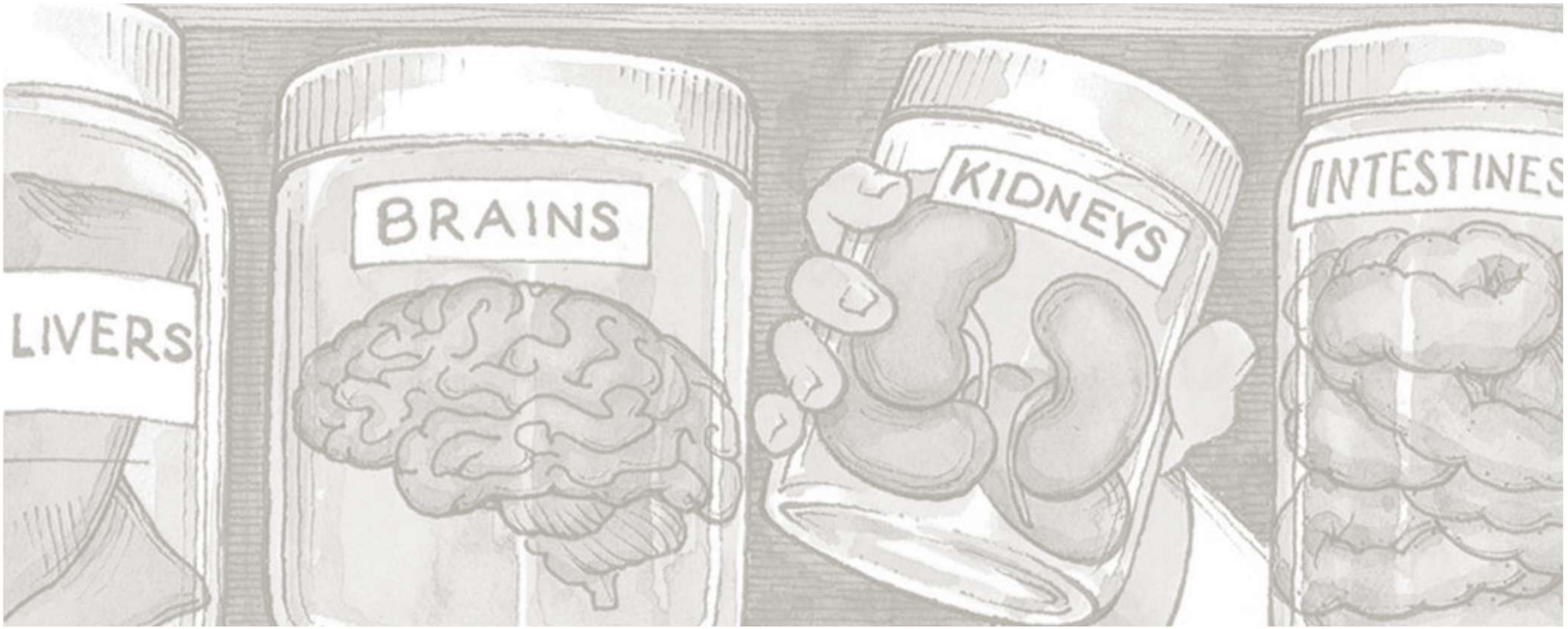
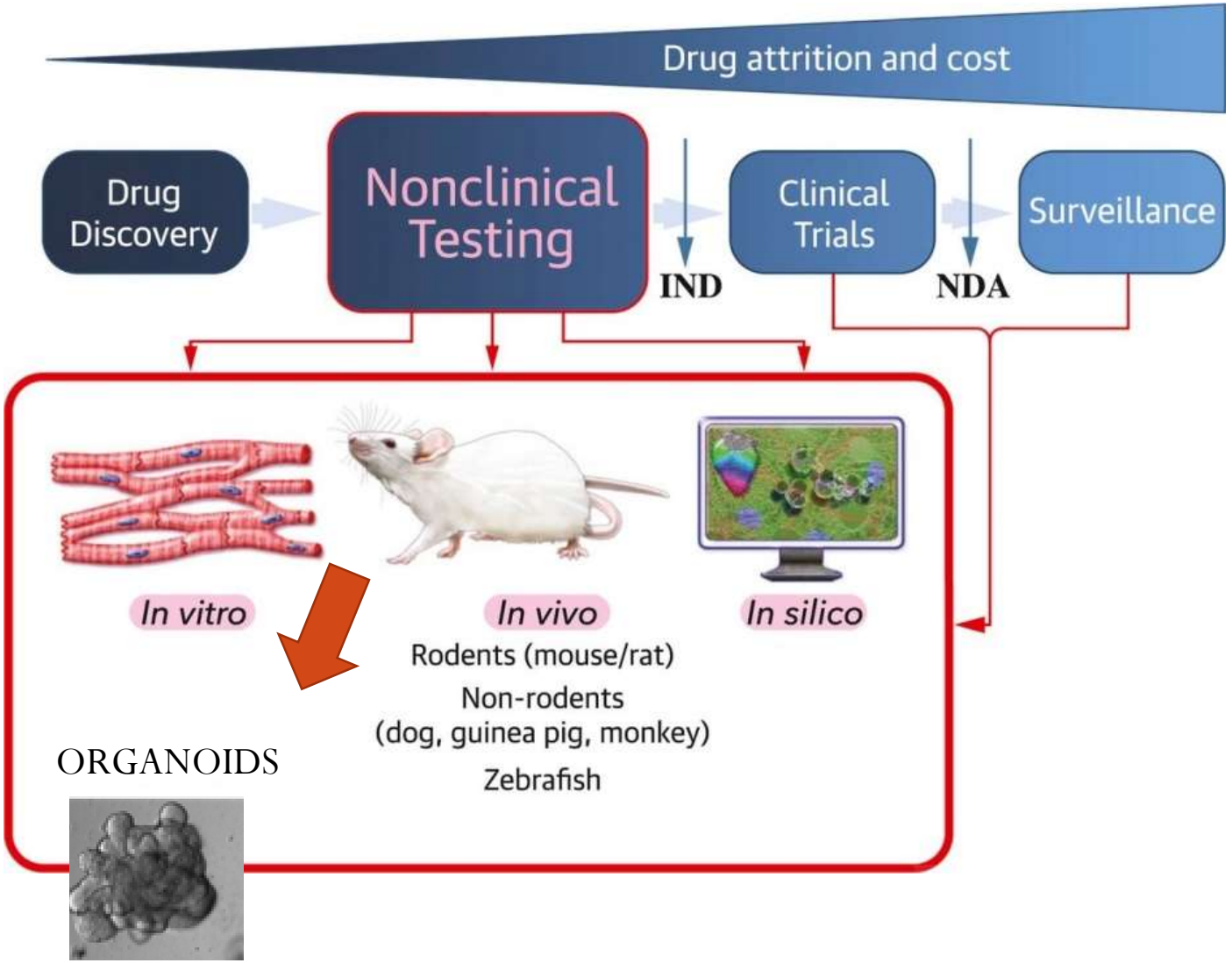
Three-dimensional Organoids as preclinical models



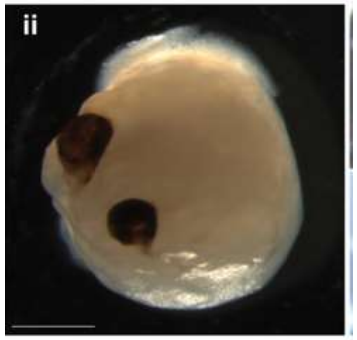
Preclinical models

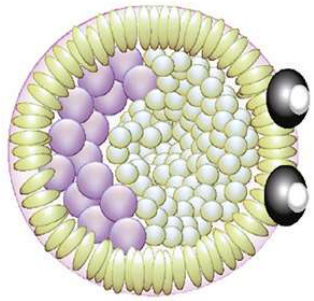


Calvin Chen Sheng et al., JACC VOL. 1, NO. 5, 2016

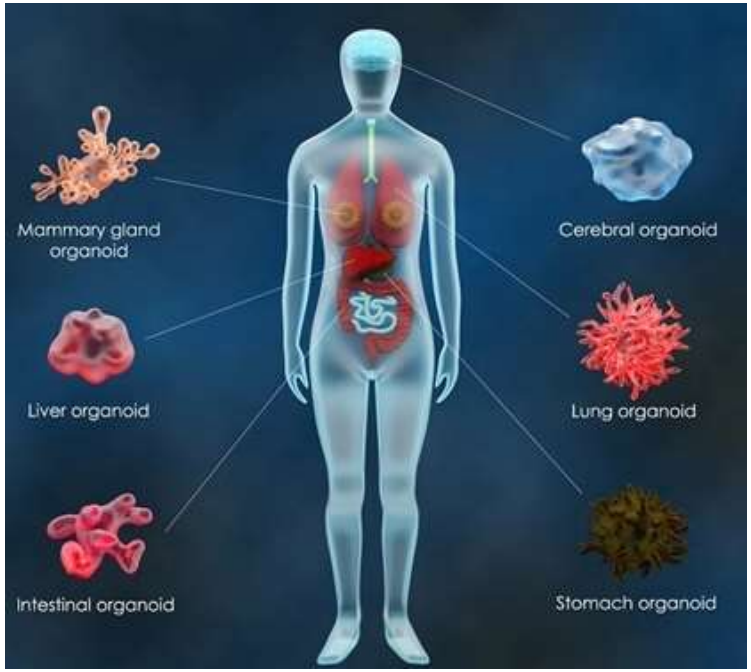
An **ORGANOID** is defined as a cellular structure containing multiple organ-specific cell types, capable of recapitulating some specific function of the organ, and spatially organized similarly to an organ.

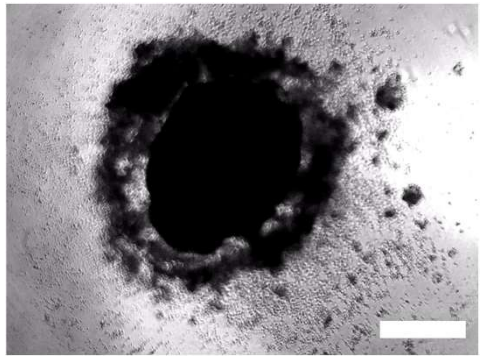
ORGANOIDS can be derived from pluripotent stem cells or adult stem cells.



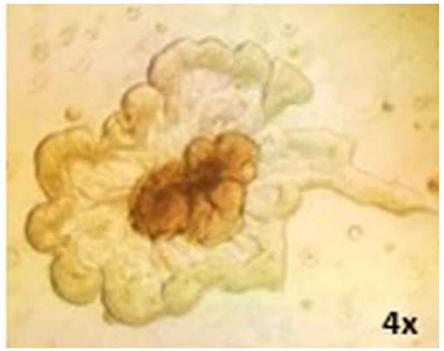


optic vesicle-containing brain organoids



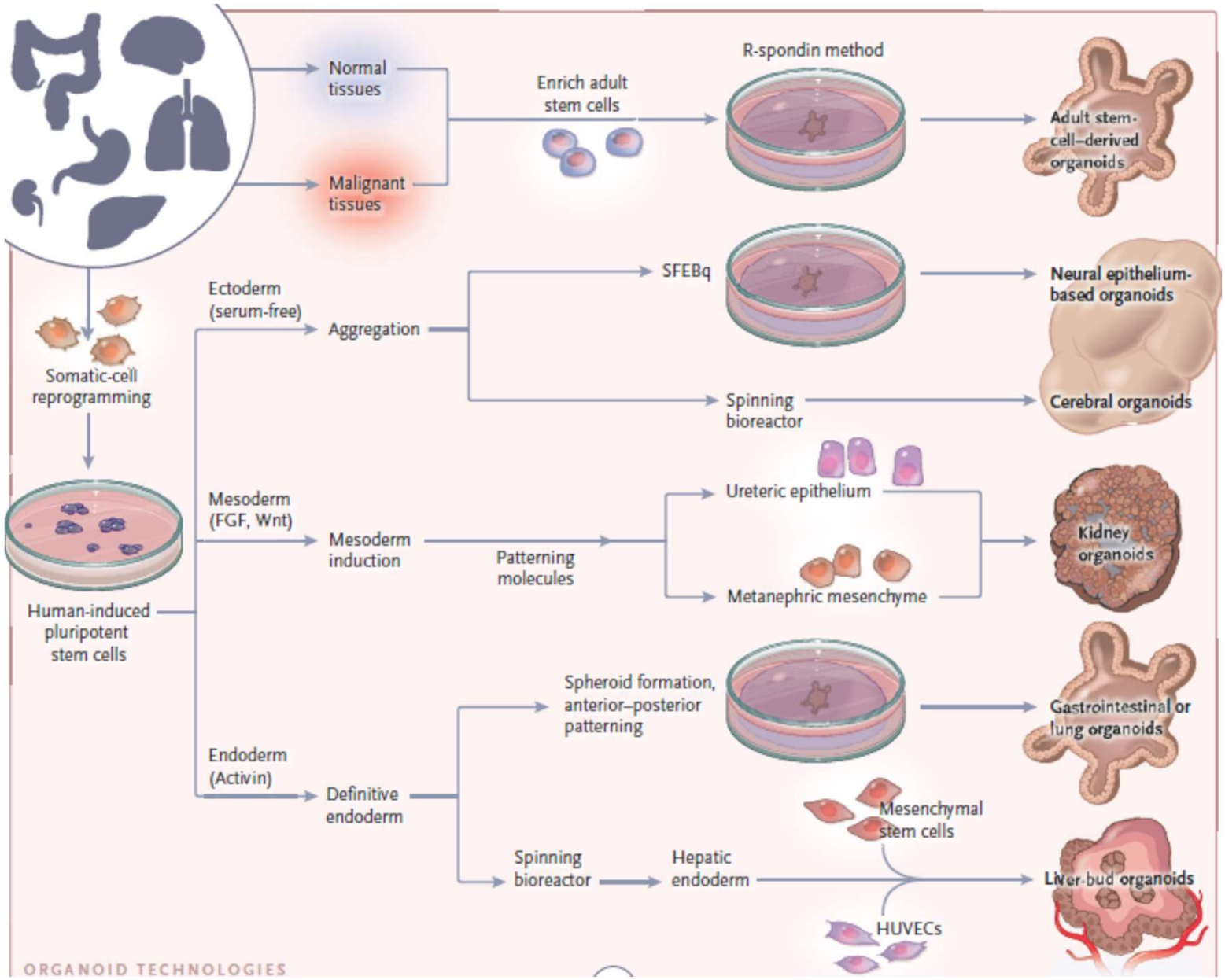


Heart organoid



Kidney organoid

Mainstream Human Organoid Models

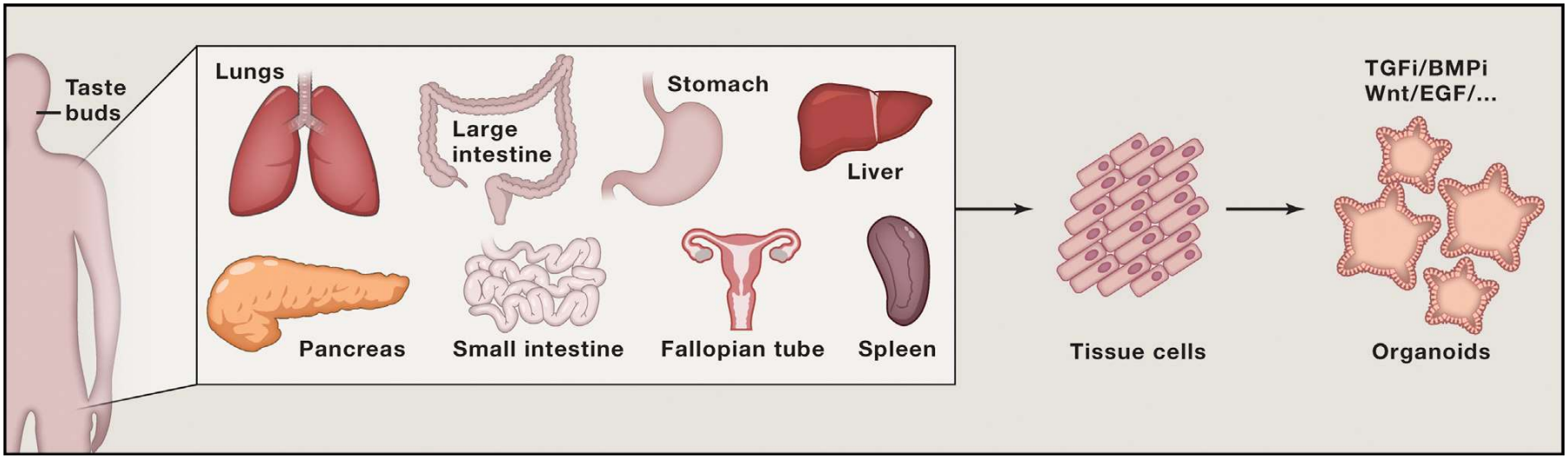


Mo Li and Juan C. Izpisua Belmonte
NEJM, 2019

Modeling Development and Disease with Organoids

Hans Clevers[1,*]

Cell



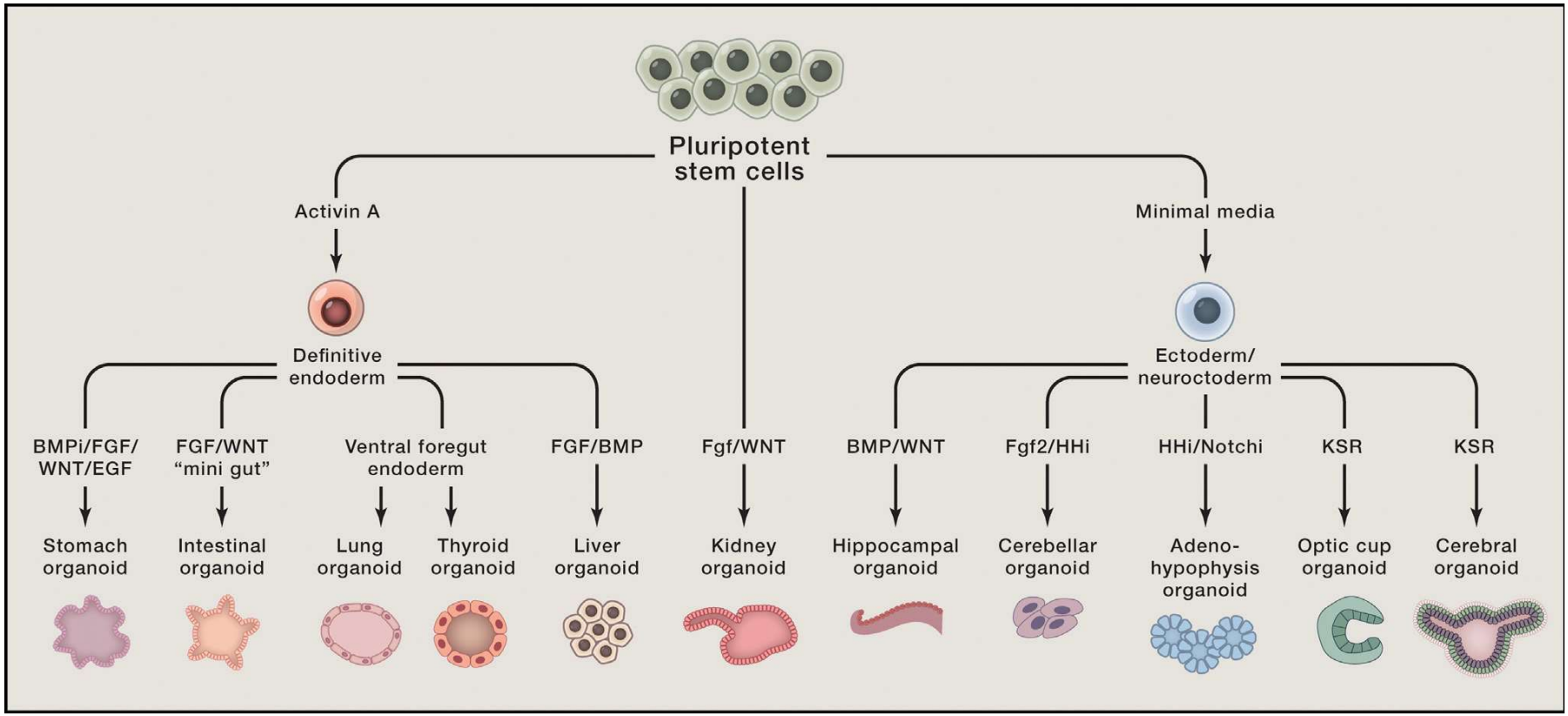
Schematic of the Various Regions of the Body that Can Be Cultured as aSC-Derived Organoids

Cell 165, June 16, 2016

Modeling Development and Disease with Organoids

Hans Clevers[1,*]

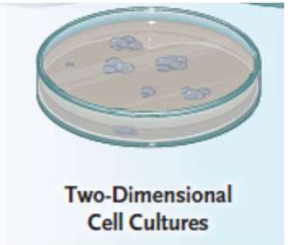
Cell

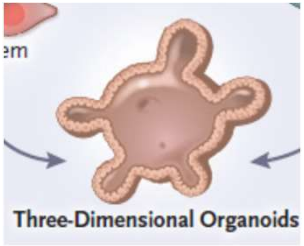


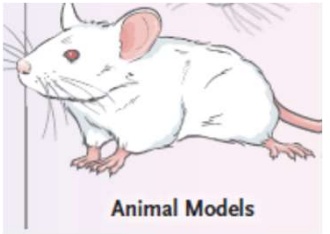
Schematic of the Various Organoids that Can Be Grown from PSCs and the Developmental Signals that Are Employed

Cell 165, June 16, 2016

Organoids – Preclinical models of human disease







Physiologic representation	Limited	Semiphysiologic	Physiologic
Vascularization and immune system	No	No	Yes
High-throughput screening	Yes	Yes	No
Manipulability	Excellent	Good, but may have experimental variability	Limited
Biobanking	Yes	Yes	Yes, but only at the cellular level
Genome editing	Yes	Yes	Yes, but may require generation of embryonic stem cells
Modeling organogenesis	Poor	Suitable for study of cell–cell communication, morphogenesis; reduced complexity	Yes, but often confounded by complex tissue environment
Modeling human development and disease	Poor owing to over-simplified nonphysiologic conditions	Yes	Yes

Mo Li and Juan C. Izpisua Belmonte
NEJM, 2019

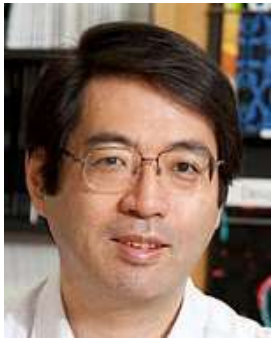
History of organoid methodologies

REVIEW

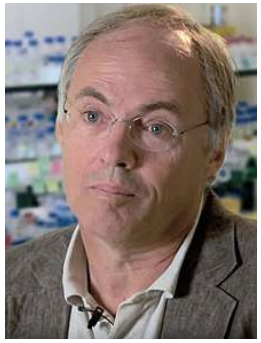
Organogenesis in a dish: Modeling development and disease using organoid technologies

Madeline A. Lancaster[1], Juergen A. Knoblich[1,*]
+ See all authors and affiliations

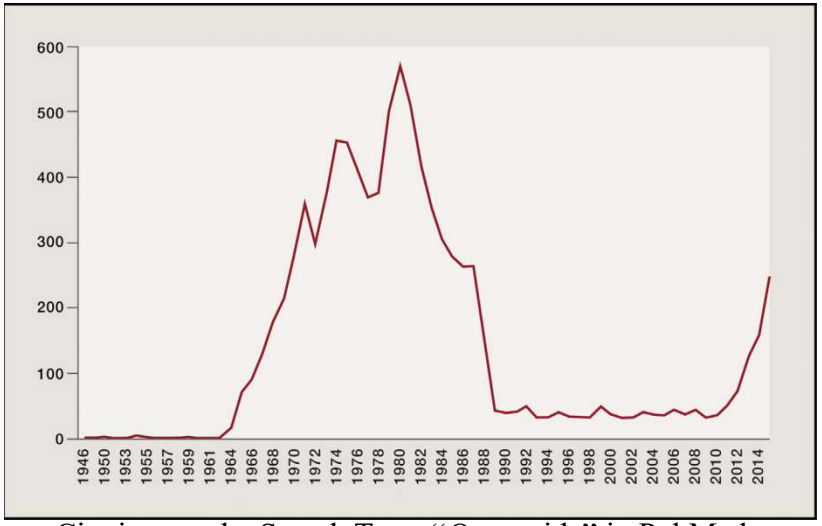
Science 18 Jul 2014:
Vol. 345, Issue 6194, 1247125
DOI: 10.1126/science.1247125



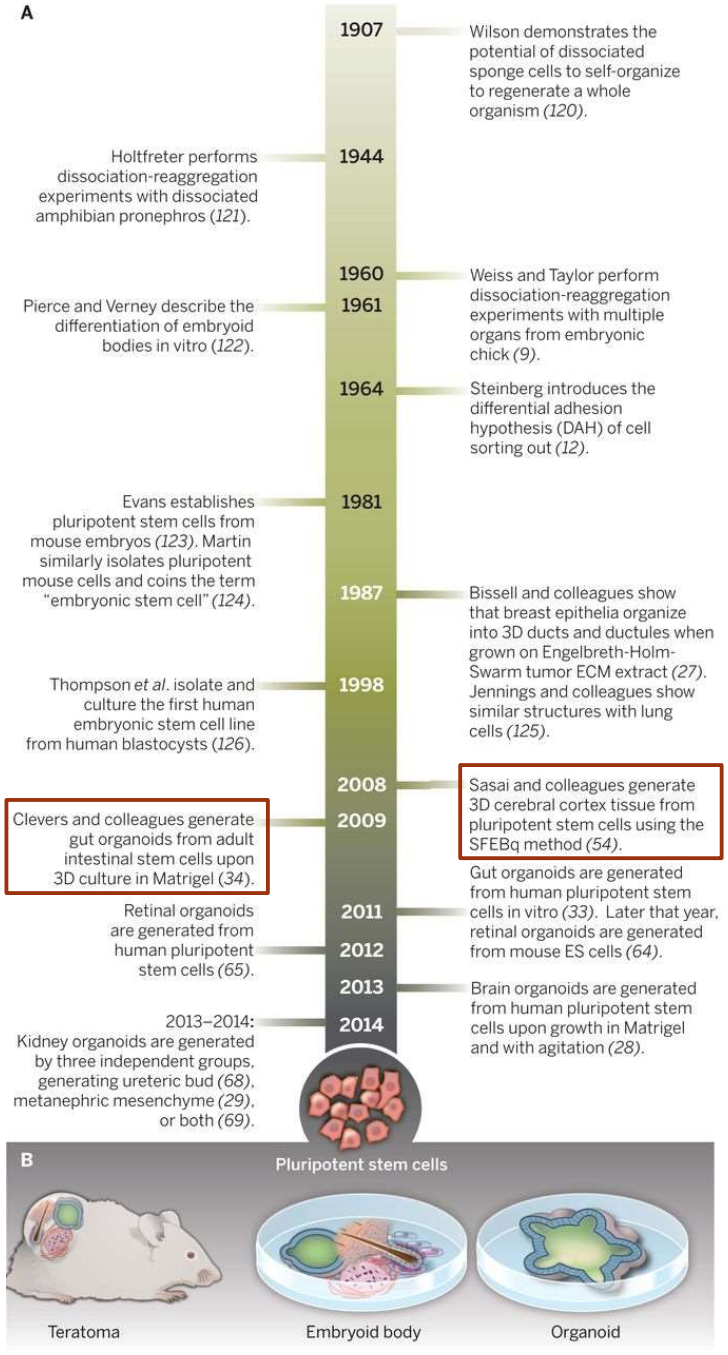
Yoshiki Sasai



J.C. Clevers



Citations to the Search Term ‘‘Organoids’’ in PubMed



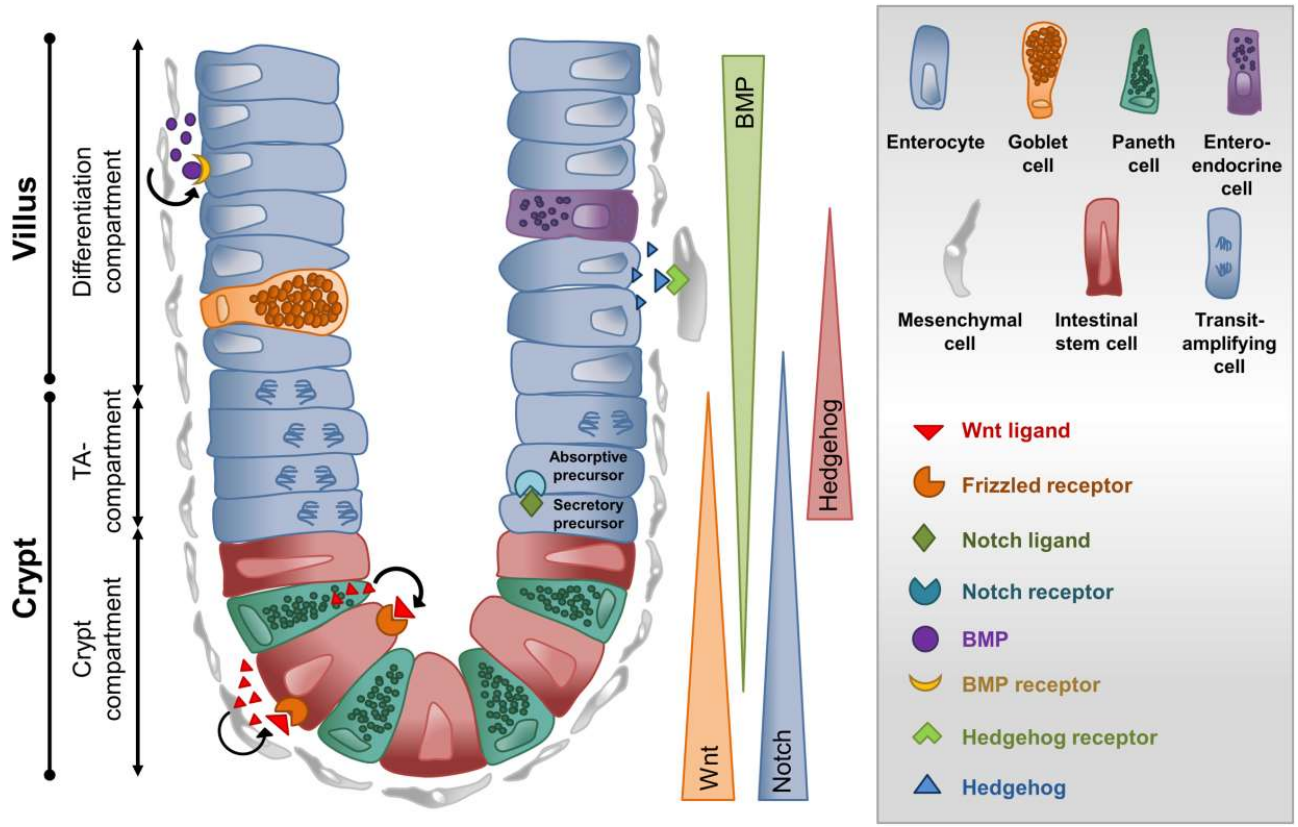
Editorial | Published: 03 January 2018

Method of the Year 2017: Organoids

Nature Methods **15**, 1 (2018) | Download Citation

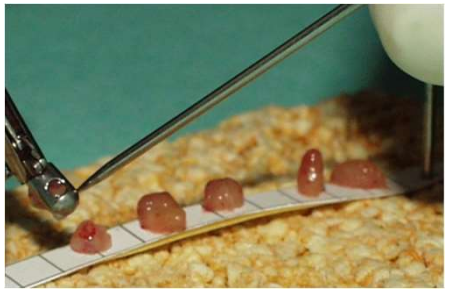
The ability to prod stem cells into three-dimensional tissue models makes for a powerful way to study human biology. But these exciting tools are still works in progress.

Intestinal organoid culture method



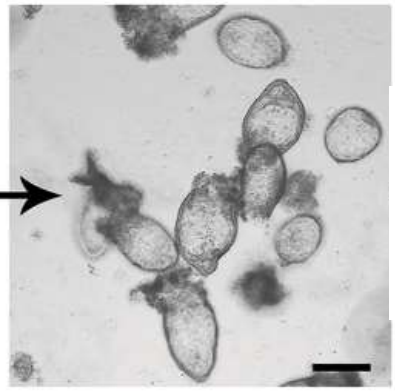
- Lgr5 crypt stem cells divide constantly;
- Stem cells numbers remain fixed because stem cells compete ‘neutrally’ for niche space;
- Daughters of the intestinal stem cells, the Paneth cells, serve as crypt niche cells by providing Wnt, Notch and EGF signals.

Intestinal organoid culture method

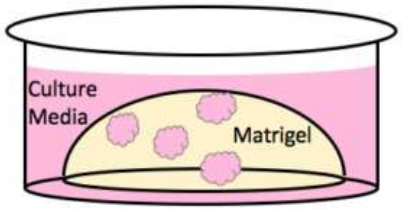


1. Intestinal biopsies

2. Crypt Isolation

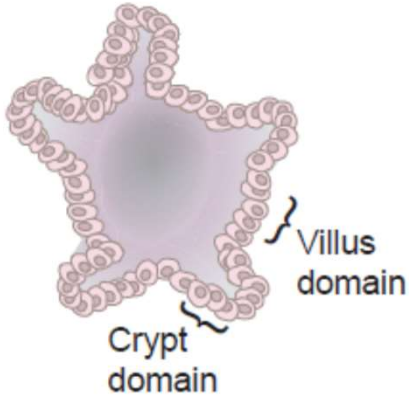


3. Crypt Culture

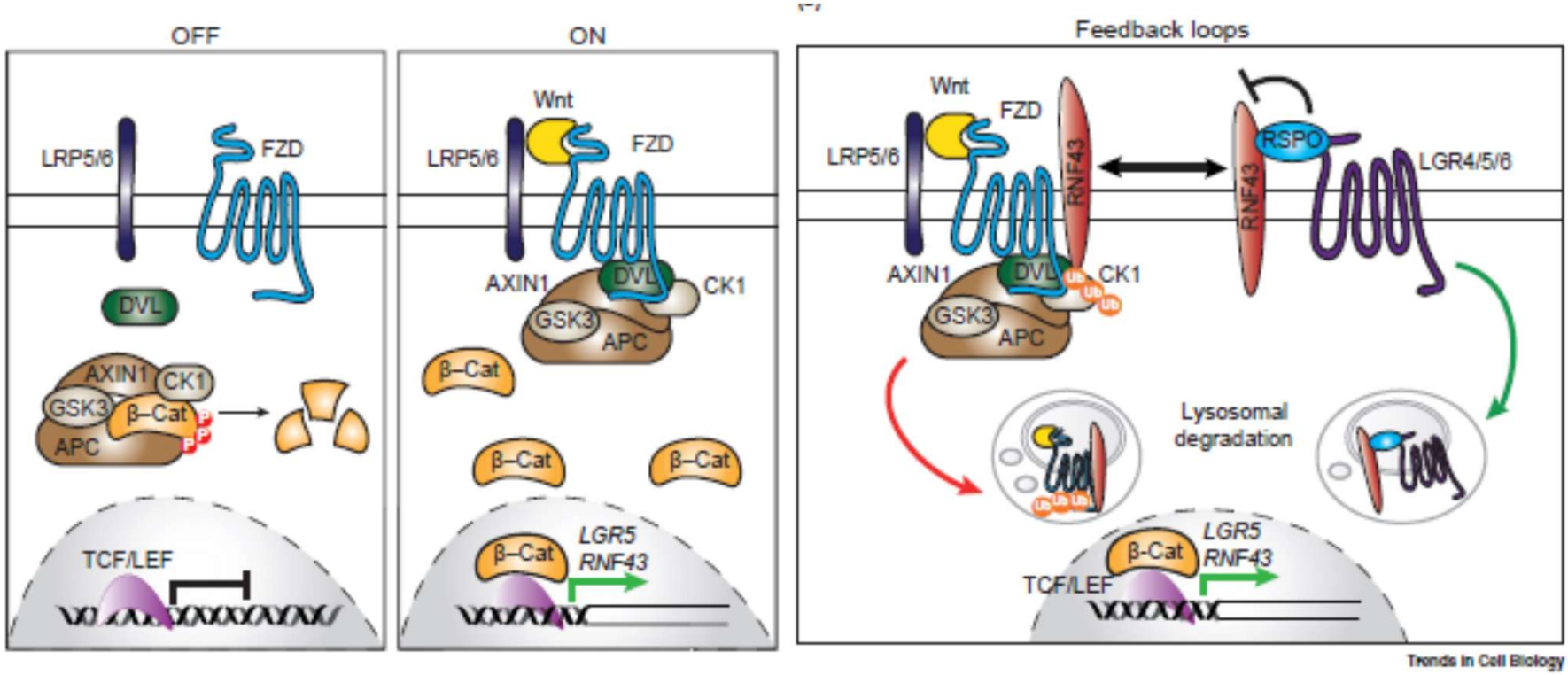


1)Wash
2)Fungizone+Normocin+Gentamicin
3)EDTA incubation
4)Shake vigorously

Stem Organoid Medium:
R-spondin
WNT3a
Noggin
+ 12 reagents



Wnt Signaling



The Wnt/b-Catenin Signaling Pathway and Its Regulatory Loops.

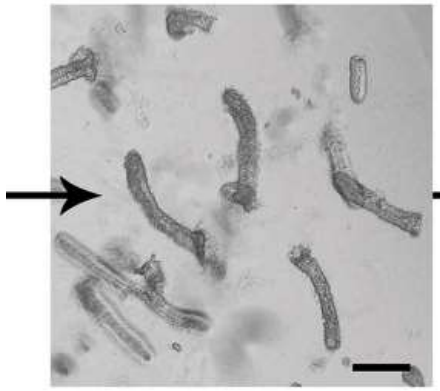
Merenda et al., 2020 Trends in Cell Biology

Table 1. Frequently used growth media constituents, their working mechanisms and effects, as well as applications.

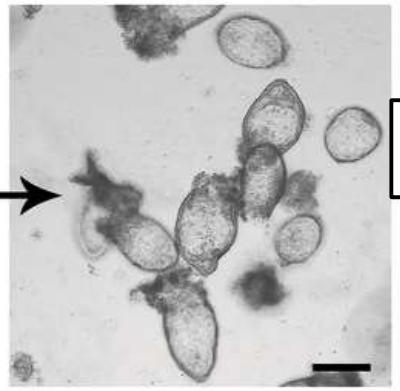
Growth medium constituents	Working mechanism in ISCs	Effect on ISCs and application
WNT3a[a]	Activates canonical WNT signaling (Clevers & Nusse, 2012)	Stimulates crypt cells proliferation and maintains the stem cell state (Clevers & Nusse, 2012; Farin *et al*, 2012; Krausova & Korinek, 2014)
R-spondin 1[a]	Augments WNT/β-catenin signaling (de Lau *et al*, 2014)	Stimulates crypt cell proliferation and maintains stem cell state (Farin *et al*, 2012; Krausova & Korinek, 2014; de Lau *et al*, 2014)
CHIR99021	Stimulates canonical WNT signaling (Yin *et al*, 2014)	Stimulates stem cell proliferation and can be used in combination with VPA, when growing single mouse ISCs in absence of Paneth cells (Yin *et al*, 2014)
Valproic acid	Inhibits histone deacetylase and activates Notch signaling (Yin *et al*, 2014)	Maintains proliferative crypts and blocks secretory differentiation (Sato *et al*, 2011b). Can be used in combination with CHIR99021 when growing single mouse ISCs in absence of Paneth cells (Yin *et al*, 2014)
Noggin[a]	Inhibits BMP signaling (Haramis *et al*, 2004)	Stimulates crypt formation (Haramis *et al*, 2004)
Jagged-1	Activates Notch signaling (Sato *et al*, 2009)	Maintains the stem cell state, and promotes proliferation, while blocking secretory differentiation, thereby maintaining proliferative crypts (Stanger *et al*, 2005; Van Dussen *et al*, 2012) Used in the early phase of single-cell cultures in absence of Notch signaling from adjacent supportive cells (Sato *et al*, 2009; Grabinger *et al*, 2014)
EGF[a]	Activates RAS/RAF/MEK/ERK signaling pathway (Suzuki *et al*, 2010; Date & Sato, 2015)	Stimulates stem cell migration, proliferation, and inhibits apoptosis (Frey *et al*, 2004; Suzuki *et al*, 2010)
PGE_2	Enhances canonical WNT signaling (Buchanan & DuBois, 2006)	Prevents anoikis as well as promotes stem cell survival and proliferation, thereby improving culture efficiency. Stimulates spheroid morphology (Cohn *et al*, 1997; Joseph *et al*, 2005)
Nicotinamide	Inhibits the activity of sirtuins (Denu, 2005)	Improves ISC maintenance when cultured > 1 week (Sato *et al*, 2011a). Often used for long-term human intestinal organoid cultures (Sato *et al*, 2011a), but can be omitted (Fujii *et al*, 2015)
Gastrin-17	Not decisively concluded	Marginally increases culture efficiency (Sato *et al*, 2011a)
A83-01 or SB431542[a]	Inhibits TGF-β signaling (Sato *et al*, 2011a)	Inhibits differentiation and allows human intestinal stem cell cultures to be sustained in the long term (Sato *et al*, 2011a)
SB202190[a]	Inhibits P38 MAPK (Sato *et al*, 2011a)	Inhibits secretory differentiation, increases plating efficiency, and decreases degradation of the EGF receptor (Frey *et al*, 2006; Sato *et al*, 2011a; Date & Sato, 2015). Allows human intestinal stem cell cultures to be sustained in the long term (Sato *et al*, 2011a)
Y-27632 or thiazovivin	Inhibition of caspase-3 (Wu *et al*, 2015)	Prevents anoikis after single-cell dissociation (Watanabe *et al*, 2007). Used in the early phase of single-cell cultures
IL-22	JAK/STAT signaling (Lindemans *et al*, 2015)	ISC proliferation and organoid growth. Can potentially further increase ISC expansion and make EGF redundant (Lindemans *et al*, 2015)

[a]Mandatory growth medium components for long-term culturing human intestinal stem cells as organoids.

Intestinal organoid culture method

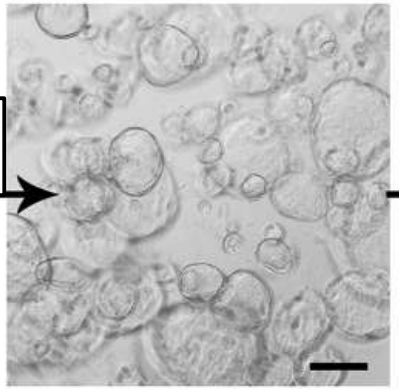


2. Crypt Isolation



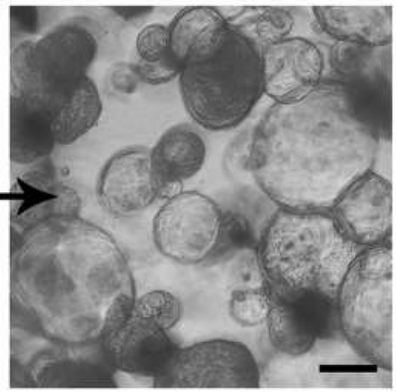
3. Crypt Culture

48/ 72h



4. Epithelial Organoid cultures

5 D



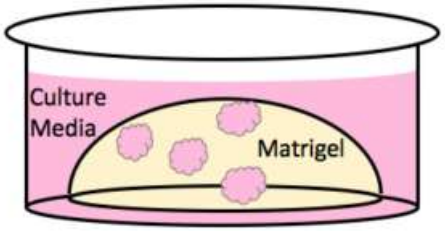
5. Differentiated Epithelial Organoid cultures (enterocytes, goblet cells, Paneth cells, enteroendocrine cells)

Stem Organoid Medium:
R-spondin
WNT3a
Noggin
+ 12 reagents

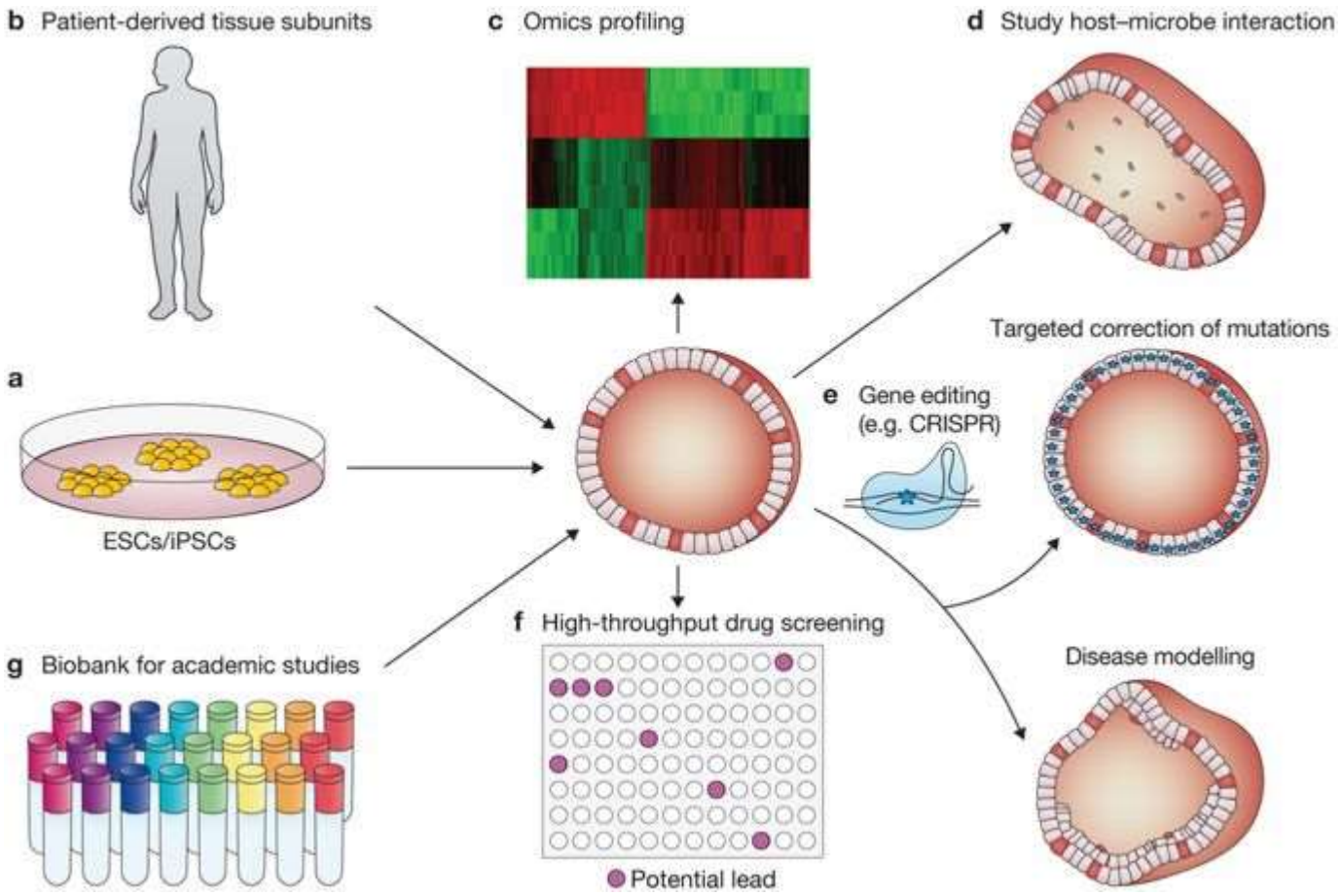
Organoid passaging:
1)Recovery solution
2)Disaggregation solution (dispase or trypsin)

EXPANSION/ ANALYSES

ANALYSES



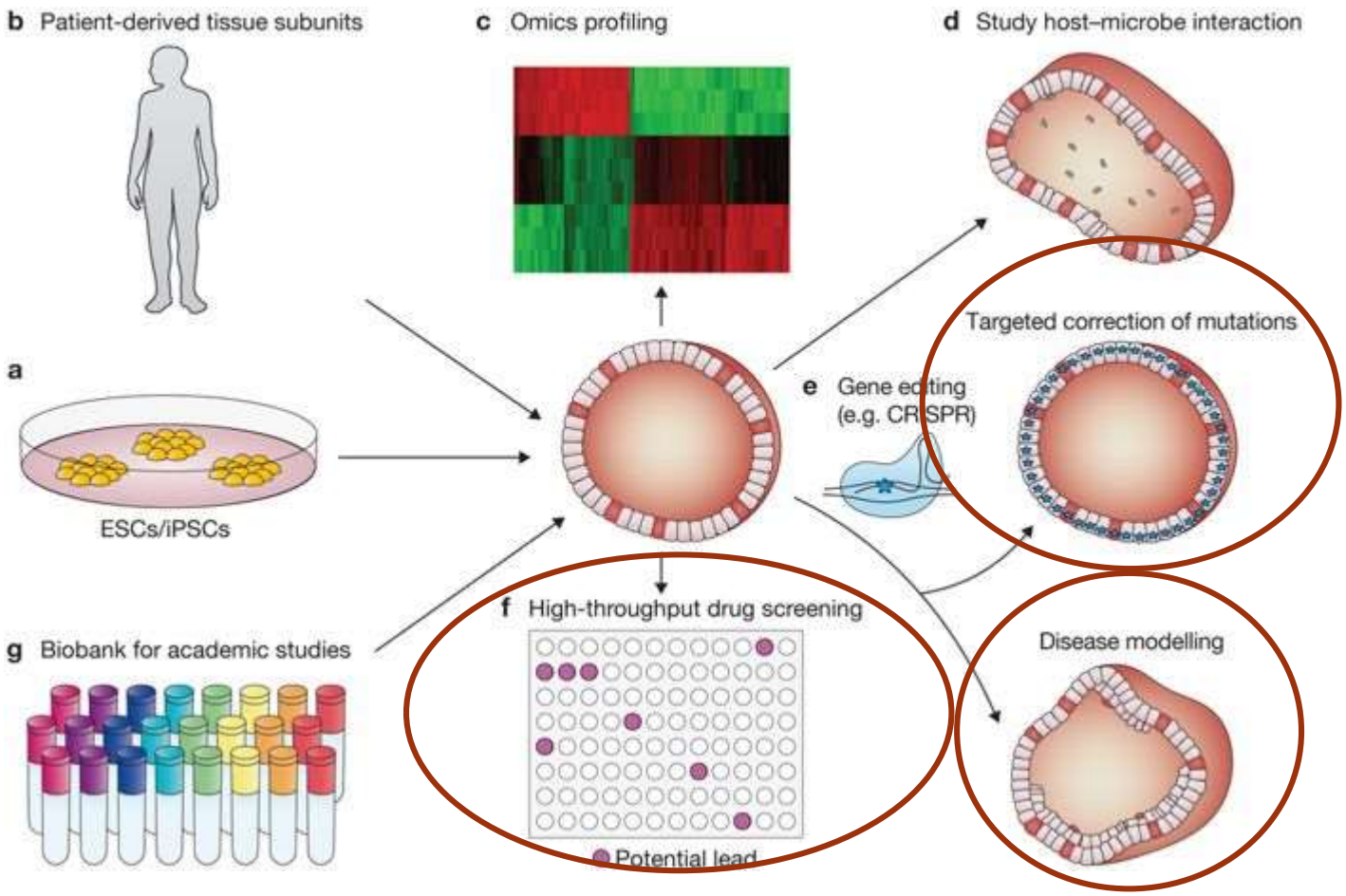
Applications of organoid technology



Applications of organoid technology

https://www.hubrecht.eu/research-groups/clevers-group/

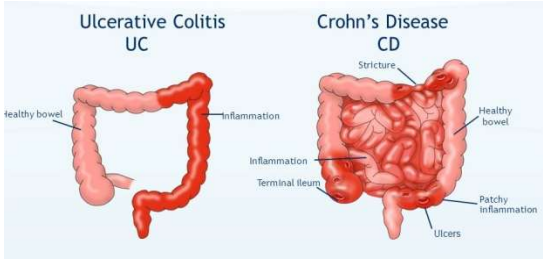
Applications of organoid technology

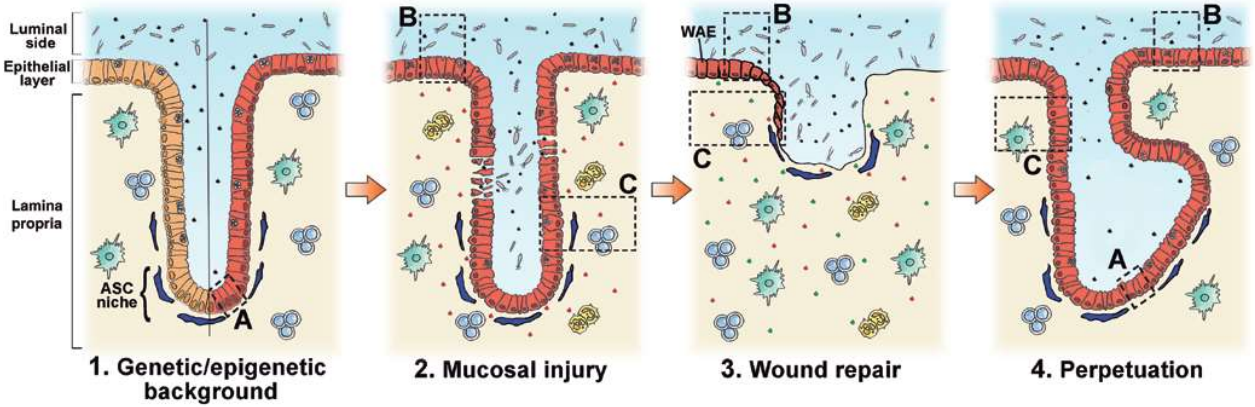


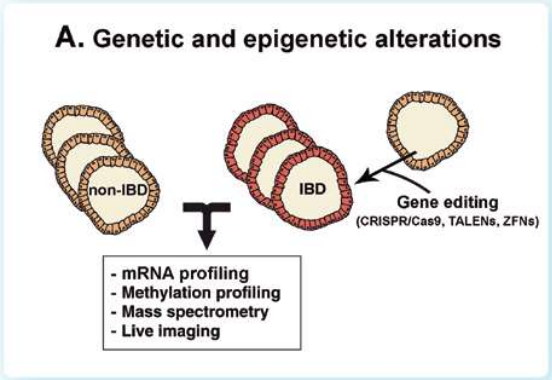
Potential Use of Human Stem Cell–Derived Intestinal Organoids to Study Inflammatory Bowel Diseases

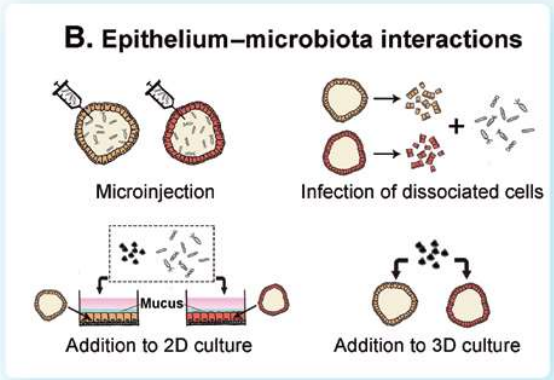
Isabella Dotti, PhD, and Azucena Salas, PhD

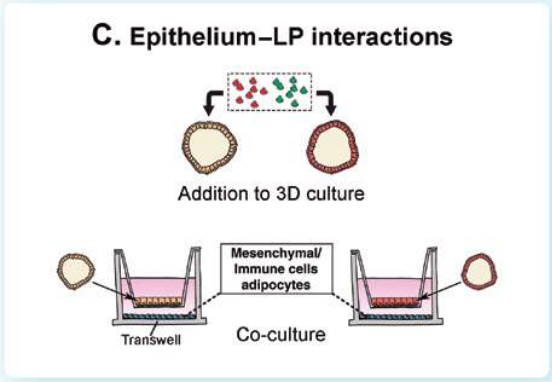
Inflammatory Bowel Diseases

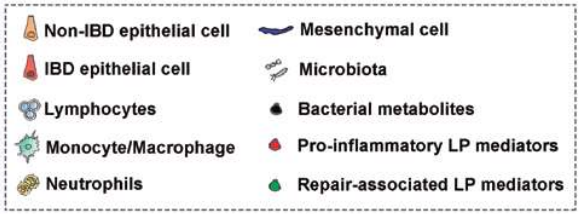












Lamina propria (LP)

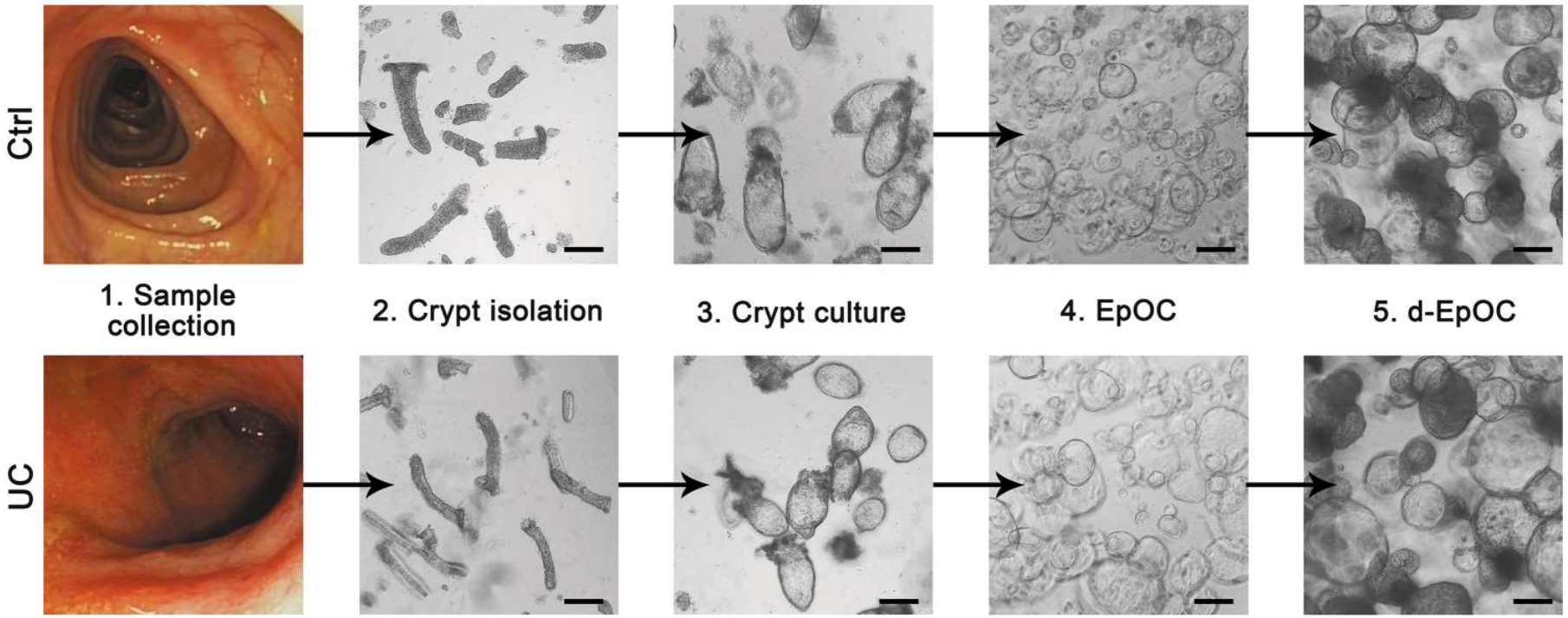
Epithelial organoid cultures from patients with Inflammatory Bowel Diseases

Inflammatory bowel disease

ORIGINAL ARTICLE

Alterations in the epithelial stem cell compartment could contribute to permanent changes in the mucosa of patients with ulcerative colitis

Isabella Dotti,[1] Rut Mora-Buch,[1] Elena Ferrer-Picón,[1] Núria Planell,[1,2] Peter Jung,[3,4] M Carme Masamunt,[1] Raquel Franco Leal,[1,5] Javier Martín de Carpi,[6] Josep Llach,[7] Ingrid Ordás,[1] Eduard Batlle,[3,8] Julián Panés,[1] Azucena Salas[1]



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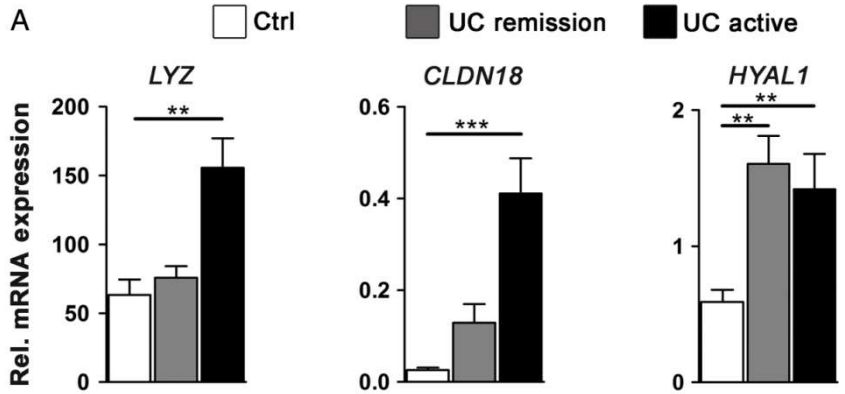
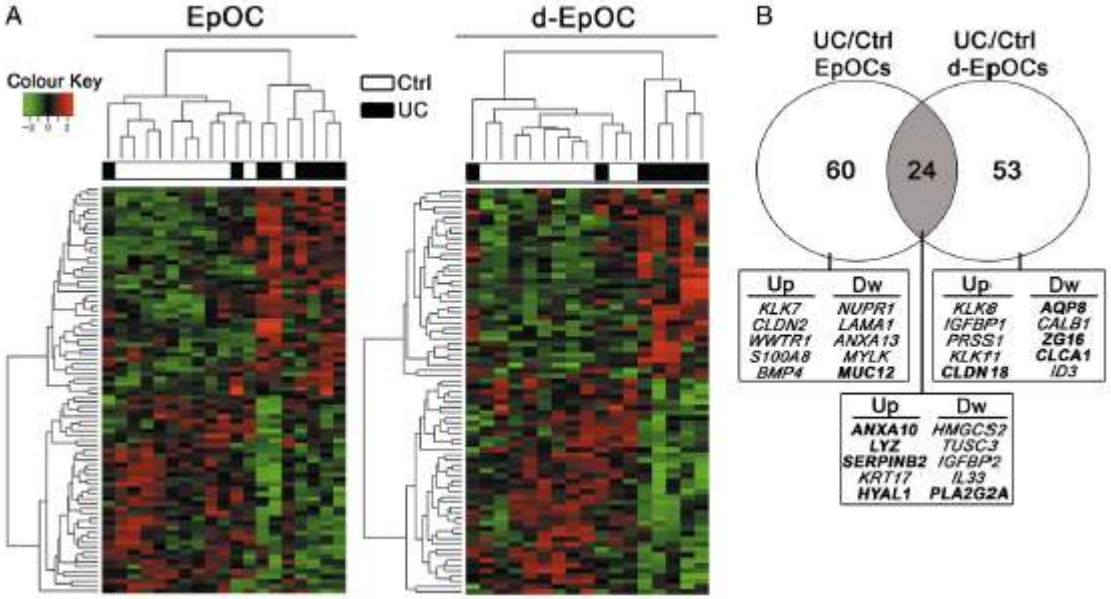




Epithelial Organoid cultures (EpOC)

Differentiated Epithelial Organoid cultures (d-EpOC)

- **A specific expression signature characterizes EpOCs and d-EpOCs from patients with UC compared with non-IBD controls (antimicrobial defense, secretory and absorptive functions);**

-Whole biopsies and organoid cultures from patients with UC show common expression features (>66%);

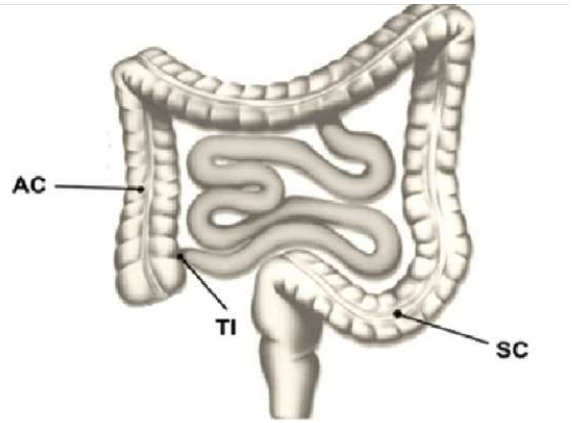
Epithelial organoid cultures from patients with IBDs

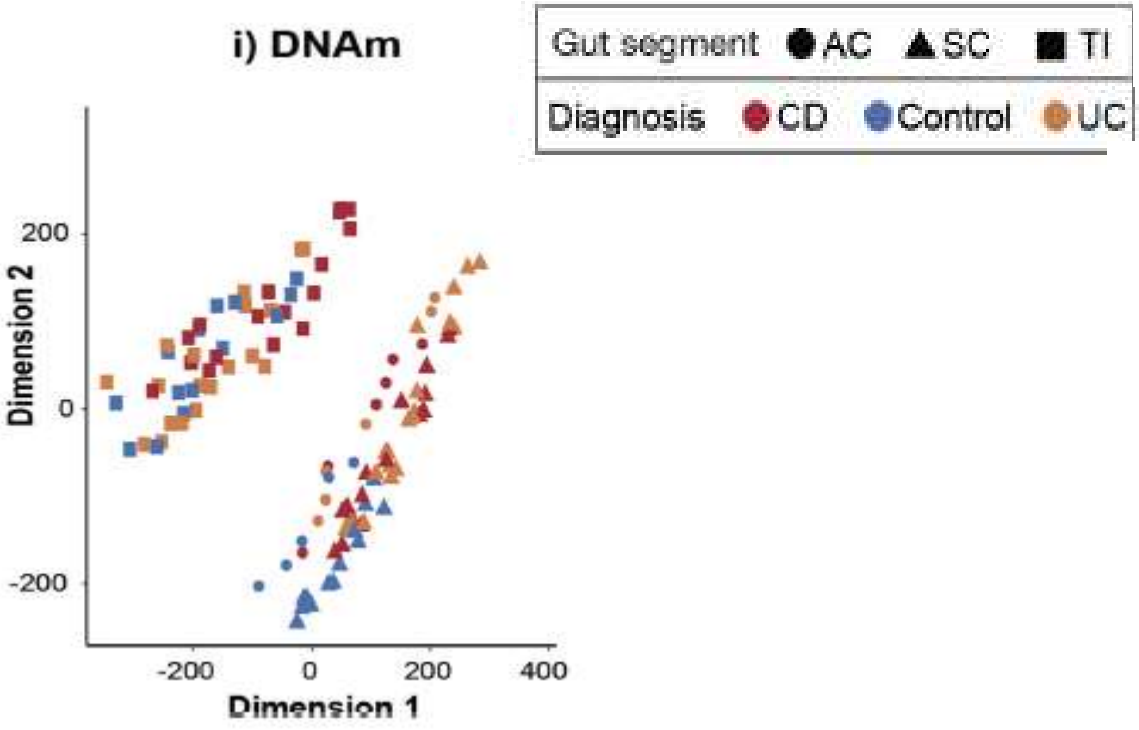
Gastroenterology 2018;154:585–598

BASIC AND TRANSLATIONAL—ALIMENTARY TRACT

DNA Methylation and Transcription Patterns in Intestinal Epithelial Cells From Pediatric Patients With Inflammatory Bowel Diseases Differentiate Disease Subtypes and Associate With Outcome

Kate Joanne Howell,[1,3,*] **Judith Kraiczy**,[1,*] Komal M. Nayak,[1] Marco Gasparetto,[1,2] Alexander Ross,[1,4] Claire Lee,[1,2] Tim N. Mak,[1] Bon-Kyoung Koo,[4] Nitin Kumar,[5] Trevor Lawley,[5] Anupam Sinha,[6] Philip Rosenstiel,[6] Robert Heuschkel,[2] Oliver Stegle,[3,§] and Matthias Zilbauer[1,2,4,§]





TI = Terminal ileum
AC = Ascending colon
SC = Sigmoid colon

Epithelial organoid cultures from patients with IBDs

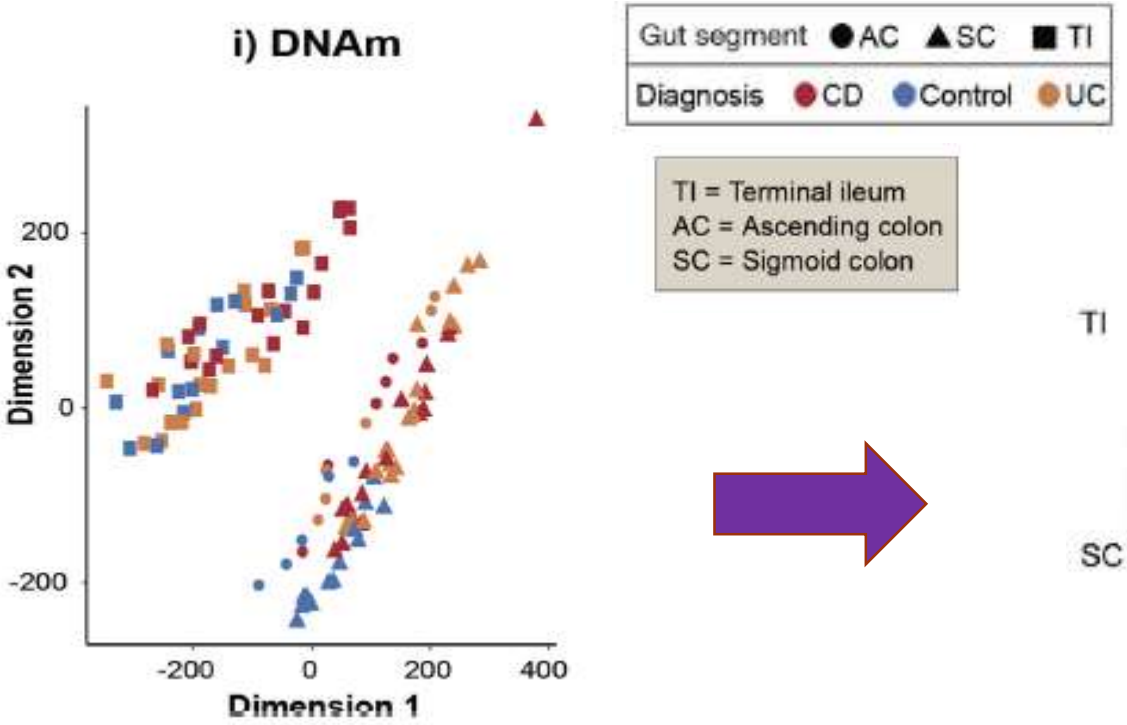
Gastroenterology 2018;154:585–598

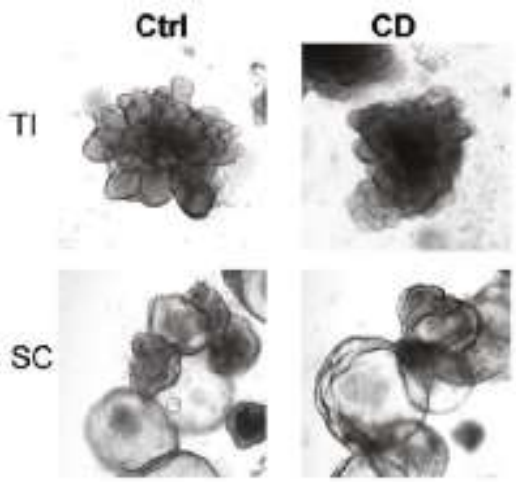
BASIC AND TRANSLATIONAL—ALIMENTARY TRACT

DNA Methylation and Transcription Patterns in Intestinal Epithelial Cells From Pediatric Patients With Inflammatory Bowel Diseases Differentiate Disease Subtypes and Associate With Outcome

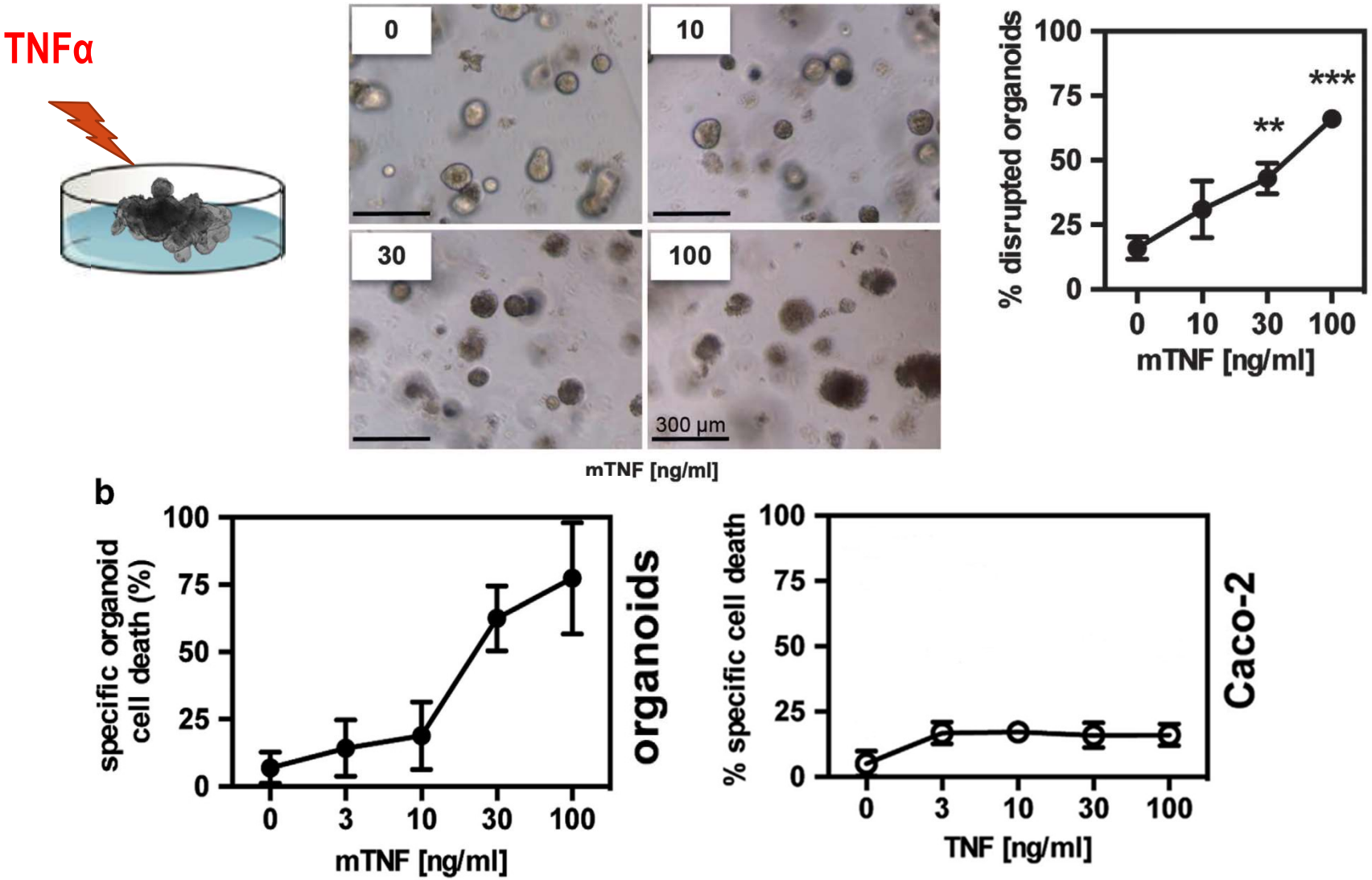
Kate Joanne Howell,[1,3,*] **Judith Kraiczy**,[1,*] Komal M. Nayak,[1] Marco Gasparetto,[1,2] Alexander Ross,[1,4] Claire Lee,[1,2] Tim N. Mak,[1] Bon-Kyoung Koo,[4] Nitin Kumar,[5] Trevor Lawley,[5] Anupam Sinha,[6] Philip Rosenstiel,[6] Robert Heuschkel,[2] Oliver Stegle,[3,§] and Matthias Zilbauer[1,2,4,§]

- Disease associated epigenetic alterations in the intestinal epithelium are stable over time and are at least in part retained in ex-vivo organoid cultures;



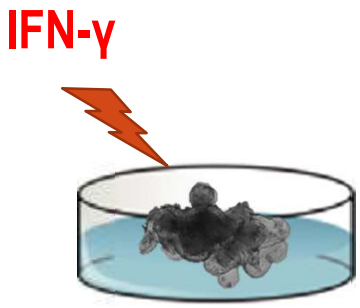


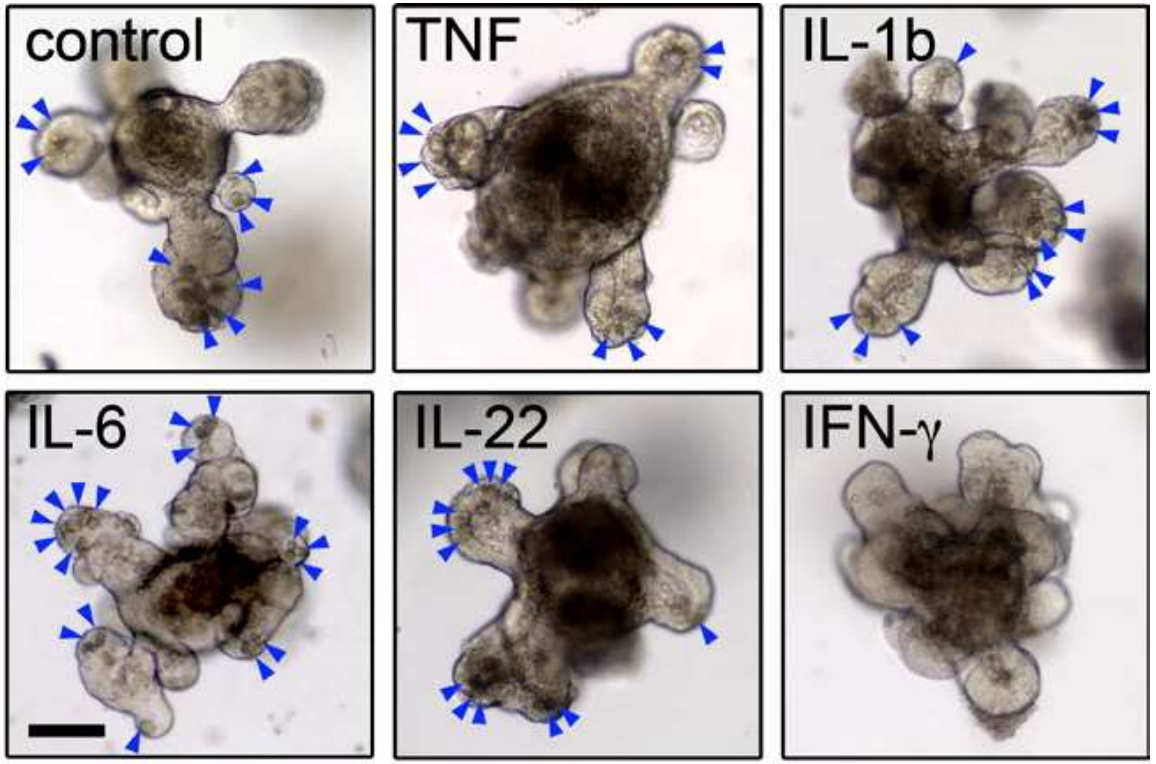
Organoids to study the interactions of the epithelium with the environment in mucosal injury

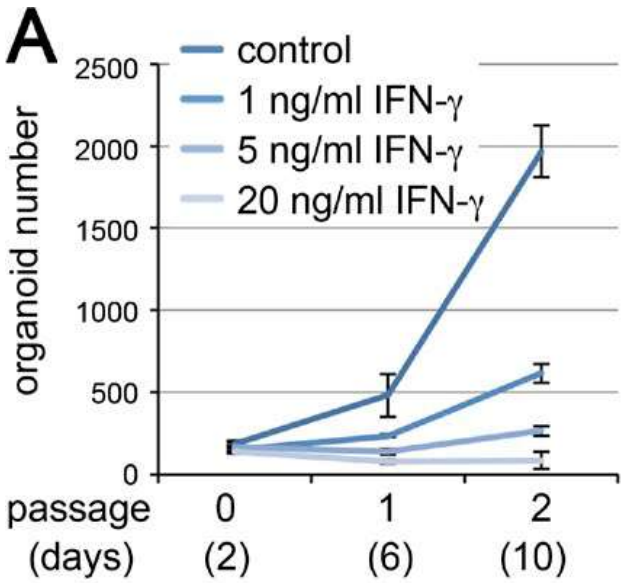


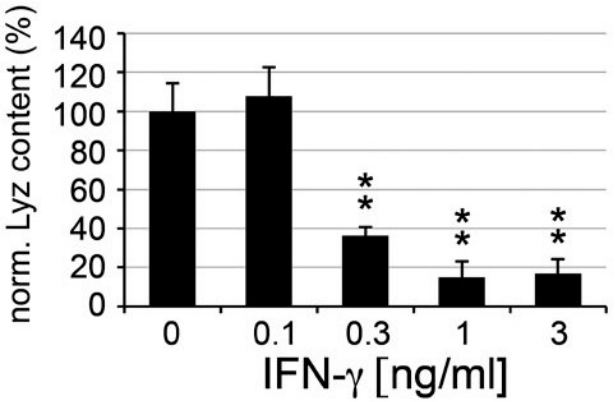
Grabinger T et al., Cell Death and Disease 2014

Organoids to study the interactions of the epithelium with the environment in mucosal injury and wound repair









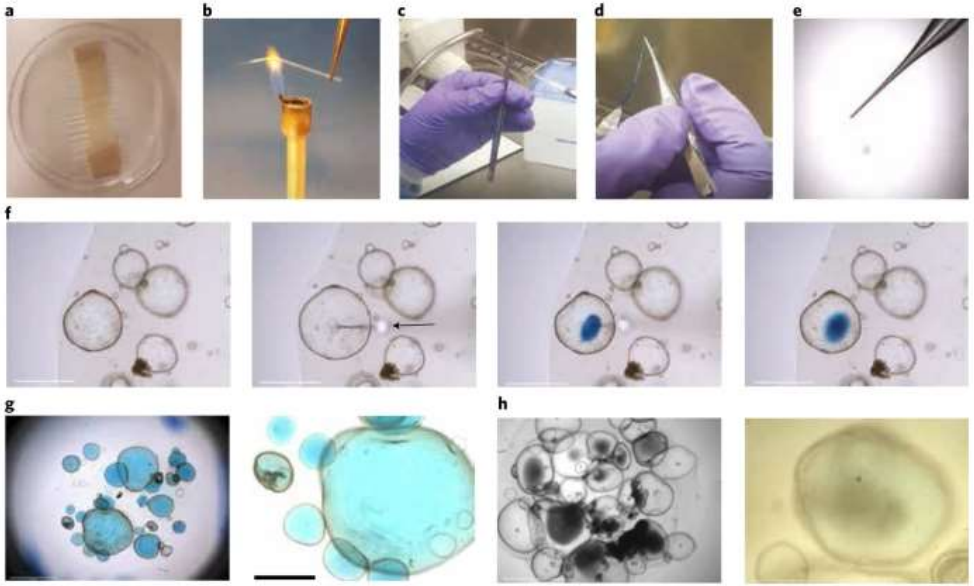
Farin HF et al., J. Exp. Med. 2014

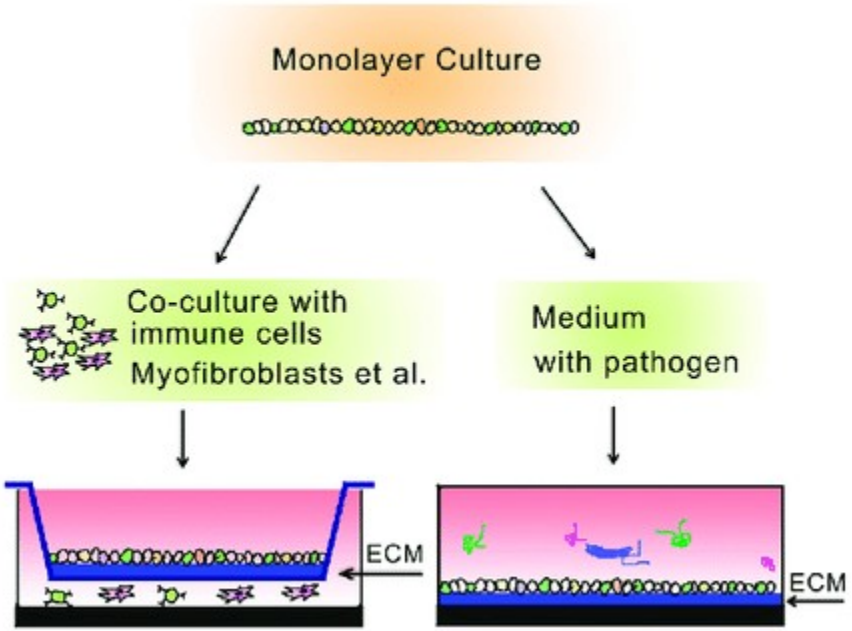
Intestinal organoid cocultures with microbes

Intestinal organoid cocultures with microbes

Jens Puschhof, Cayetano Pleguezuelos-Manzano, Adriana Martinez-Silgado, Ninouk Akkerman, Aurelia Saftien, Charelle Boot, Amy de Waal, Joep Beumer, Devanjali Dutta, Inha Heo & Hans Clevers

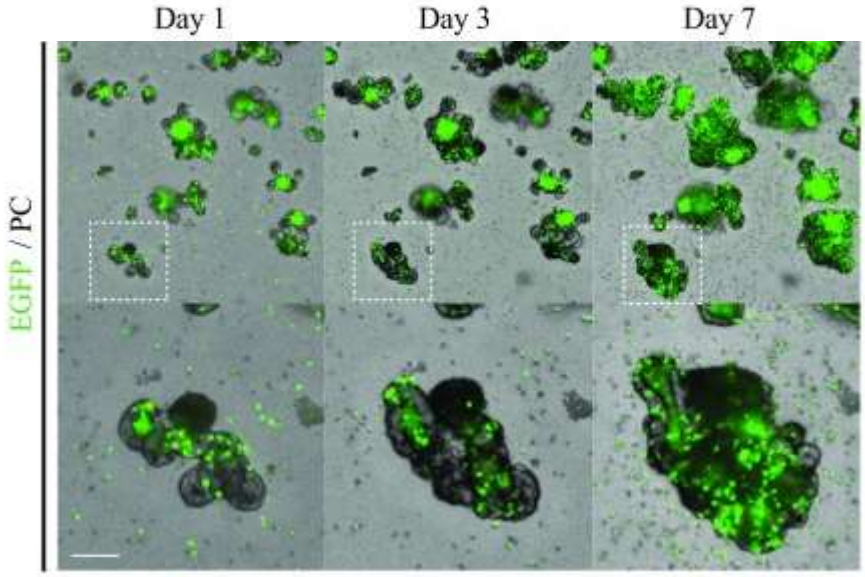
Nature Protocols (2021) | Cite this article



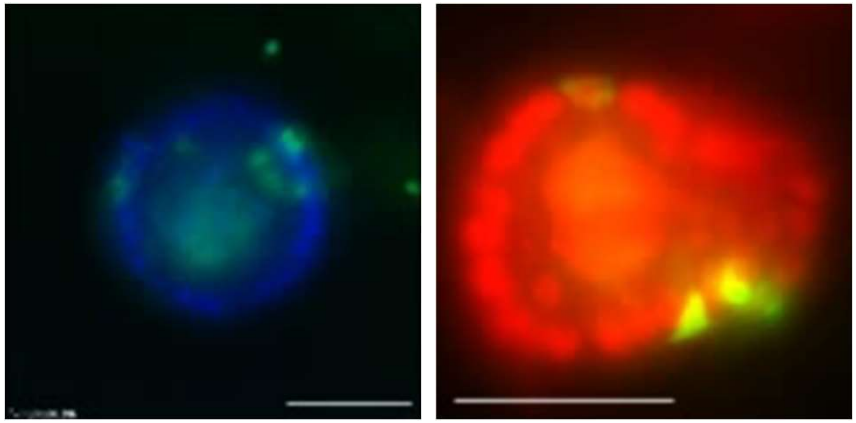


Co-culture with intestinal epithelial organoids

Lymphocytes can be expanded with epithelial organoids and efficiently maintained within and outside a 3-D system for a period of 2 weeks



IL-2/-7/-15 enhances expansion of αβT and γδT of intraepithelial lymphocytes



Motility analysis of intraepithelial lymphocytes in the co-culture system

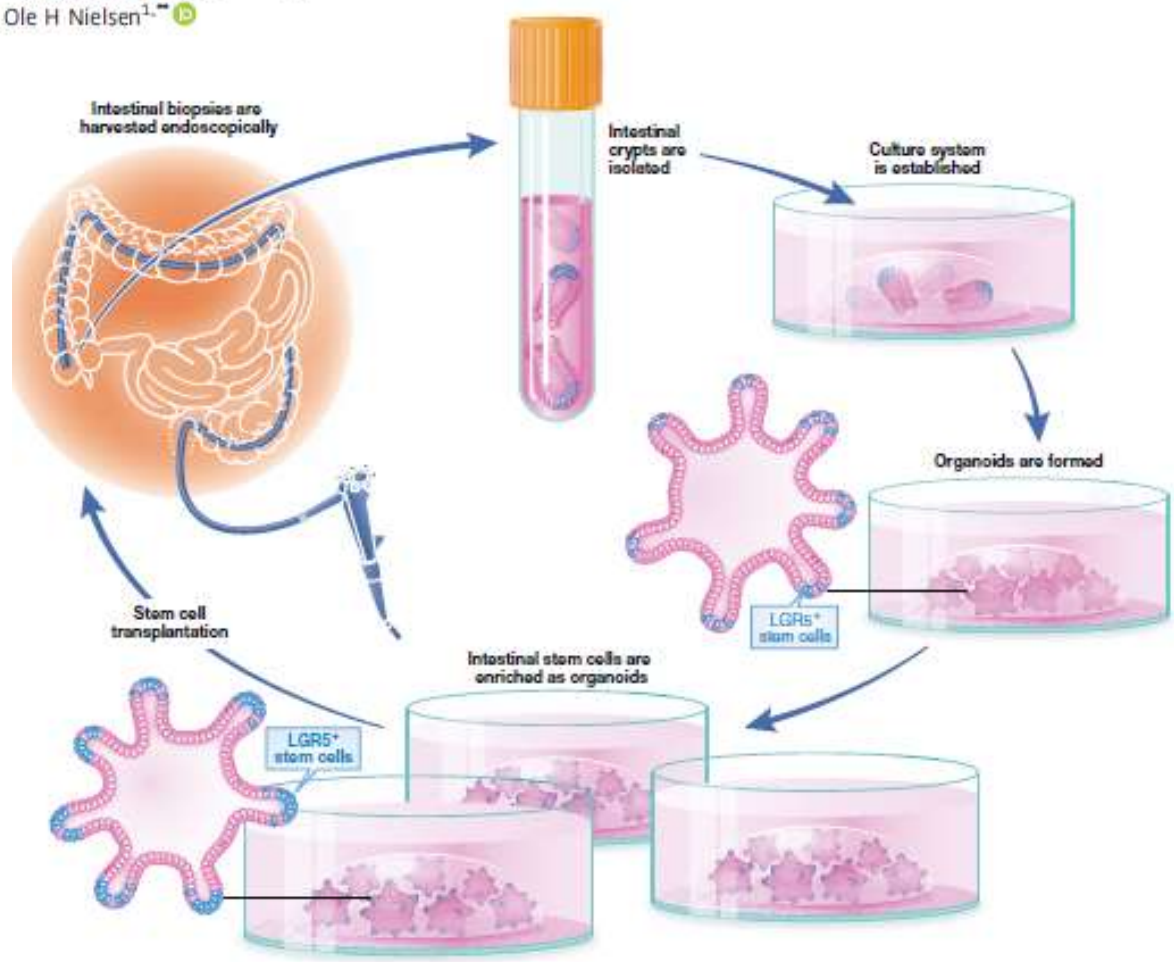
Nozaki K et al., J Gastro 2016

Review

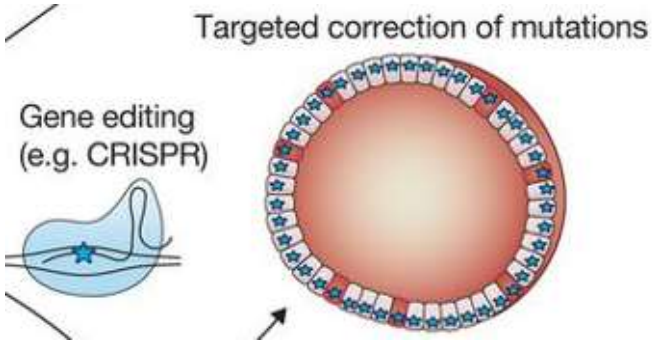
EMBO Molecular Medicine

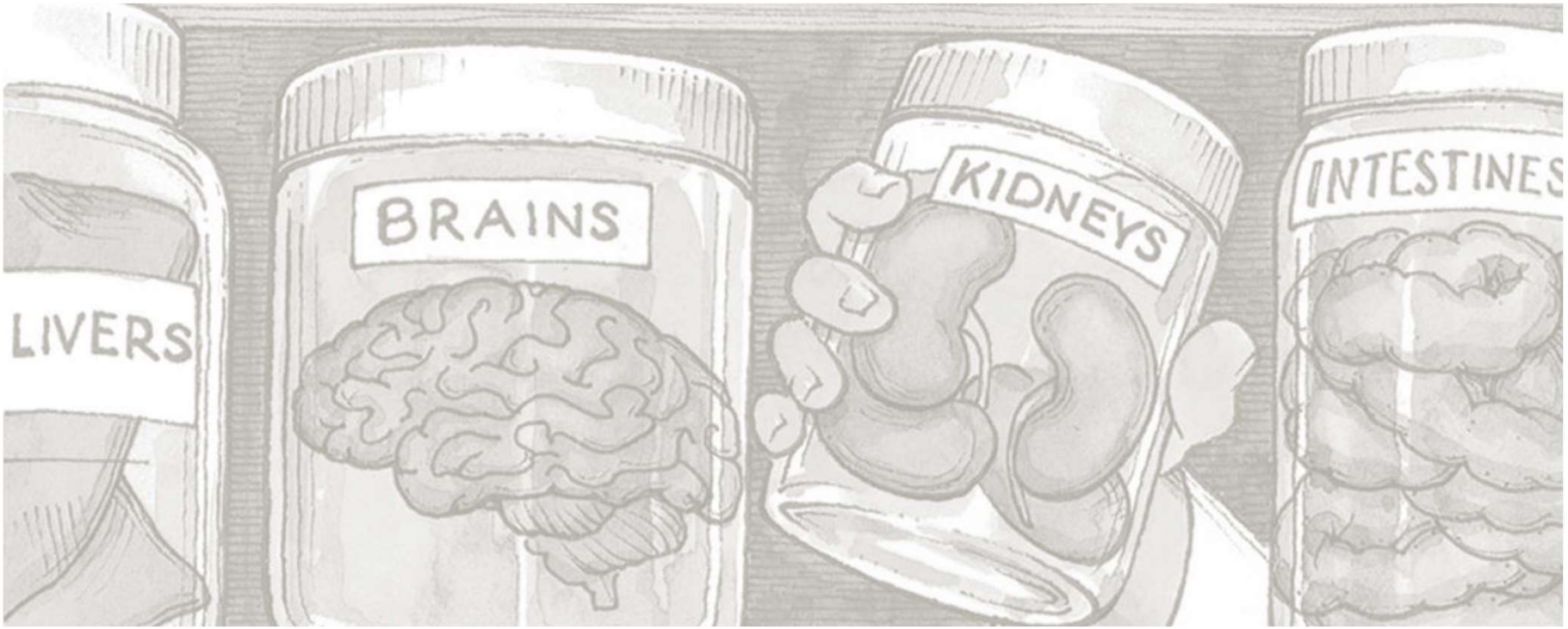
Culturing human intestinal stem cells for regenerative applications in the treatment of inflammatory bowel disease

Fredrik EO Holmberg[1], Jakob B Seidelin[1], Xiaolei Yin[2,3,4,5,6], Benjamin E Mead[2,3,4,5,6,7], Zhixiang Tong[2,3,4,5], Yuan Li[1], Jeffrey M Karp[2,3,4,5,6,7,*] & Ole H Nielsen[1,**]



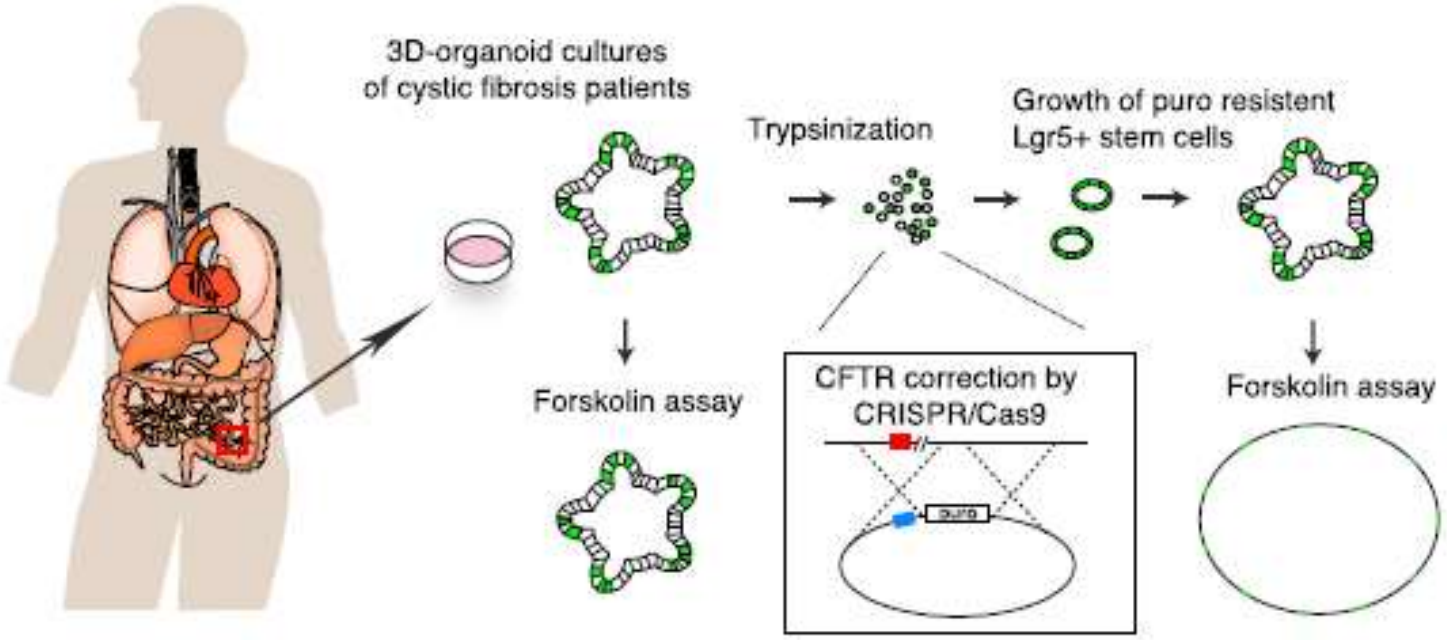
CRISPR/Cas9-Mediated Genome Editing in Adult Stem Cells





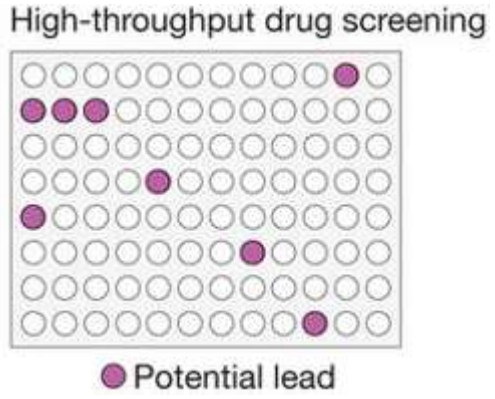
Functional Repair of CFTR by CRISPR/Cas9 in Intestinal Stem Cell Organoids of Cystic Fibrosis Patients

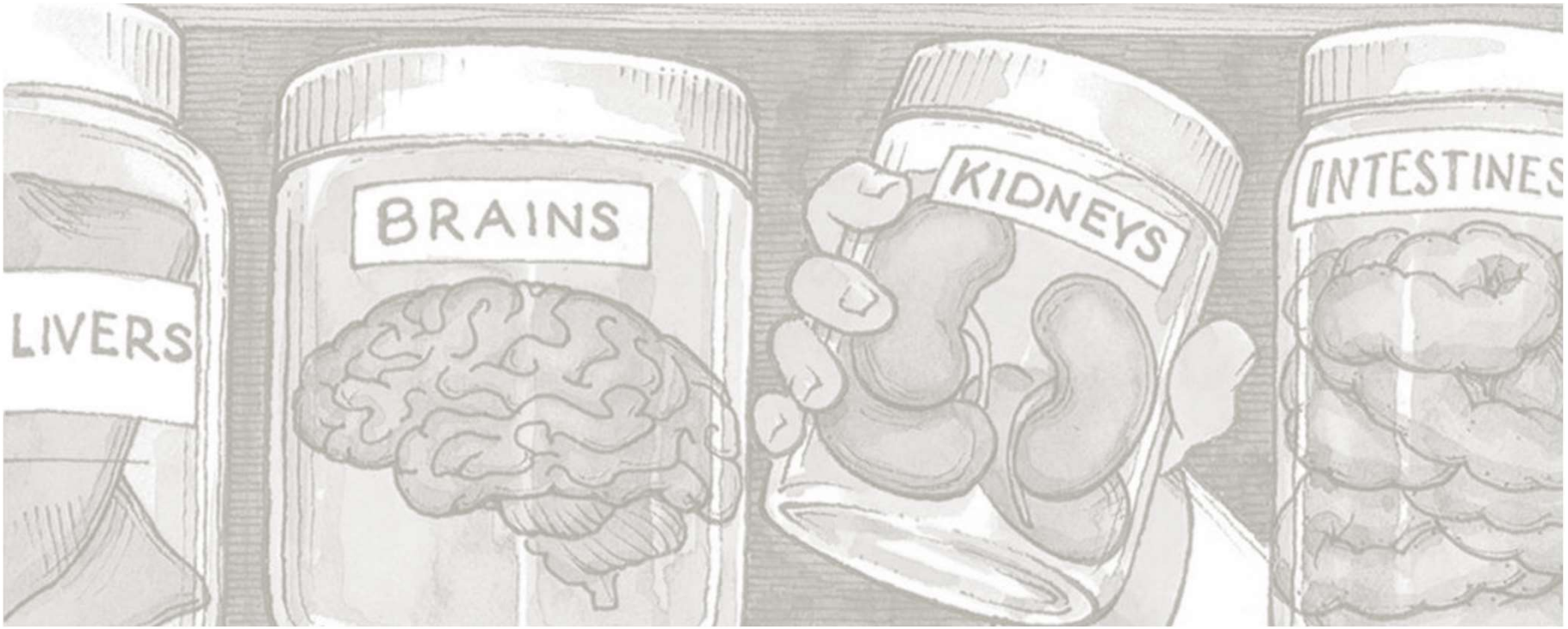
Gerald Schwank,[1,2,7] Bon-Kyoung Koo,[1,2,7,8] Valentina Sasselli,[1,2] Johanna F. Dekkers,[3,4] Inha Heo,[1,2] Turan Demircan,[1] Nobuo Sasaki,[1,2] Sander Boymans,[1] Edwin Cuppen,[1,6] Cornelis K. van der Ent,[3] Edward E.S. Nieuwenhuis,[5] Jeffrey M. Beekman,[5,6] and Hans Clevers[1,2,*]



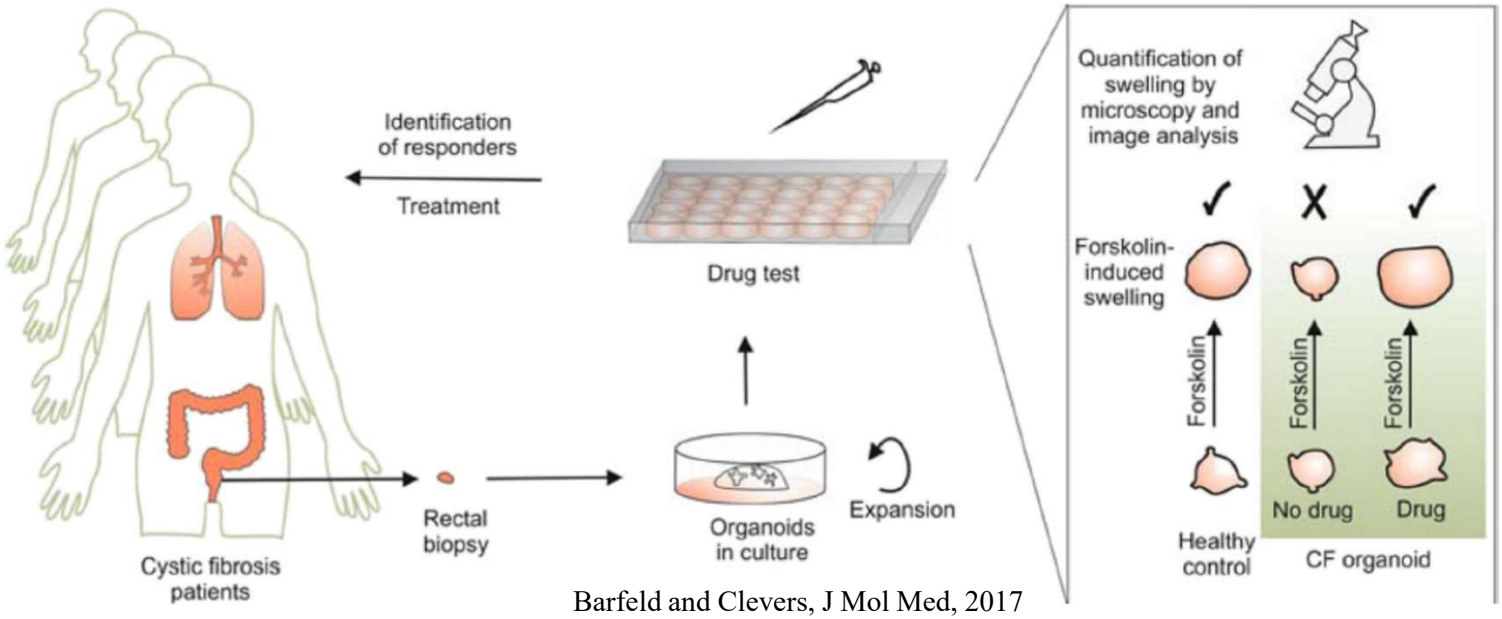
Forskolin induces swelling of organoids from healthy subjects, but the swelling is absent in organoids from patients with CF.

Drug response in patient-derived organoids



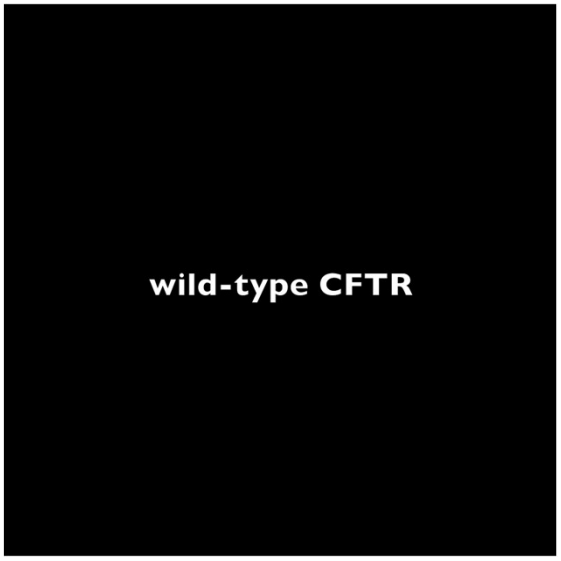


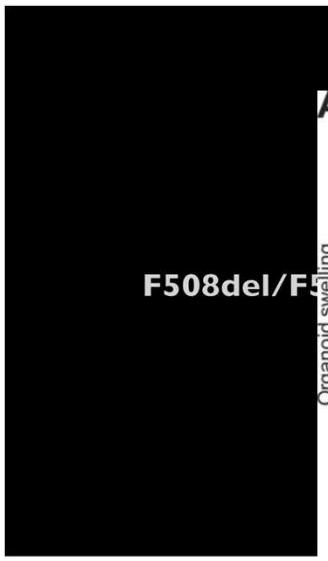
Patient-derived organoids in personalized medicine



Barfeld and Clevers, J Mol Med, 2017

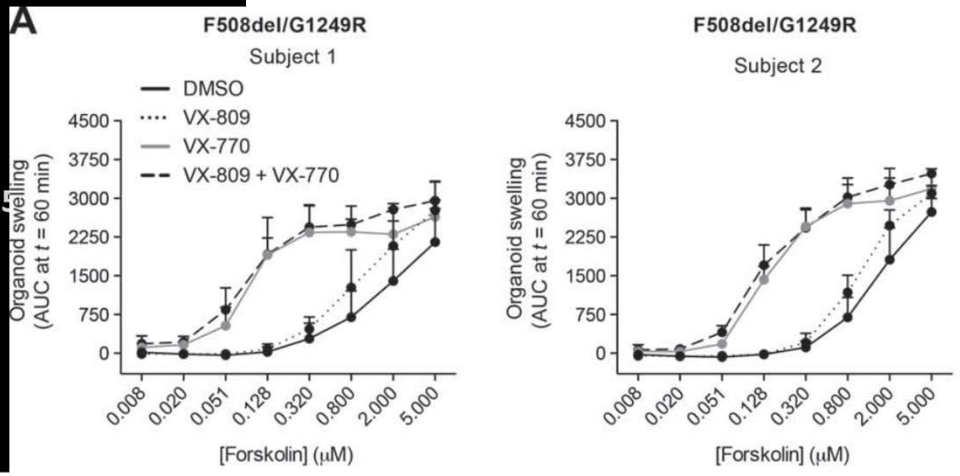
CFTR ASSAY





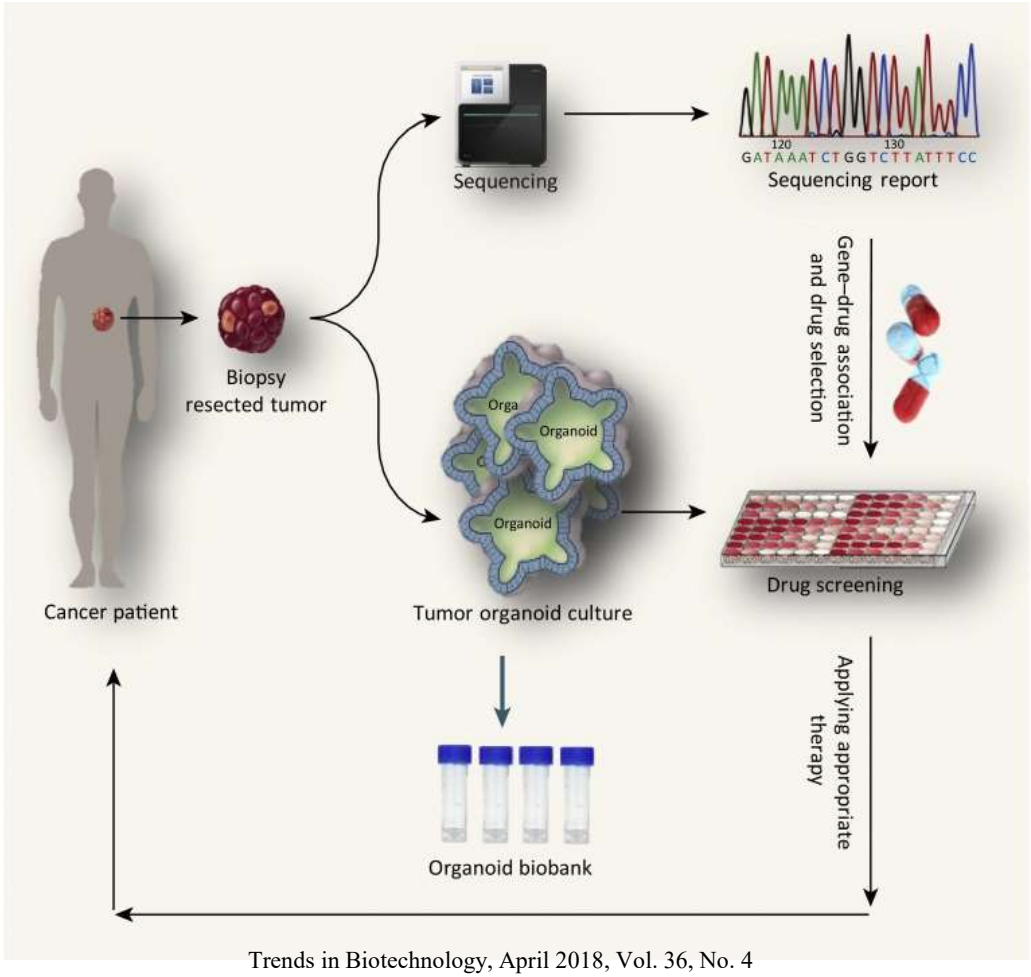
Dekkers et al., Nature Med, 2013

Characterizing responses to CFTR-modulating drugs using rectal organoids derived from subjects with cystic fibrosis



Dekkers et al., Sci Trans Med, 2016

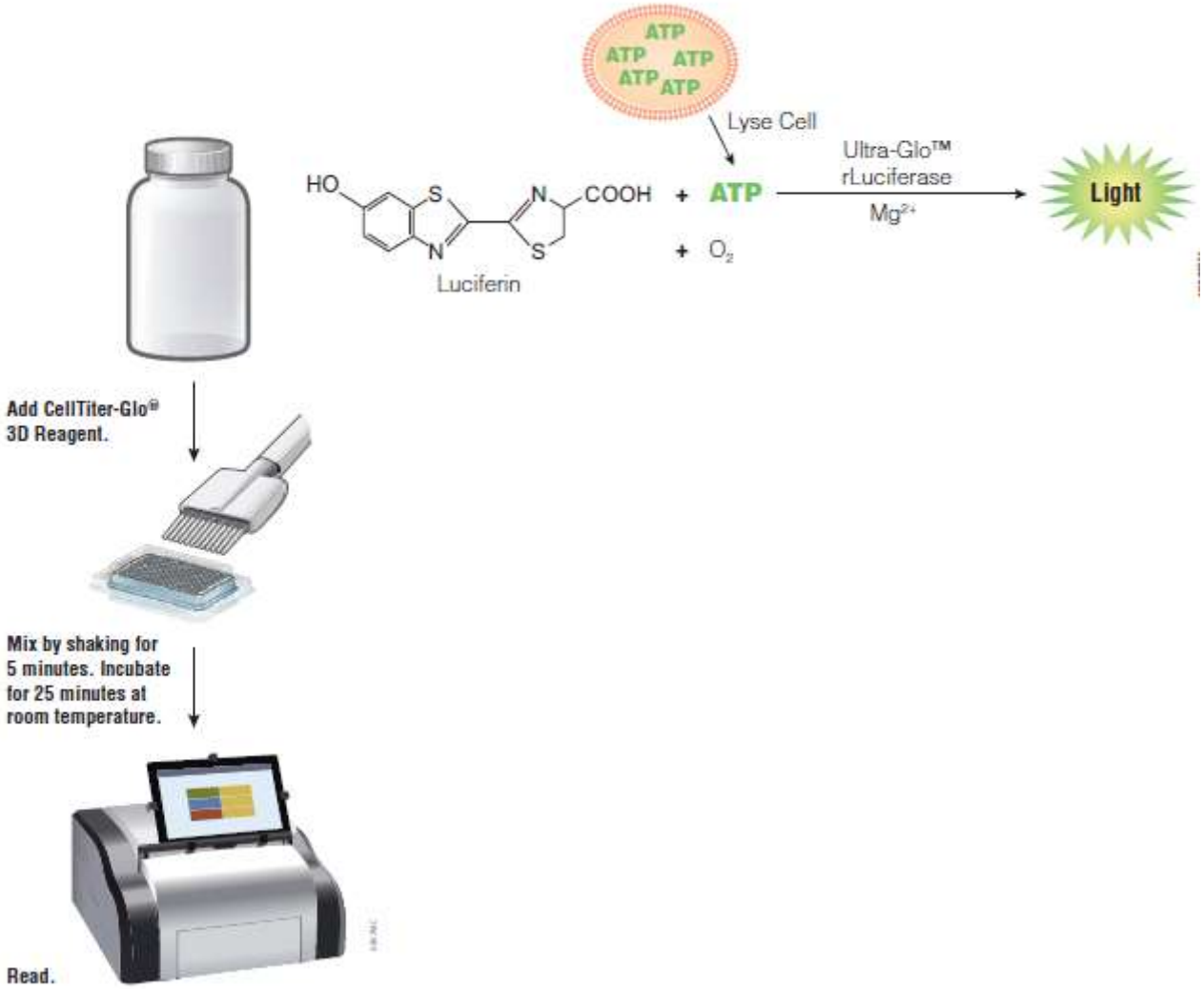
Drug response in patient-derived organoids



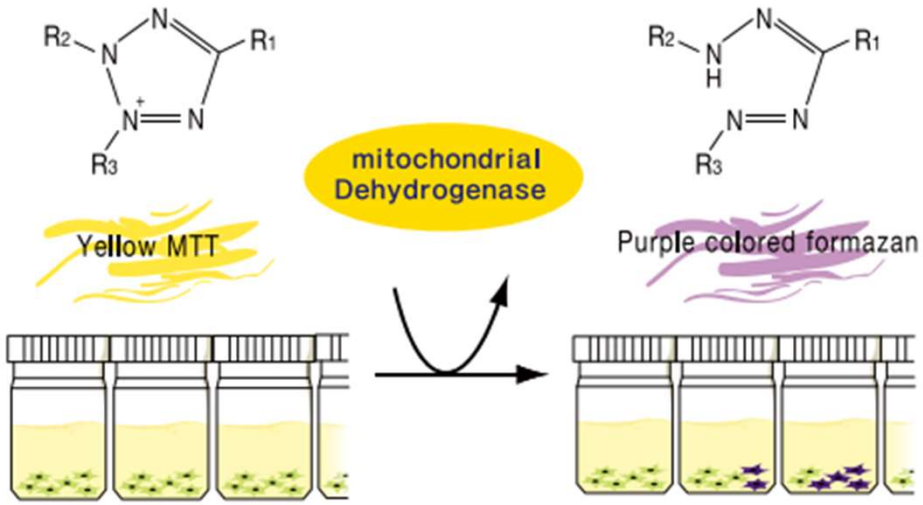
Trends in Biotechnology, April 2018, Vol. 36, No. 4

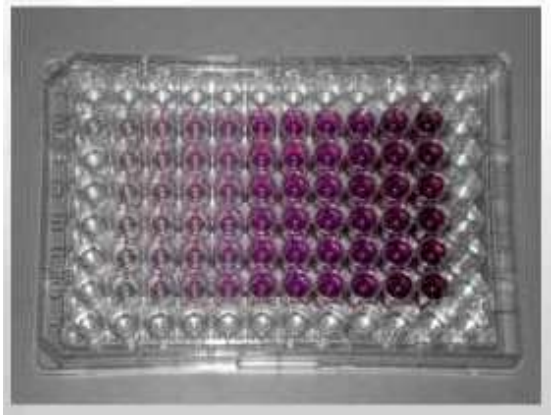
In this approach, the procedure begins with sequencing tumor biopsies or dissected samples by using the next-generation sequencing method and continues with culturing patient-derived tumor organoids, which will be histologically and pathologically compared with the primary tumors before they are subjected to drug screening. In parallel, part of the derived organoids will be preserved as a biobank. To determine effective therapeutic strategies, based on the sequencing results and gene–drug association links, high-throughput drug screening of candidate drugs that include standard chemotherapy and targeted therapy agents can be performed in a replicative process.

Cell Viability Assay: CellTiter-Glo® 3D

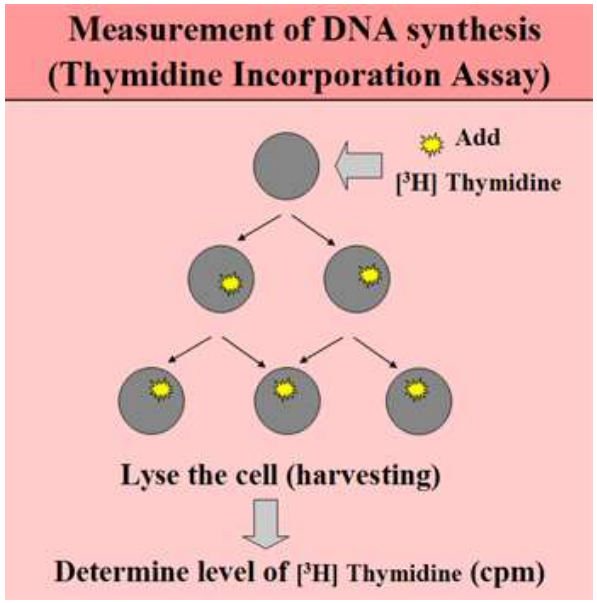


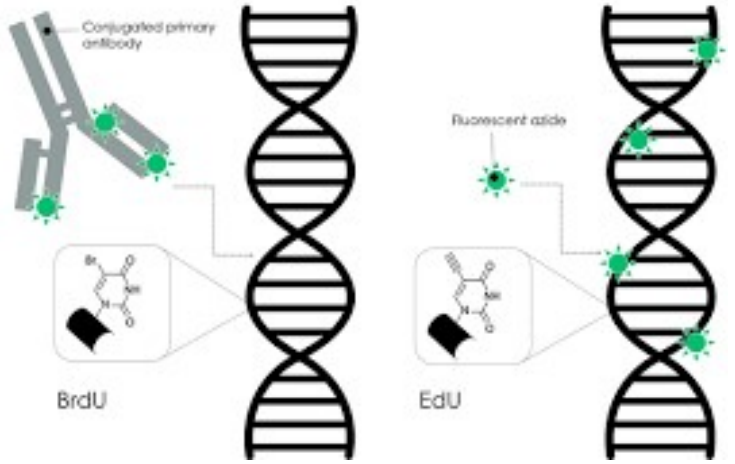
Cell Viability Assay: MTT Reagent





Cell Viability Assay: Thymidine incorporation, BrdU and EdU



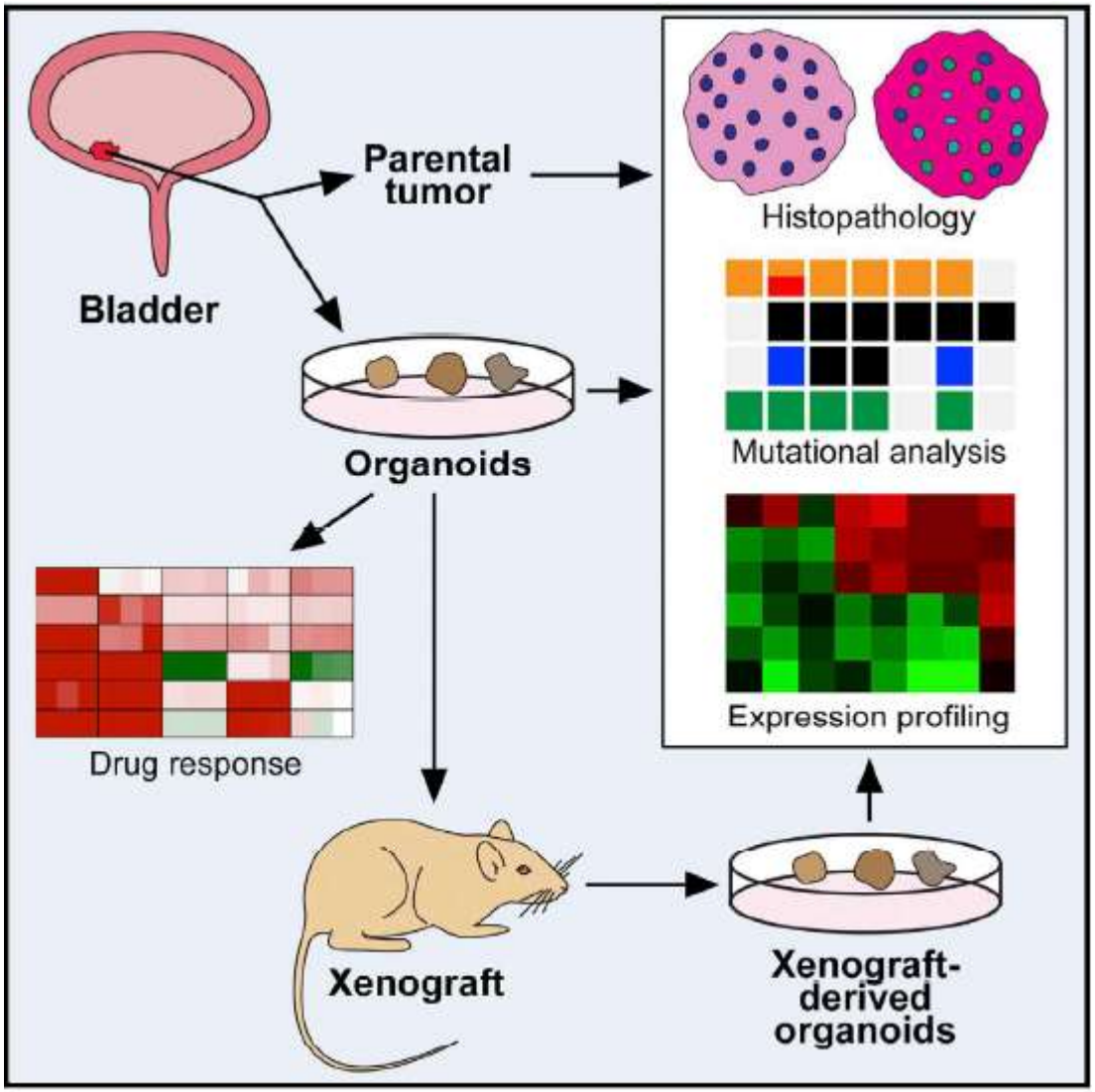


Tumor Evolution and Drug Response in Patient-Derived Organoid Models of Bladder Cancer

Lee et al., 2018, Cell *173*, 515–528

CellPress

22 patient-derived bladder cancer organoid lines:
-histopathological and molecular concordance with their corresponding parental tumors;
-display changes in their mutational profiles during culture and xenografting consistent with clonal evolution.

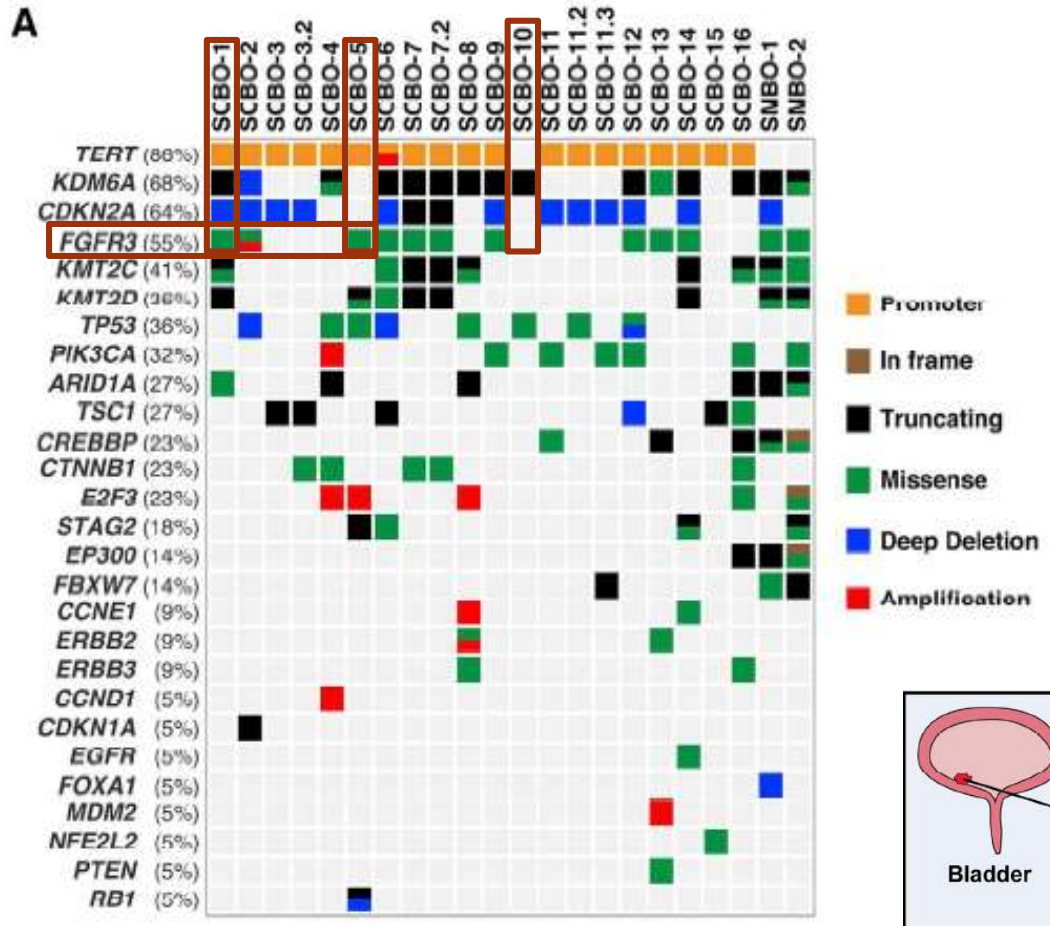


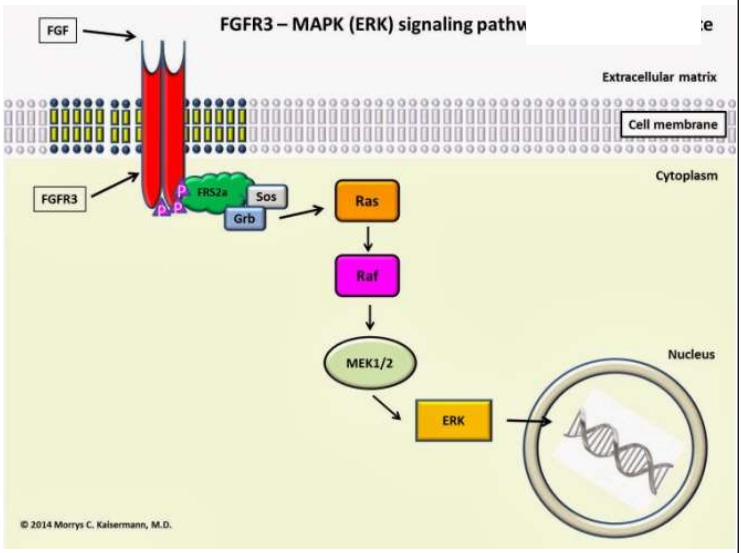
Tumor Evolution and Drug Response in Patient-Derived Organoid Models of Bladder Cancer

Lee et al., 2018, Cell *173*, 515–528

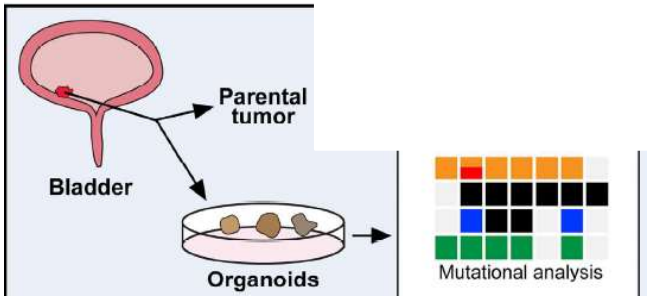
CellPress

PRESENCE OF ACTIVATING MUTATIONS IN **FGFR3**





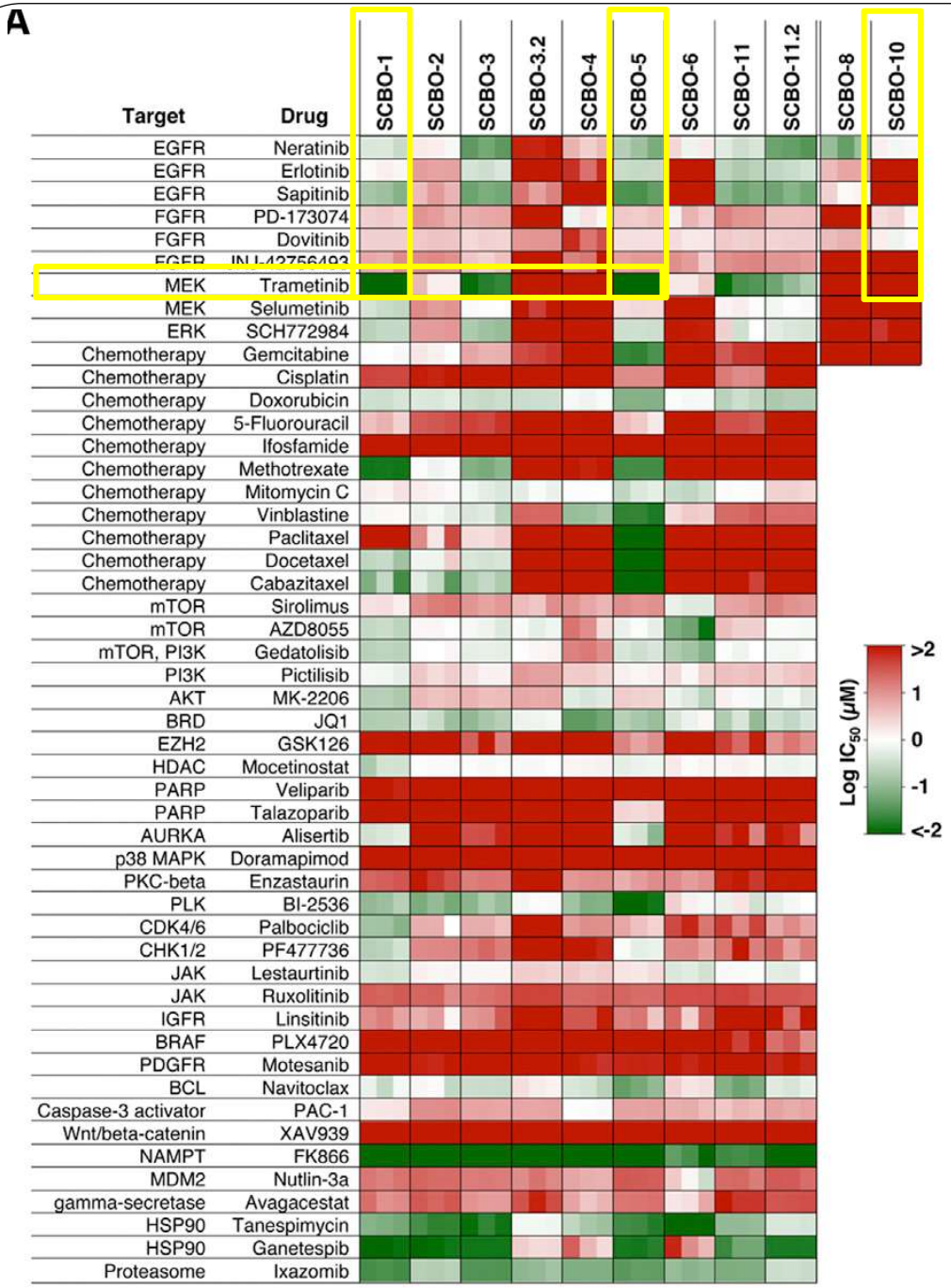
Proliferation

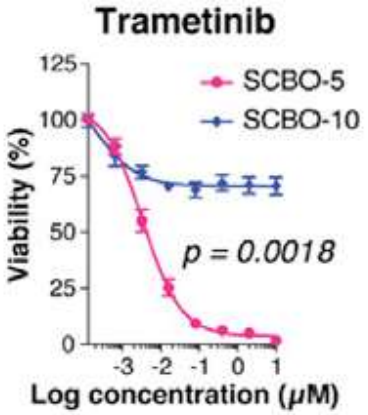


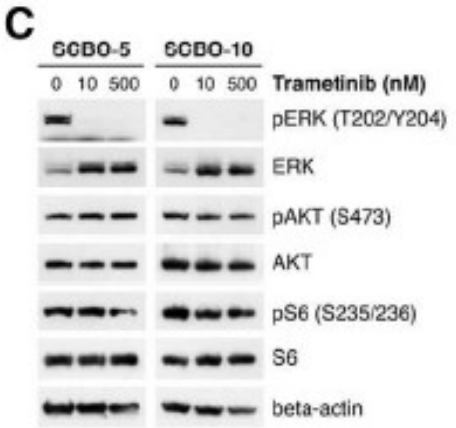
Tumor Evolution and Drug Response in Patient-Derived Organoid Models of Bladder Cancer

Lee et al., 2018, Cell *173*, 515–528

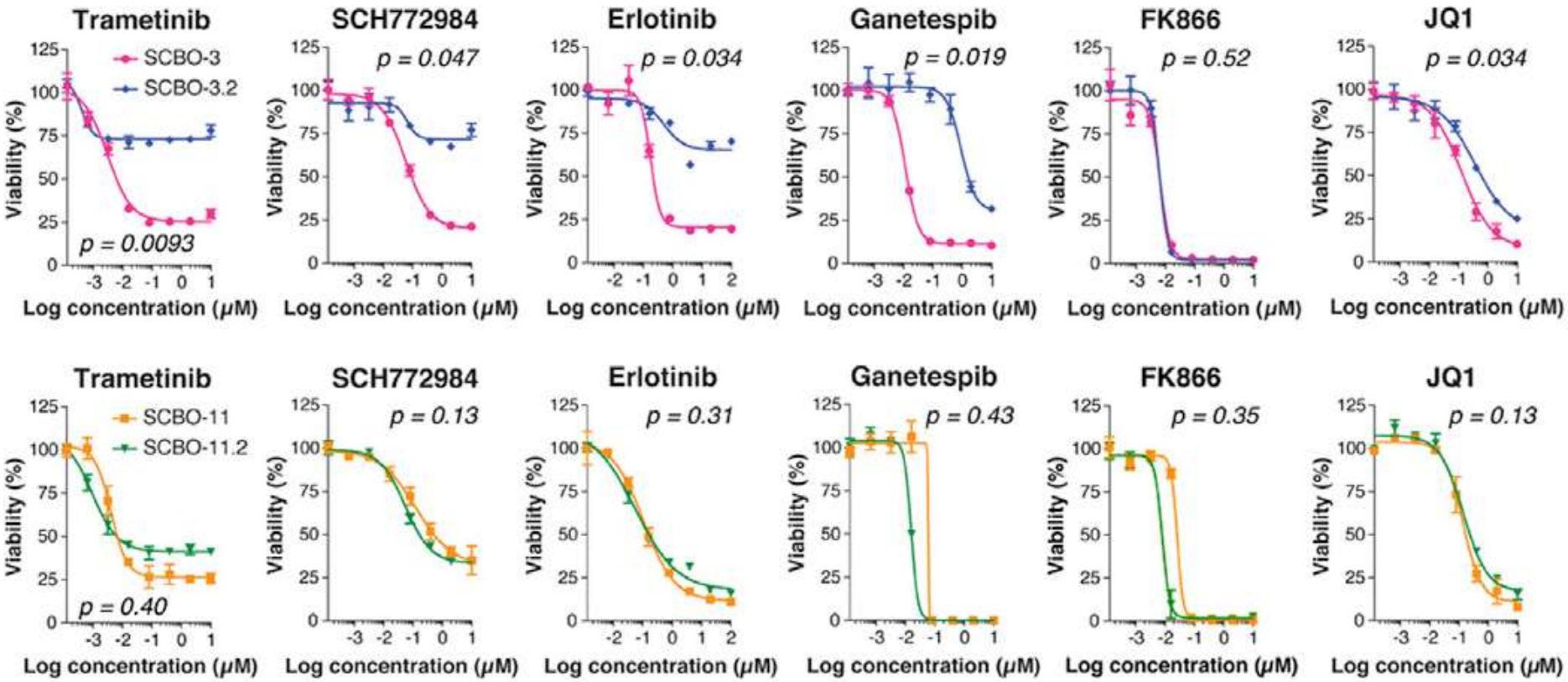
Effects of 40 compounds (standard therapies and agents being tested in clinical trials)







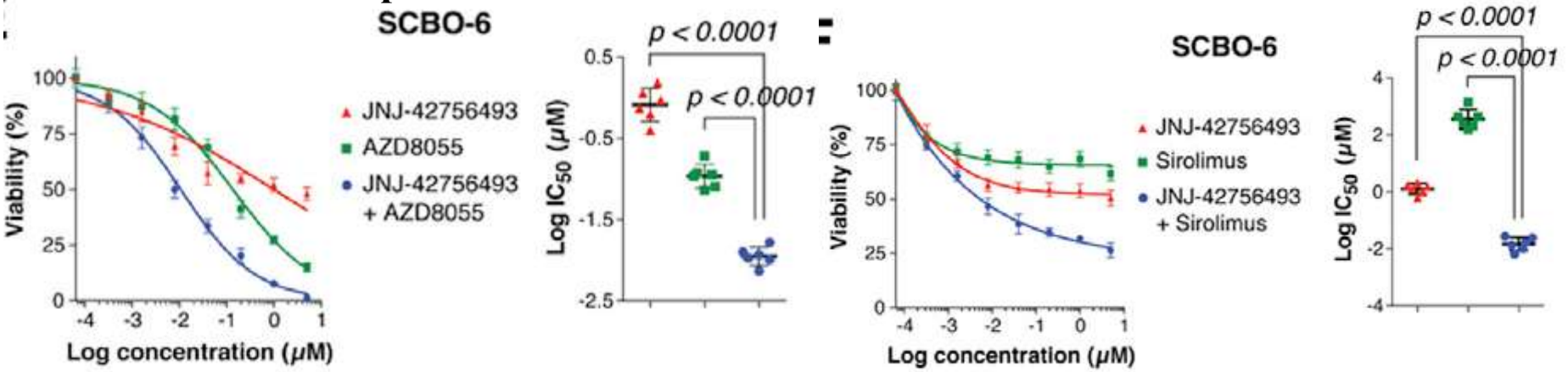
DRUG RESPONSE IN PATIENTS WITH RECURRENT BLADDER CANCER



These results suggest that drug responses in the SCBO-3.2 organoid line are likely to reflect changes in drug response of its parental tumor as a consequence of treatment.

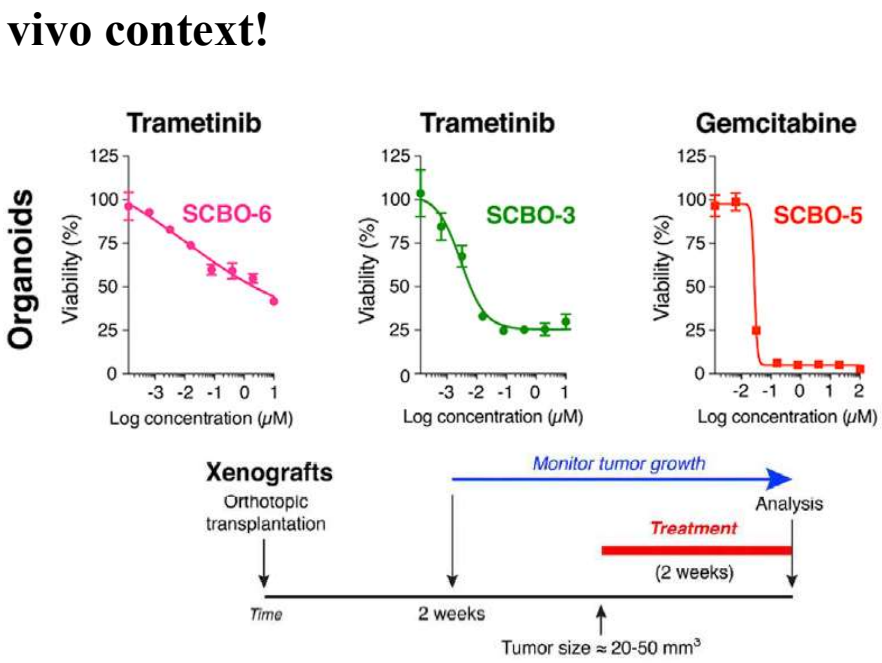
Lee et al., 2018, Cell *173*, 515–528

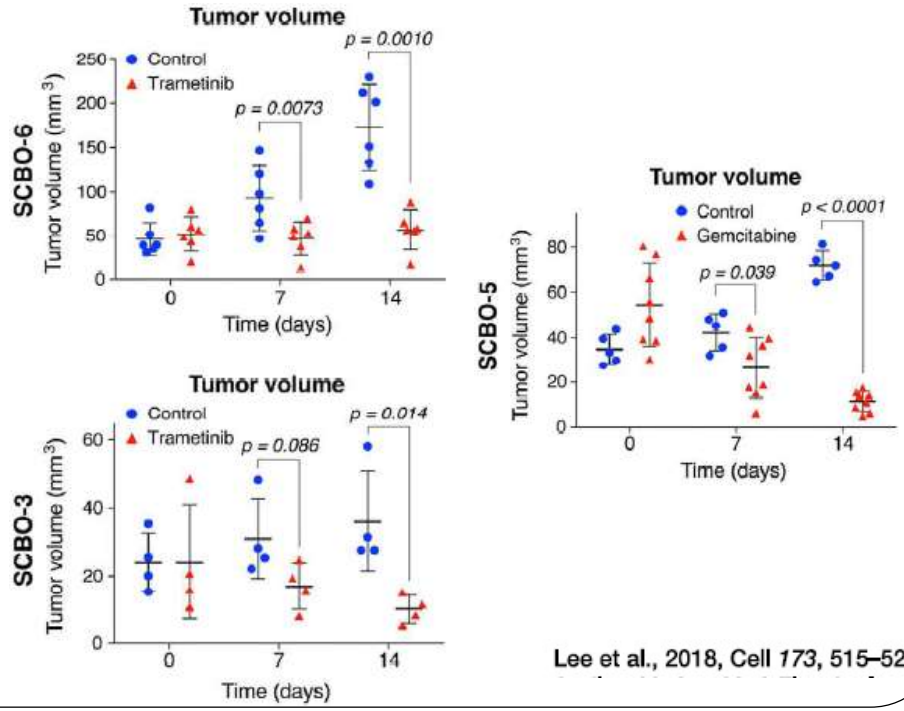
The molecular profiles of the organoid lines can be useful for identification of potential combinatorial therapies!



SCBO-6 displayed an additive response to treatment with the FGFR inhibitor JNJ-42756493 and the mTOR inhibitor AZD8055, consistent with the presence of both an activating FGFR3 mutation and a nonsense mutation in TSC1.

Drug response observed in organoid culture can be recapitulated when assayed in an in vivo context!

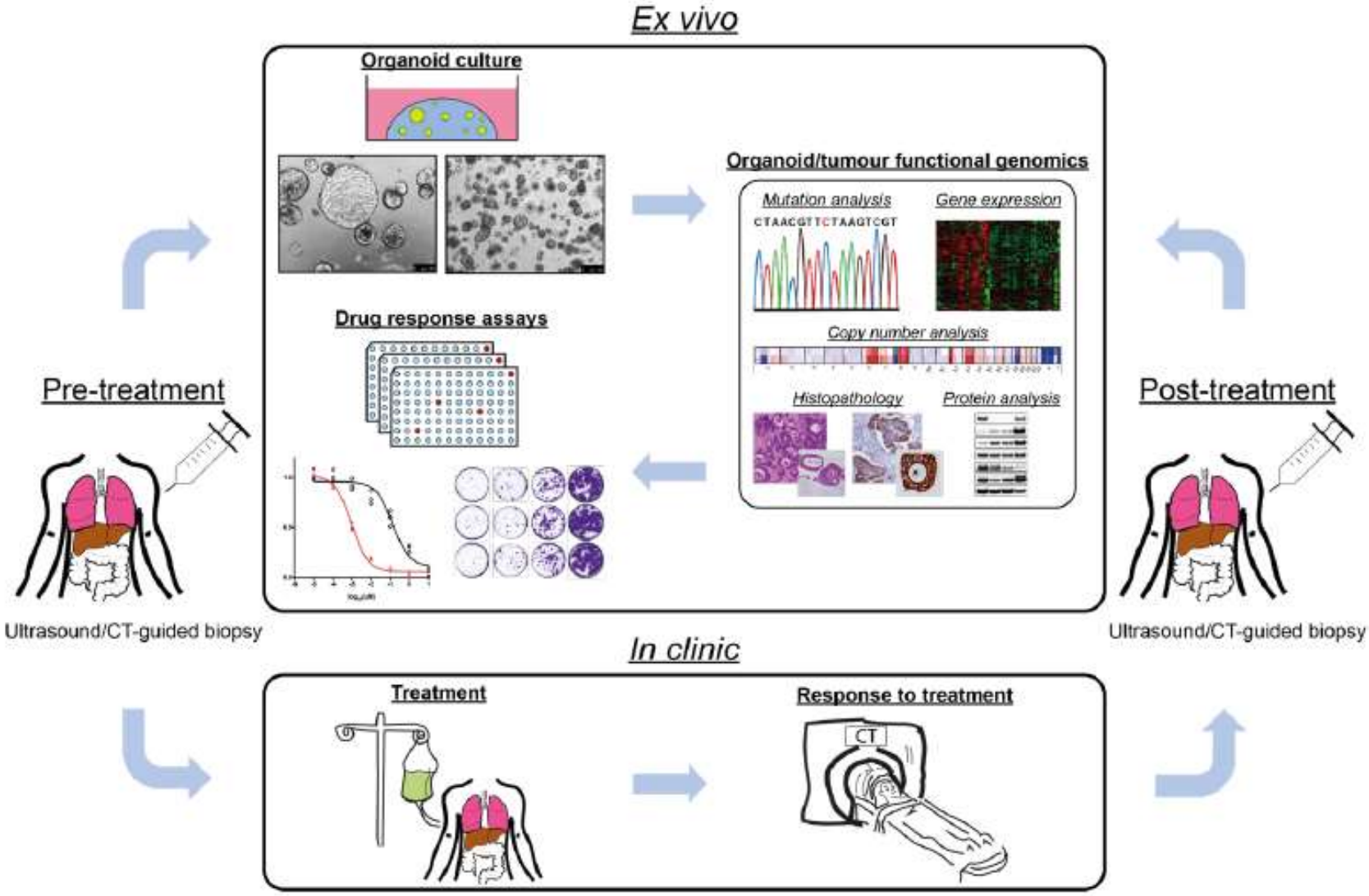




Lee et al., 2018, Cell *173*, 515–528

Patient-derived organoids model treatment response of metastatic gastrointestinal cancers

Vlachogiannis et al. *Science*. 2018



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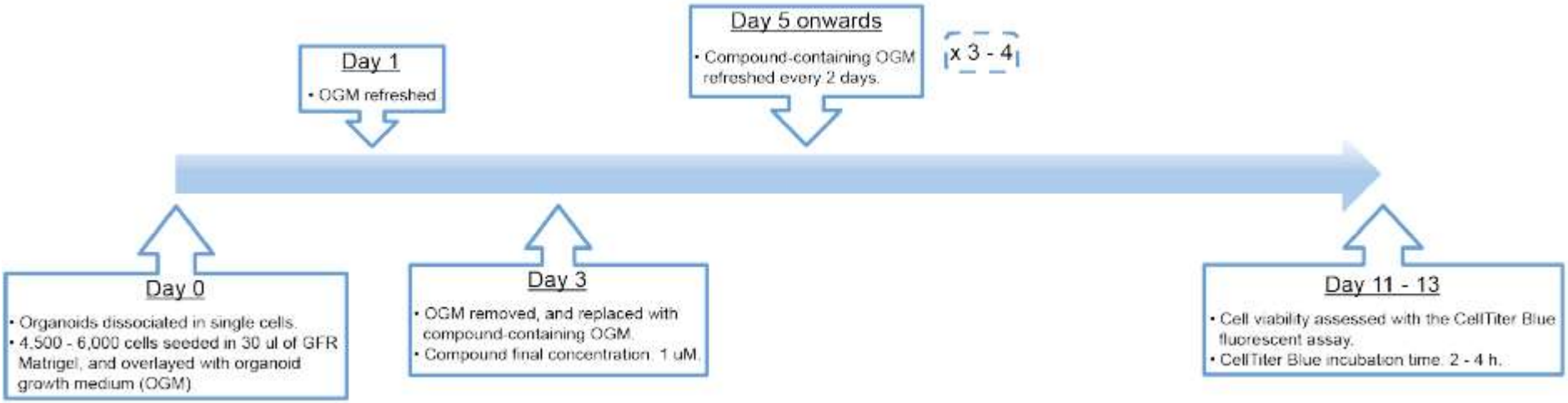

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		Nilotinib	Crizotinib	XL147	Afatinib	Erlotinib HCl	AUY922	NVP-TAE684	Everolimus	Bortezomib	Gefitinib	
C		Brivanib	DMSO	Imatinib Mesylate	Olaparib	SGX-523	Sorafenib Tosylate	GSK690693	Saracatinib	Selumetinib	MK-2206	
D		OSI-906 (Linsitinib)	Palbociclib	Dasatinib	DMSO	Tandutinib	OSI-930	DMSO	Regorafenib	Oxaliplatin	ENMD-2076	
E		Tivozanib	5-FU	Vemurafenib	Barasertib	AZD6482	AZD7762	Erismodegib	KU-60019	Rigosertib	MK-1775	
F		Lapatinib	CP-466722	LY2157299	GDC-0980	Dacomitinib	Irinotecan	Trametinib	DMSO	S-Ruxolitinib	LY2109761	
G		Torin 2	CHIR-99021	FR 180204	LGK-974	SCH772984	DMSO	SB 265610	CCT251455	CCT252422	CCT241736	
H												

Patient-derived organoids model treatment response of metastatic gastrointestinal cancers

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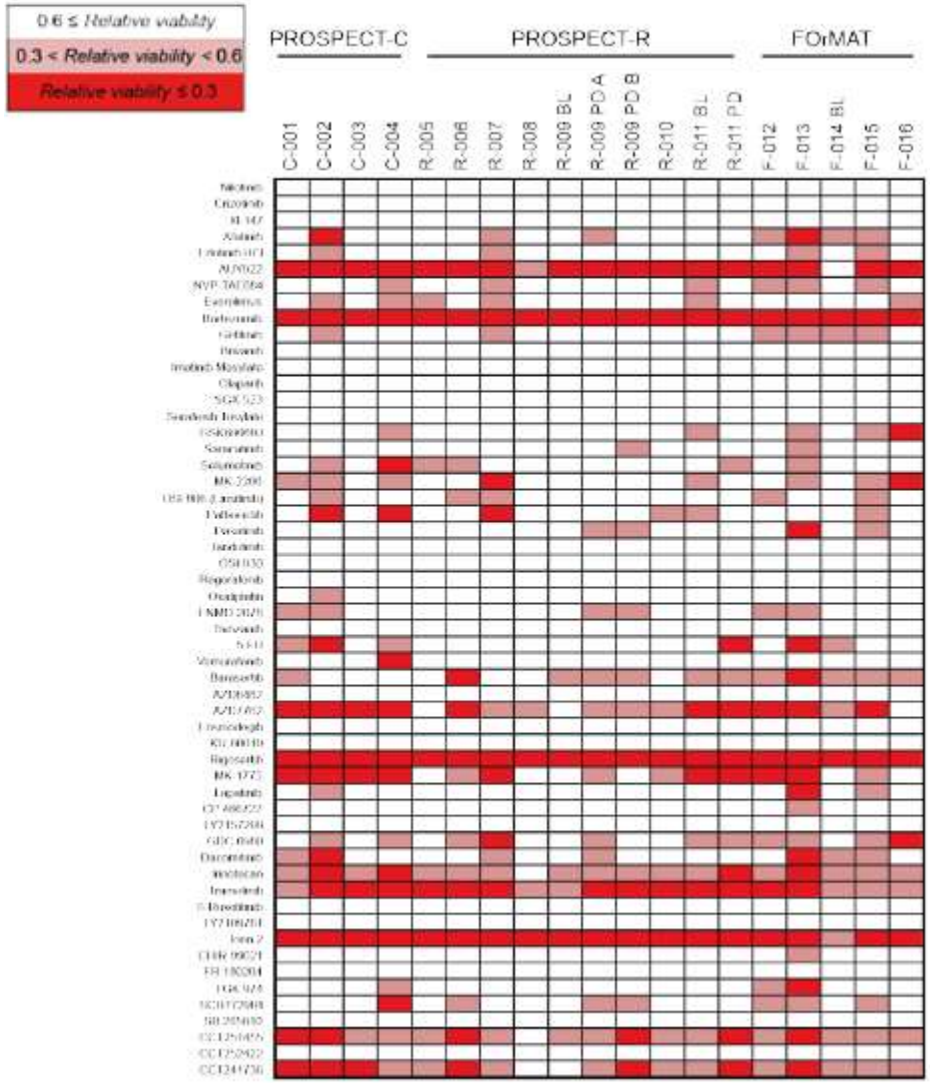

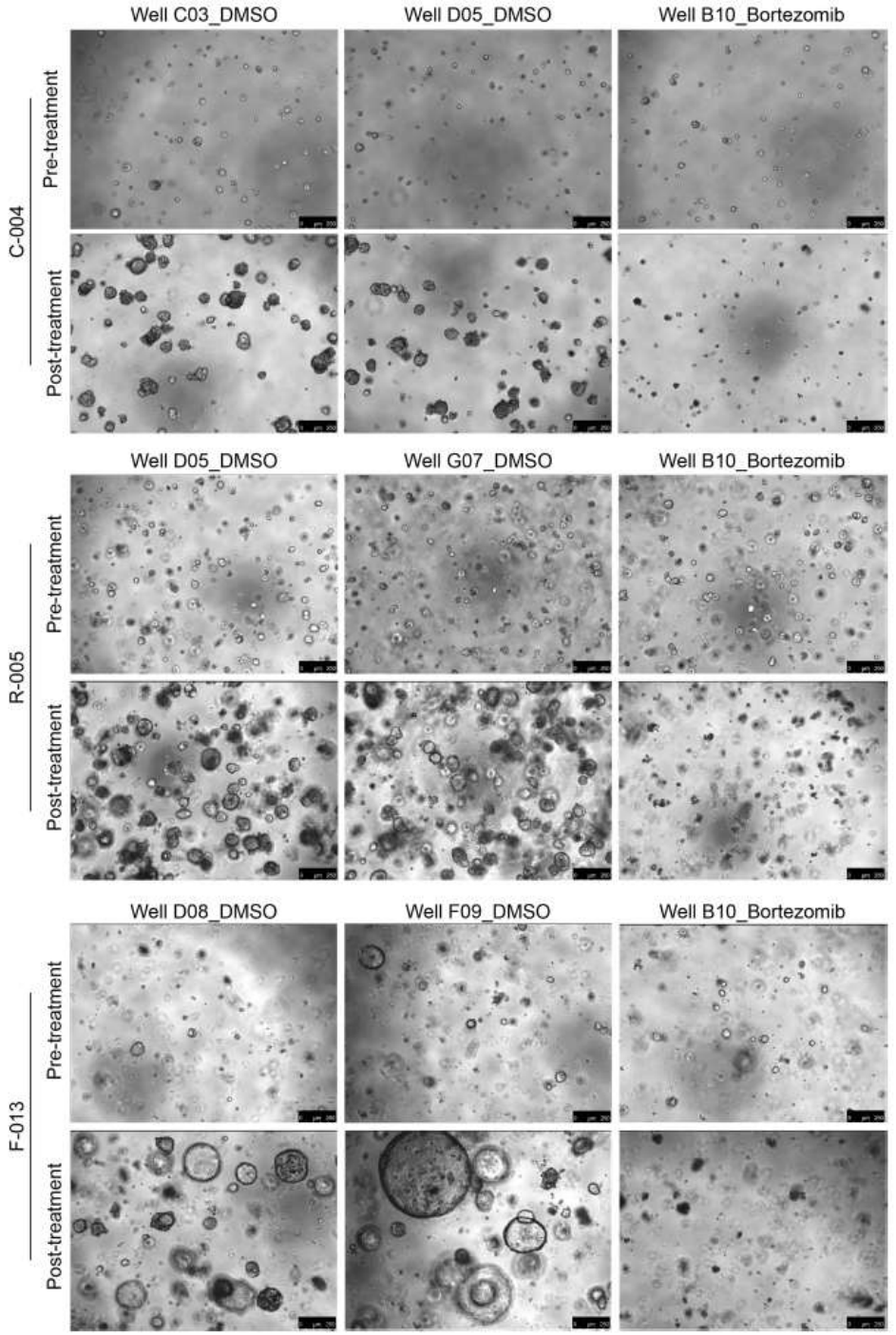

Patient-derived organoids model treatment response of metastatic gastrointestinal cancers

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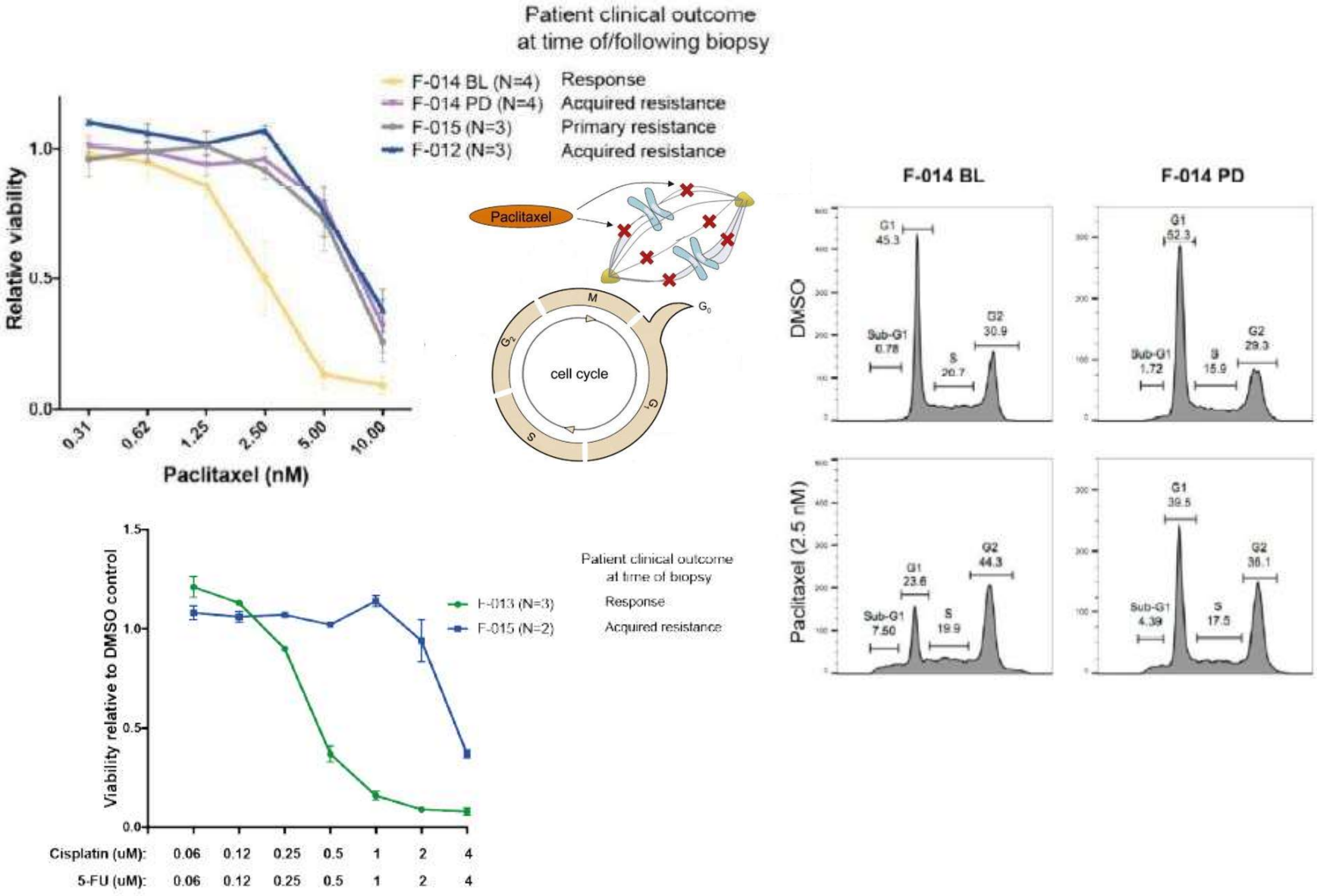

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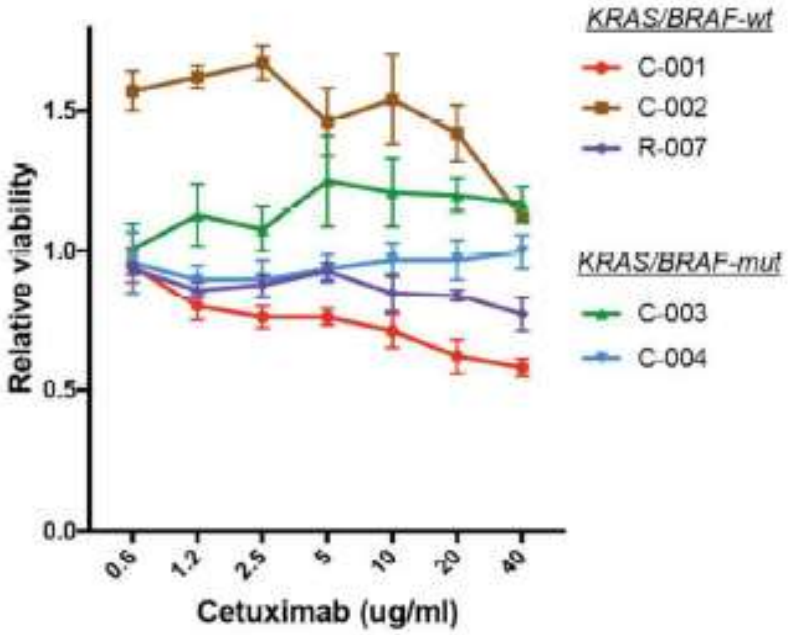
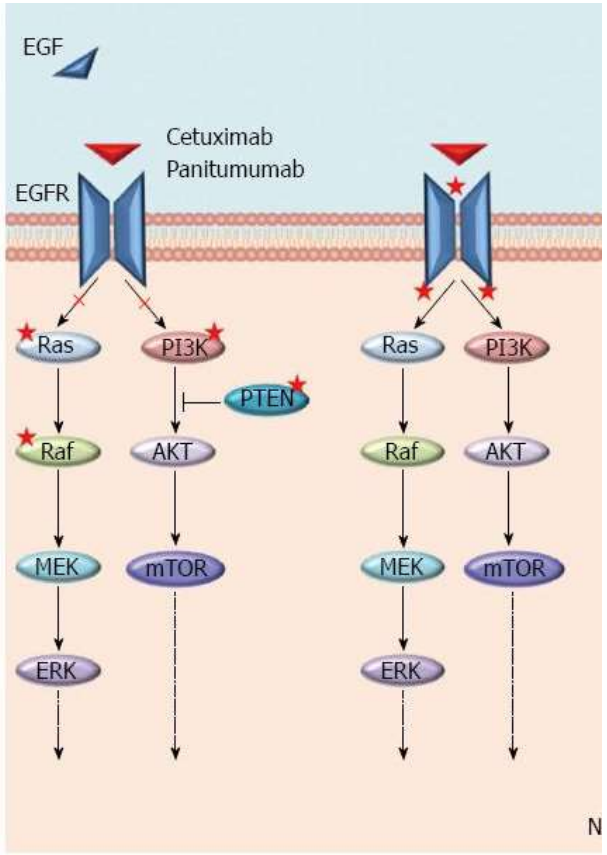
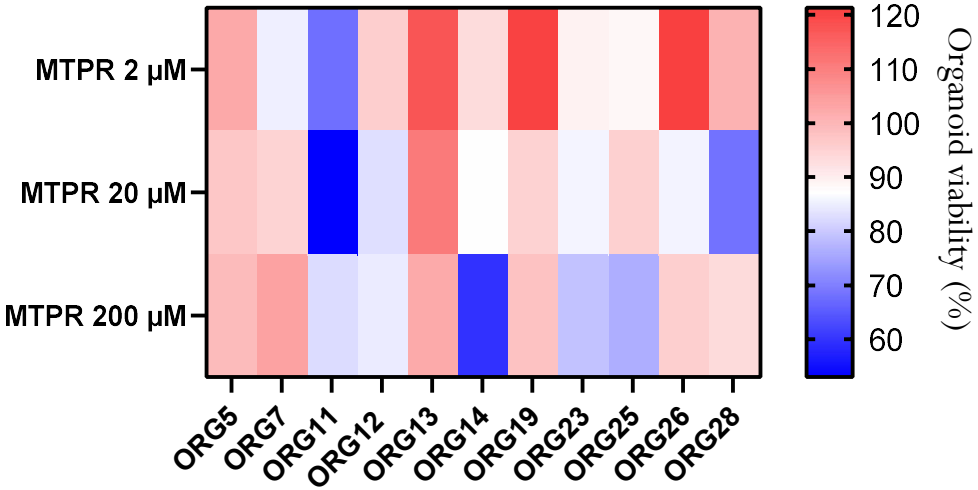


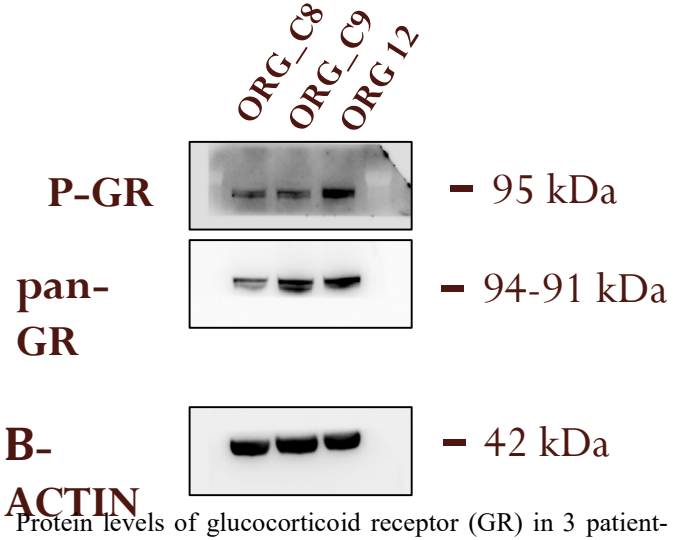


Evaluation of the cytotoxicity of drugs used in the treatment of IBD

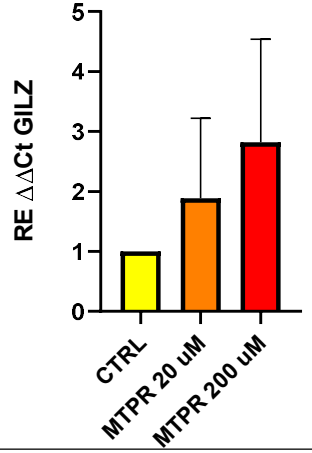
GLUCOCORTICOIDS

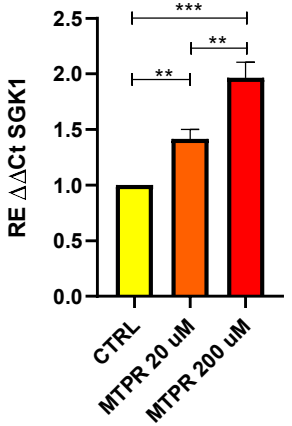


Cytotoxic effect of methylprednisolone (MTPR) on 11 patient-derived organoids. Cells were exposed for 72 h to drug and cytotoxicity was evaluated by the Cell-titer Glo assay. The value is the percentage of treated cells vs untreated controls.



Protein levels of glucocorticoid receptor (GR) in 3 patient-derived organoids.

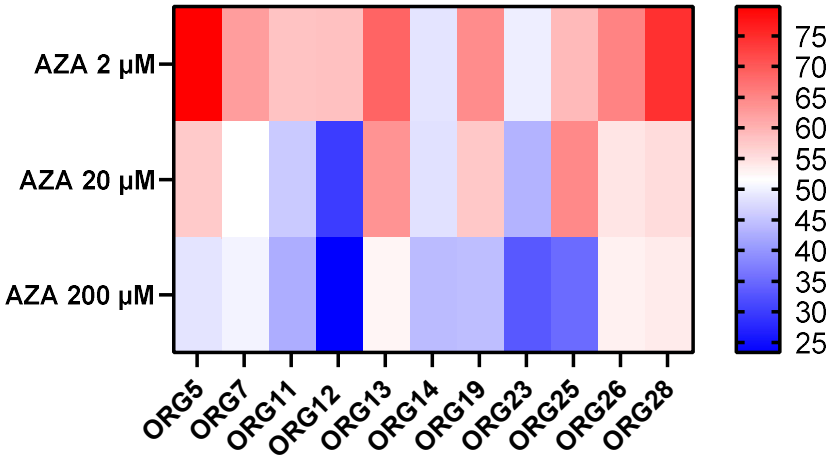




Relative expression (RE) of GILZ AND sgk1after incubation for 72 h with MTPR. One-way ANOVA ($p = 0.004$) and Bonferroni post-test **p value < 0.001; ***p value < 0.0001. The data are reported as means ± SE of three independent patient-derived organoids.

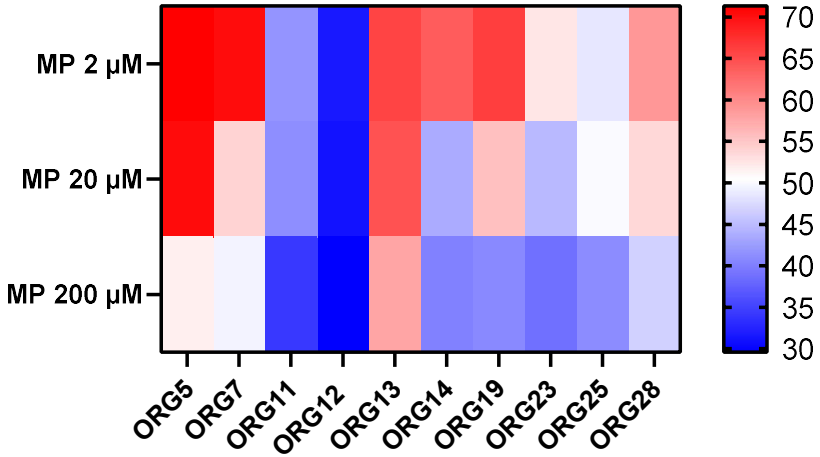
Evaluation of the cytotoxicity of drugs used in the treatment of IBD

THIOPURINES



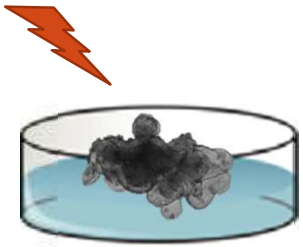
Cytotoxic effect of azathioprine (AZA) on 11 patient-derived organoids. Cells were exposed for 72 h to drug and cytotoxicity was evaluated by the Cell-titer Glo assay. The value is the percentage of treated cells vs untreated controls.

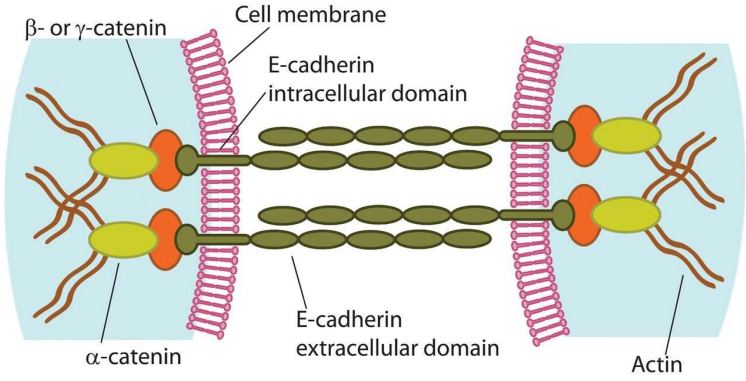
Cytotoxic effect of mercaptopurine (MP) on 11 patient-derived organoids. Cells were exposed for 72 h to drug and cytotoxicity was evaluated by the Cell-titer Glo assay. The value is the percentage of treated cells vs untreated controls.

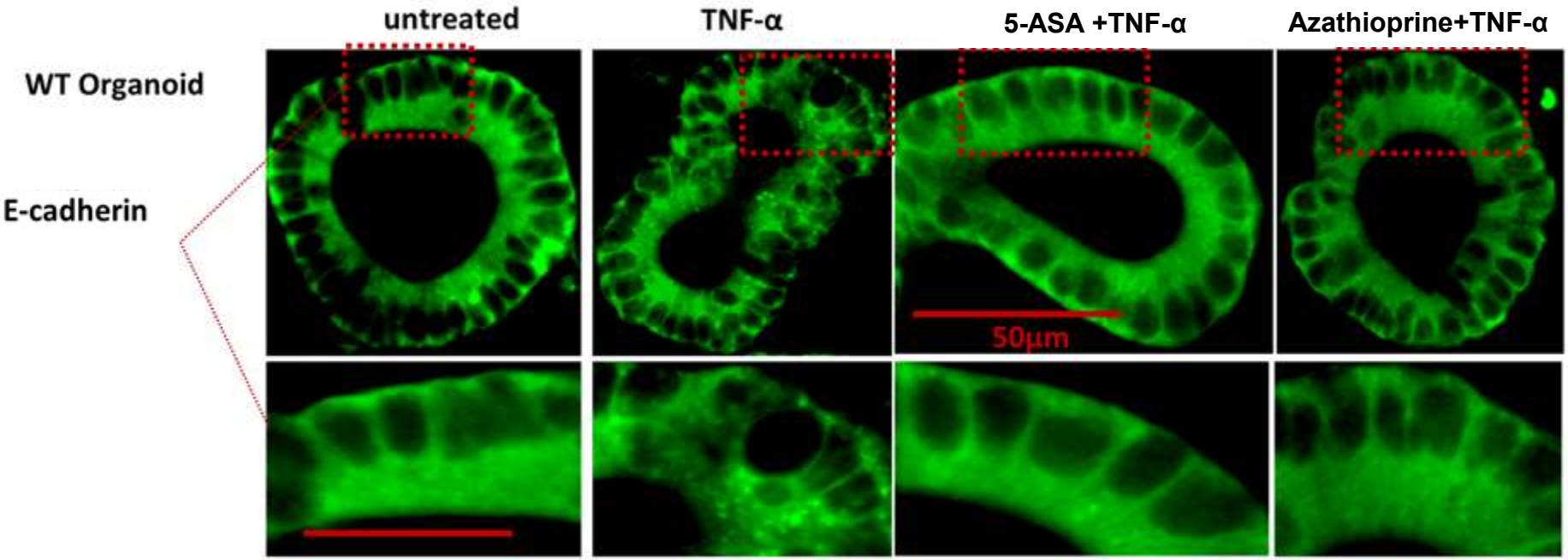


Organoids to study the effect of anti-inflammatory and immunomodulator drugs on epithelial barrier restoration

5-ASA
Azathioprine

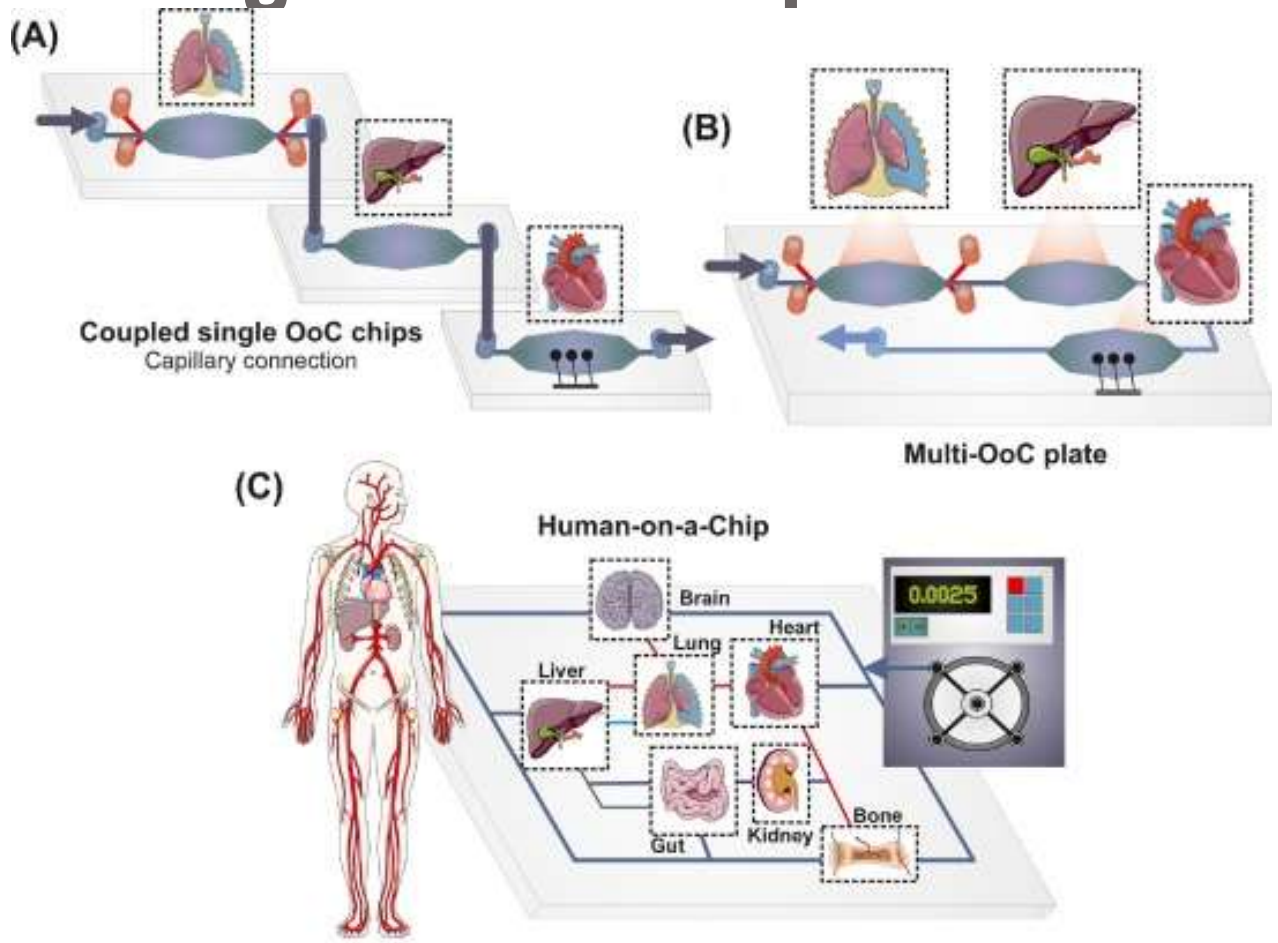






Vineeta Khare et al., Scien Report 2019

The “MultiOrgan-on-a-Chip” Models



Picollet-D’hahan et al., Trends of Biotechnology 2021

Schematic Representation of the Two Main Approaches for Developing Multi-OoC Systems:
(A) Through coupling of single OoC devices, each modeling a different organ, via capillary connection or a microfluidic motherboard (B); and (C) by integrating different organ models in a single plate, an approach that is more in line with the body-on-a-chip philosophy.