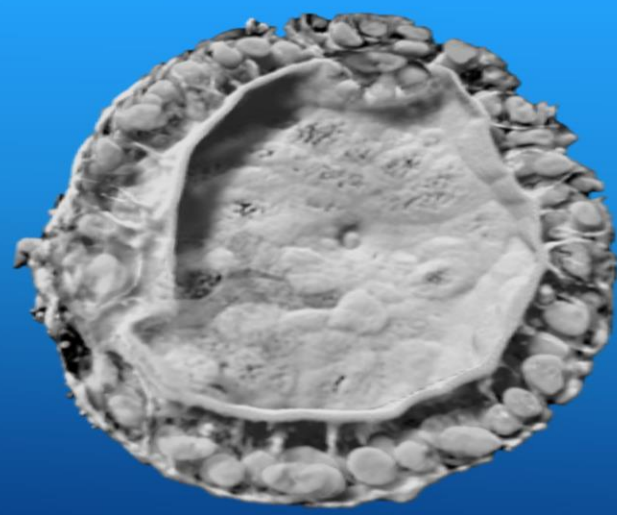
3D intestinal organoid model



Audrey FERRAND, PhD
Head of the Organoid core facility
Group: Intestinal Stem Cells & Associated Pathologies
Team: Pathophysiology of the intestinal epithelium
Institut de Recherche en Santé Digestive (IRSD)
INSERM U.1220, INRA UMR1416, ENVT, UT3
Toulouse, France

What is an organoid ?

an artificially grown mass of cells or tissue that resembles an organ

The ability to grow human tissues from stem cells in 3D culture has the potential to revolutionize the drug discovery process and regenerative medicine.

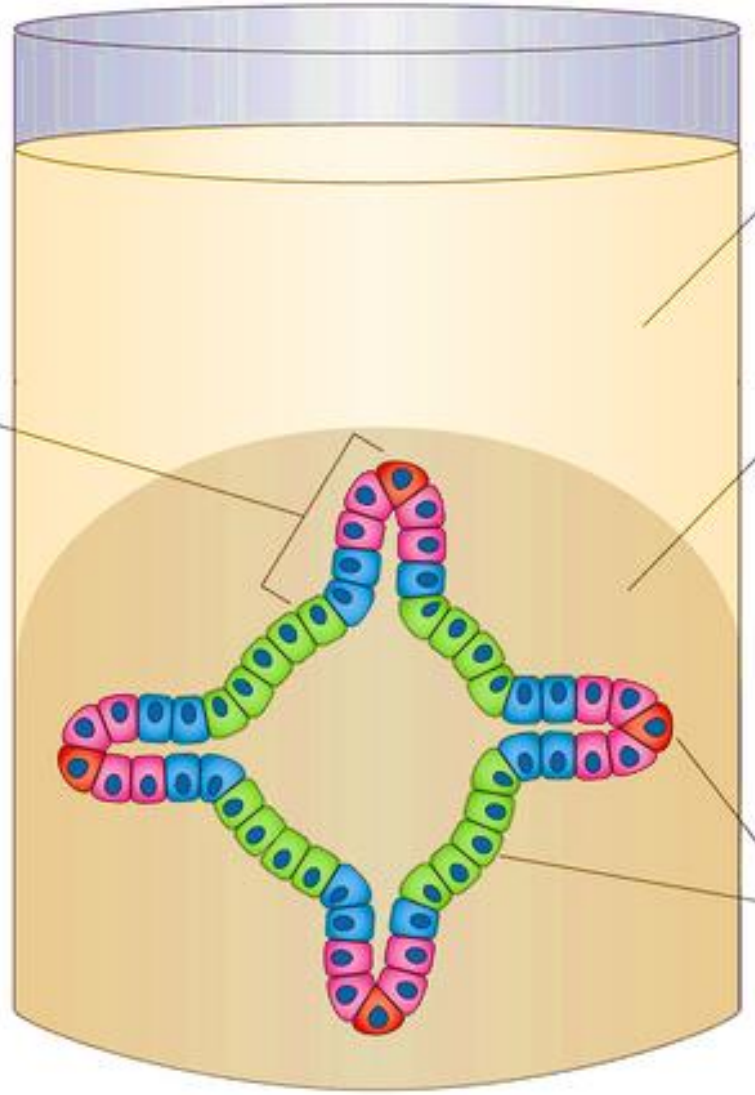
3D organoid models

Organotypic
Organoids resemble the *in vivo* situation as closely as possible

Multiple cell types
Organoids consist of multiple cells and contain at least two cell types

Self-organization
Organoids spontaneously assemble into ordered structures and do not require preformed patterns

3D organization
Maintains cell differentiation and enables expansion of progenitor cells



Growth factors
Selected on the basis of their role(s) in health and disease or their effect in 2D cultures

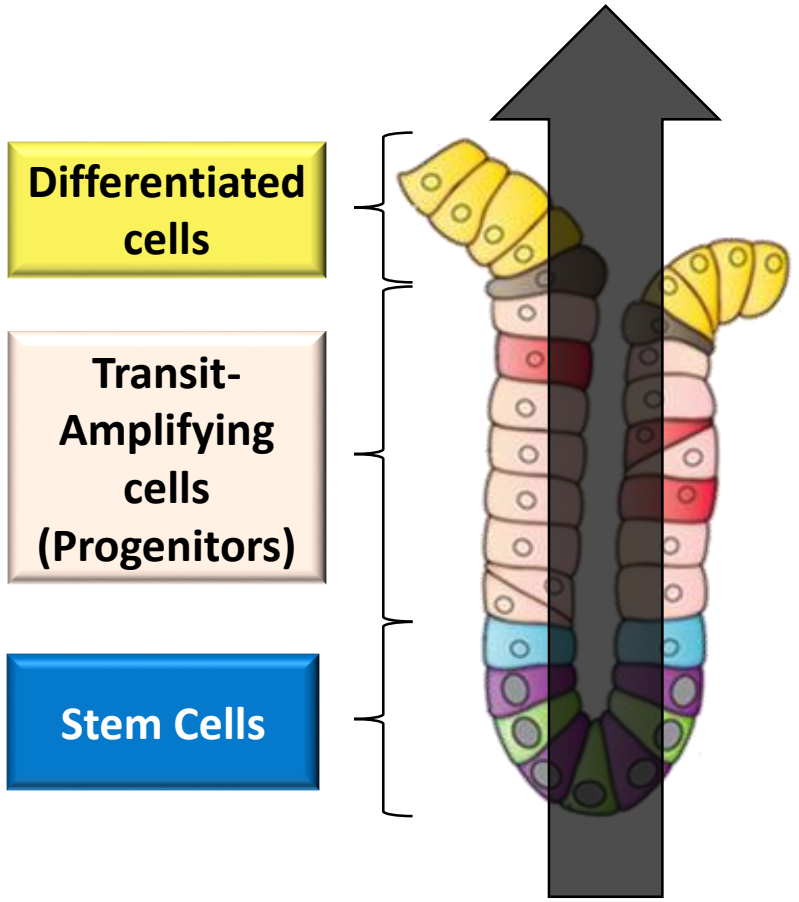
Extracellular matrix
Provides cues for differentiation and cell orientation, but does not limit self-organization by enforcing an architecture

Adult organ derived
Enhances clinical applicability and enables patient-specific study of disease and personalized medicine

Stem or progenitor cells
Required for long-term expansion of organoids

Nature Reviews | Nephrology

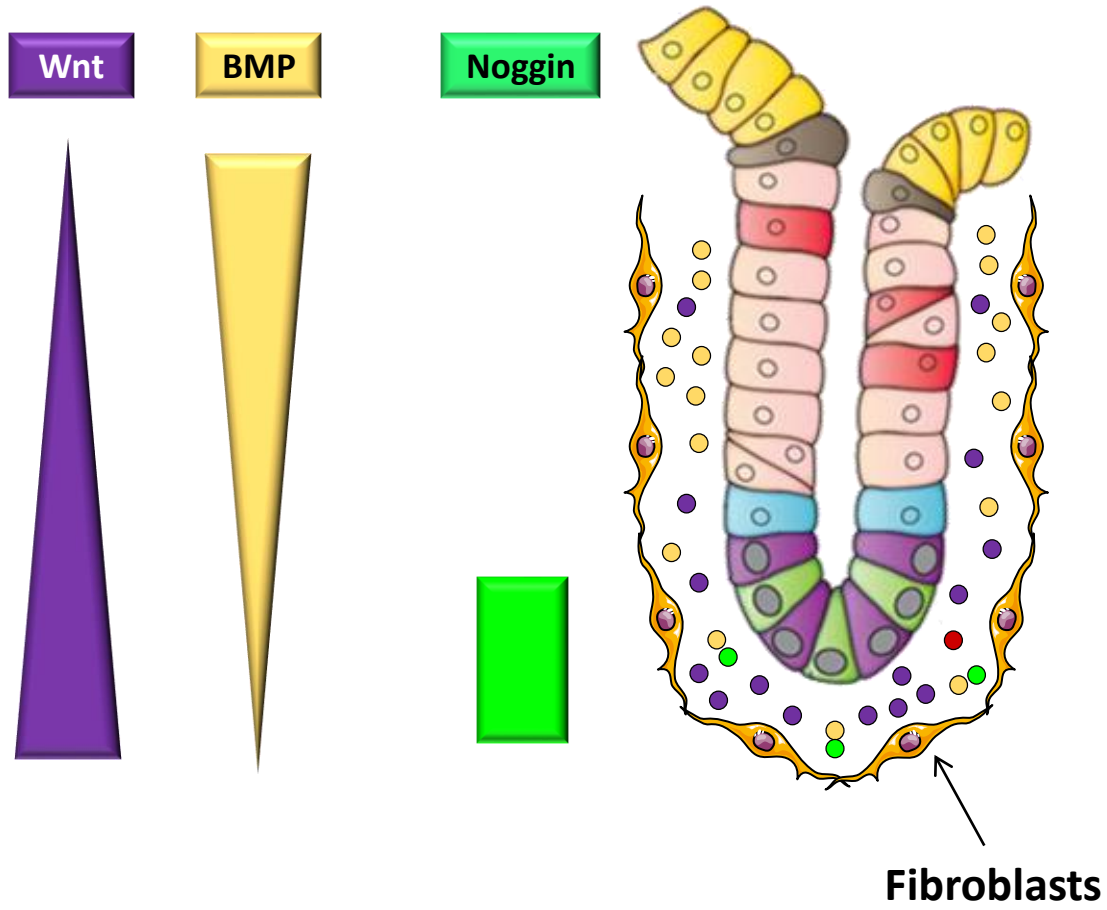
Intestinal epithelium renewal – Intestinal Stem Cells



DIFFERENTIATION

Yan, et al., PNAS 2012

Intestinal epithelium renewal – Intestinal Stem Cells

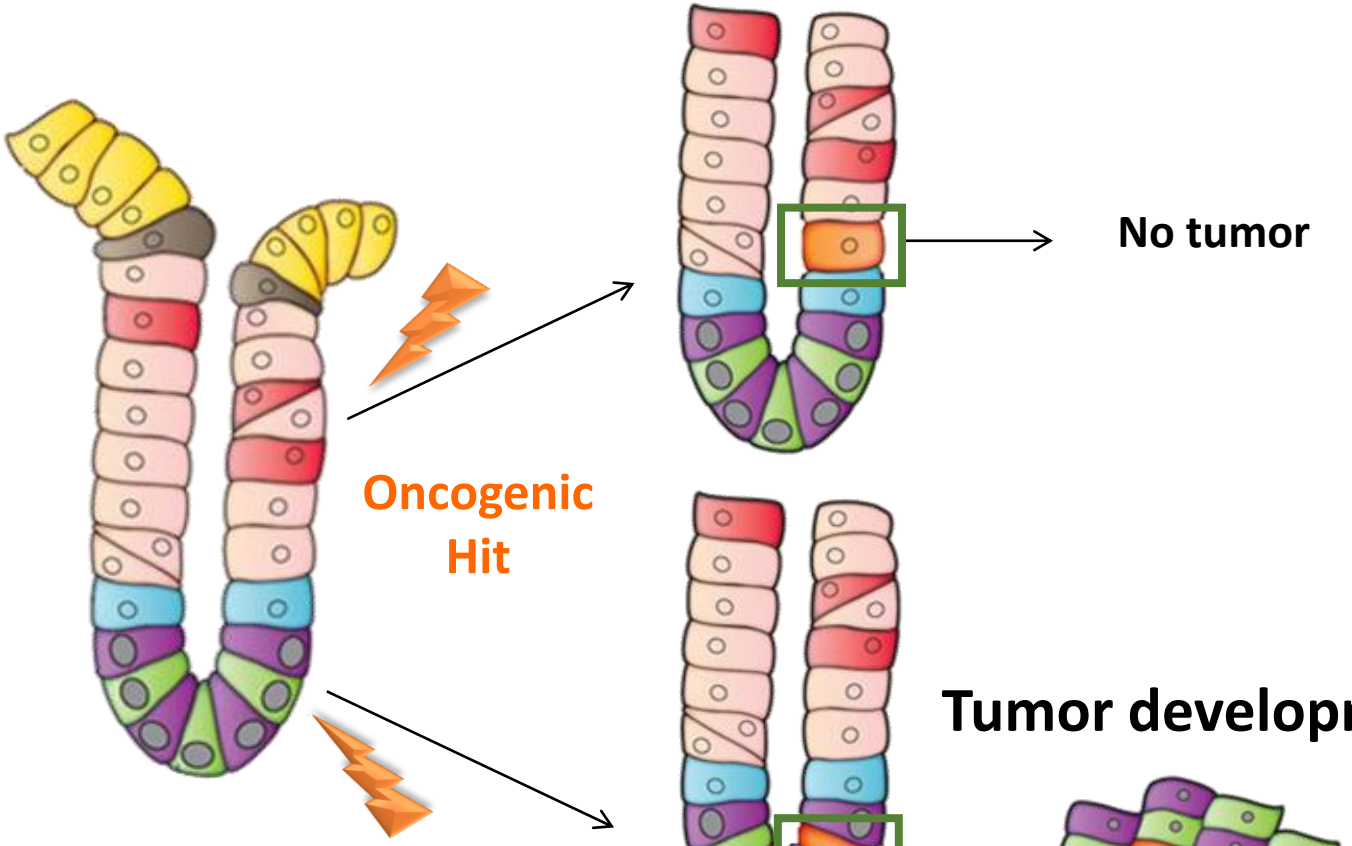


Differentiation

Self Renewal
Proliferation

Yan, et al., PNAS 2012

Intestinal crypt : origin of the cancer stem cells



No tumor

Oncogenic Hit

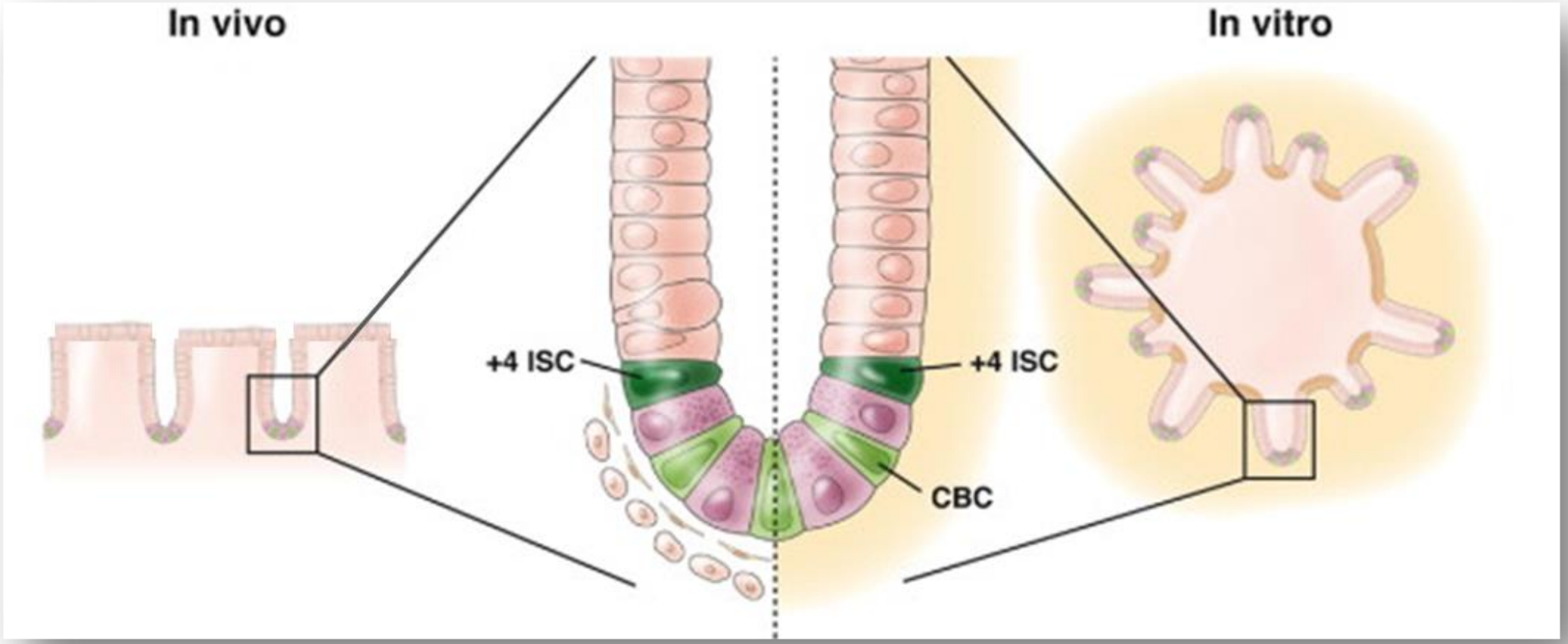
Tumor development => Adenocarcinoma (cancer)

Normal Stem Cells ⟹ Cancer Stem Cells

Barker et al., Nature 2009

The 3D colon organoid model

Adapted from Barker, Nature 2014



The 3D colon organoid model

Adapted from Barker, Nature 2014

Hôpitaux de Toulouse

OrganoCan consortium

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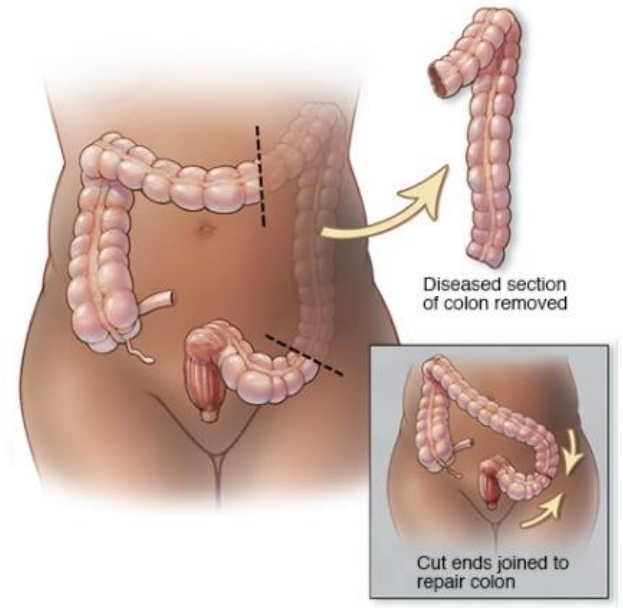
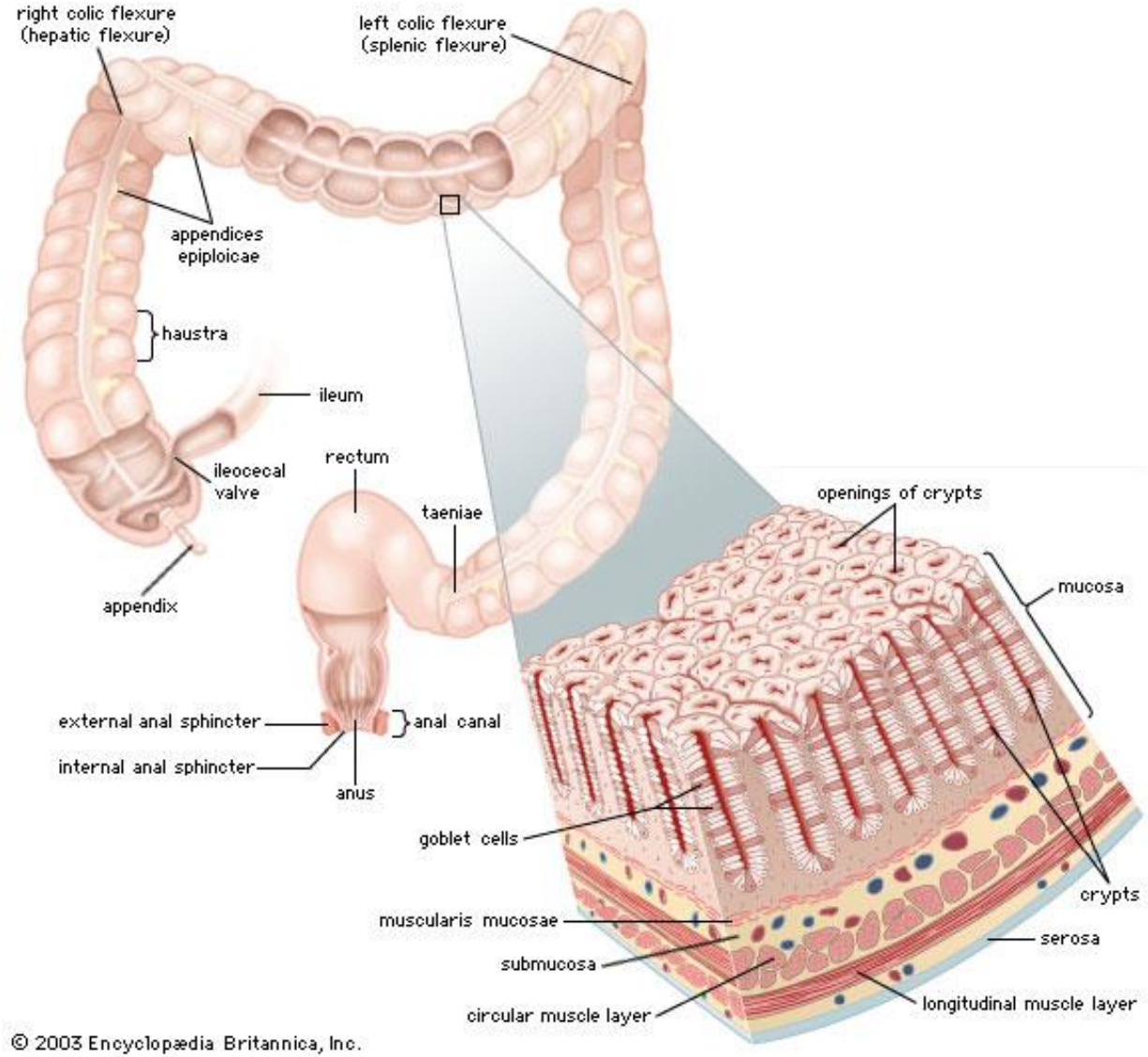
Patients
(IBD, CRC,…)

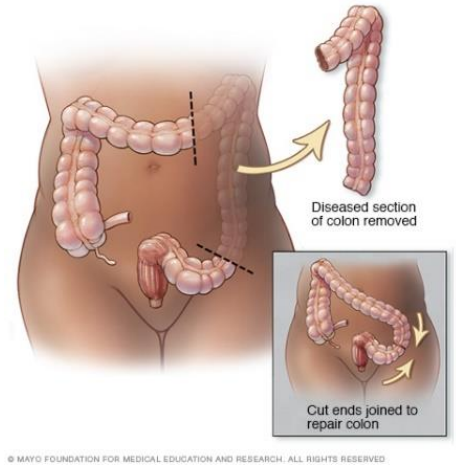
Resections, biopsies





The 3D colon organoid model

1) Colorectal resection / biopsie

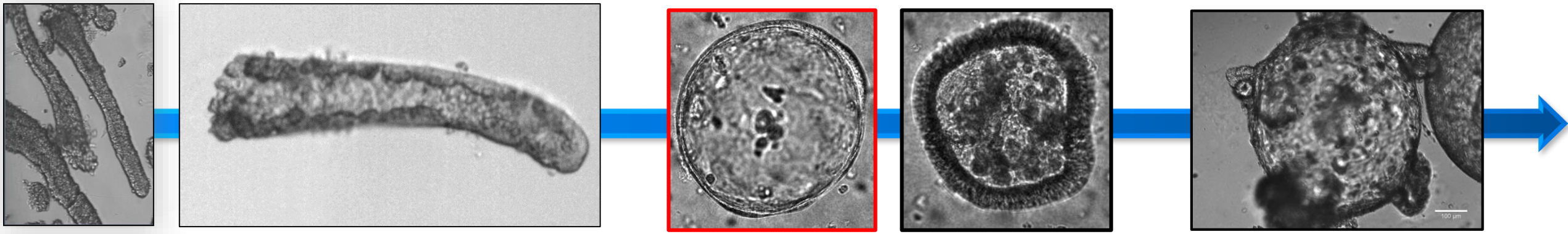


2) Crypt extraction

3) Plating in matrigel

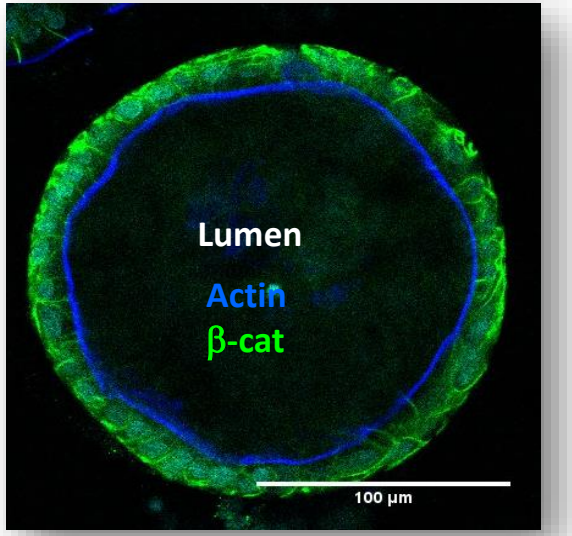
Specific medium
(Wnt3A, Noggin, R-spondin, EGF…)

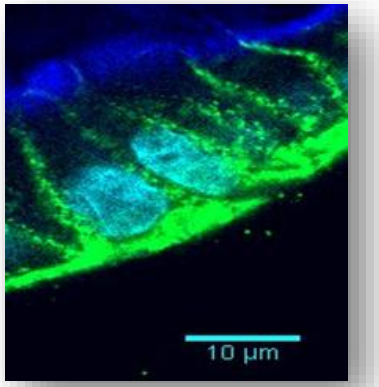
Day 1 — **From day Day 3 to 14**

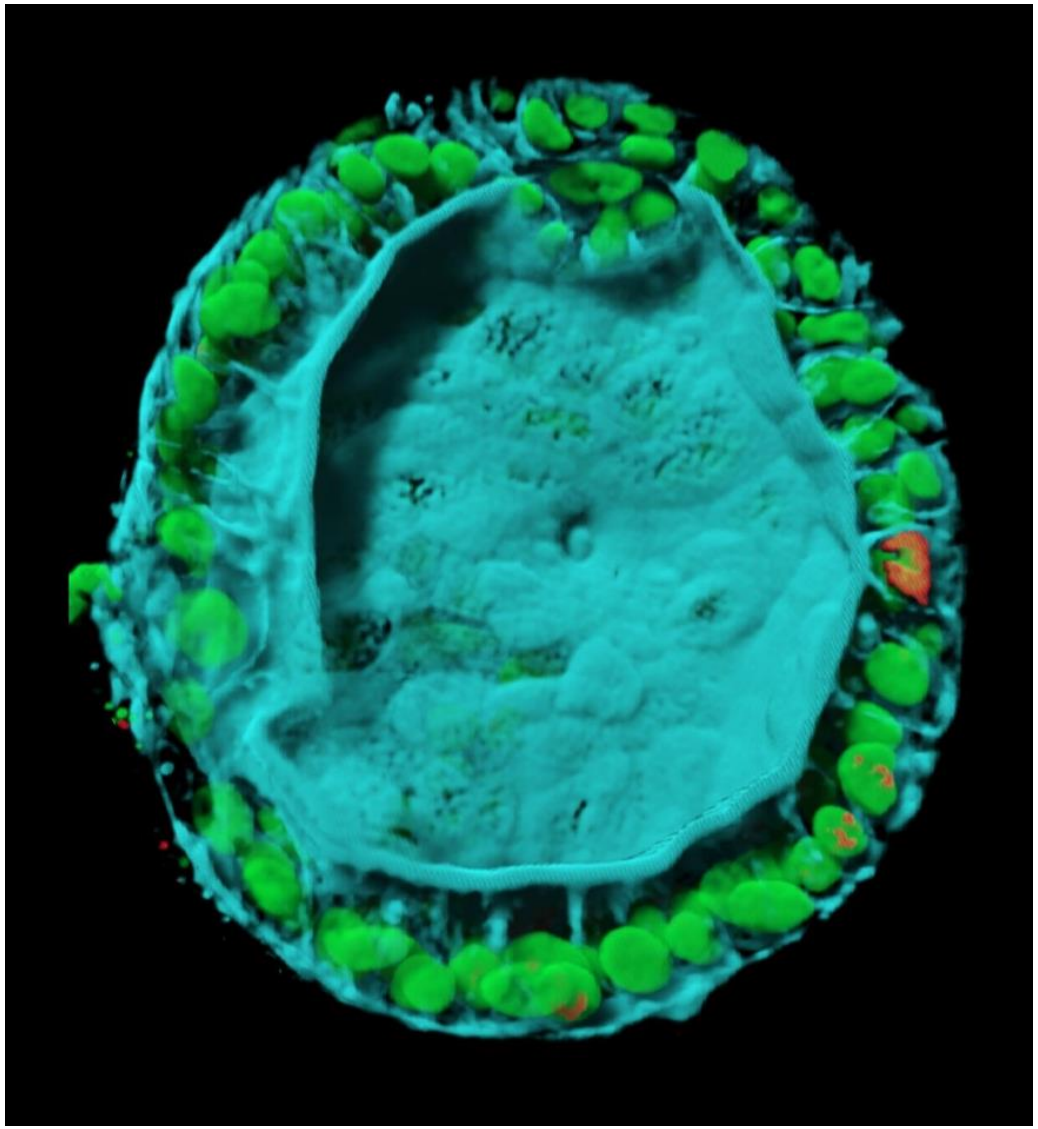


The 3D colon organoid model

1) Is a polarized structure

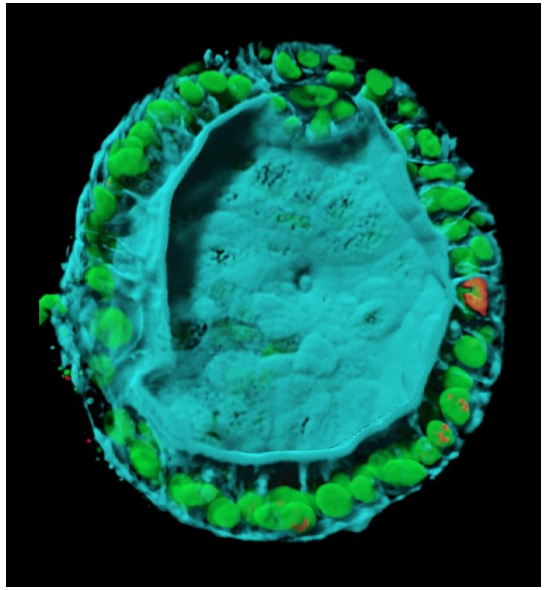


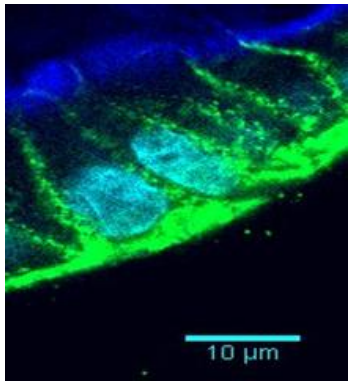




The 3D colon organoid model

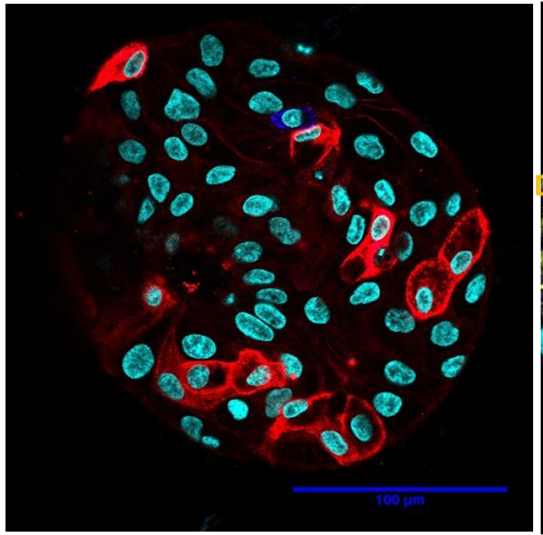
1) Is a polarized structure



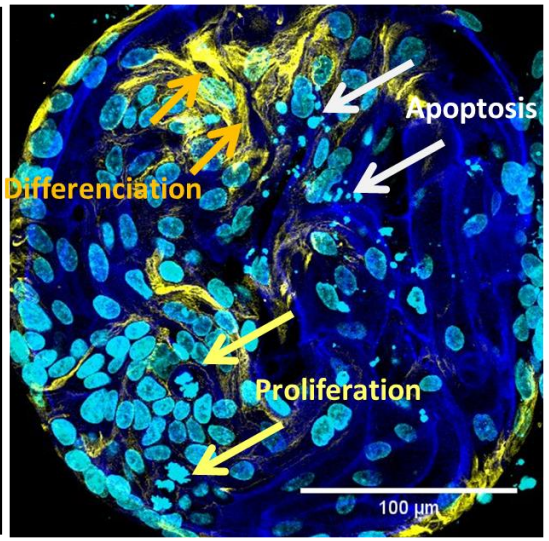


2) Reconstitutes the epithelial cell populations

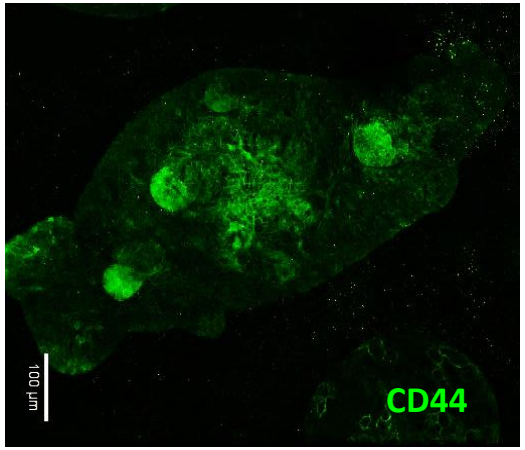
Nuclei
Mucine 2
Chromogranine A



Nuclei
Cytokeratin 20
Actin



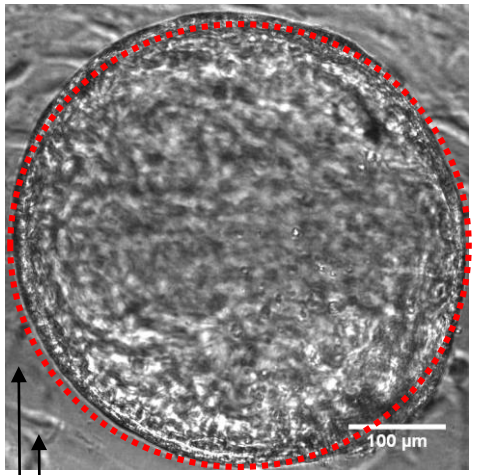
3) Reconstitutes cryptic structures



The 3D colon organoid model

➢ 3D Colorectal Human Organoids can be…

4) Co-cultured with stromal cells (here: fibroblasts)



Colonosphere

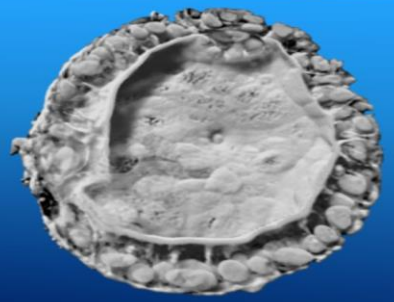
Fibroblasts

5) Be analyzed in terms of RNAs, proteins expressions, secretome…

Sample	66 : Muc2	69 : CyclD1	73 : Notch1	87 : Bcat	90 : GSK3beta
66 : Org36 J0					
67 : Org36 J10 Dif					
68 : Org36 J10 NoDif					
94 : RT-					

6) Frozen, stored and thawed

Organoid Facility



irsd
Institut de Recherche en Santé Digestive

COLIC Biobank
DC-2015-2443

Head - Scientific director

Audrey Ferrand
CR1 INSERM

Facility manager – Technical director

Aude Rubio
IE - CHU

Biobank manager

Muriel Quaranta
IE UT3

Organoid cultures: Intestinal / Colorectal, Bladder, Pancreas…

High Content Screening: Imaging, Analysis, Profiling

PerkinElmer

Healthy patients

Crohn disease
Ulcerative colitis

Colorectal Cancer

OrganoCan Project:

Development of a High Content Screening approach based on 3D human colon organoid to identify drug candidates against ColoRectal Cancer

Drug screening application

1) Patient:Mice tissue resections

Pancreas
Colon
Bladder
Prostate

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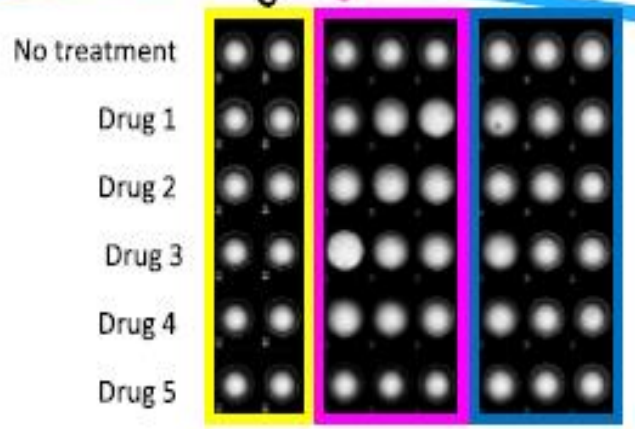
2) Epithelium extraction

3) Plating in 96 well plate for screening

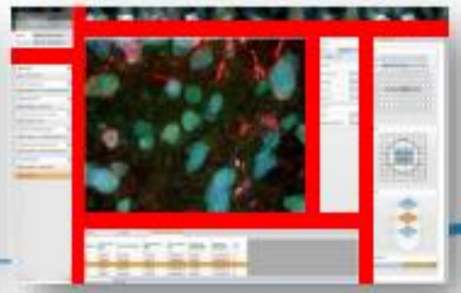
4) Plating in 96 well plate for screening



5) Plate scanning and Imaging

Data Mining
High Content Profiler

Image analysis
High Content Screening



6) Application to patient treatment protocol

THANKS TO…

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