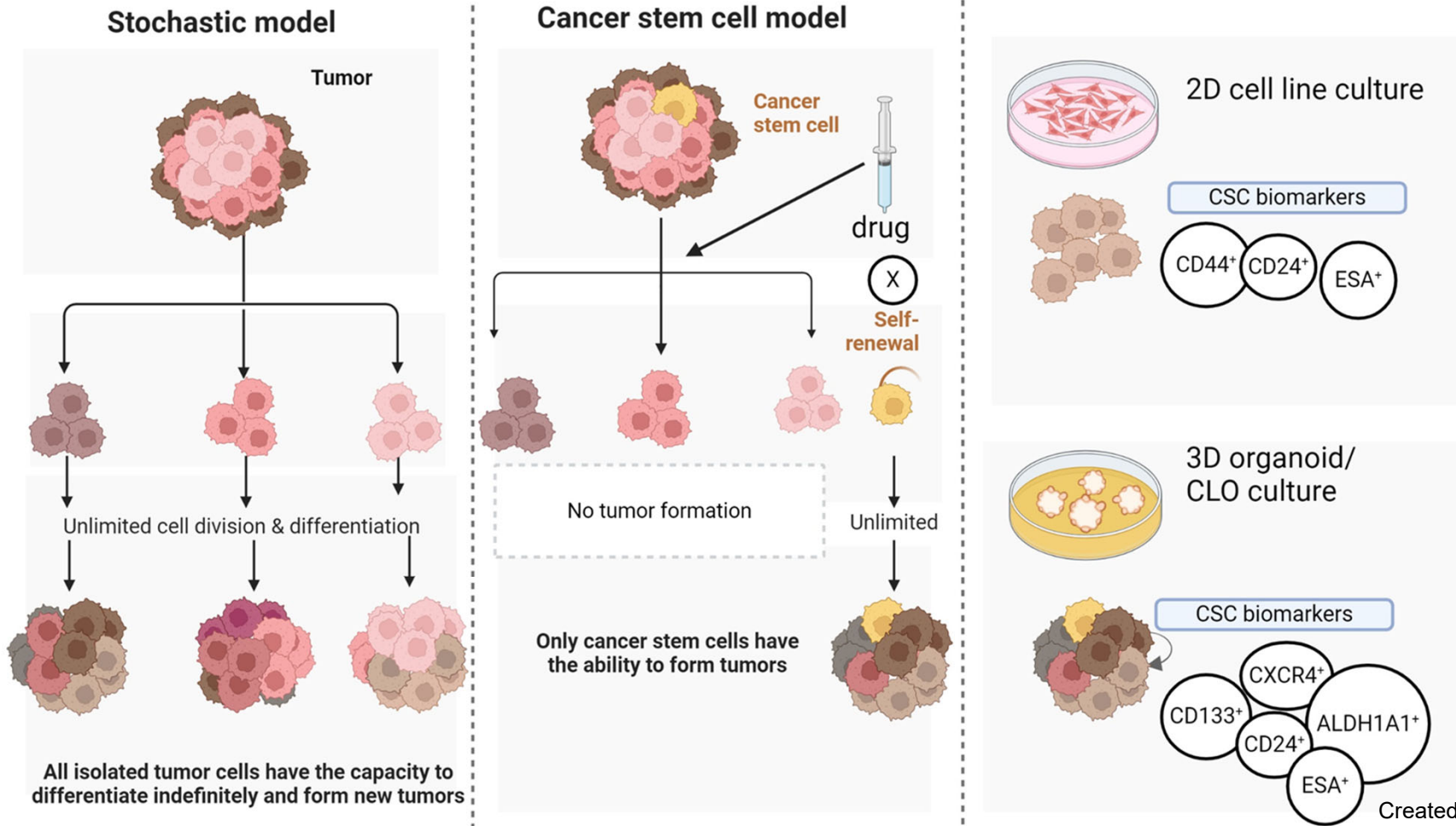
CSC expression altered between tumour and normal
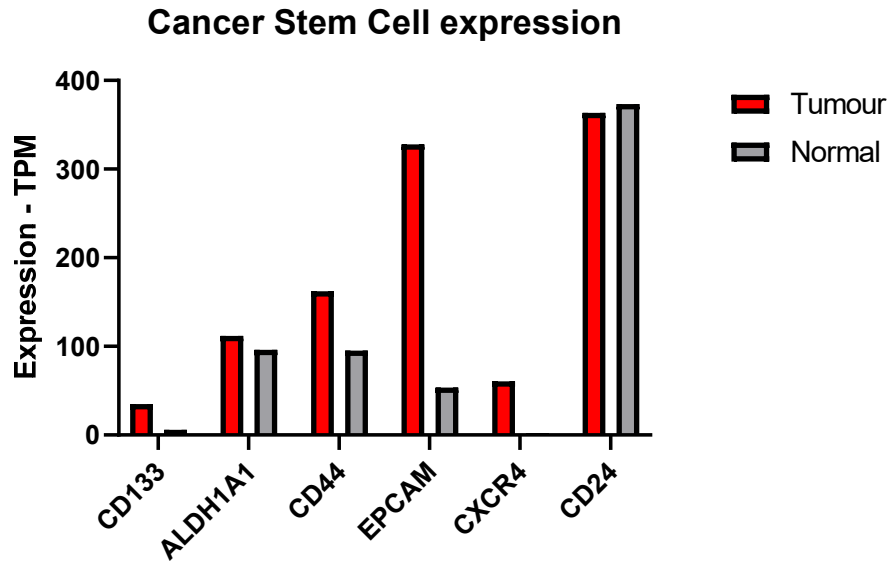
CD44 associated with overall survival

Cancer Stem Cell expression

Legend: ■ Tumour (red), ■ Normal (grey)
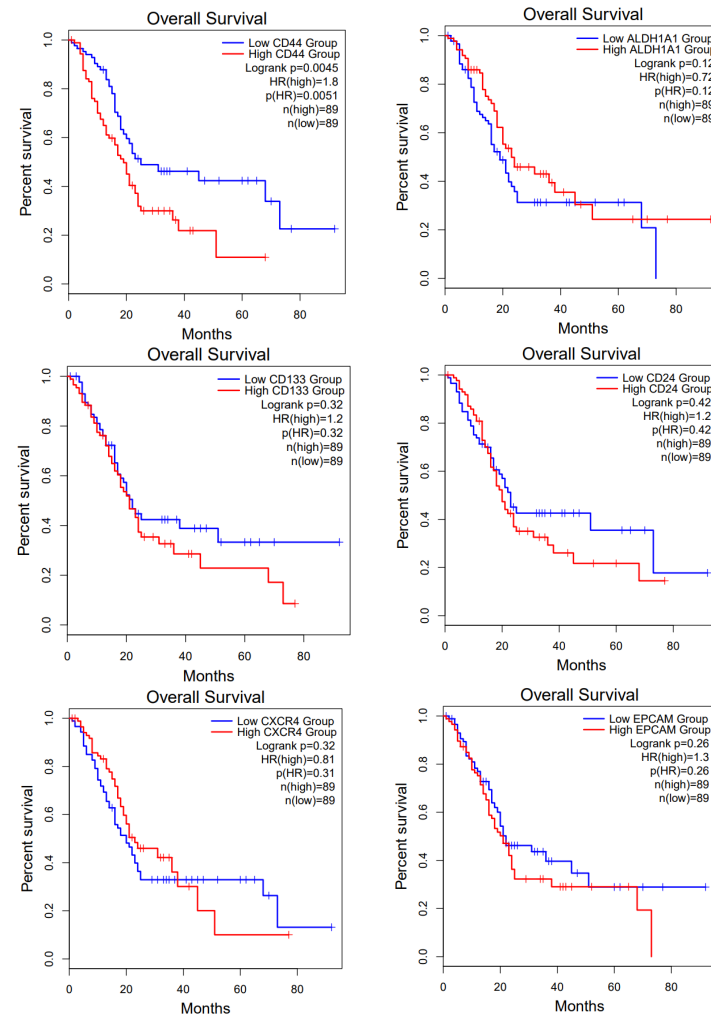
Modelling drug resistance *in vitro*:

- Poor long-term survival rates of pancreatic cancer are the consequences of rapidly acquired chemoresistance and represent a major therapeutic challenge

- Studying the emergence of resistance to therapeutics would allow us to identify key markers to guide treatment strategies

Created with BioRender.com
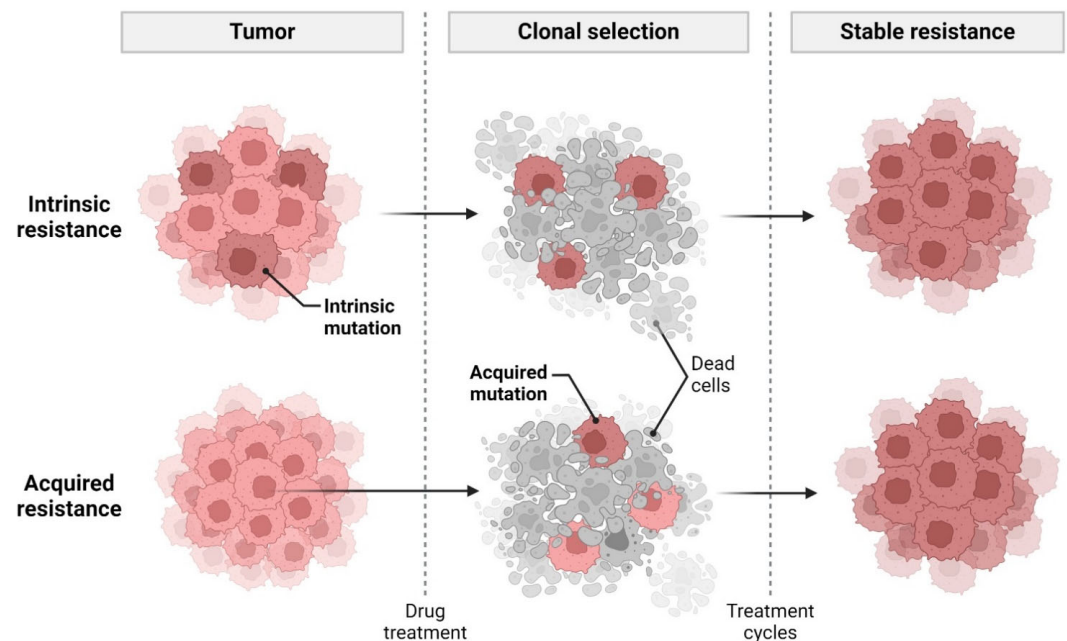
Long-term drug selection derived organoids

Advanced *in vitro* organoid models
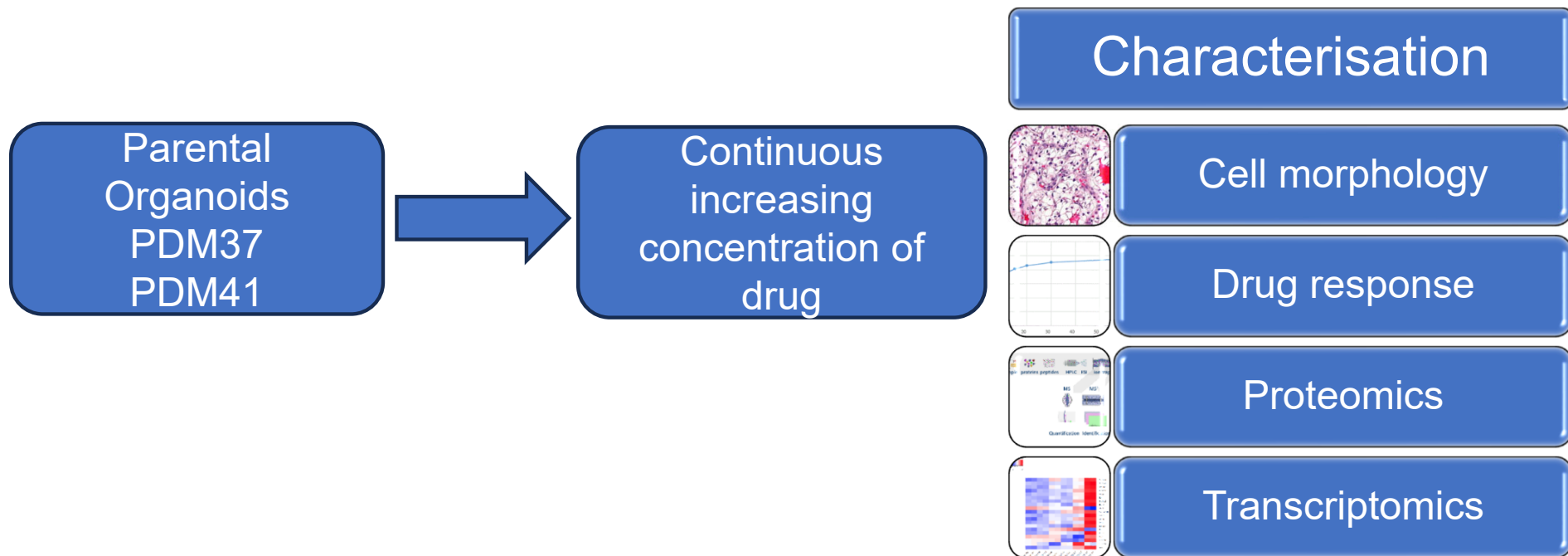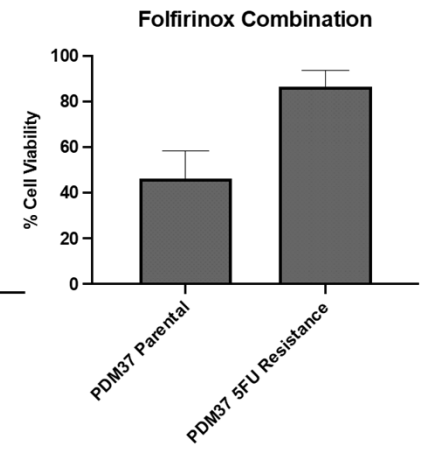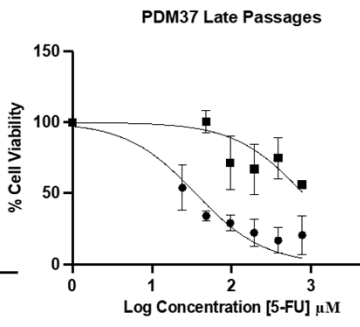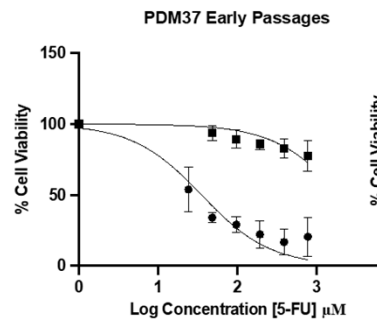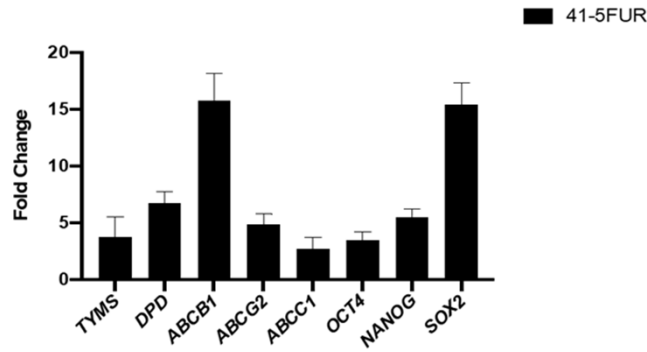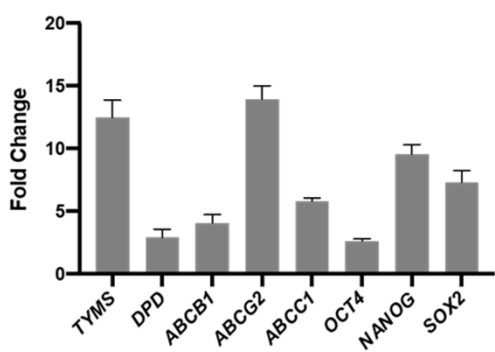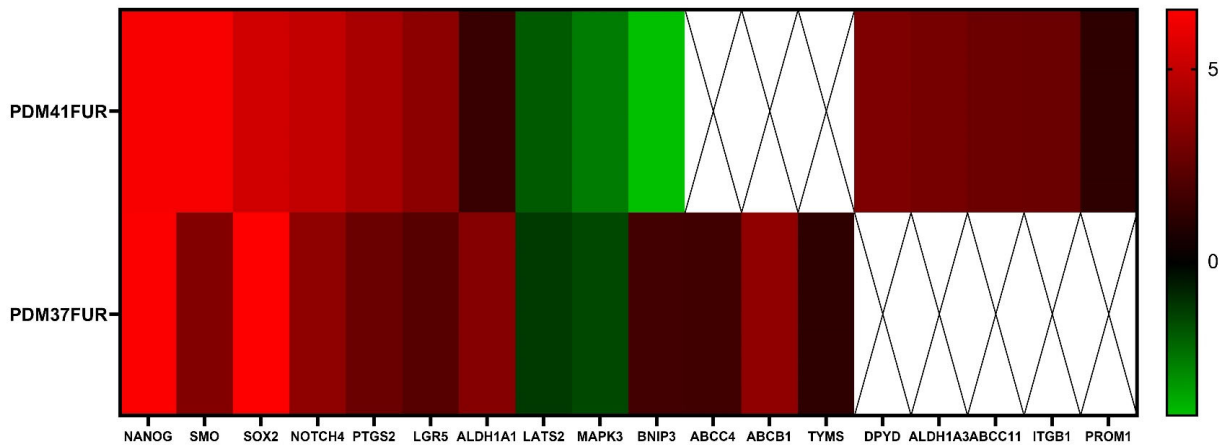
Establishment of 5-FU resistant PDAC organoids
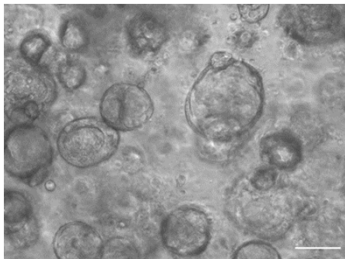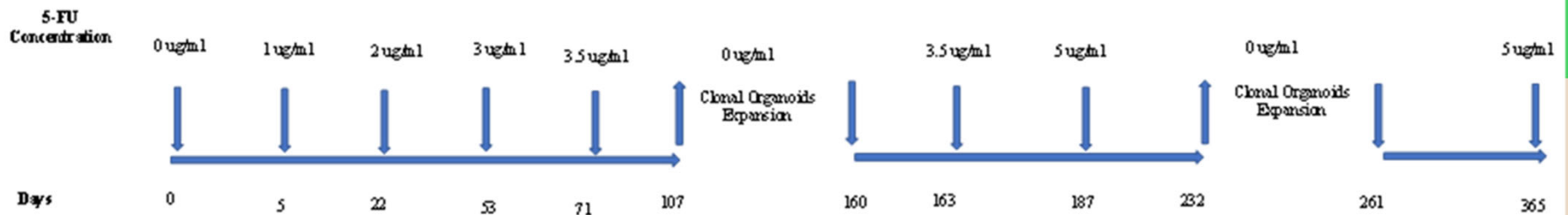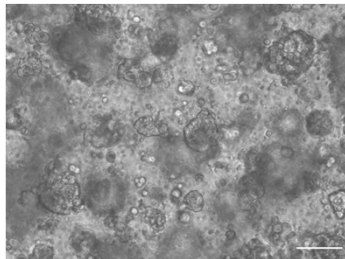
Transcriptomic identification of differentially regulated genes involved in 5-FU resistance
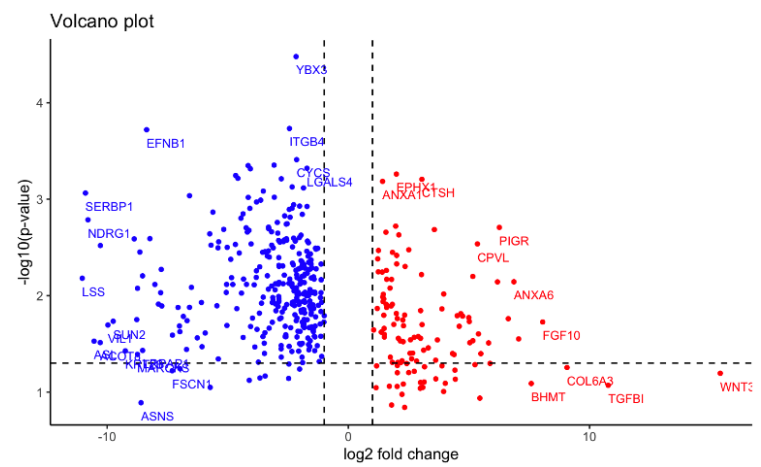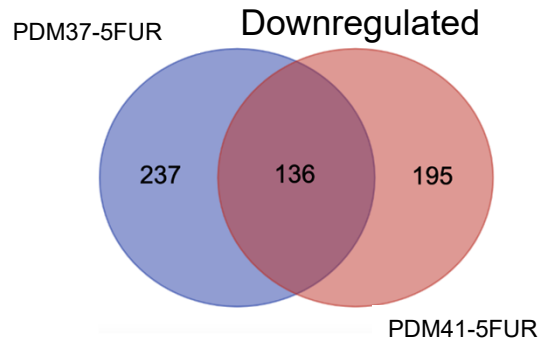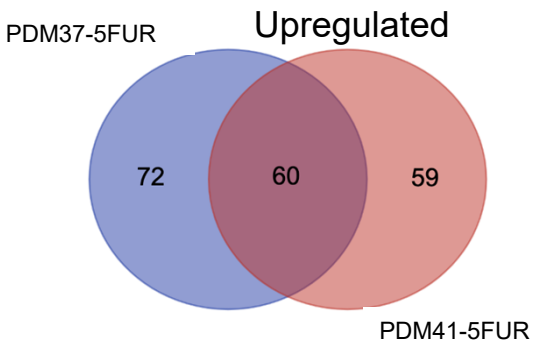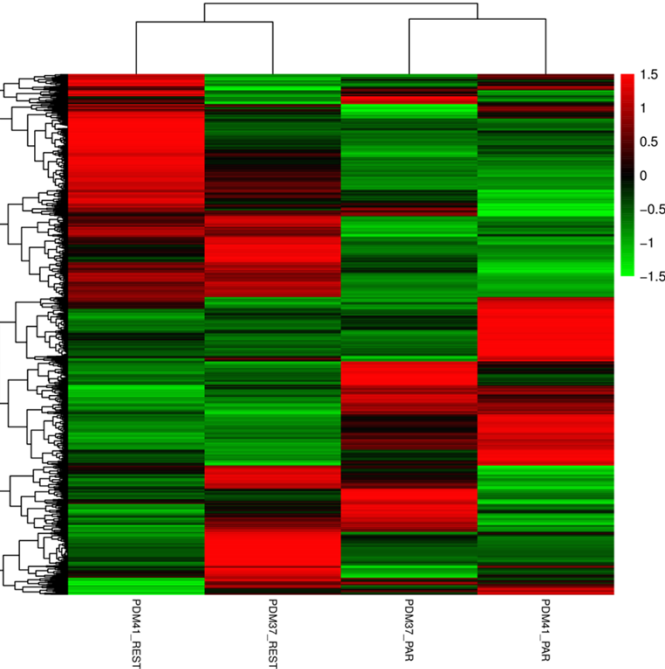
Common and differential mediators of 5FU drug resistance
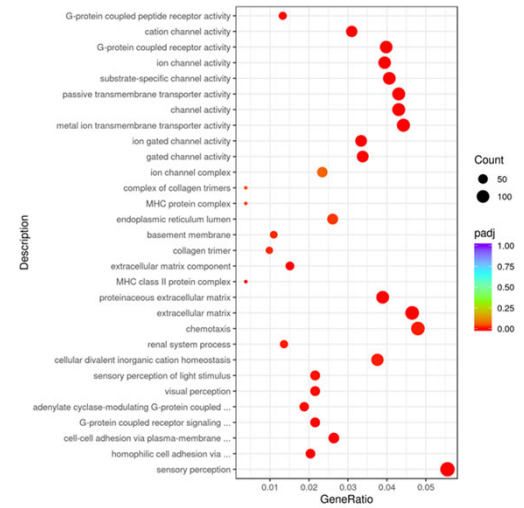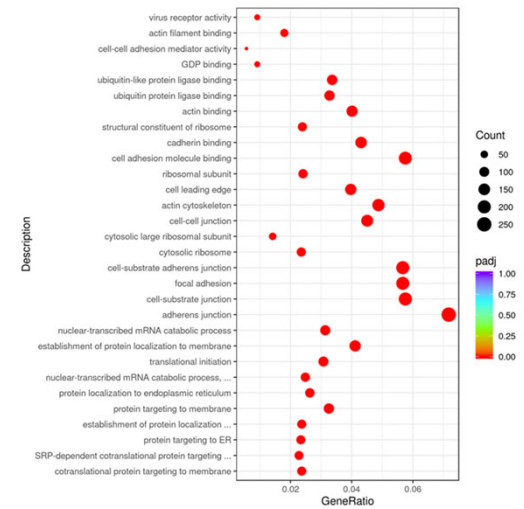
Conclusions

- Organoids can be used as an **emerging technology to advance of personalized medicine**

- Model tumorigenesis, and recapitulate critical features of original cancer tissue

- CLOs are flexible, expandable, traceable models used for high-throughput screening of sensitive drugs to provide individualized treatment options

- Tools for understanding the mechanisms of drug resistance
  - Identify markers of resistance
  - Develop novel therapeutics to overcome drug resistance
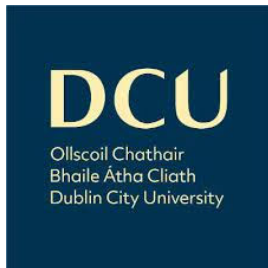
Acknowledgements

Dr. Shannon Nelson (past PhD)

Ms. Sara Noorani (PhD candidate)

Ms. Letizia Palanga (PhD candidate)

Ms. Jojanneke Stoof (PhD candidate, TCD)

Mr. Panos Sarameditis (PhD candidate)

Dr. Charlotte Andrieu

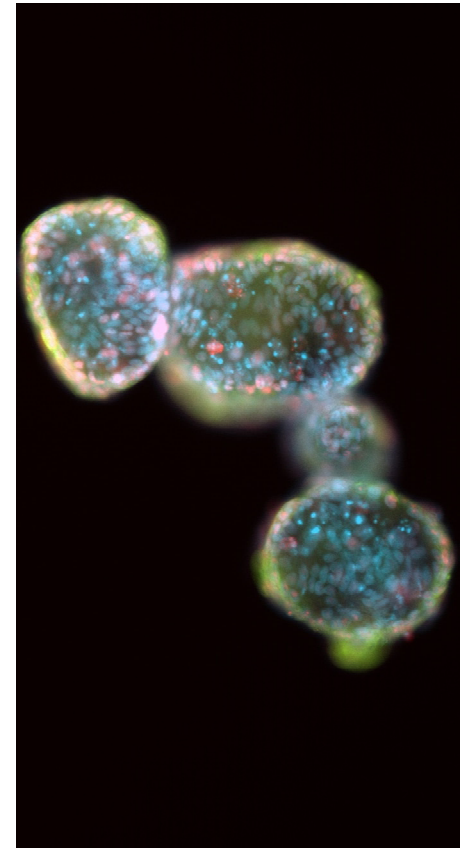# Learn more: www.atcc.org/HCMI

AACR Annual Meeting 2024

- San Diego Convention Center, San Diego, California
- April 07 - April 10, 2024, **booth #1413**