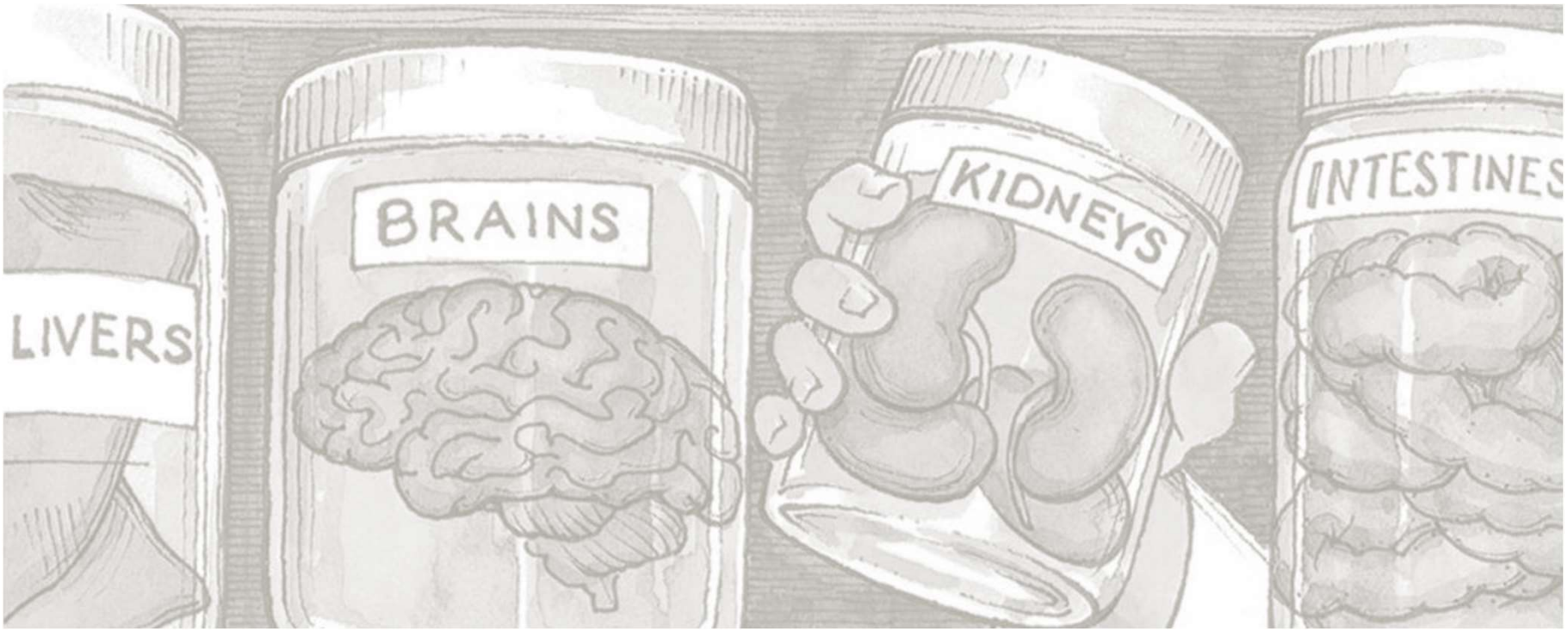
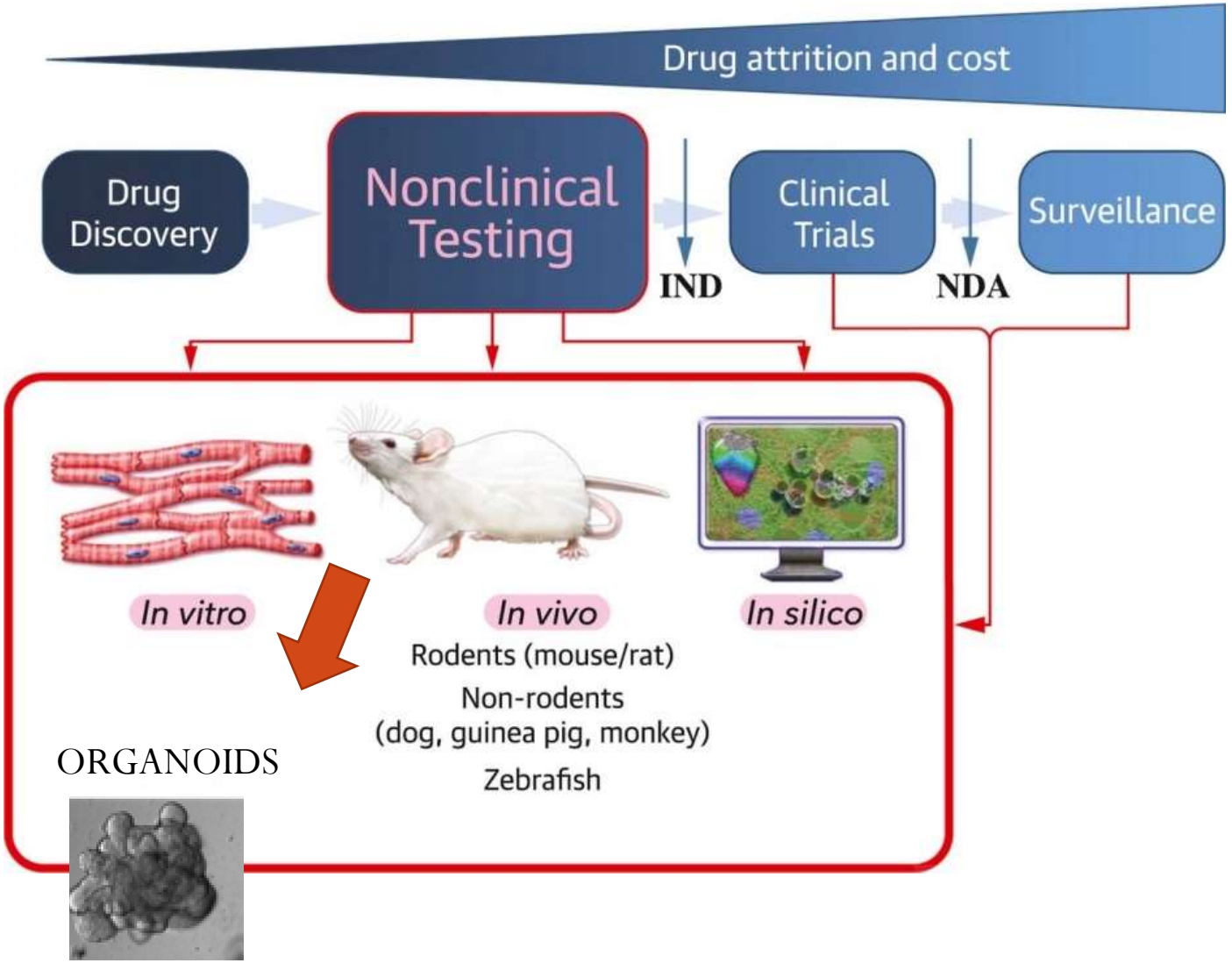
Three-dimensional Organoids as preclinical models



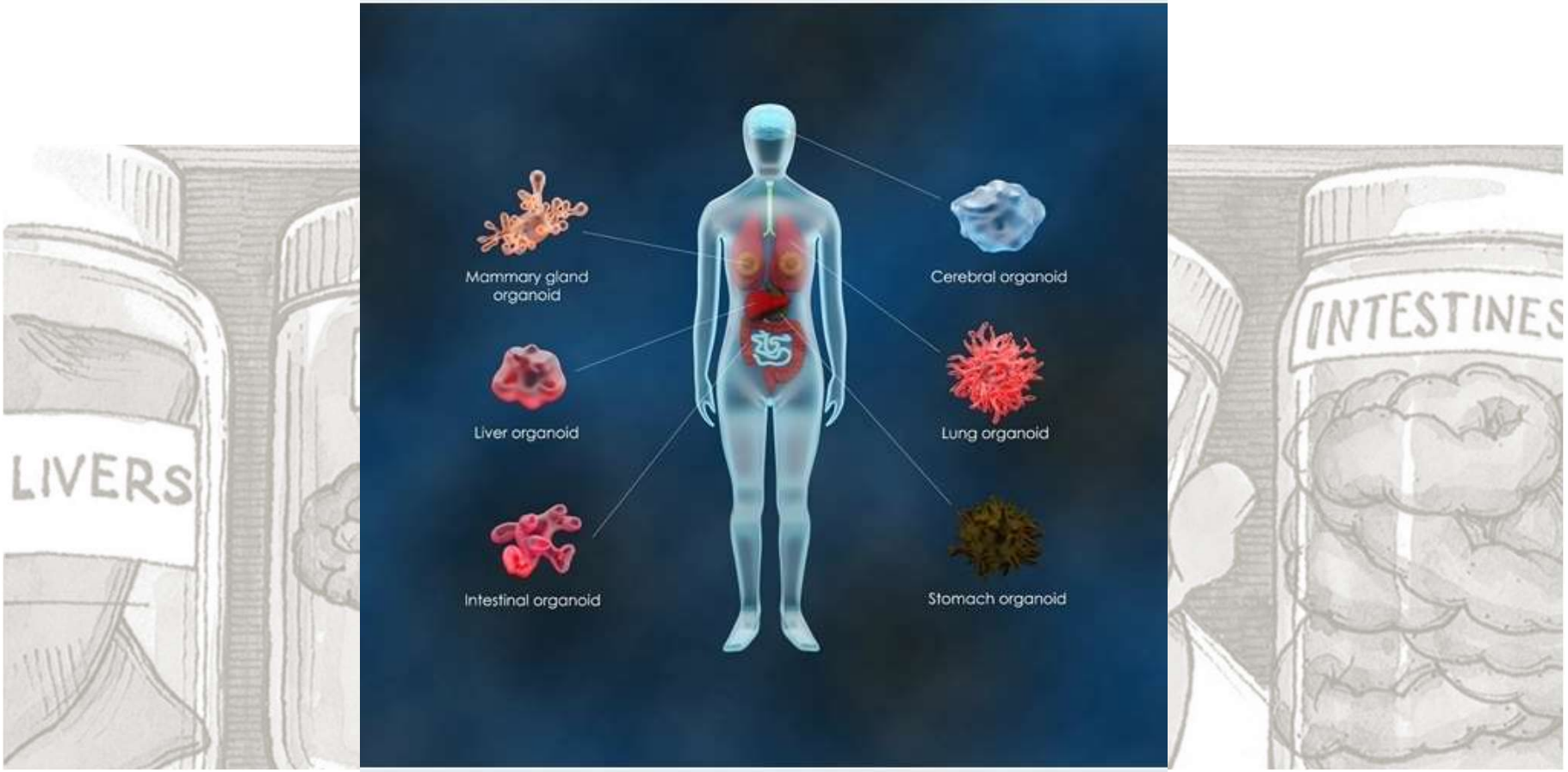
Preclinical models



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.

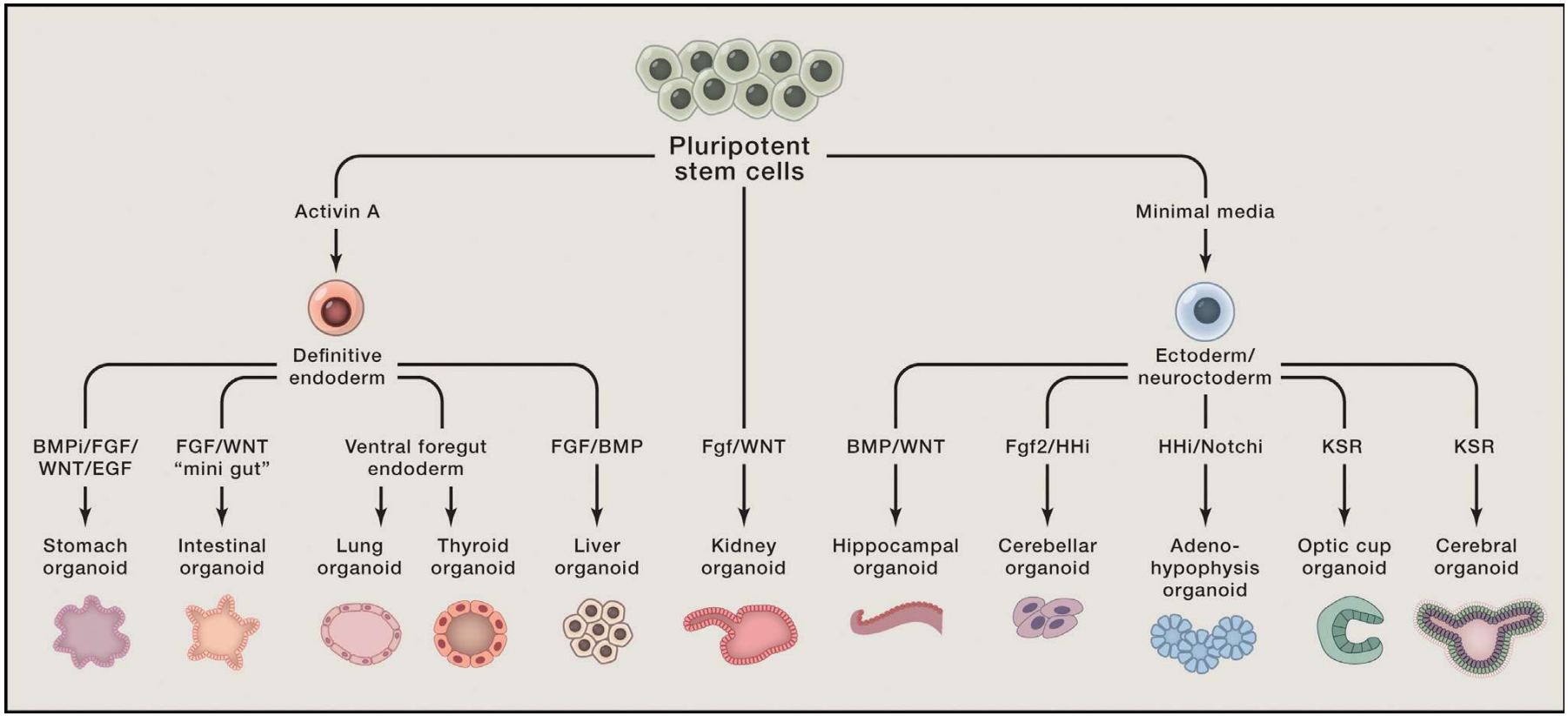
ORGANOIDS can be derived from pluripotent stem cells or adult stem cells.



Modeling Development and Disease with Organoids

Hans Clevers[1,*]

Cell



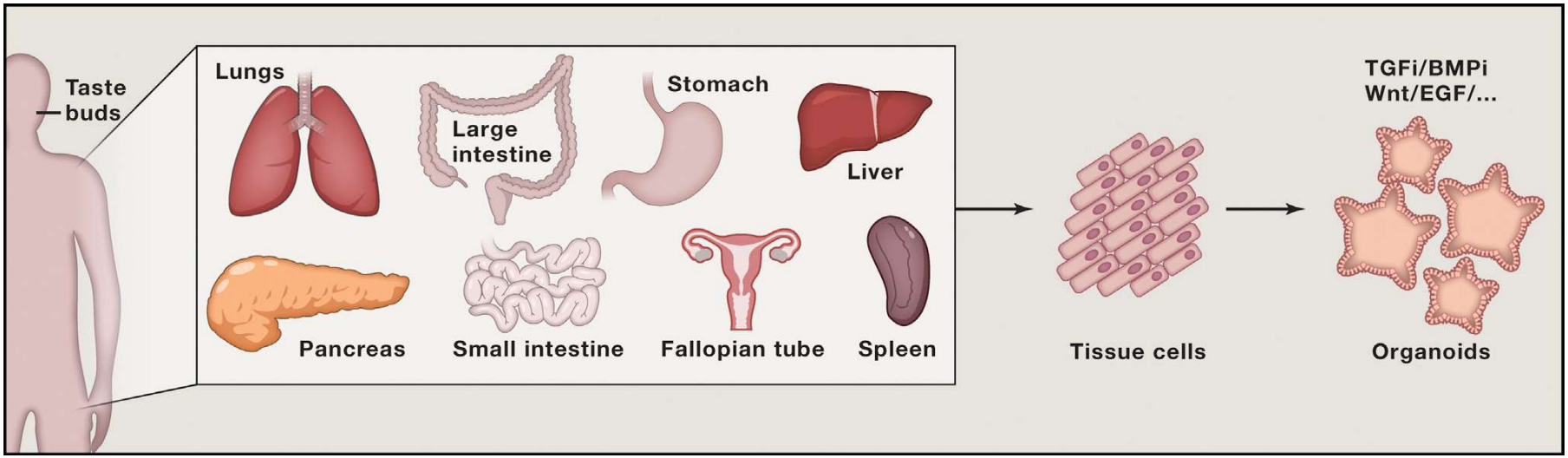
Schematic of the Various Organoids that Can Be Grown from PSCs and the Developmental Signals that Are Employed

Cell 165, June 16, 2016

Modeling Development and Disease with Organoids

Hans Clevers[1,*]

Cell

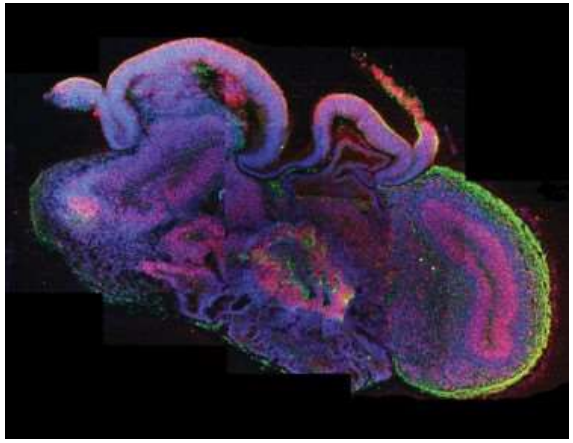


Schematic of the Various Regions of the Body that Can Be Cultured as aSC-Derived Organoids

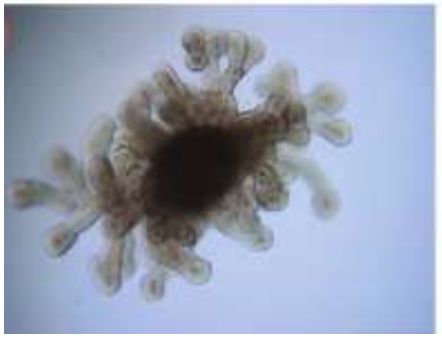
Cell 165, June 16, 2016

ORGANOIDS

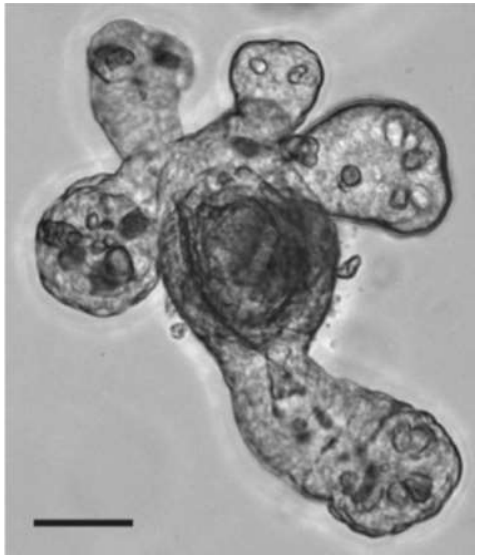
CEREBRAL ORGANOID

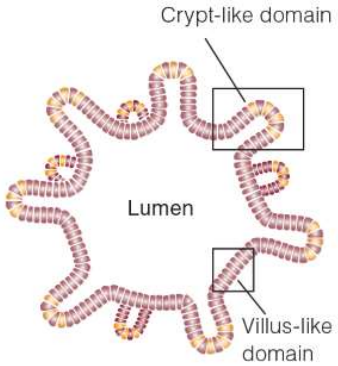


LUNG ORGANOID



INTESTINAL ORGANOID





LIVER ORGANOID

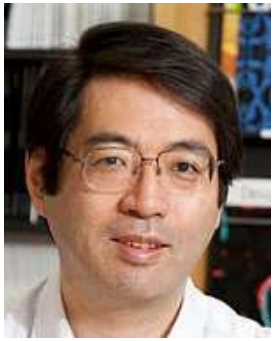
History of organoid methodologies

REVIEW

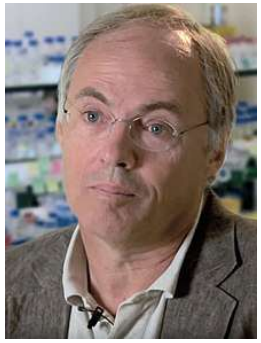
Organogenesis in a dish: Modeling development and disease using organoid technologies

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

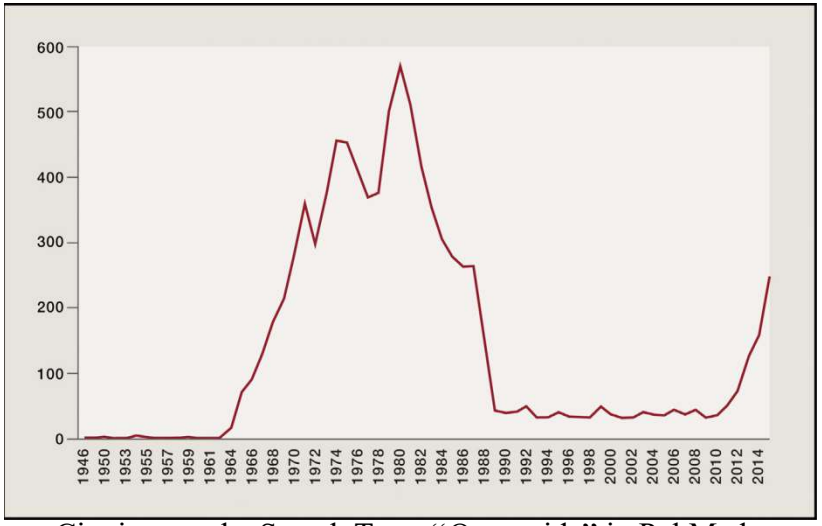
Science 18 Jul 2014:
Vol. 345, Issue 6194, 1247125
DOI: 10.1126/science.1247125



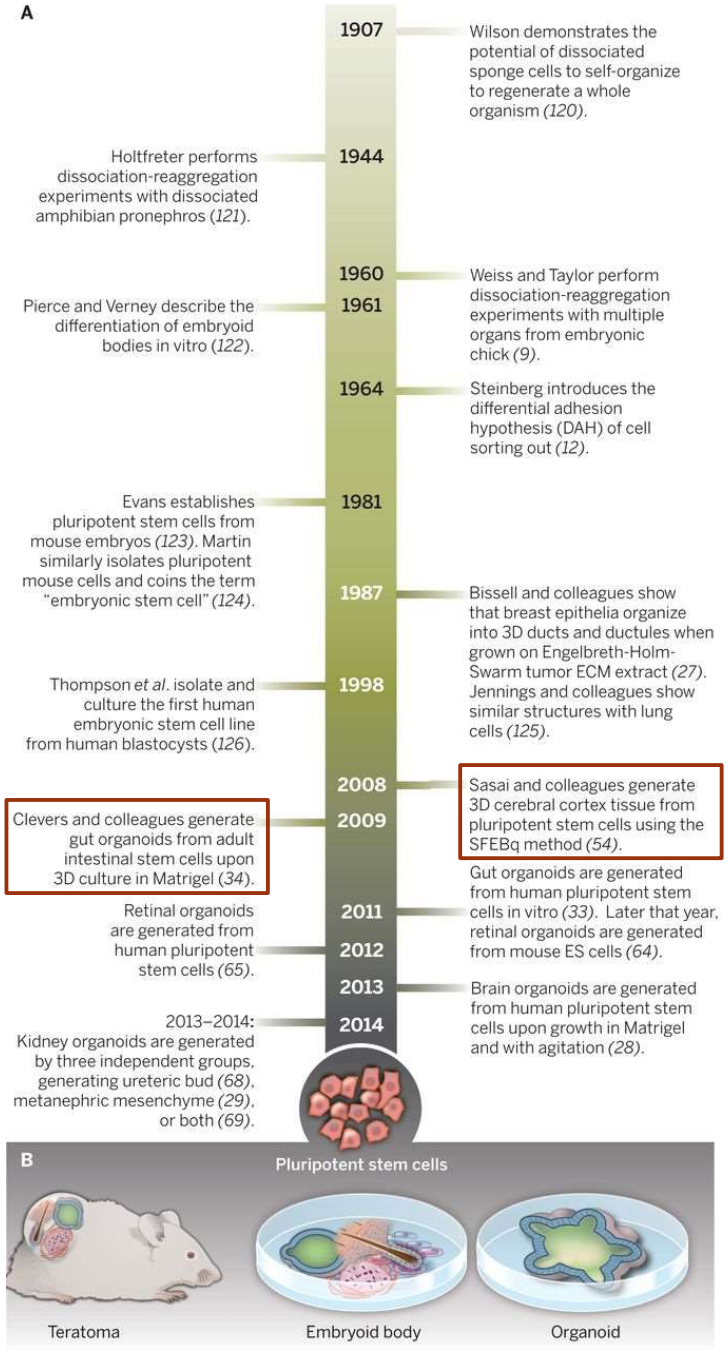
Yoshiki Sasai



J.C. Clevers



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



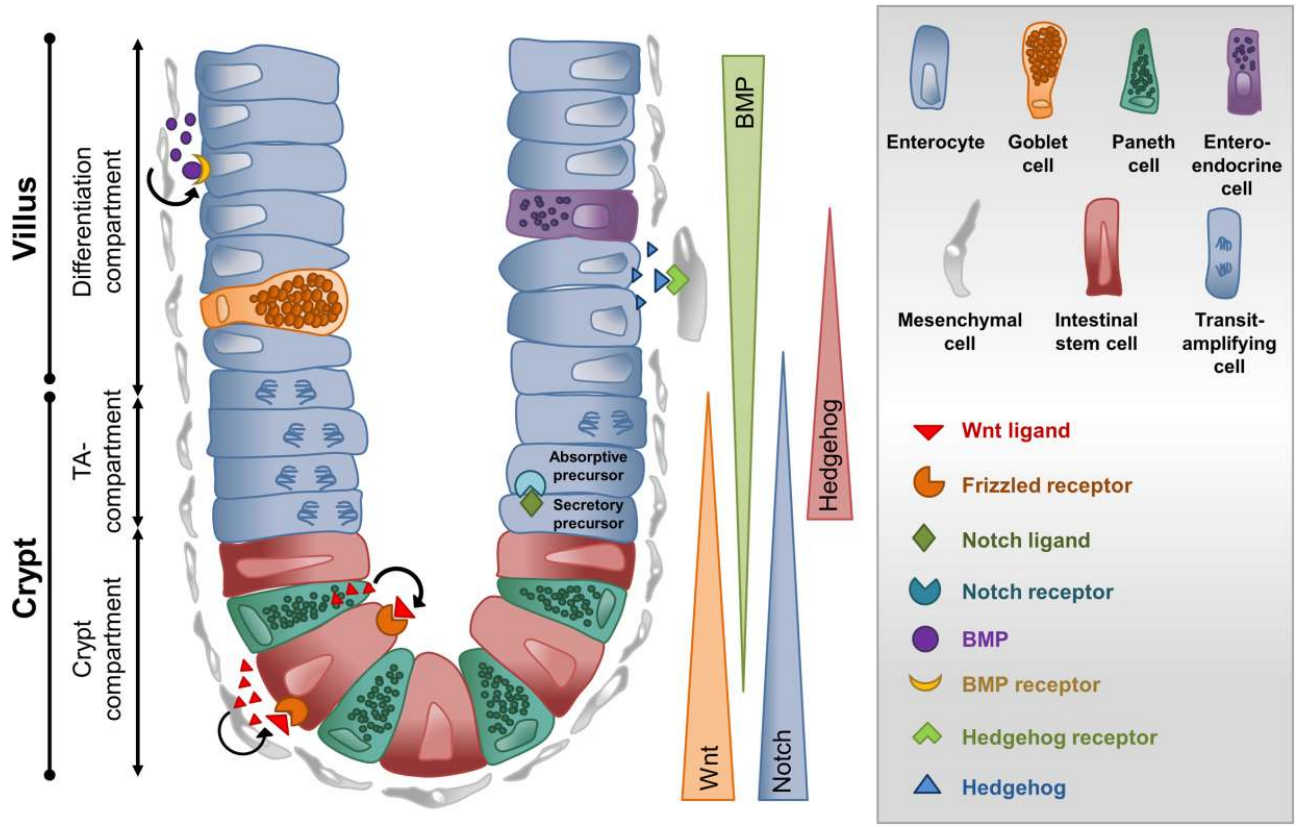
Editorial | Published: 03 January 2018

Method of the Year 2017: Organoids

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

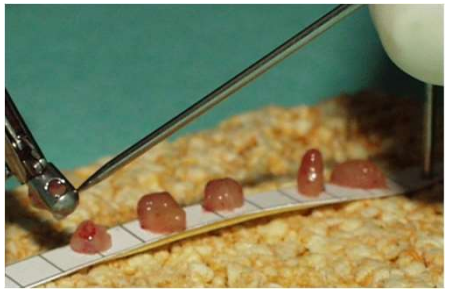
The ability to prod stem cells into three-dimensional tissue models makes for a powerful way to study human biology. But these exciting tools are still works in progress.

Intestinal organoid culture method



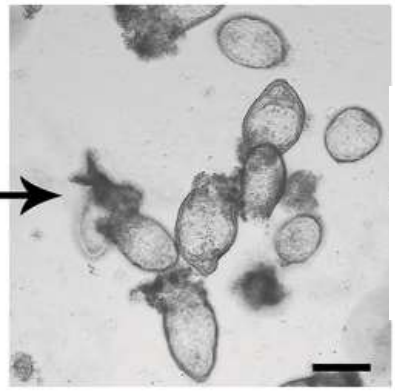
- Lgr5 crypt stem cells divide constantly;
- Stem cells numbers remain fixed because stem cells compete ‘neutrally’ for niche space;
- Daughters of the intestinal stem cells, the Paneth cells, serve as crypt niche cells by providing Wnt, Notch and EGF signals.

Intestinal organoid culture method

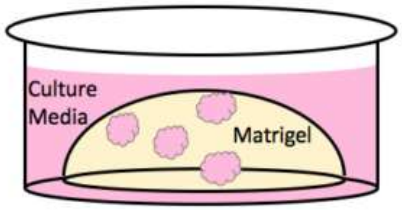


1. Intestinal biopsies

2. Crypt Isolation



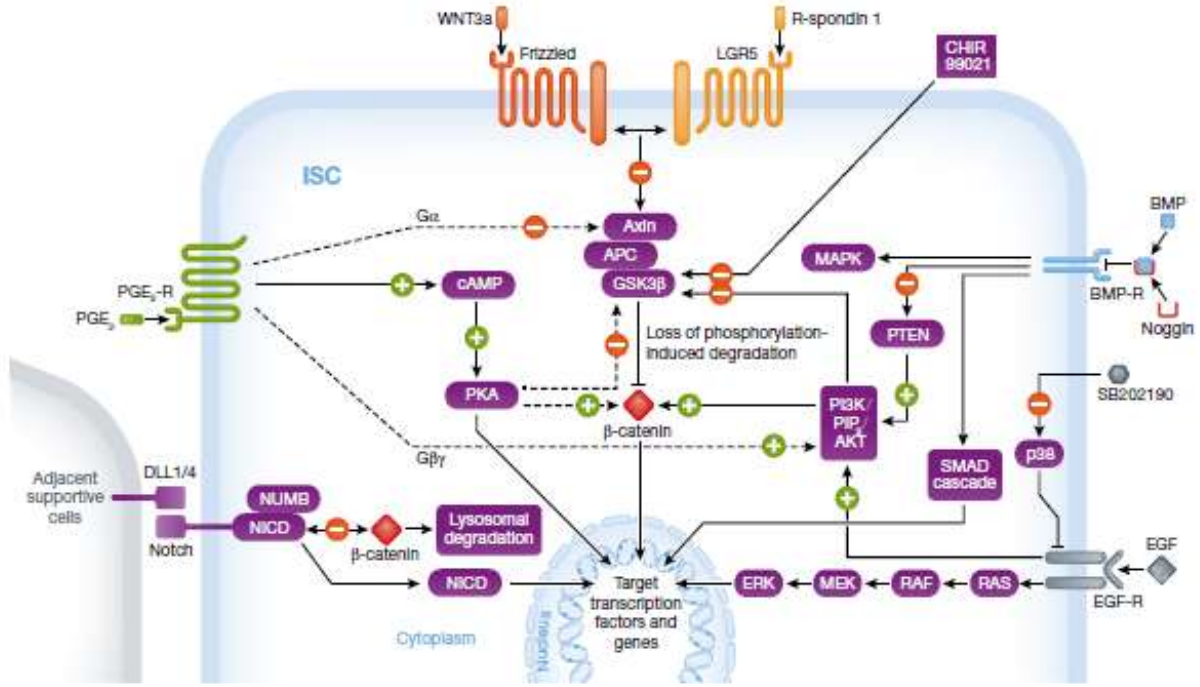
3. Crypt Culture



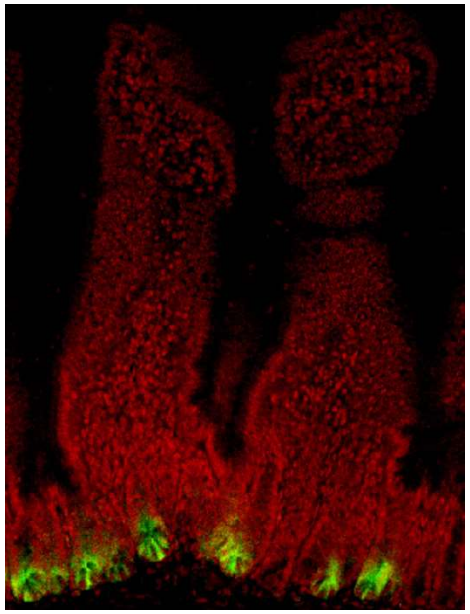
1)Wash
2)Fungizone+Normocin+Gentamicin
3)EDTA incubation
4)Shake vigorously

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

Intestinal organoid: an adult stem cell-based organoid



Lgr5 as adult stem cell marker



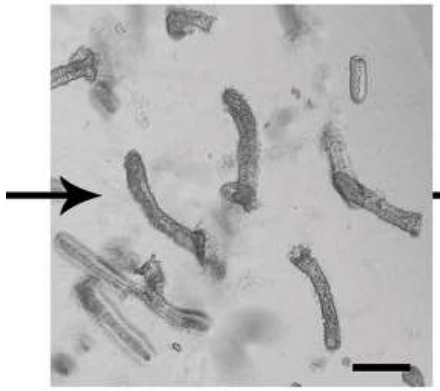
A GFP knock-in into the Lgr5 locus visualizes the stem cells of the small intestine of mice at the base of crypts.

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

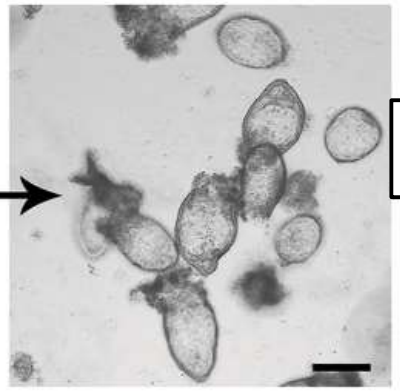
Growth medium constituents	Working mechanism in ISCs	Effect on ISCs and application
WNT3a[a]	Activates canonical WNT signaling (Clevers & Nusse, 2012)	Stimulates crypt cells proliferation and maintains the stem cell state (Clevers & Nusse, 2012; Farin *et al*, 2012; Krausova & Korinek, 2014)
R-spondin 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

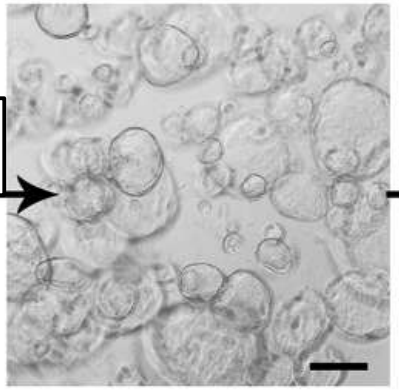


2. Crypt Isolation



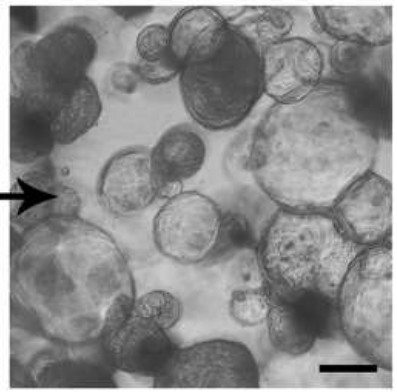
3. Crypt Culture

48/ 72h



4. Epithelial Organoid cultures

5 D



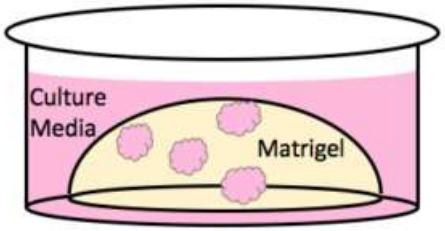
5. Differentiated Epithelial Organoid cultures (enterocytes, goblet cells, Paneth cells, enteroendocrine cells)

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

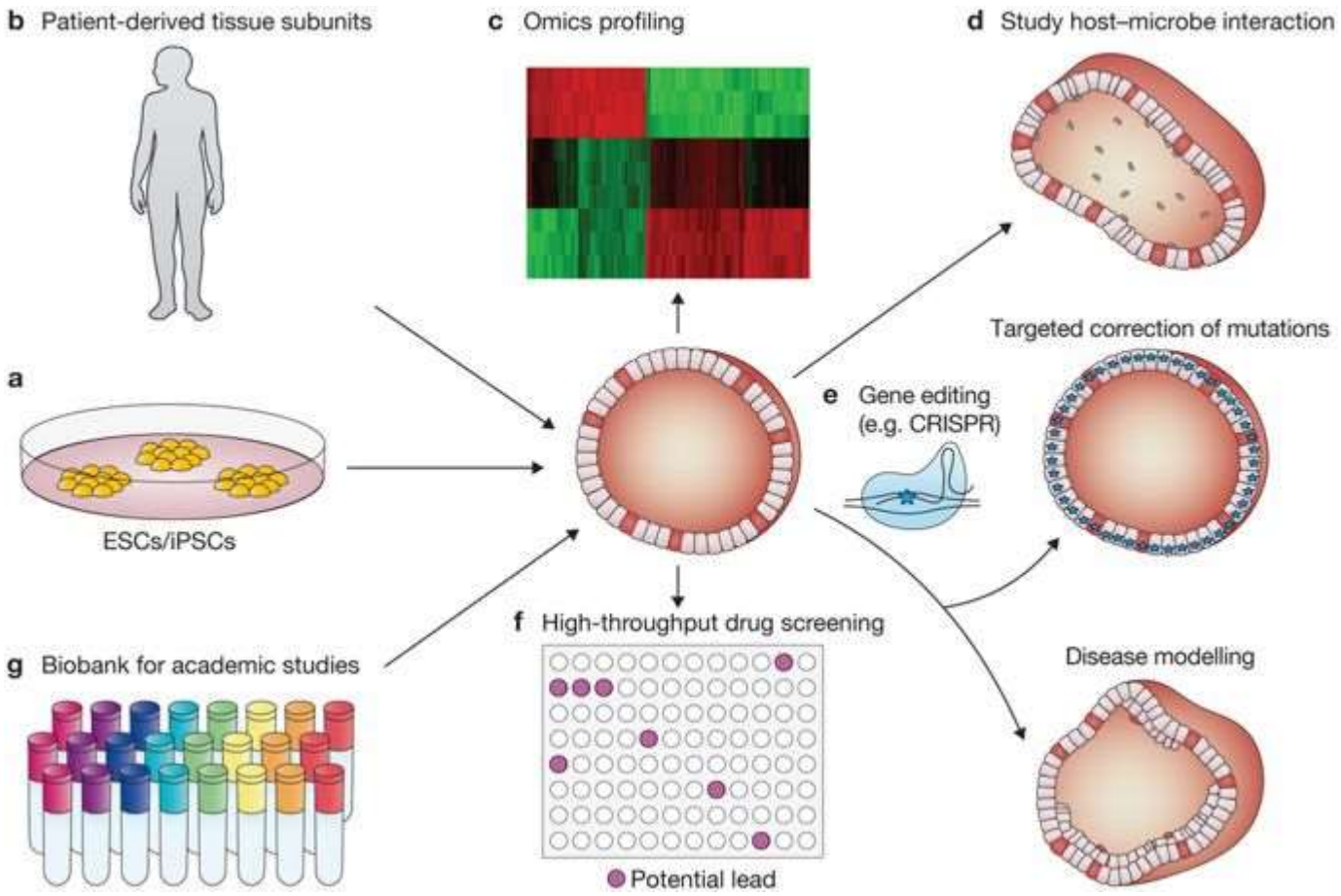
Organoid passaging:
1)Recovery solution
2)Disaggregation solution (dispase or trypsin)

EXPANSION/ ANALYSES

ANALYSES



Applications of organoid technology



Applications of organoid technology

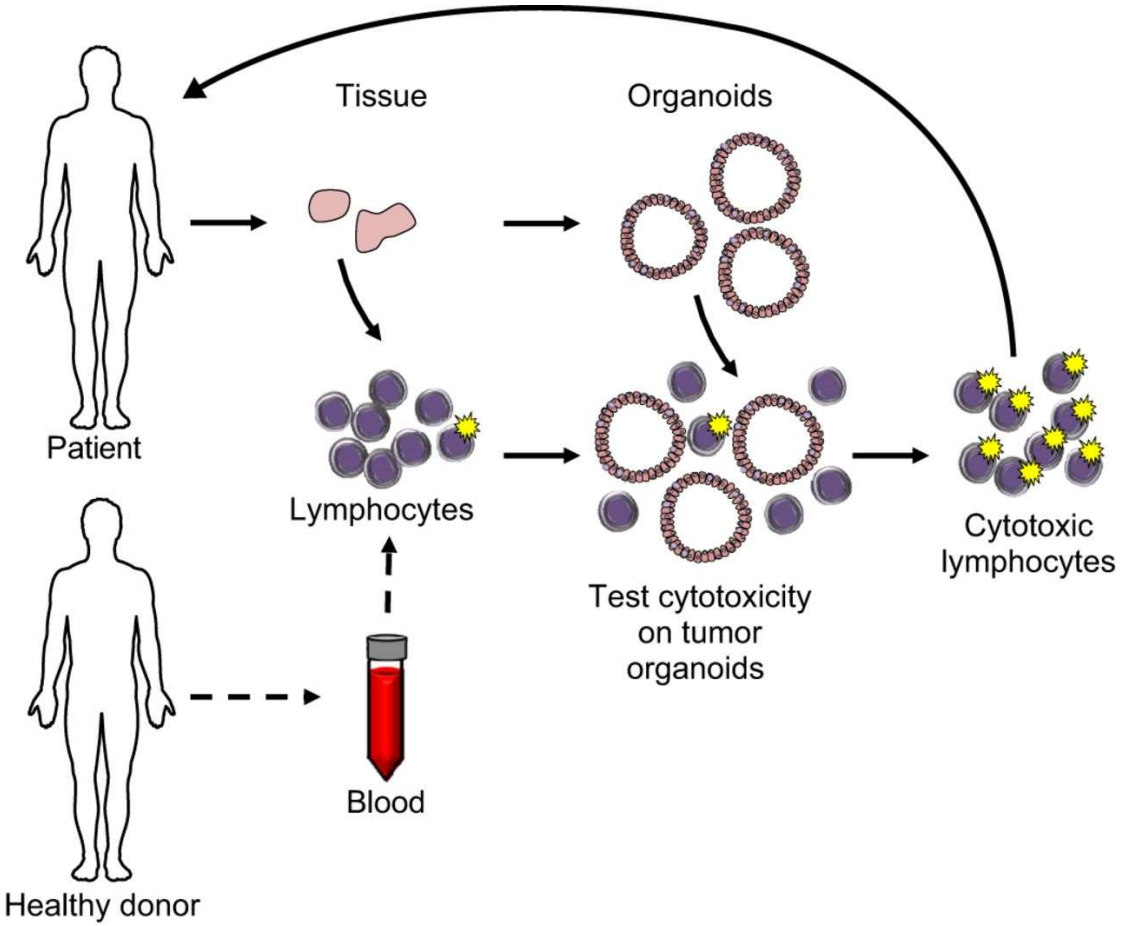
https://www.hubrecht.eu/research-groups/clevers-group/

Applications of organoid technology

Translational applications of adult stem cell-derived organoids

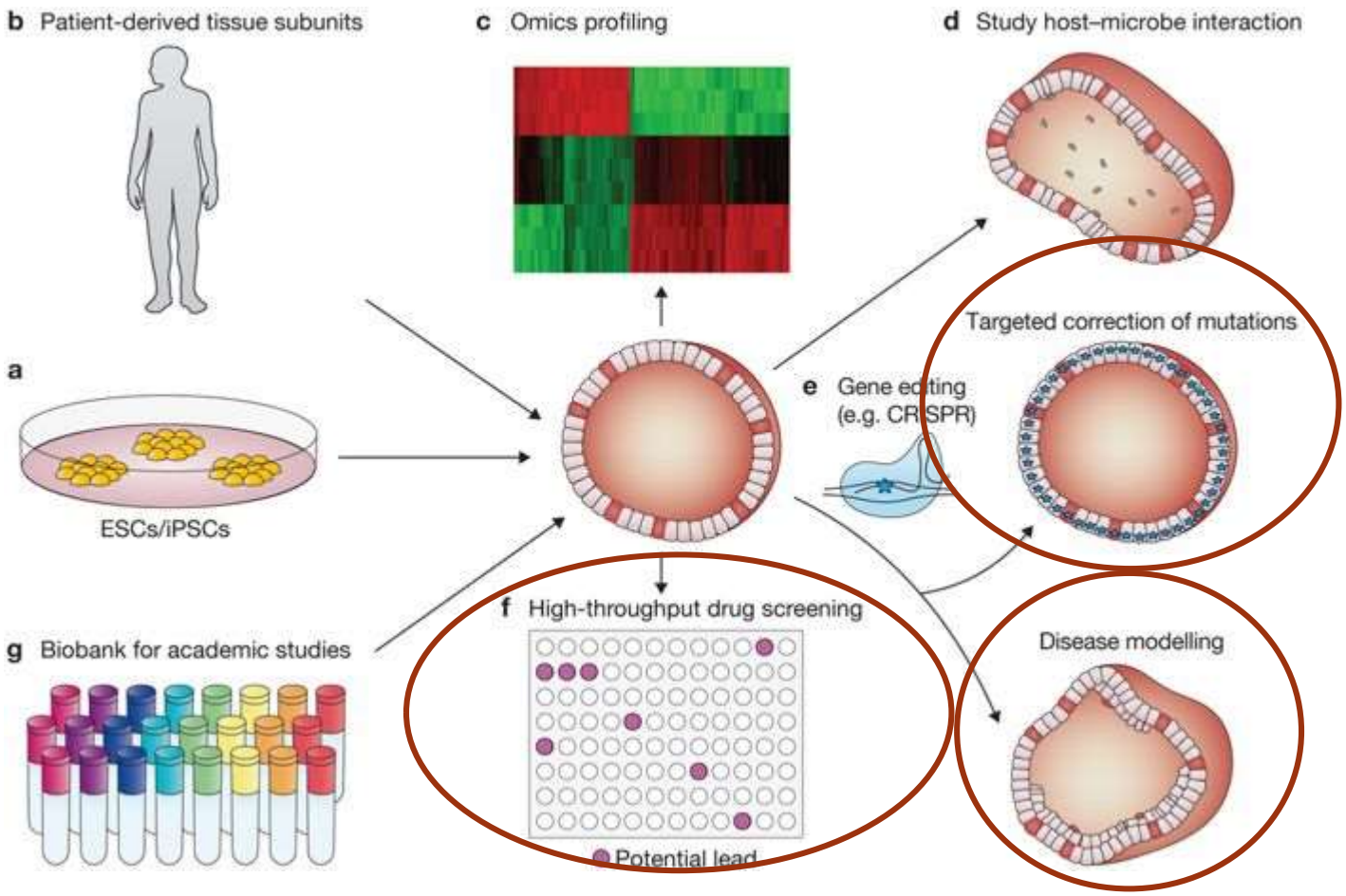
Jarno Drost, Hans Clevers

Development 2017 144: 968-975; doi: 10.1242/dev.140566

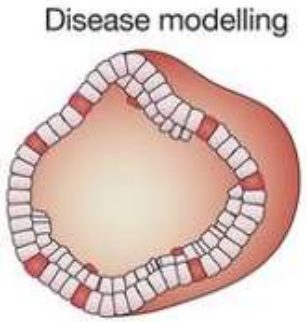


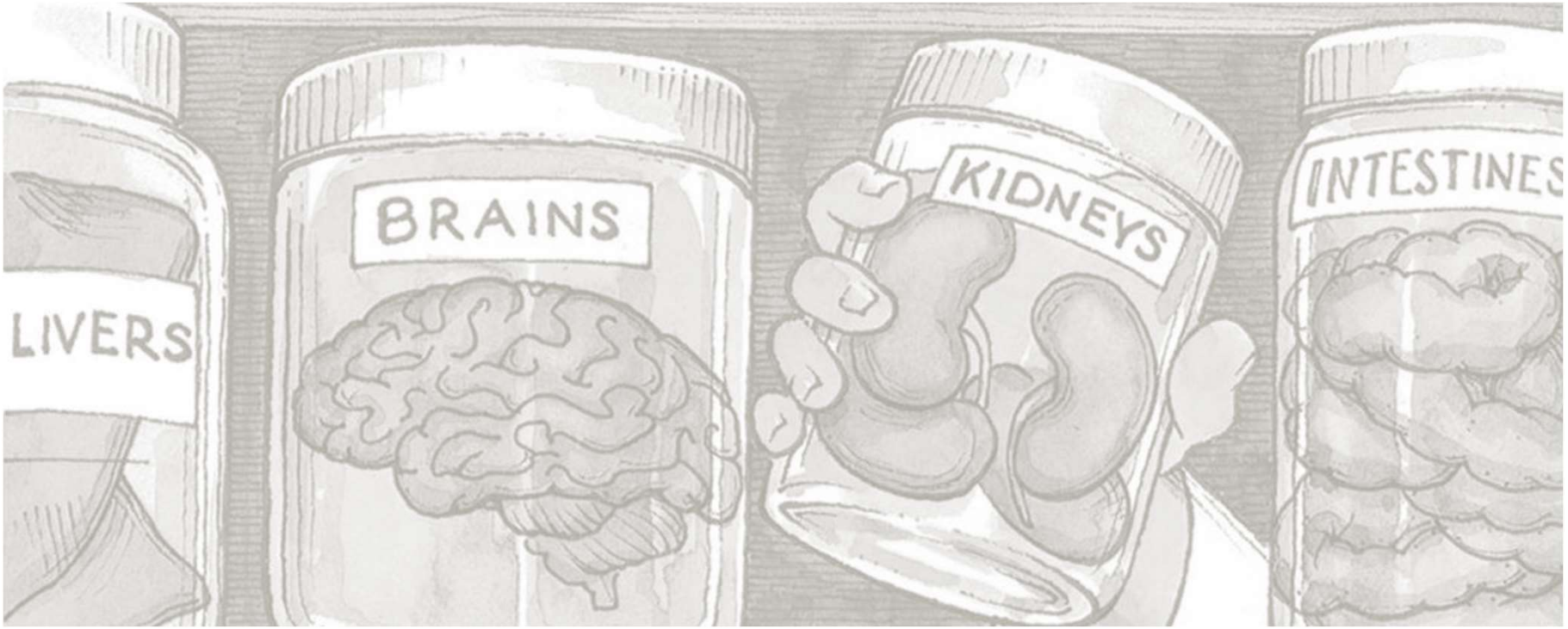
Exploiting adult stem cell-derived organoids for immunotherapy

Applications of organoid technology



Intestinal Organoids: New frontiers in the study of intestinal disease

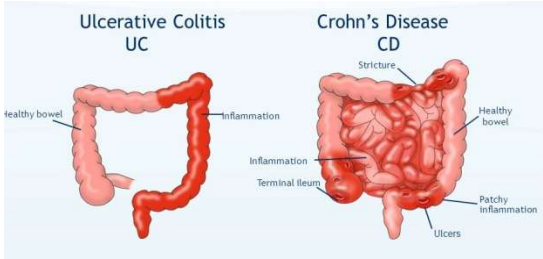


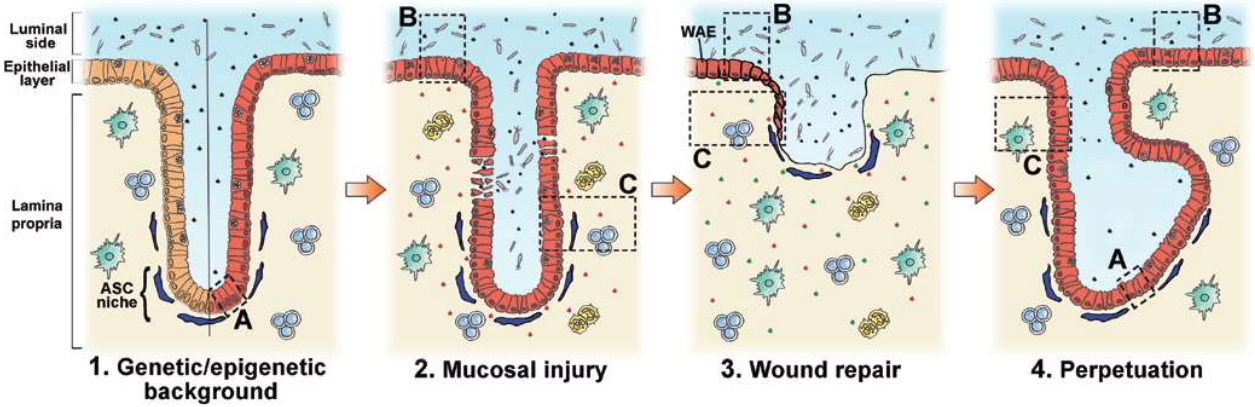


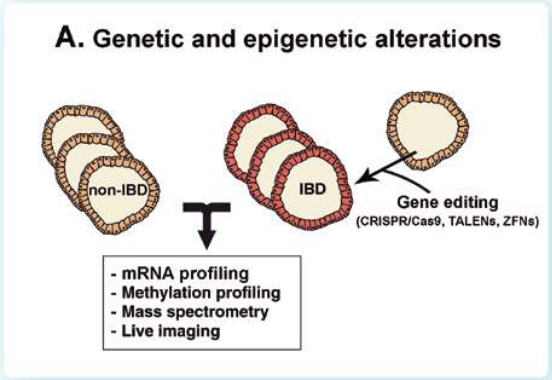
Potential Use of Human Stem Cell–Derived Intestinal Organoids to Study Inflammatory Bowel Diseases

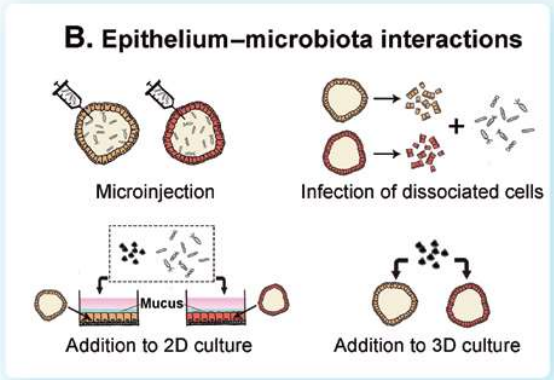
Isabella Dotti, PhD, and Azucena Salas, PhD

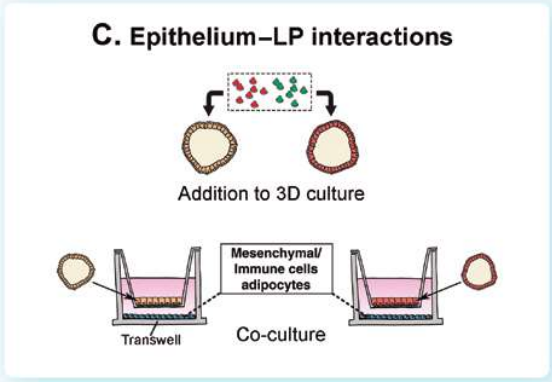
Inflammatory Bowel Diseases

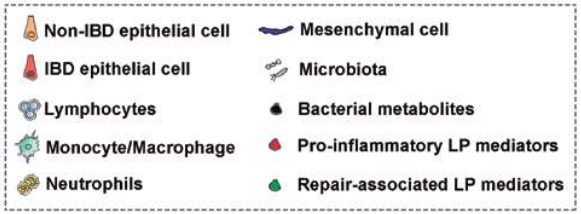












Lamina propria (LP)

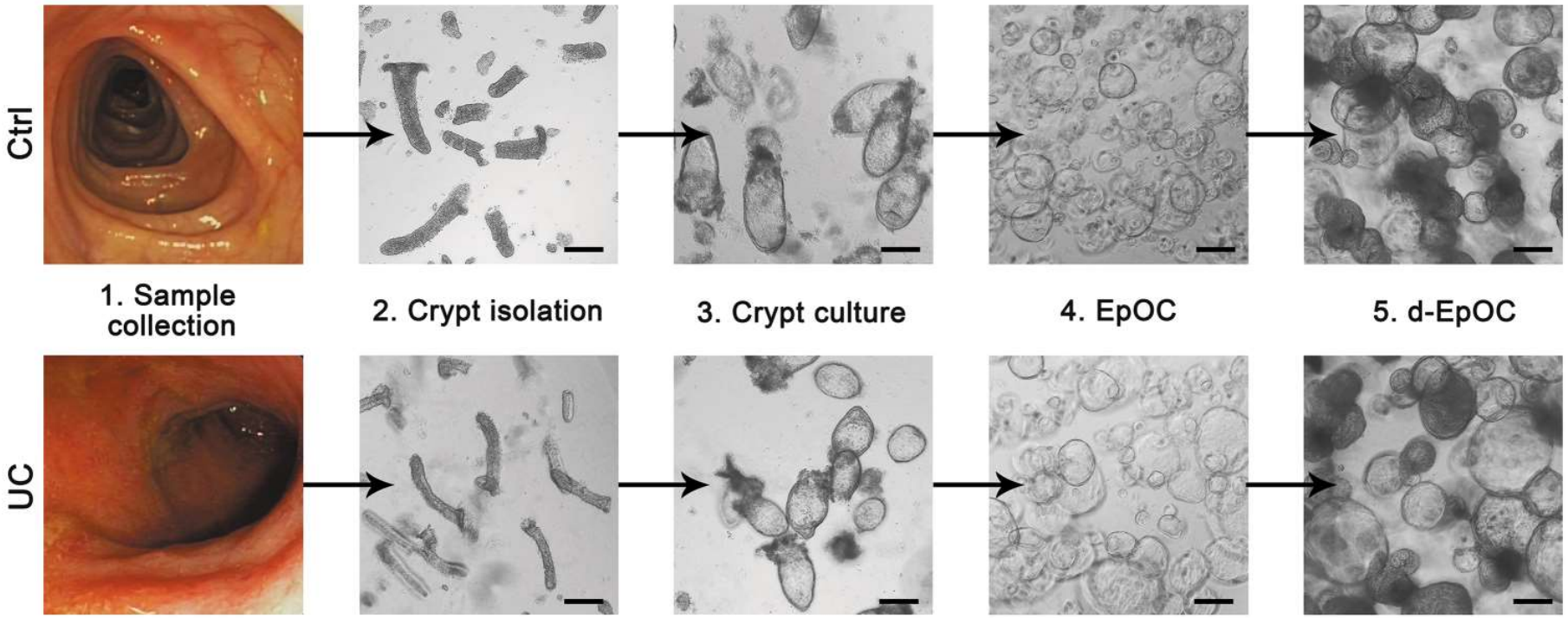
Epithelial organoid cultures from patients with Inflammatory Bowel Diseases

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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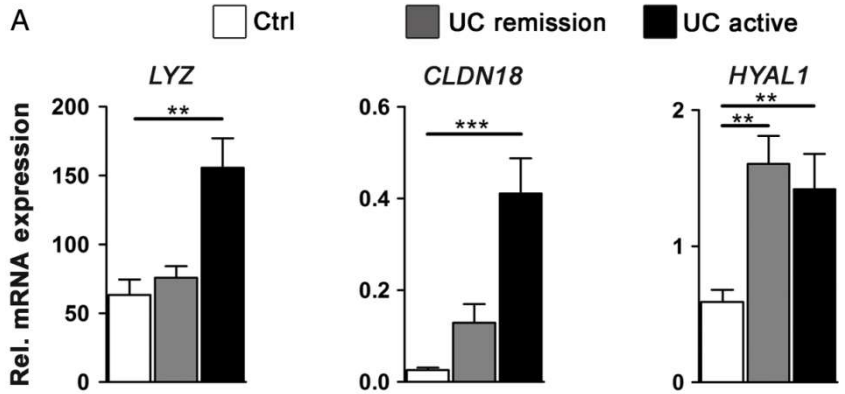
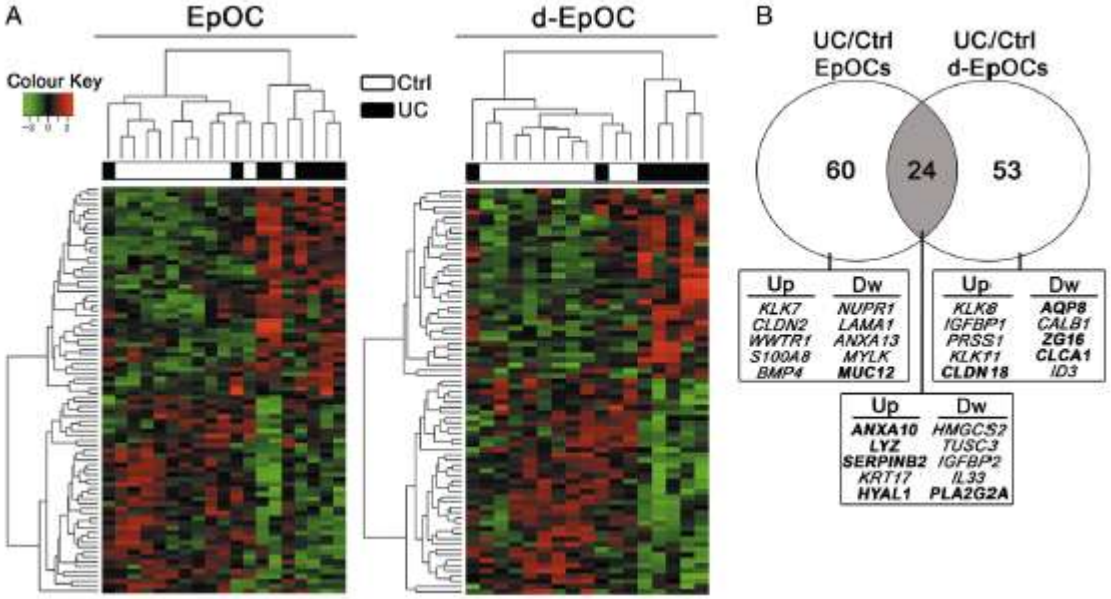




Epithelial Organoid cultures (EpOC)

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%);

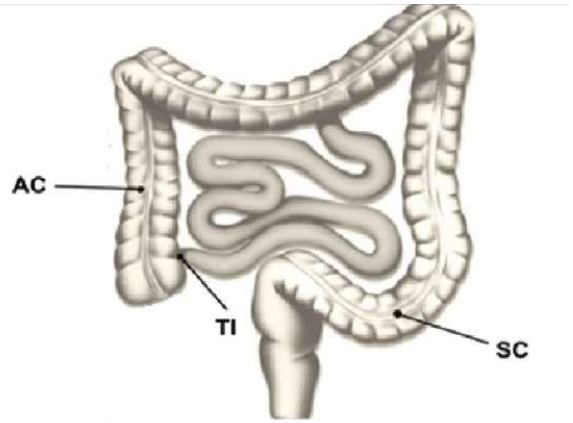
Epithelial organoid cultures from patients with IBDs

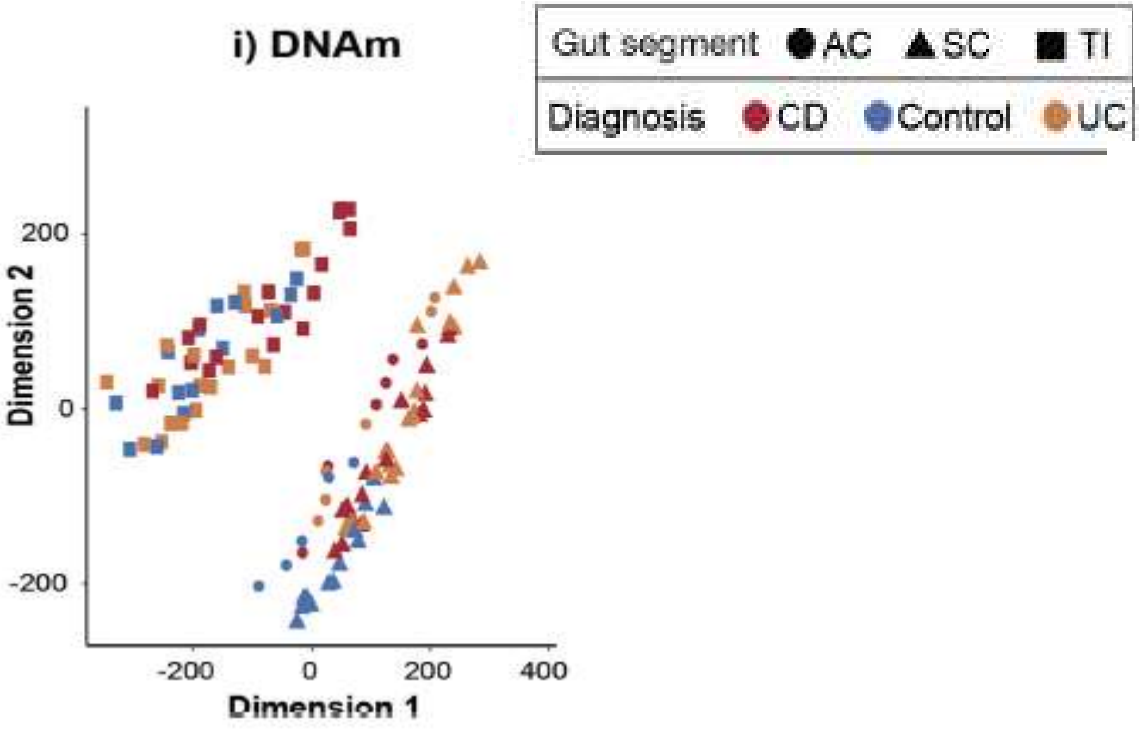
Gastroenterology 2018;154:585–598

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

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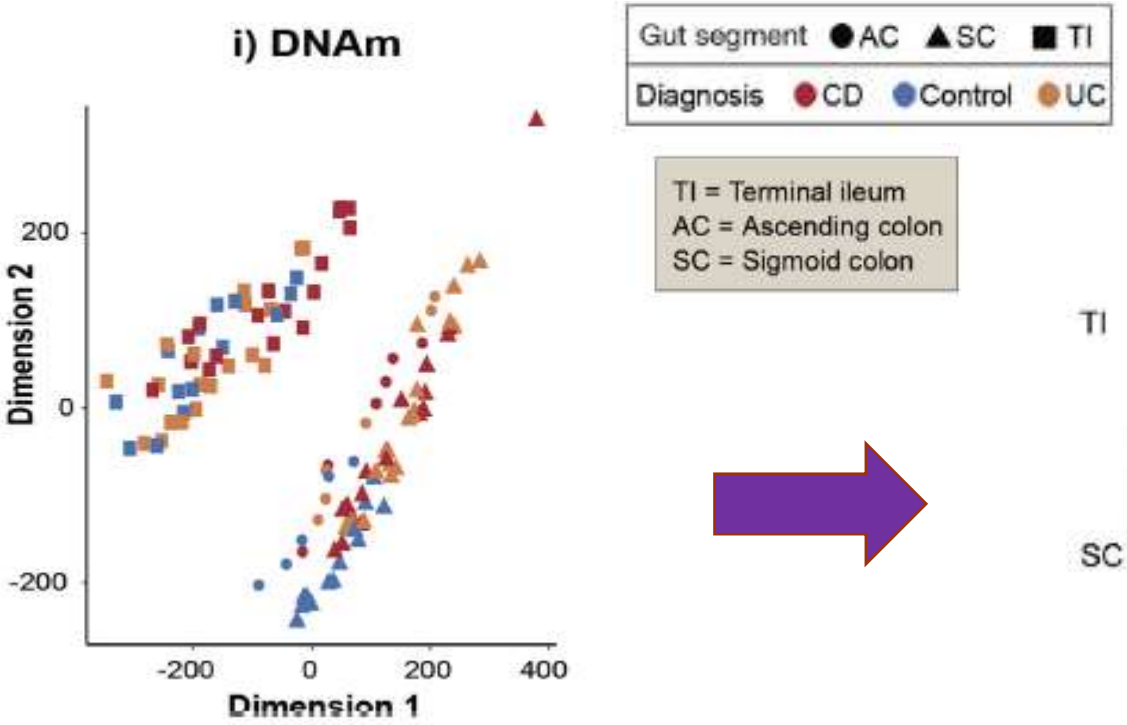
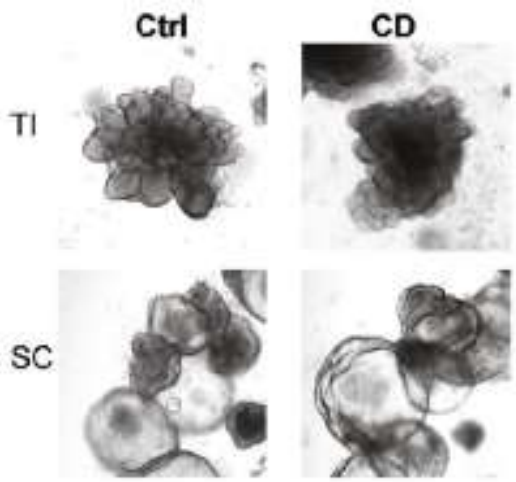
- Disease associated epigenetic alterations in the intestinal epithelium are stable over time and are at least in part retained in ex-vivo organoid cultures;



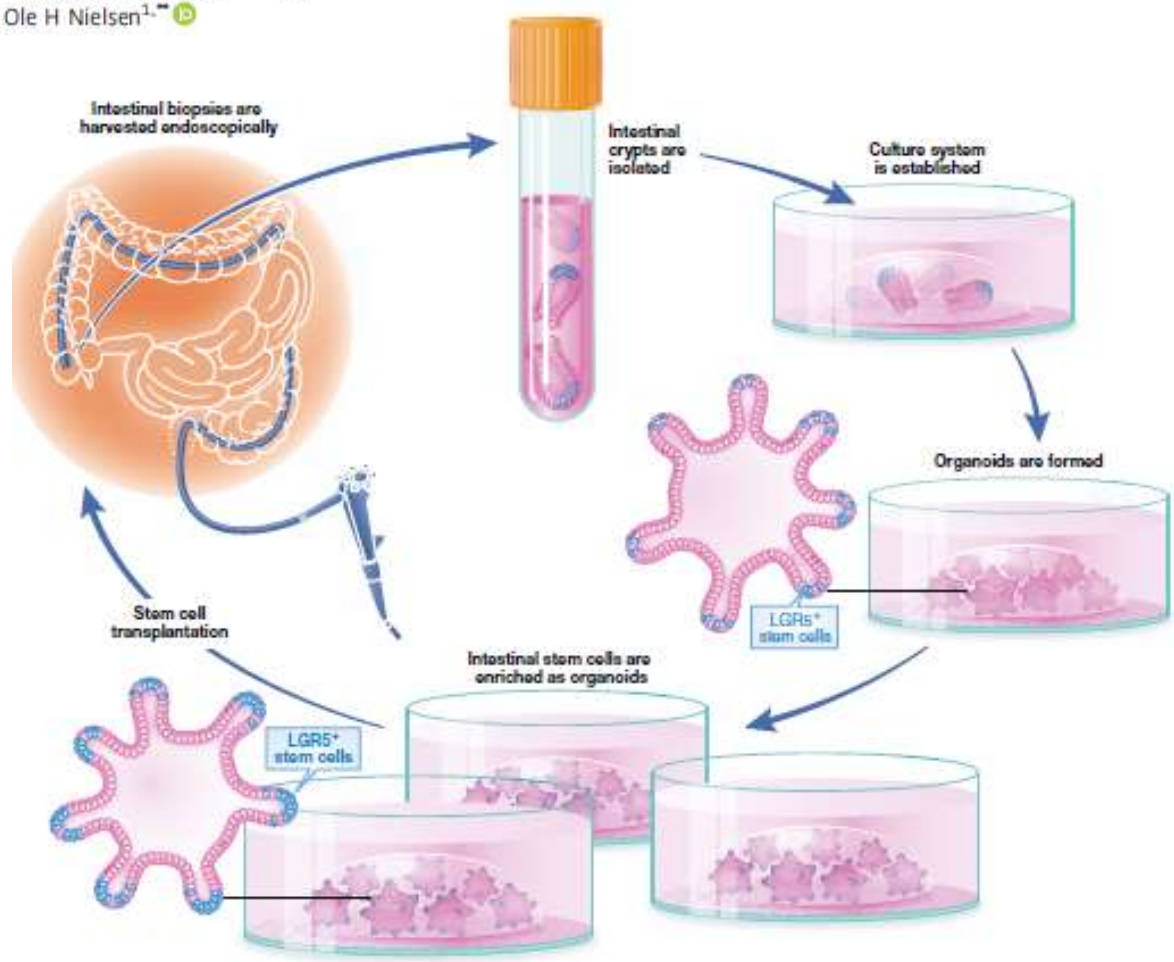


Review

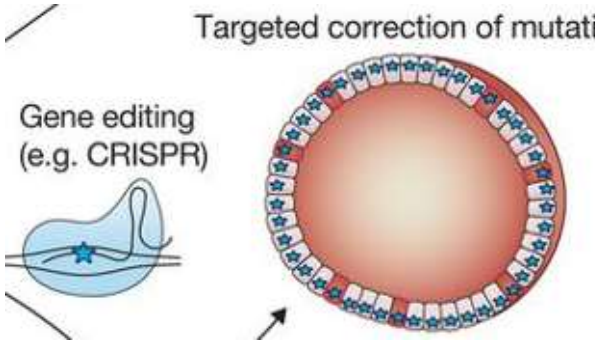
EMBO Molecular Medicine

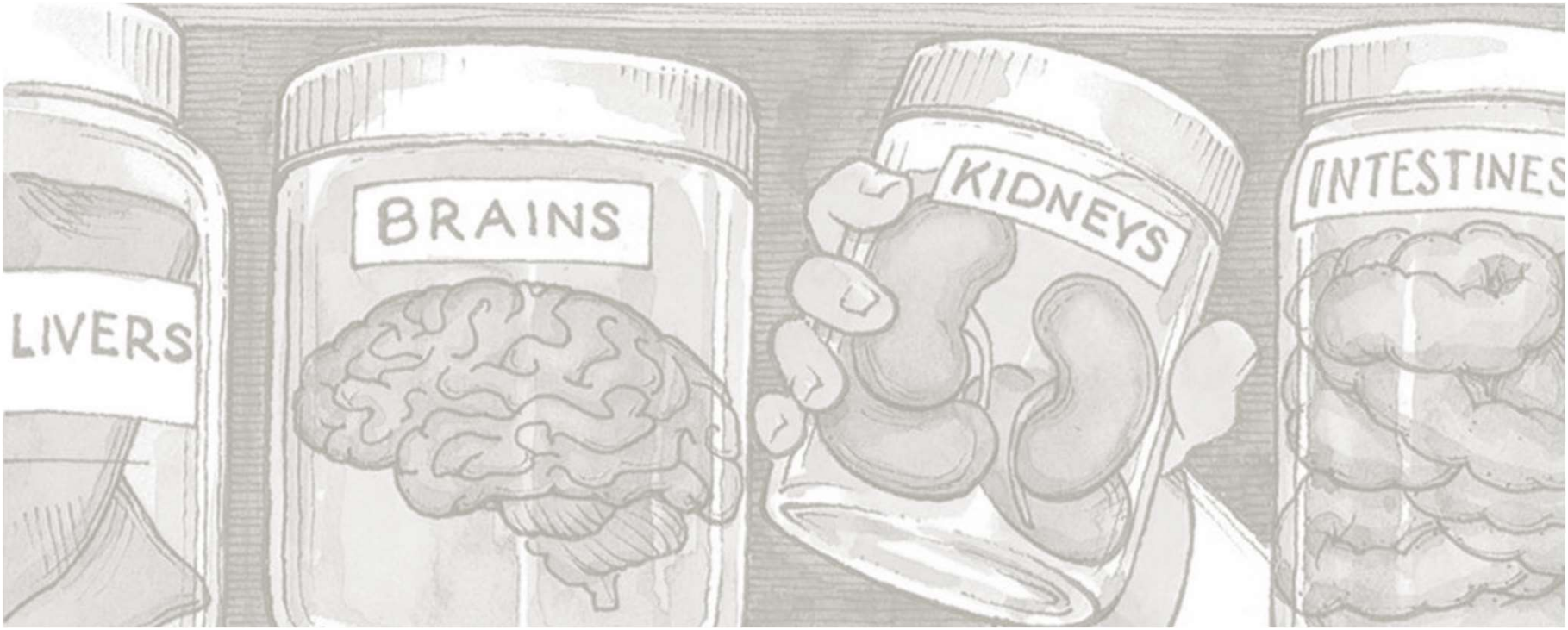
Culturing human intestinal stem cells for regenerative applications in the treatment of inflammatory bowel disease

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,**]



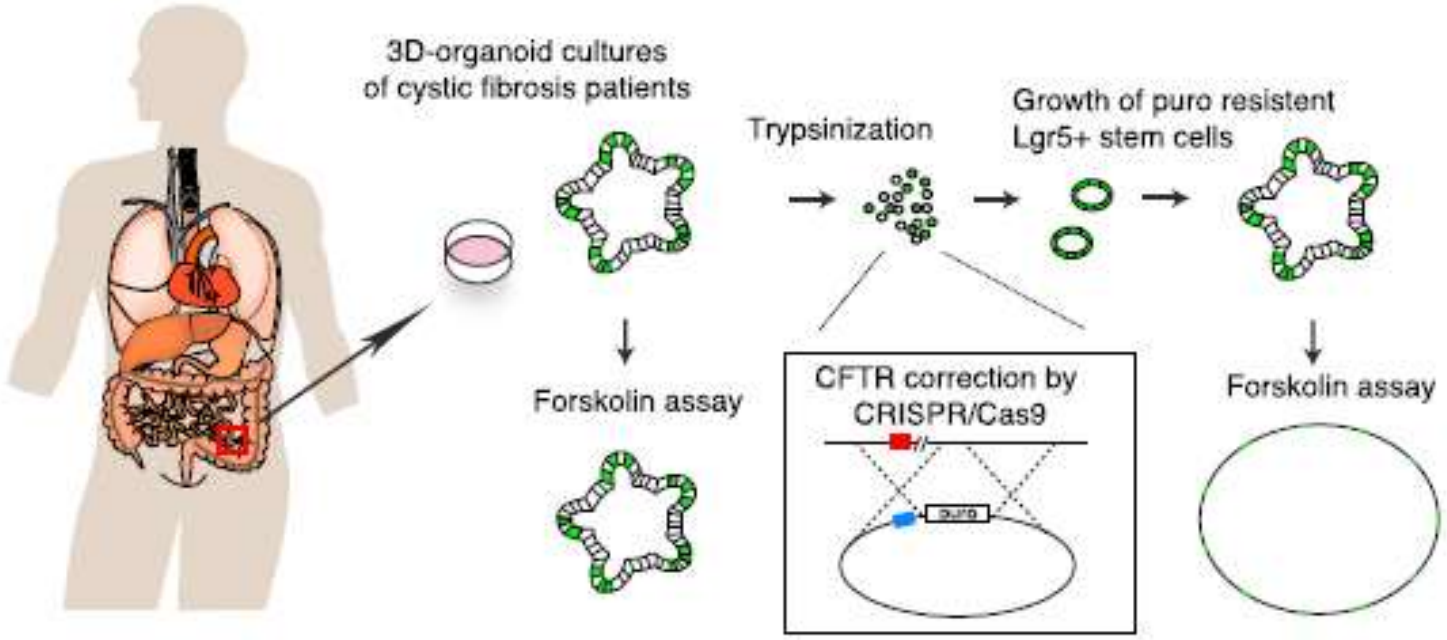
CRISPR/Cas9-Mediated Genome Editing in Adult Stem Cells





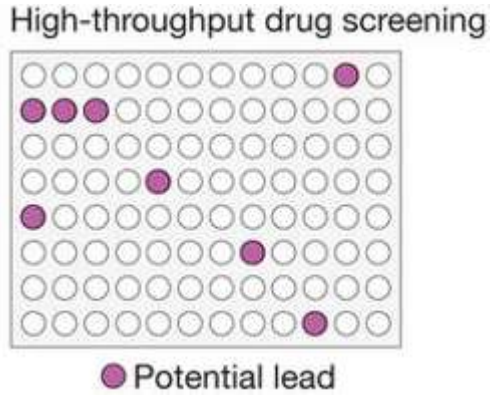
Functional Repair of CFTR by CRISPR/Cas9 in Intestinal Stem Cell Organoids of Cystic Fibrosis Patients

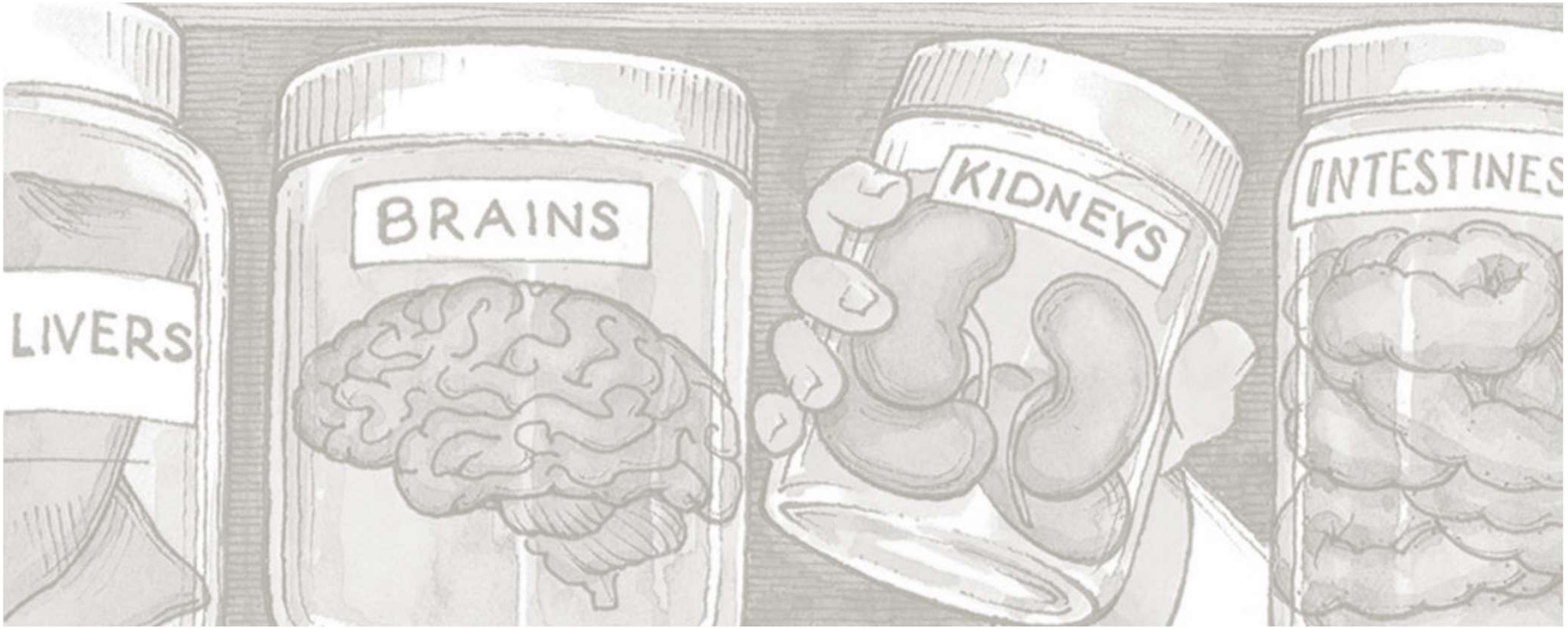
Gerald Schwank,[1,2,7] Bon-Kyoung Koo,[1,2,7,8] Valentina Sasselli,[1,2] Johanna F. Dekkers,[3,4] Inha Heo,[1,2] Turan Demircan,[1] Nobuo Sasaki,[1,2] Sander Boymans,[1] Edwin Cuppen,[1,6] Cornelis K. van der Ent,[3] Edward E.S. Nieuwenhuis,[5] Jeffrey M. Beekman,[5,6] and Hans Clevers[1,2,*]



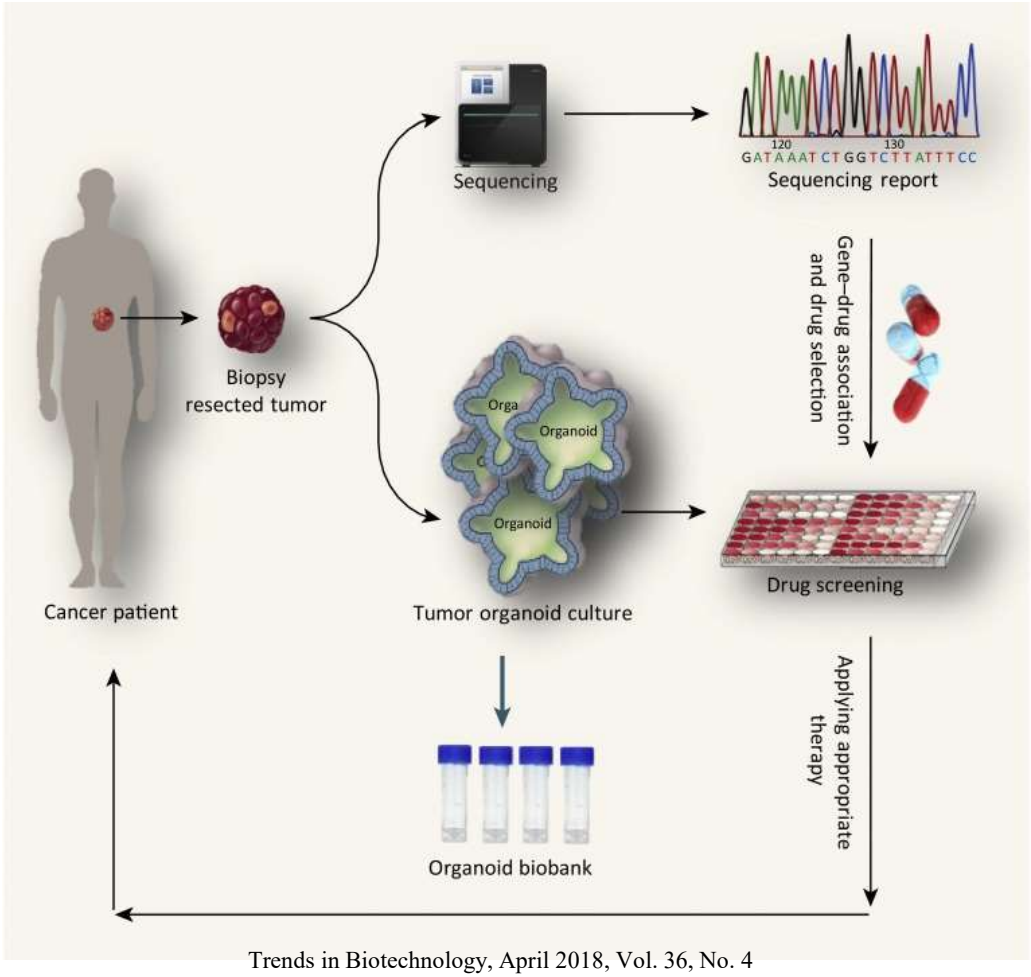
Forskolin induces swelling of organoids from healthy subjects, but the swelling is absent in organoids from patients with CF.

Drug response in patient-derived organoids





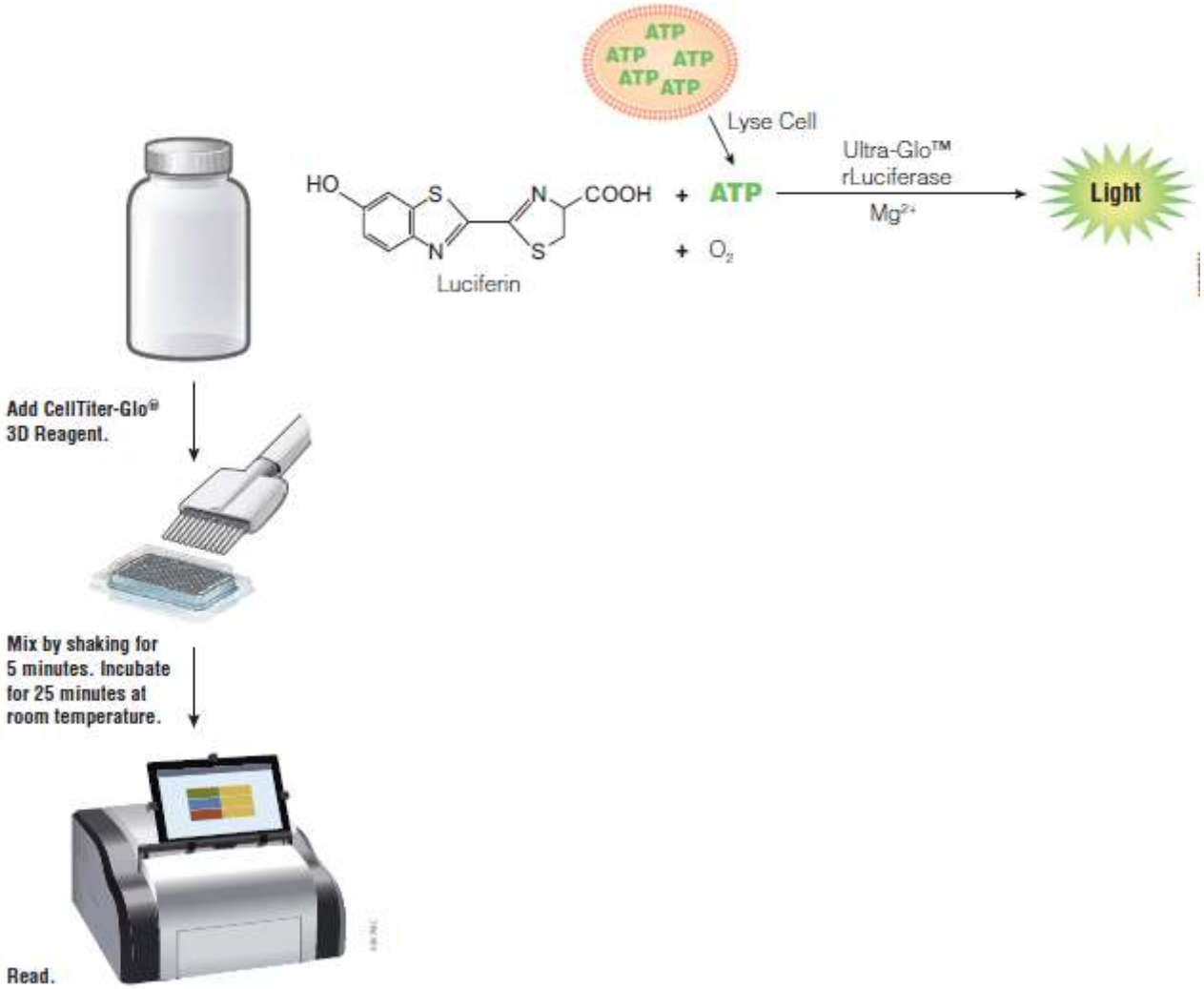
Drug response in patient-derived organoids



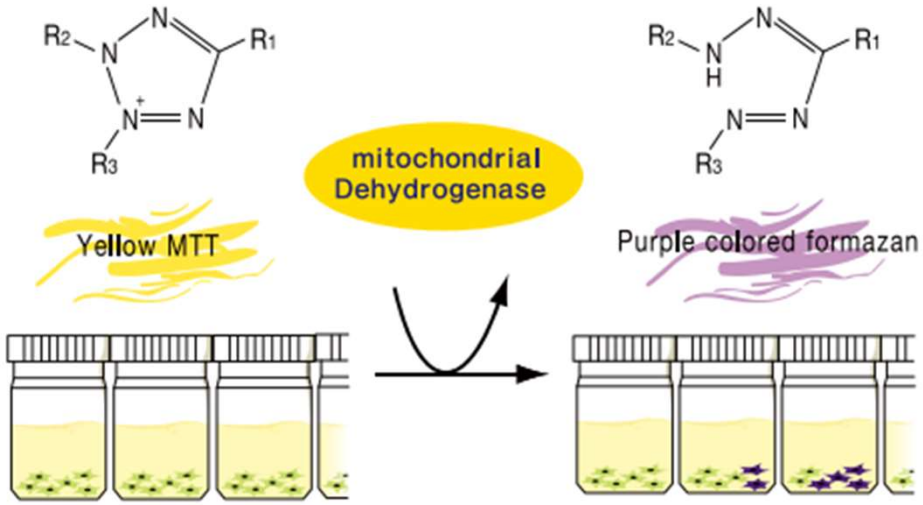
Trends in Biotechnology, April 2018, Vol. 36, No. 4

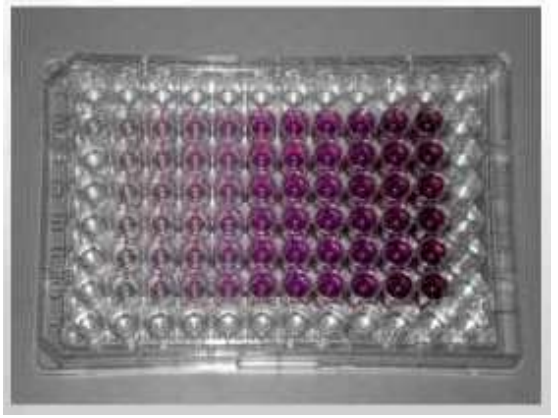
In this approach, the procedure begins with sequencing tumor biopsies or dissected samples by using the next-generation sequencing method and continues with culturing patient-derived tumor organoids, which will be histologically and pathologically compared with the primary tumors before they are subjected 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

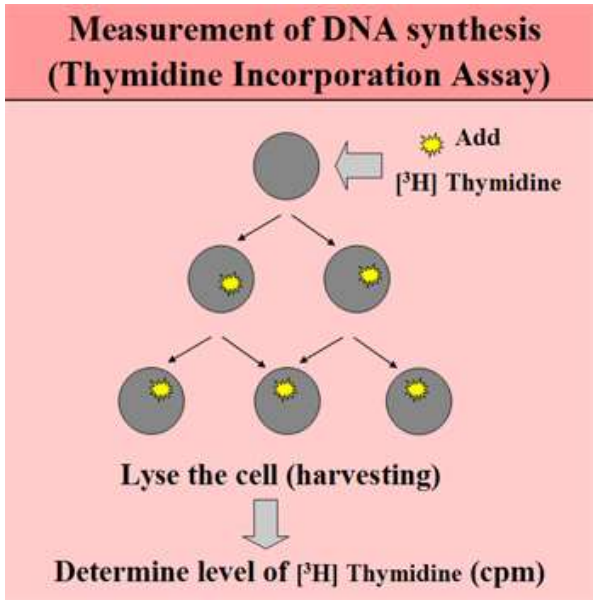


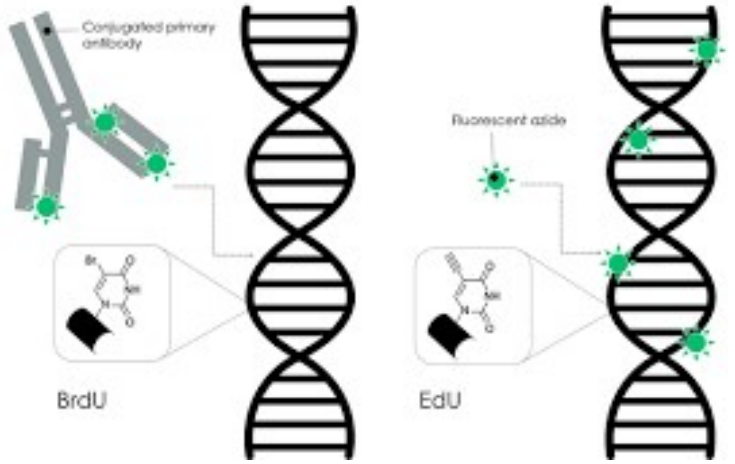
Cell Viability Assay: MTT Reagent





Cell Viability Assay: Thymidine incorporation, BrdU and EdU



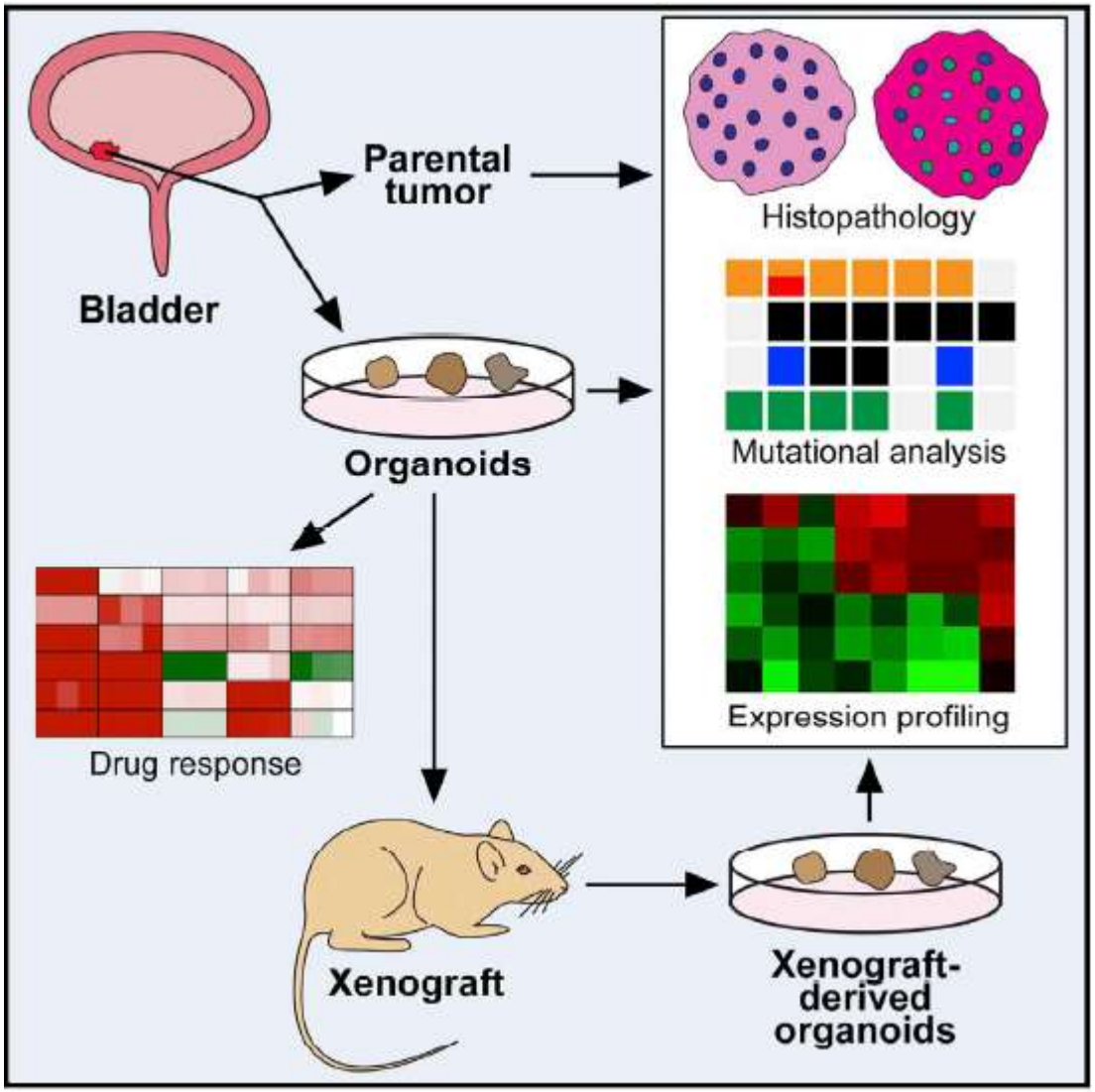


Tumor Evolution and Drug Response in Patient-Derived Organoid Models of Bladder Cancer

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.

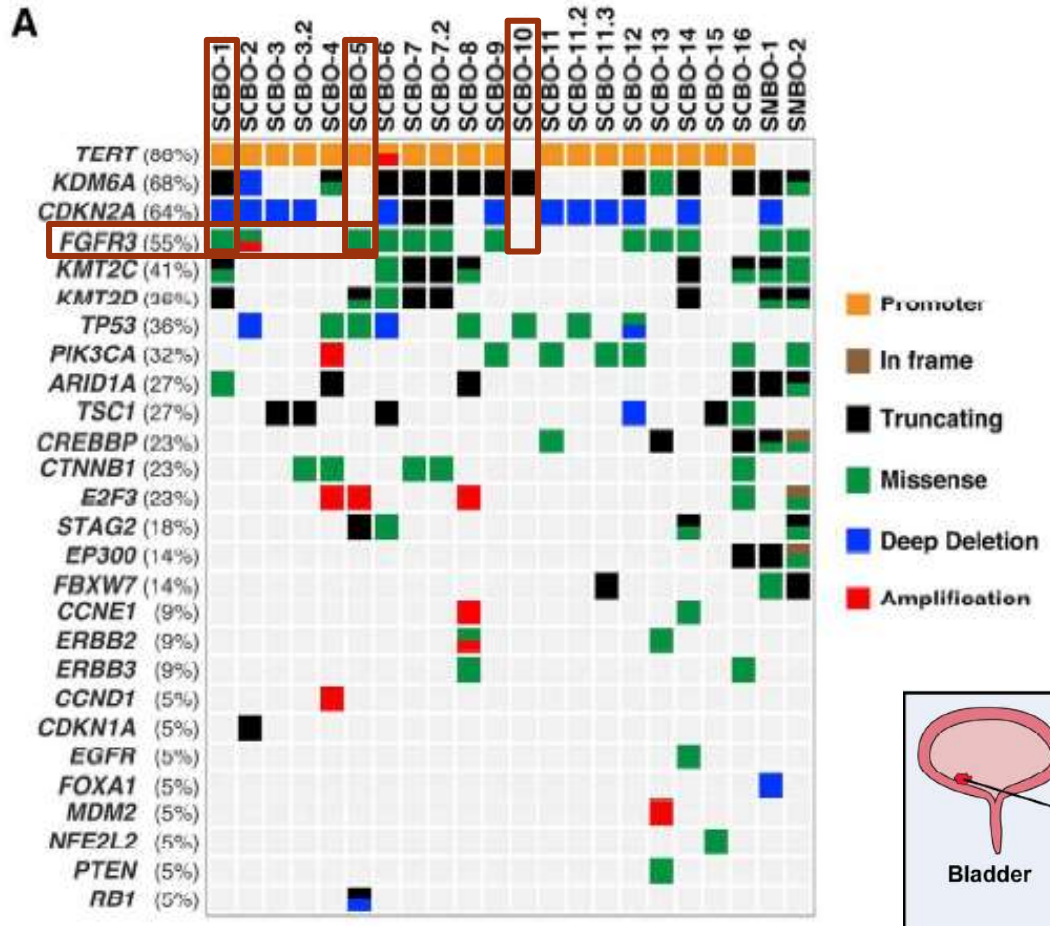


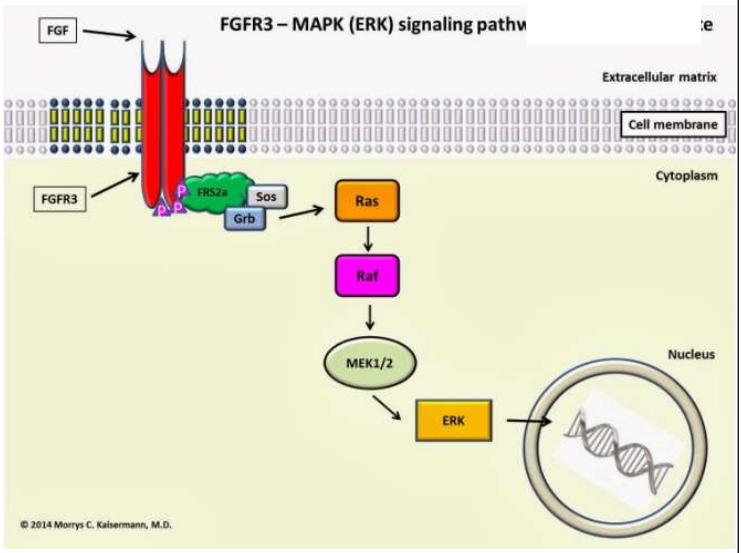
Tumor Evolution and Drug Response in Patient-Derived Organoid Models of Bladder Cancer

Lee et al., 2018, Cell *173*, 515–528

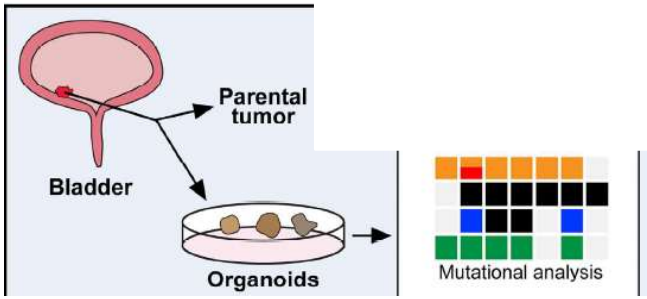
CellPress

PRESENCE OF ACTIVATING MUTATIONS IN **FGFR3**





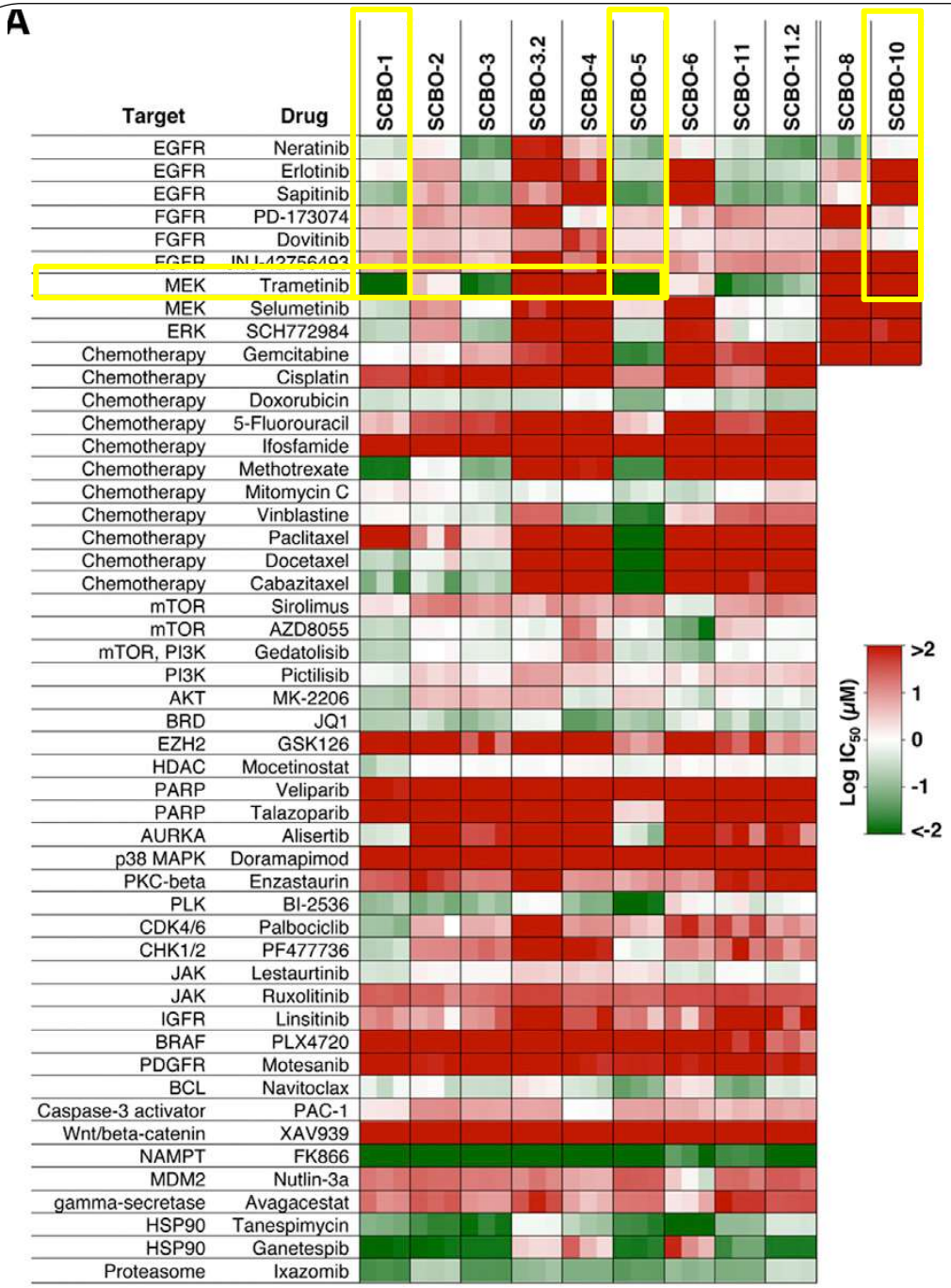
Proliferation

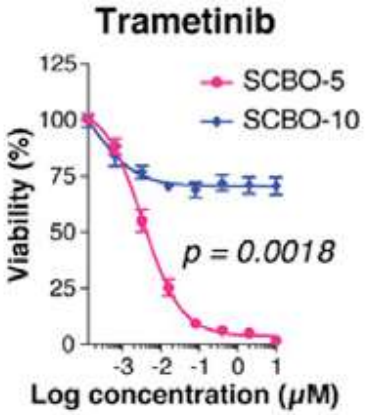


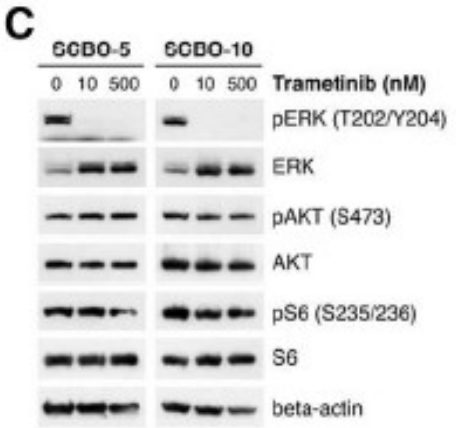
Tumor Evolution and Drug Response in Patient-Derived Organoid Models of Bladder Cancer

Lee et al., 2018, Cell *173*, 515–528

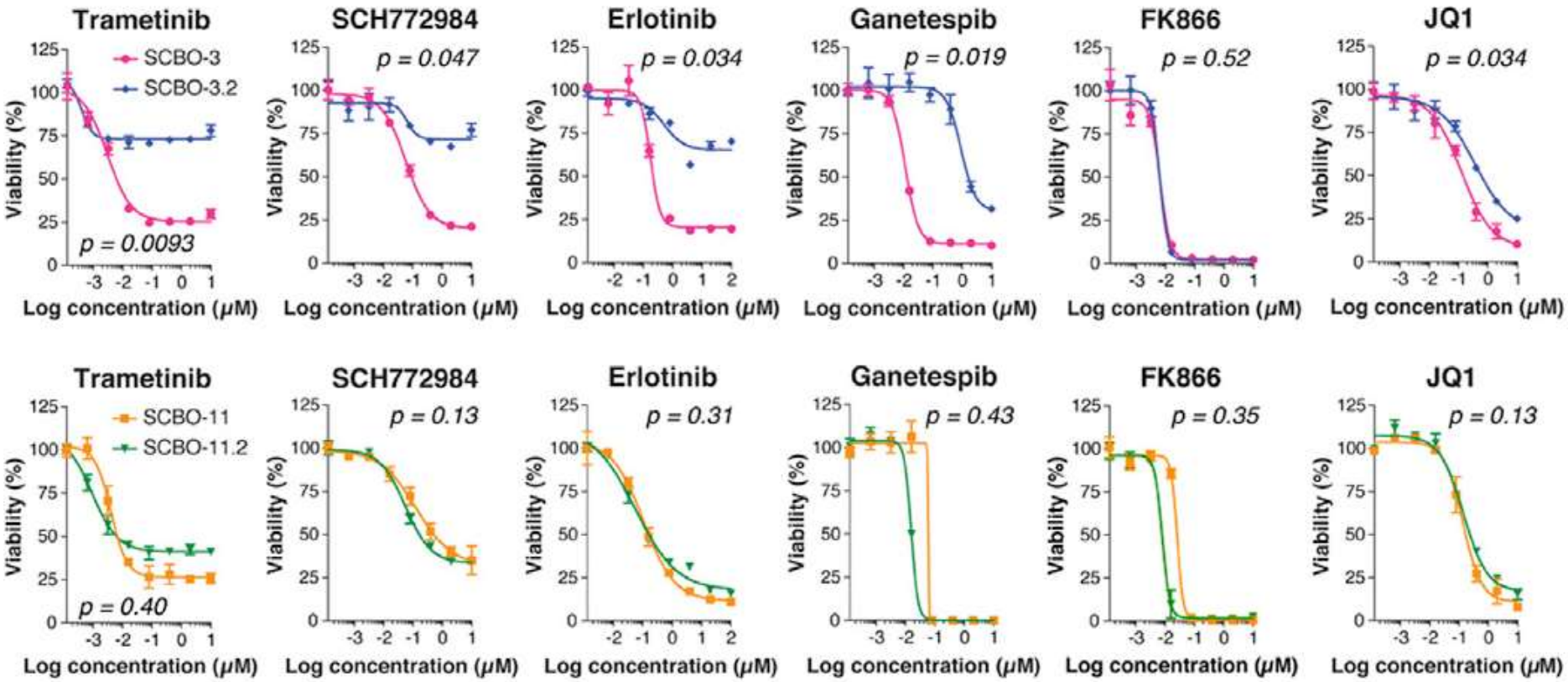
Effects of 40 compounds (standard therapies and agents being tested in clinical trials)







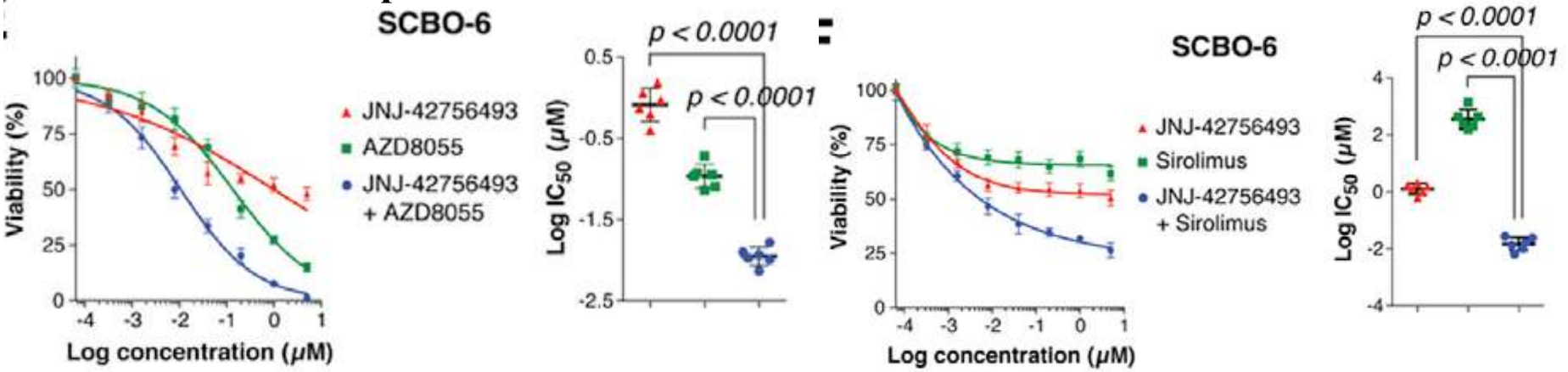
DRUG RESPONSE IN PATIENTS WITH RECURRENT BLADDER CANCER



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.

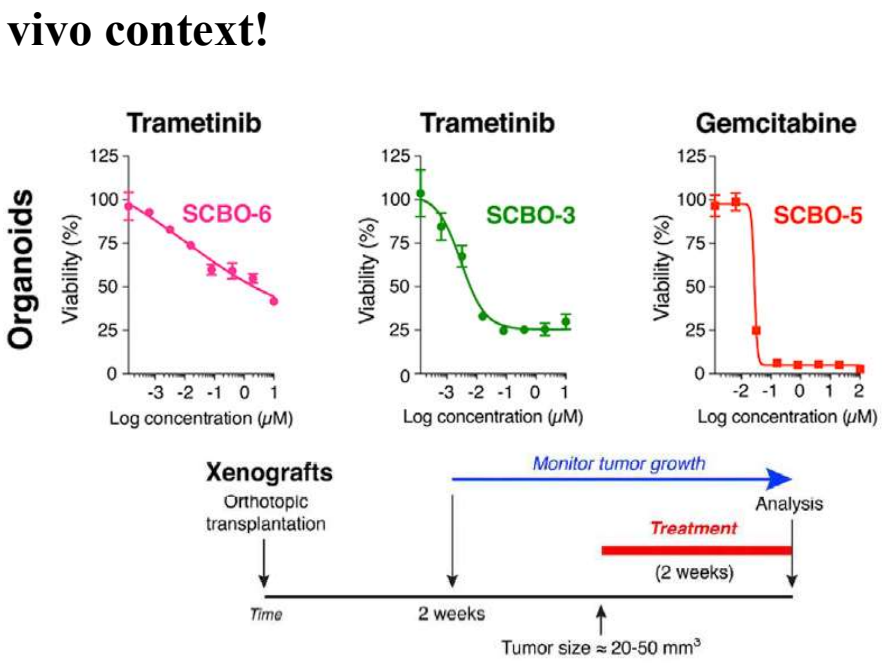
Lee et al., 2018, Cell *173*, 515–528

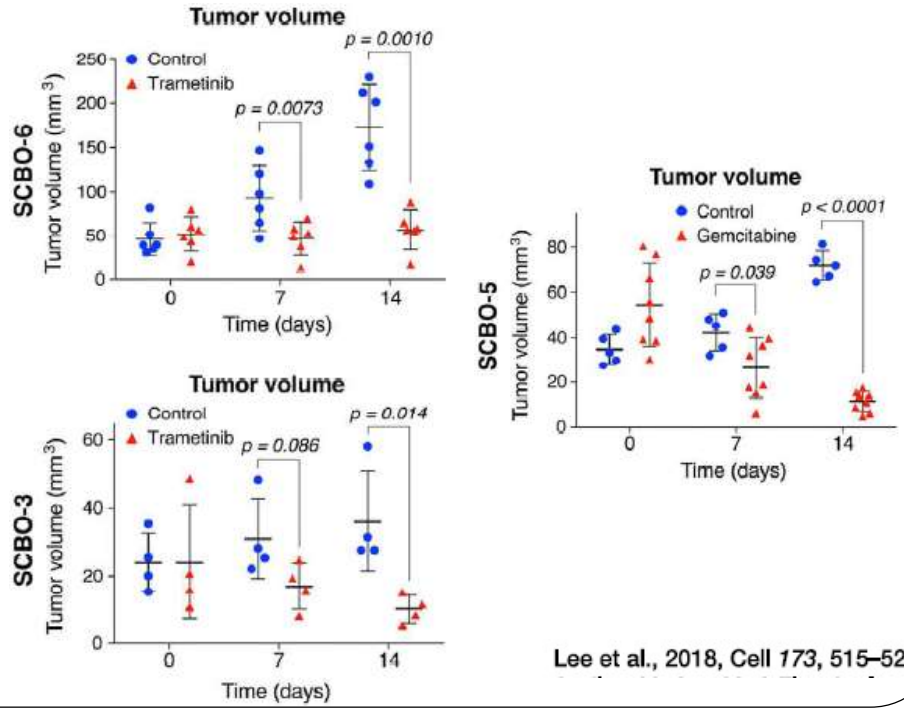
The molecular profiles of the organoid lines can be useful for identification of potential combinatorial therapies!



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!

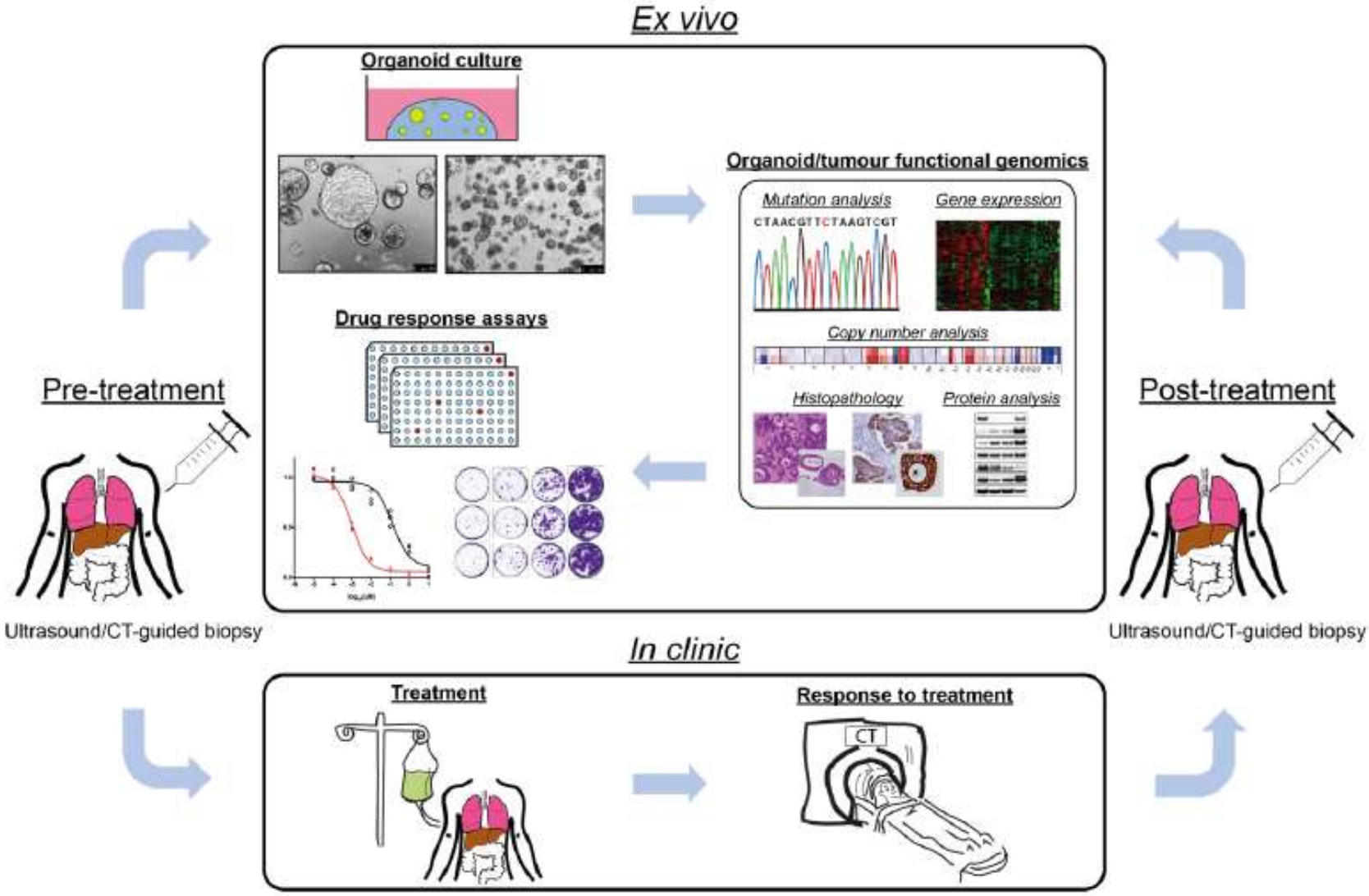




Lee et al., 2018, Cell *173*, 515–528

Patient-derived organoids model treatment response of metastatic gastrointestinal cancers

Vlachogiannis et al. *Science*. 2018



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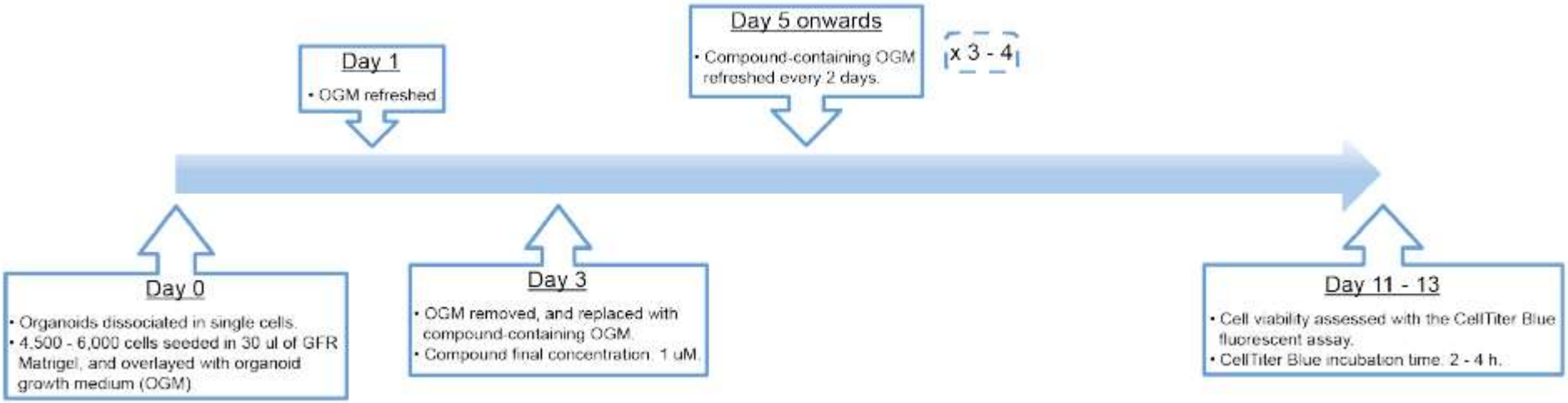

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		Nilotinib	Crizotinib	XL147	Afatinib	Erlotinib HCl	AUY922	NVP-TAE684	Everolimus	Bortezomib	Gefitinib	
C		Brivanib	DMSO	Imatinib Mesylate	Olaparib	SGX-523	Sorafenib Tosylate	GSK690693	Saracatinib	Selumetinib	MK-2206	
D		OSI-906 (Linsitinib)	Palbociclib	Dasatinib	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												

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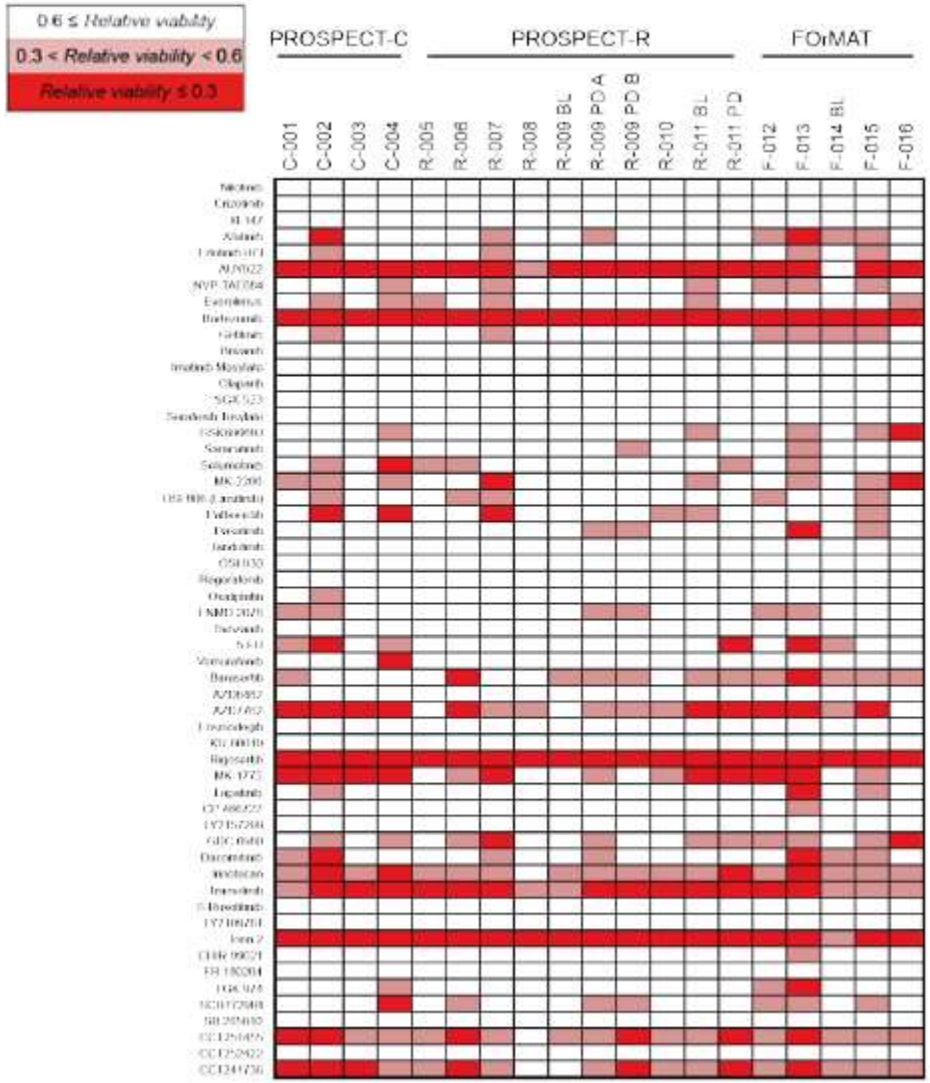

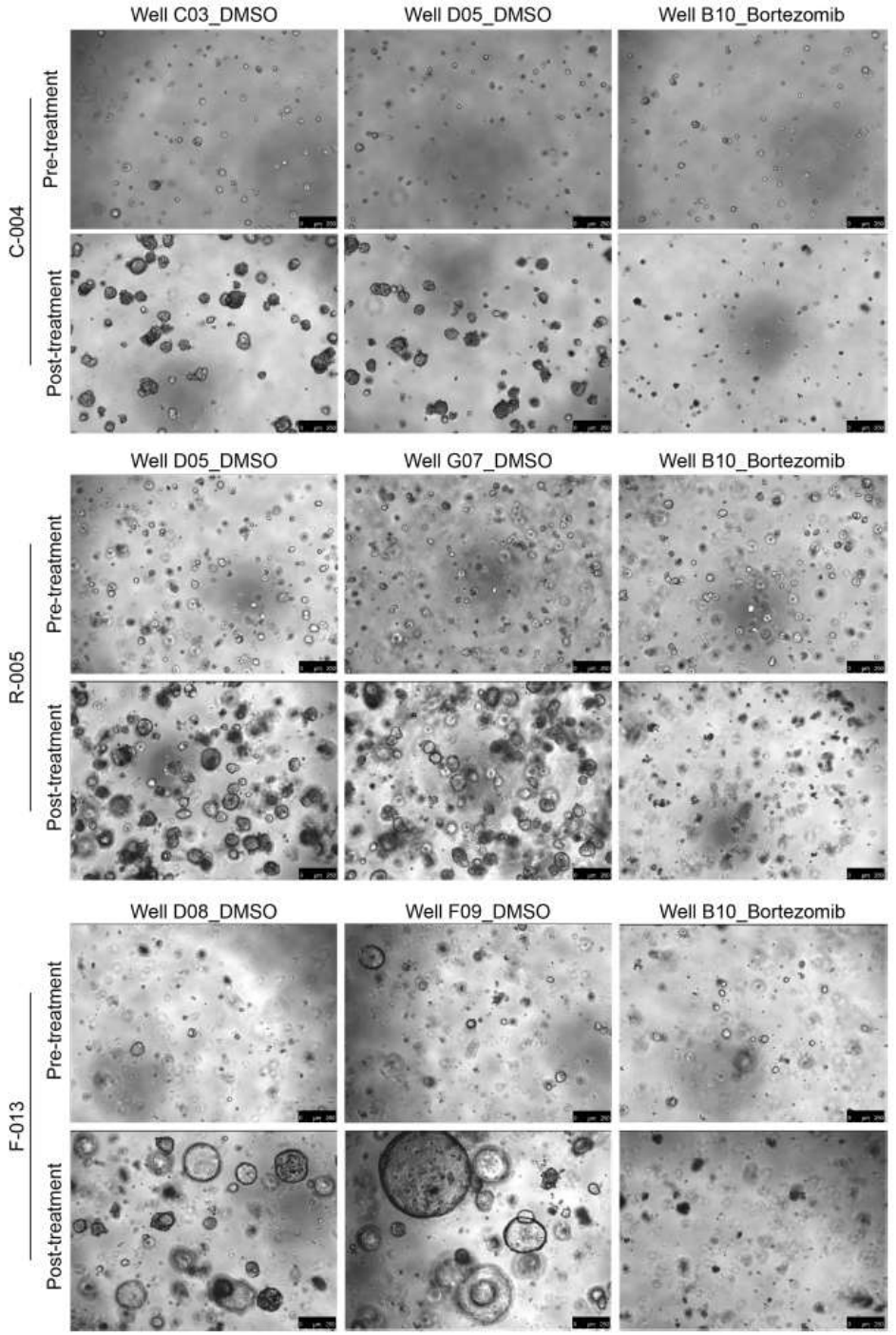

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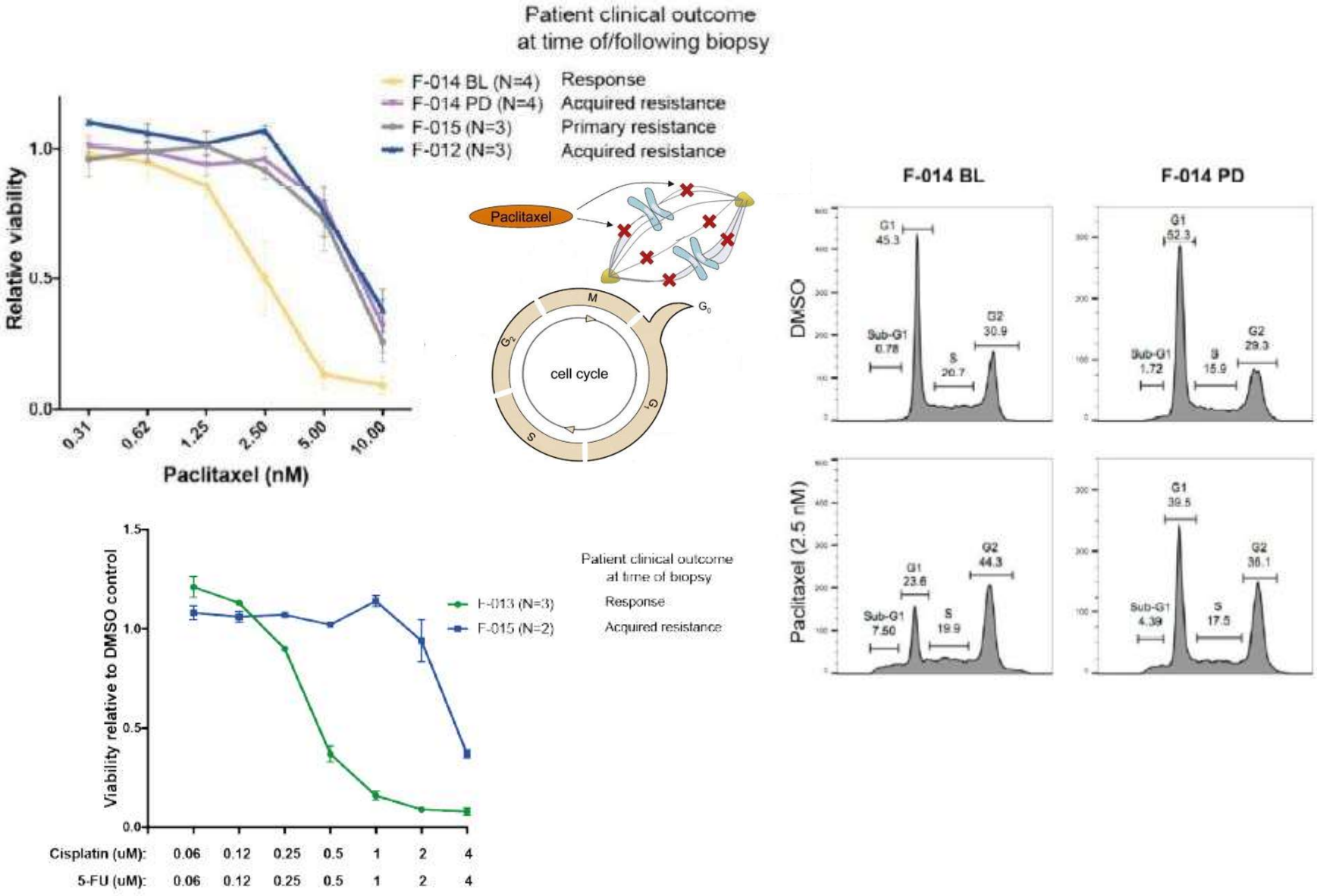

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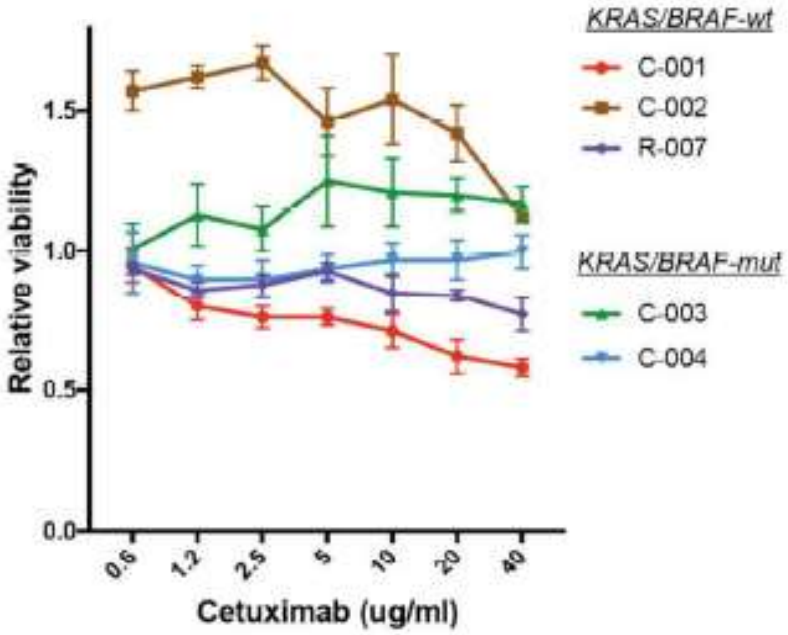

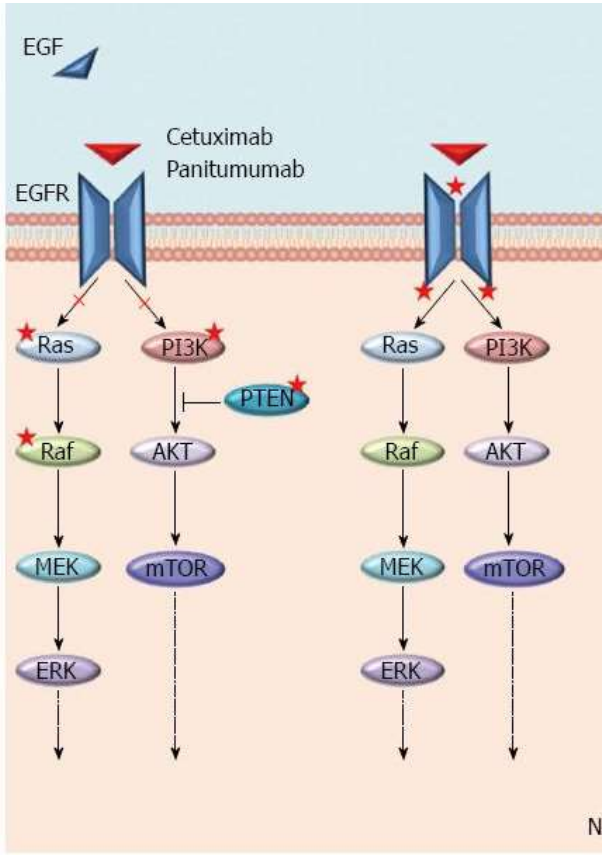

ORGAN-ON-CHIP