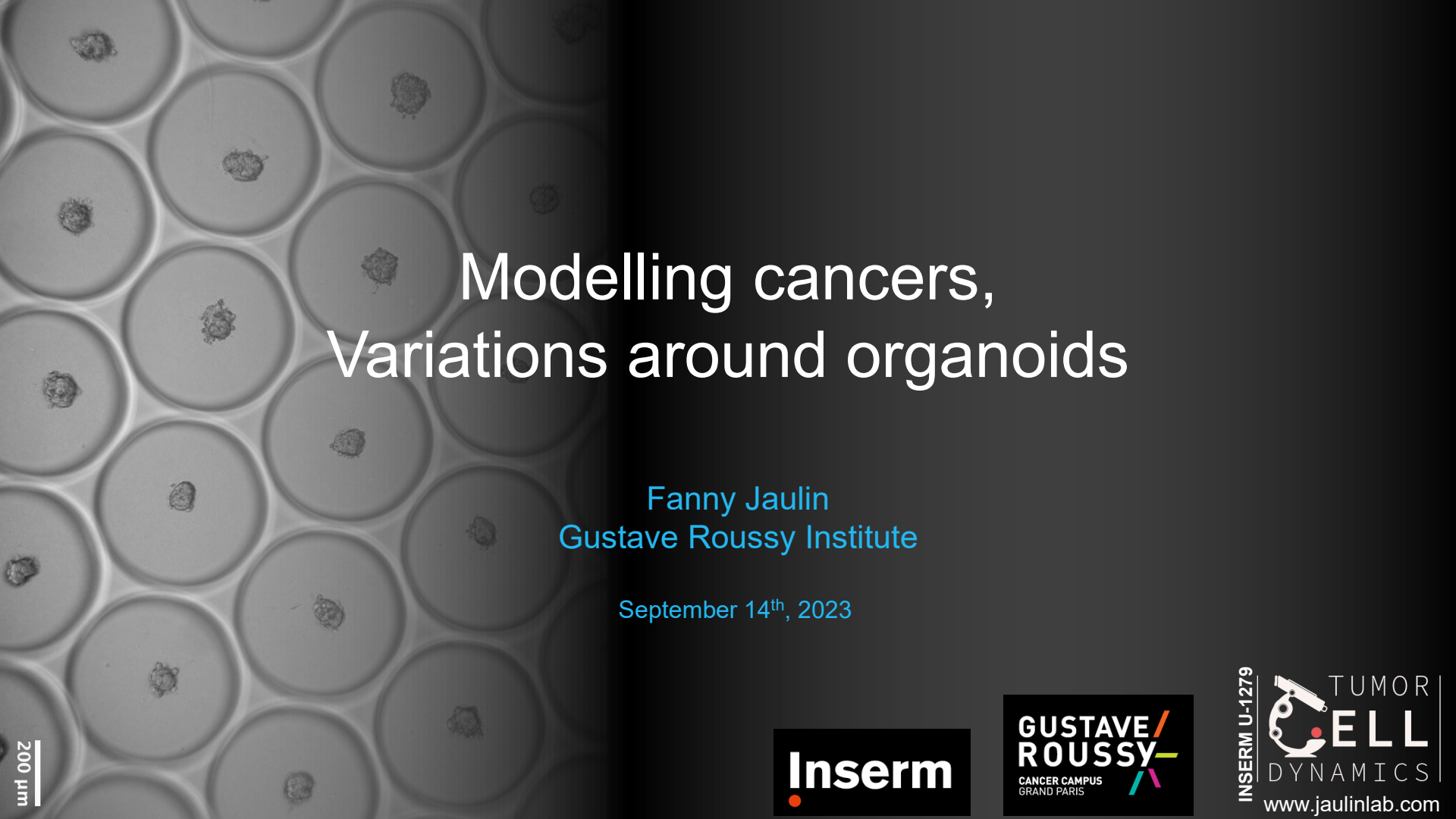
Modelling cancers, Variations around organoids

Fanny Jaulin
Gustave Roussy Institute

September 14th, 2023



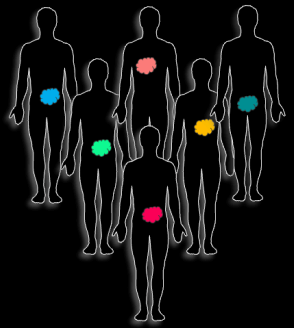
Inserm

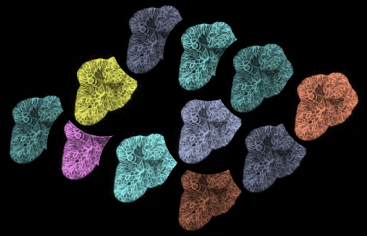
GUSTAVE ROUSSY
CANCER CAMPUS
GRAND PARIS

INSERM U-1279
TUMOR CELL DYNAMICS
www.jaulinlab.com

Relevant cancer models

To generate new knowledge
To improve translatability to patients



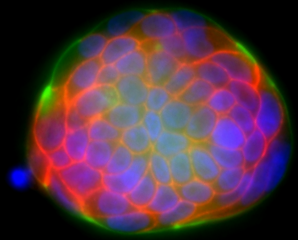


Cancer is a heterogenous
& complex tissue disease

Underperforming oncology R&D

4% of drug candidates validated in the clinic

1- Modelling cancers

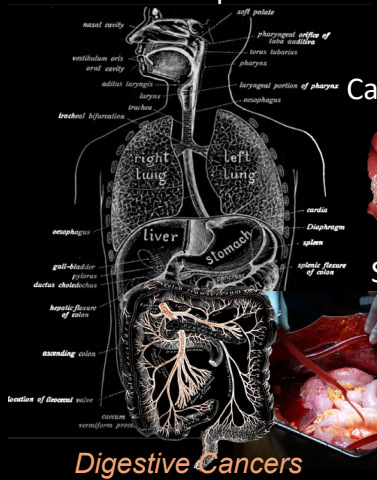


Translational Cell Biology

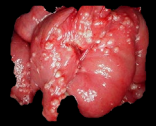
prospective systematic analyses
to reveal cancer cell behaviours

Bedside-to-bench

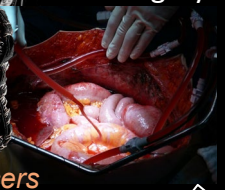
Cancer patients



Live Primary
Cancer specimens



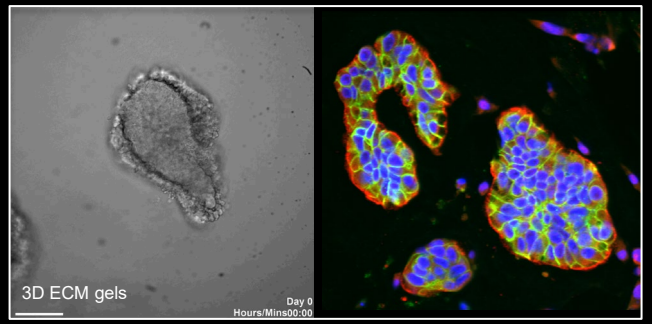
Surgery



Digestive Cancers

Digestive Pathologies committee

Explants for **immediate** and **short-term** studies



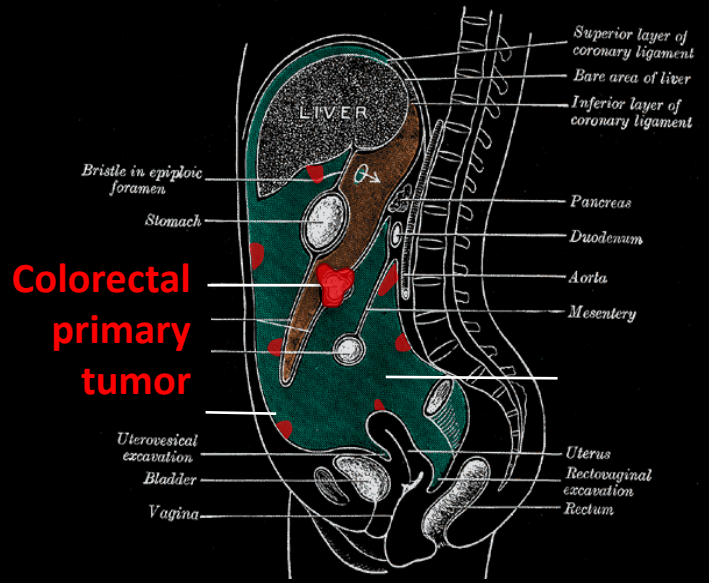
=> New and relevant knowledge

Bench-to-bedside

Biomarkers, Therapeutic targets

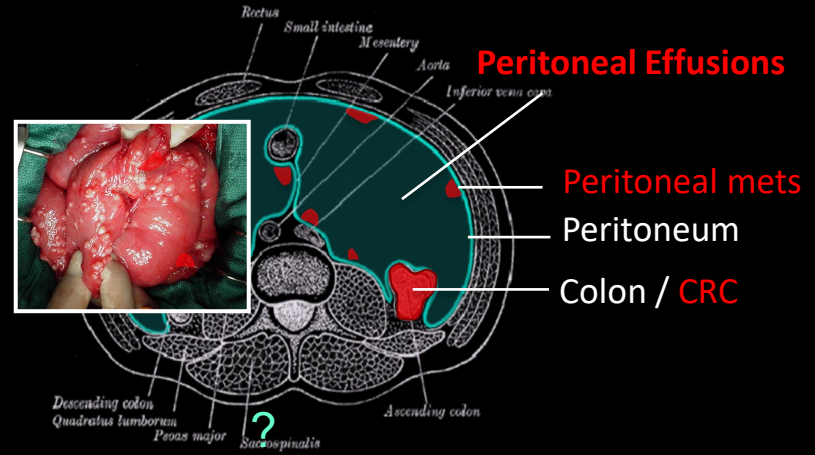
Hollistic approaches from large cohorts of CRC patients

>20 CRC patients at early stage



Libanje et al. EMBO 2019

>50 CRC patients with advanced metastatic disease

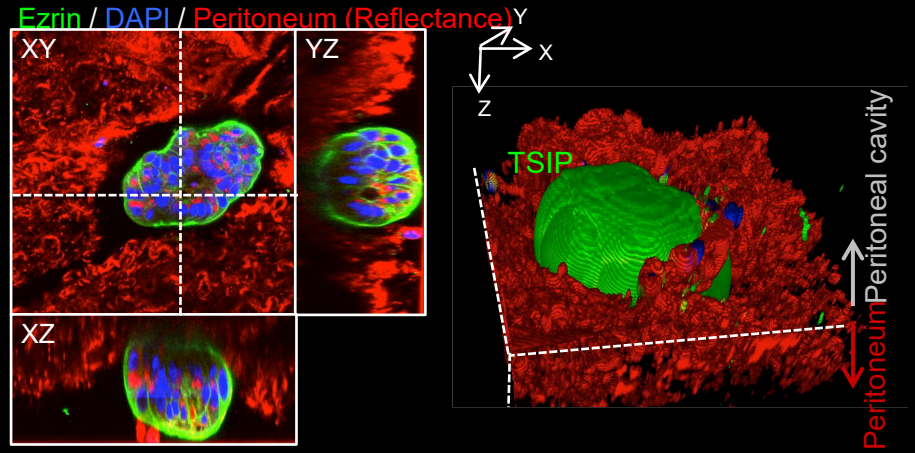


Zajac et al. Nat Cell Biol 2018

Hollistic approaches from large cohorts of CRC patients

Pros: Account for complexity & heterogeneity
Generate new & relevant knowledge (TSIPs, collective amoeboid migration)

Cons: Complicated mechanistic studies
Does not “capitalize” on this precious resources to build collection



« All Models are wrong,
but some are useful »

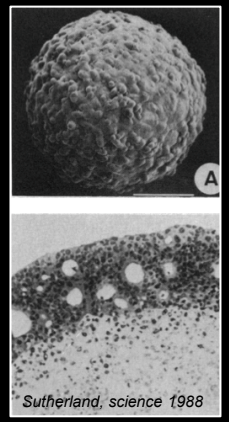
George Box
(1919 – 2013)

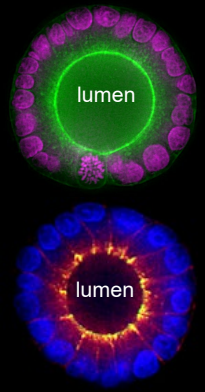
Ex vivo model systems for Research

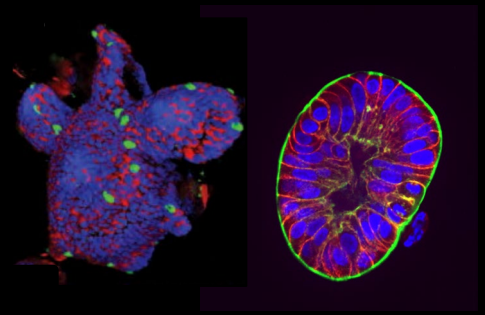
3D Models

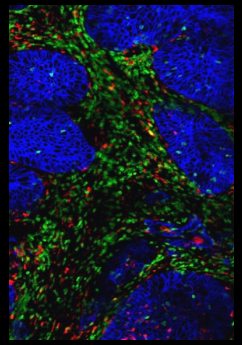
Spheroïds	Cysts/Acini	Organoïds /Tumoroids	Tissue slices	Organ-on-Chip
80's	90's (M. Bissell, J Brugge)	2009 (Clevers, Knoblich)	(E Donnadieu)	(S Decroix)

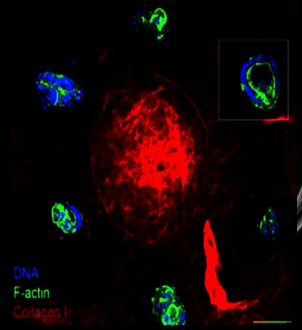
2D/stiff culture

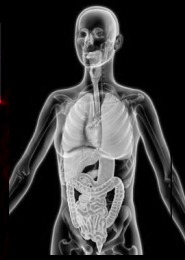








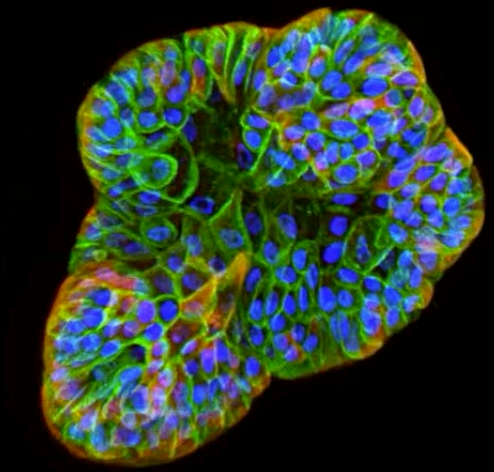




Vivo

Artificial substrates	Physiological hydrogels (ECM-based)		Native tissue	Microfluidics	Culture system
Cell lines	Stem cells / Tumor fragments		Multiple cell types		Cells
–	+	+	++	+/++	TME

Patient-derived (tumor) organoids
-PDOs-



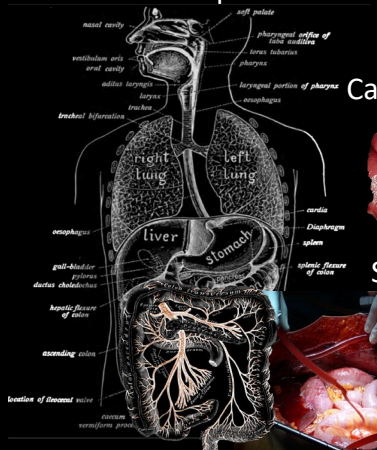
- Live specimen amplified *ex vivo* "indefinitely"
- Cryopreserved to build large collections capturing cancer heterogeneity
- Most faithfull tumor avatar to date
  - -Molecular make-up
  - -Behaviors (vs explants)
- Versatile & scalable at the population level

Translational Cell Biology

prospective systematic analyses
to reveal cancer cell behaviours

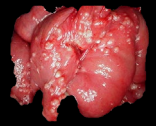
Bedside-to-bench

Cancer patients

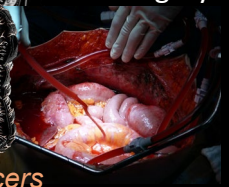


Digestive Cancers

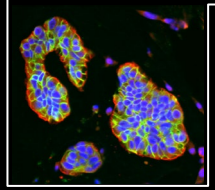
Live Primary
Cancer specimens



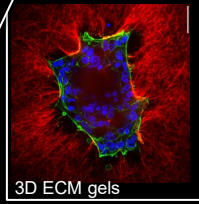
Surgery



Explants



Biological collections (>120 PDOs)



- Banked
- WES/RNAseq
- Histology
- Experiments!

Generation — Validation

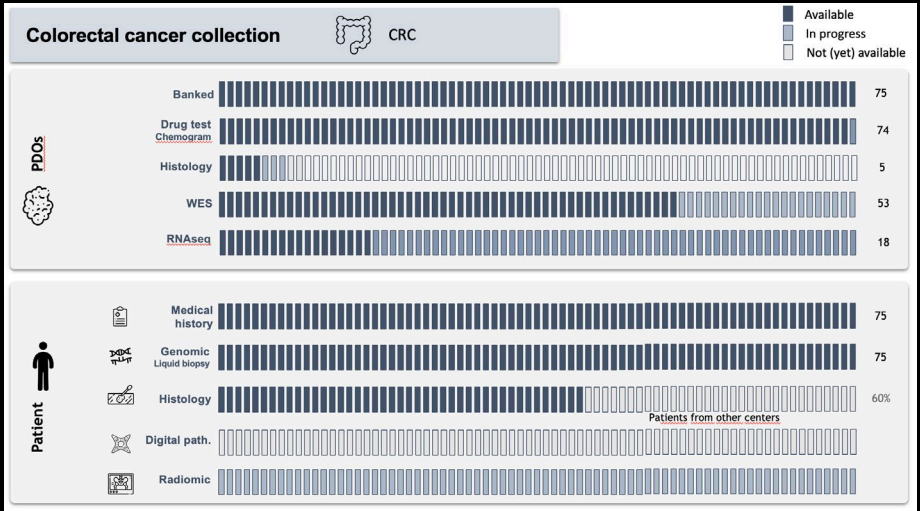
Hypotheses

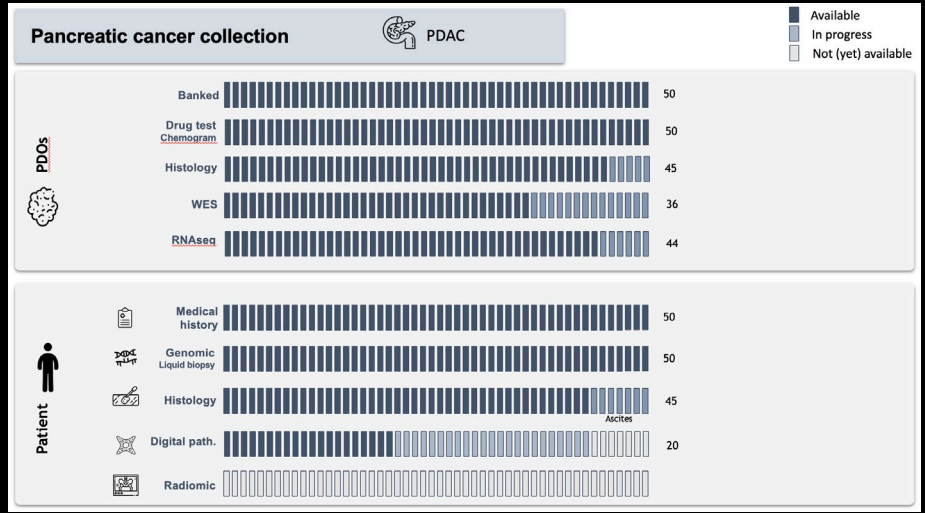
Digestive Pathologies committee

Bench-to-bedside

Biomarkers, Therapeutic targets

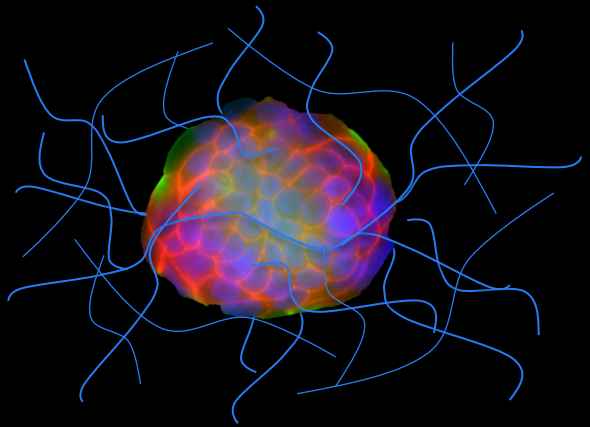
Ex vivo tumor avatars collection that captures cancer complexity & heterogeneity at the population scale





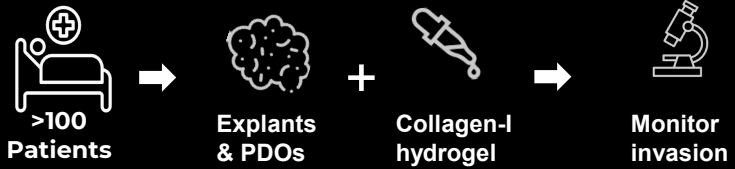
2- Fundamental research: tumor cell invasion

Collagen-I matrices

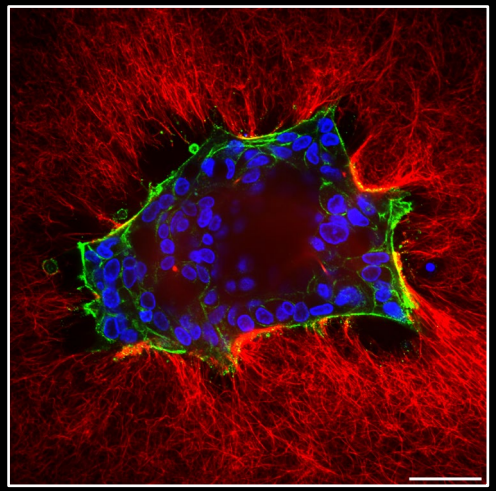


Extra-cellular matrix represents
up to 60% of the tumor mass
Henke et al, 2020

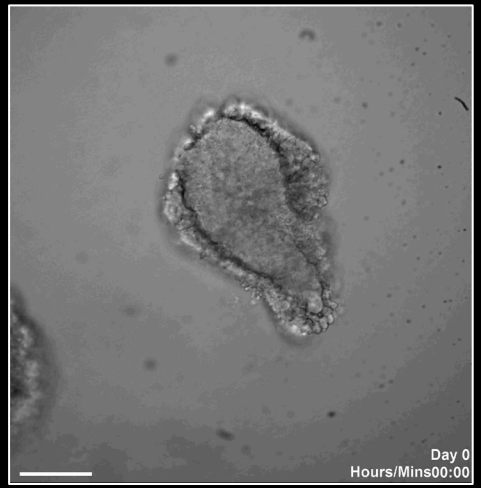
Simple collagen-I gel reveals invasive behaviors from explants and PDOs



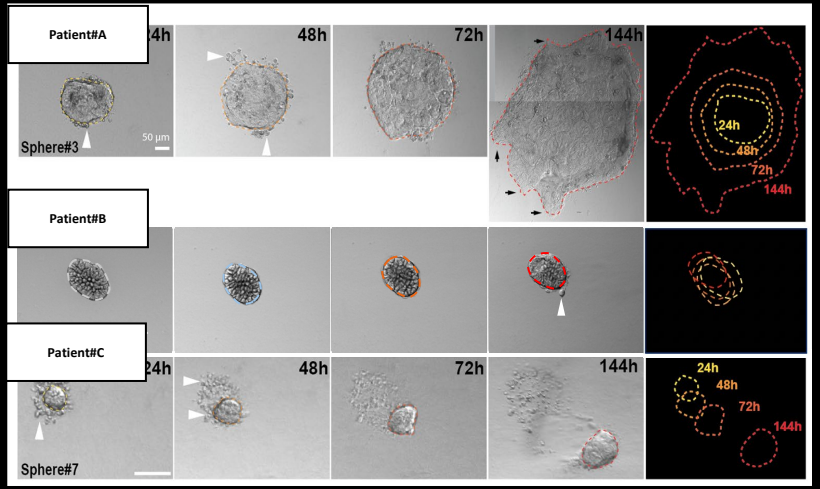
=> Profiling patterns reveals invasive behaviors & tumor heterogeneity



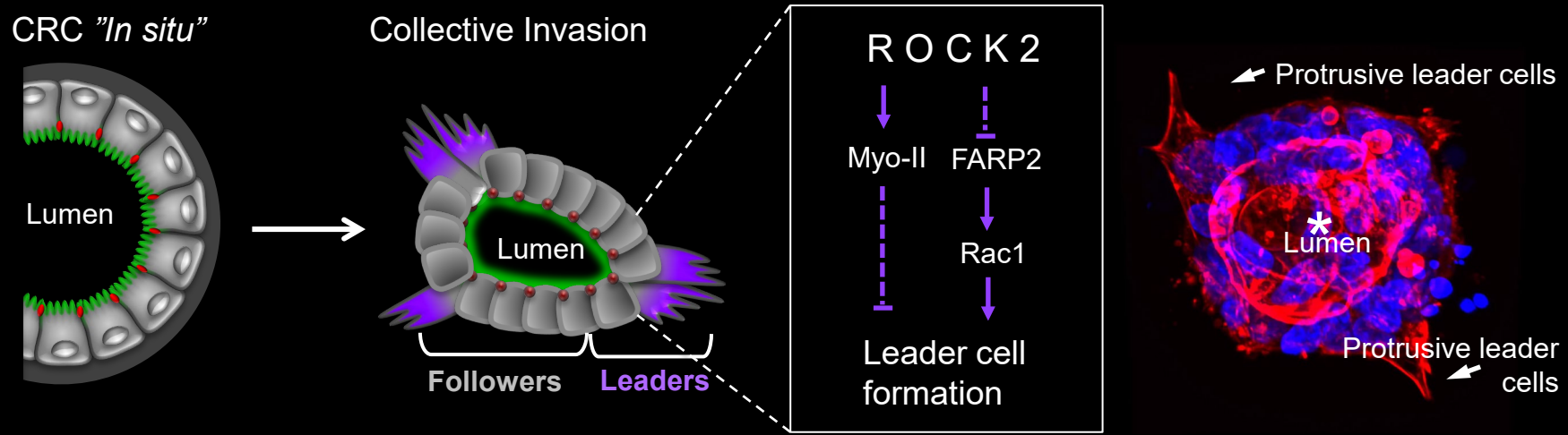
PDO



Primary explant



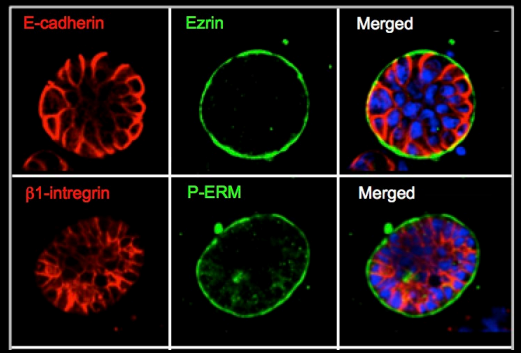
ROCK2 inhibition triggers leader cell formation & collective invasion

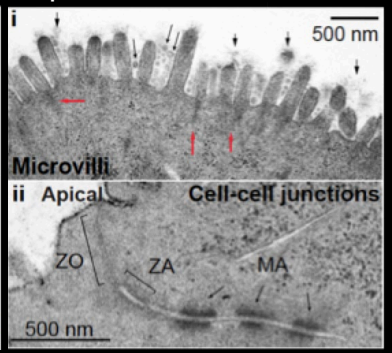


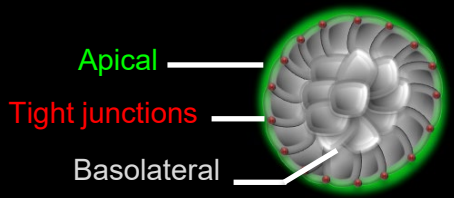
Libanje et al. EMBO 2019

TSIPs are malignant tumor intermediates underlying the metastatic spread of serrated hypermethyalted CRCs

50% of effusions from metastatic CRC patients contain TSIPs

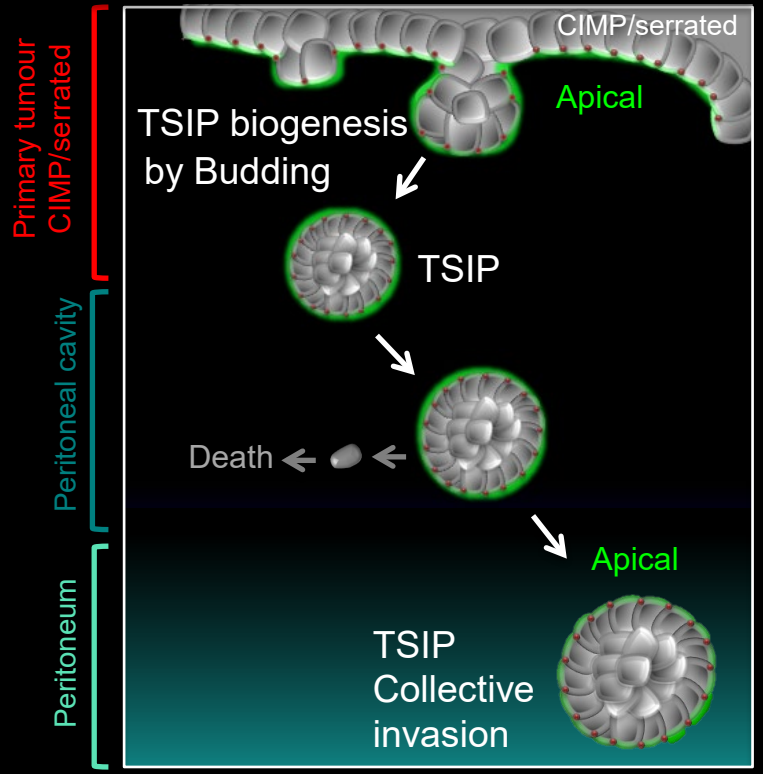






TSIPs

« Apical-Out »
Inverted topology



Zajac et al. Nat cell Biol 2018

There is a 4th mode of cell migration, that is collective & amoeboid

Mesenchymal

Amoeboid

Single cell

Rear

Front

"Traction"
Adhesion/front

"Propulsion"
Contraction/Back

Collective

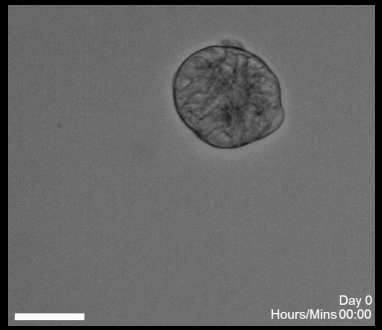
Followers

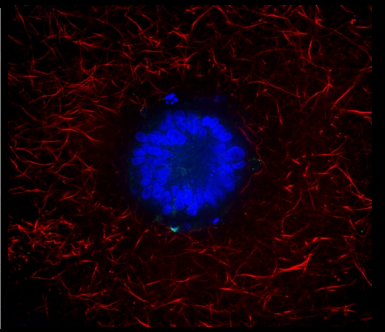
Leaders

Migration into Collagen-I gels

No protrusive leaders,

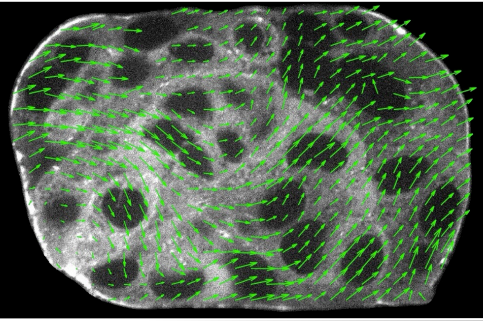
no traction forces





0 min

PIV in microfluidics



HT-29 mTurquoise MLC
30x60 µm PLL-PEG

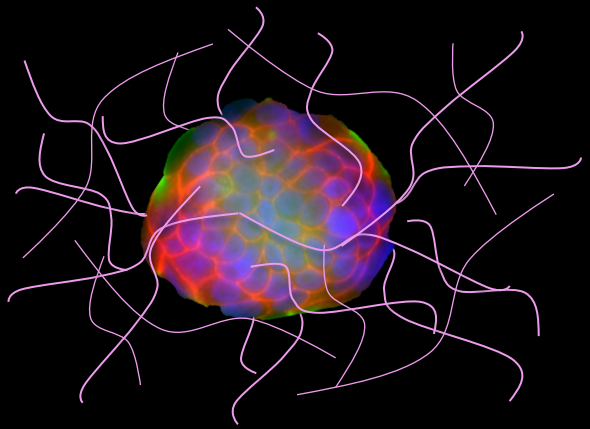
20 µm

Pages et al. Science Advances, 2022

3- Clinical applications: implement PDO to guide personalized medicine strategies

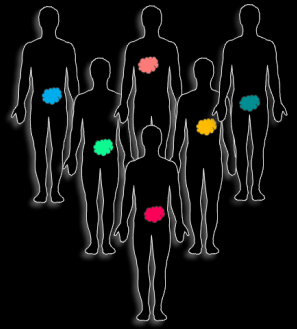
in Laminin-rich hydrogels

Matrigel derived from murin EHS tumors



Cancer patients requiere personnalized medicine

Improve patient survival & spare toxicities



Match the right drug to the right patient

Select from growing number of therapeutic options

Genomic approaches only benefit 7-15% patients*

Functional precision medicine works for leukemias**

Are PDOs relevant tumor “avatars” for solid tumors?

**Le Tourneau et al., Lancet Oncol. 2015, Massard et al., Cancer Dis. 2017, Rodon et al., Nat medicine. 2019*

***Kornauth et al. Cancer Dis. 2022 Malani et al., Cancer Dis. 2022*

Missions

1. Implement PDO technology within patient clinical path & standardize the assays
   Can we establish PDO for each patient? For the population?

2. Assess PDO clinical validity in this setting
   How faithful are PDOs to the patient's tumor?

3. Determine the clinical utility of "functional" personalized medicine
   How PDO can benefits to patient?

4. Leverage the pharmacotyping technology for all drugs
   Expand current assays (chemograms) to immuno-oncology (immunograms)

RHU-ORGANOMIC

Organoid-based assays for functional personalized medicine strategies

anr

INVESTIR L'AVENIR

FRANCE 2030

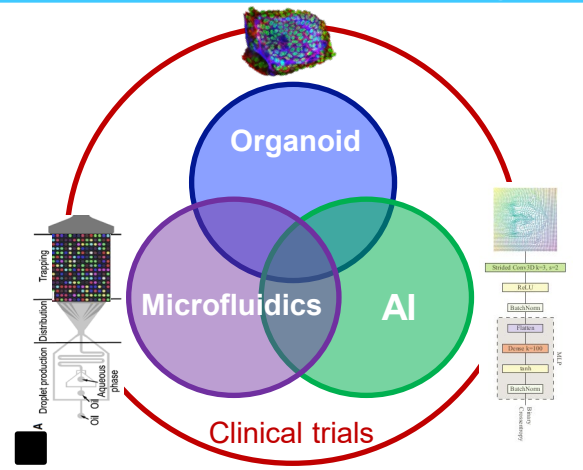
MINISTÈRE DES SOLIDARITÉS ET DE LA SANTÉ

PI: F Jaulin
Funded 2022-27

Consortium

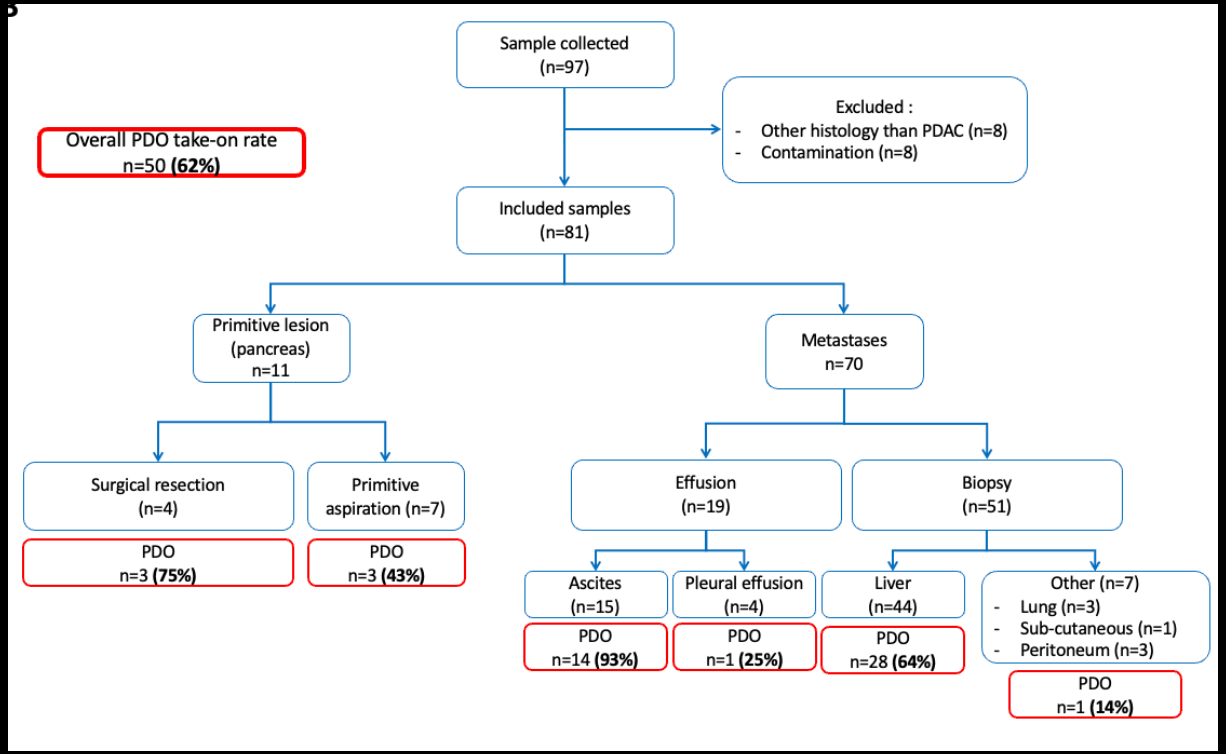
- GUSTAVE ROUSSY – CANCER CAMPUS GRAND PARIS
- INSTITUT PASTEUR
- Inserm
- OKOMERA
- CentraleSupélec
- SEngine Precision Medicine
- université PARIS-SACLAY
- AstraZeneca

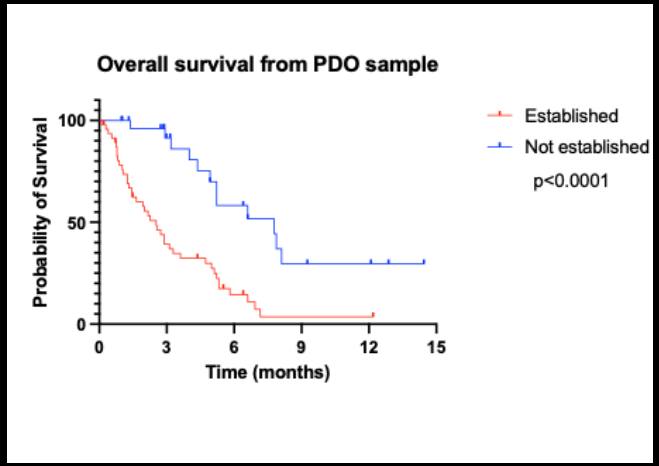
Unique combination of Expertise & ambitious clinical setting



Implement PDO technology within patient clinical path

1 We establish PDOs for all patients with different success rates



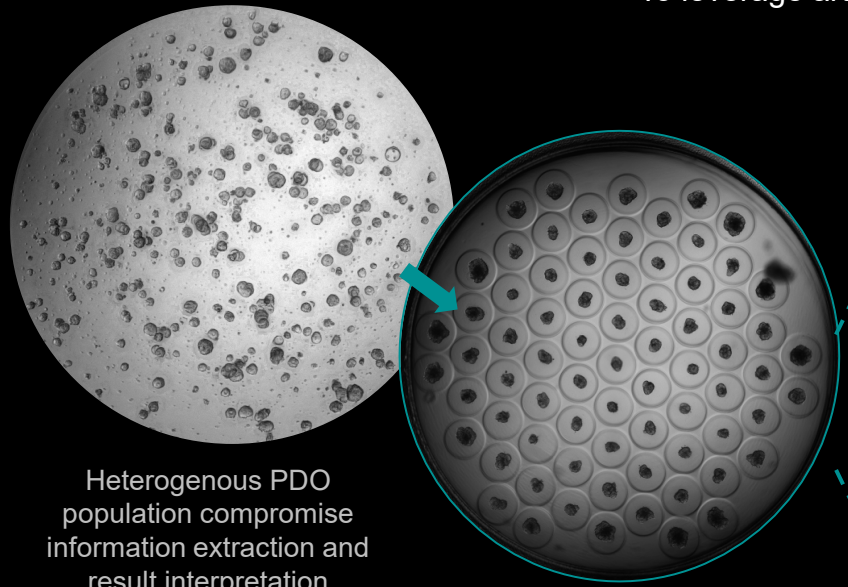


Panoramic observational study on PDAC ,Boileve et al. submitted

Standardize the assays & solve technological bottlenecks

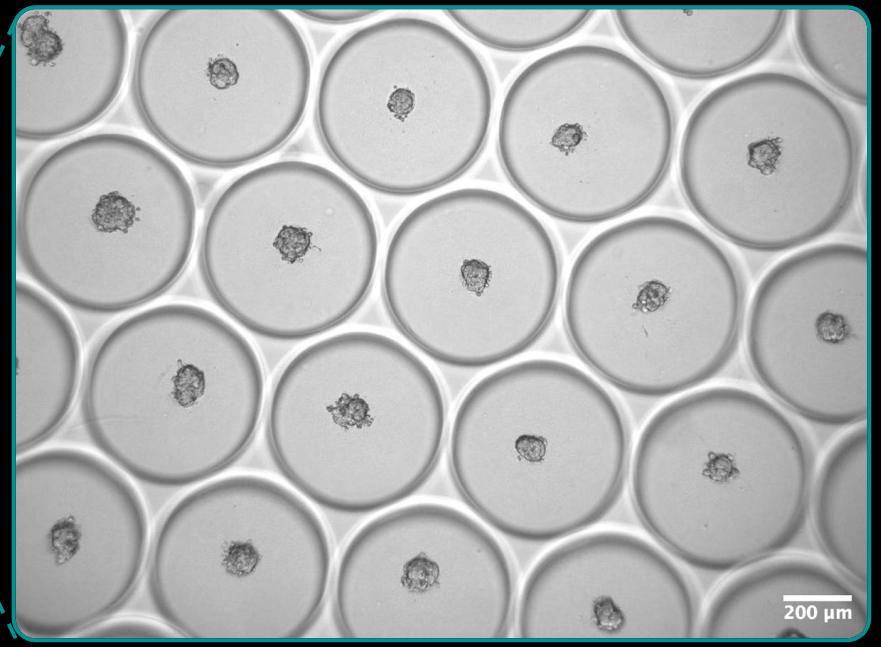
1 Time & space resolved microscopy

To standardize, automatize for scaling-up screening capacities
To resolve intratumoral heterogeneity
To leverage artificial intelligence for upgraded read-outs (dynamic)



Heterogenous PDO population compromise information extraction and result interpretation

Micro-engineering
(Microfluidics & microwells)

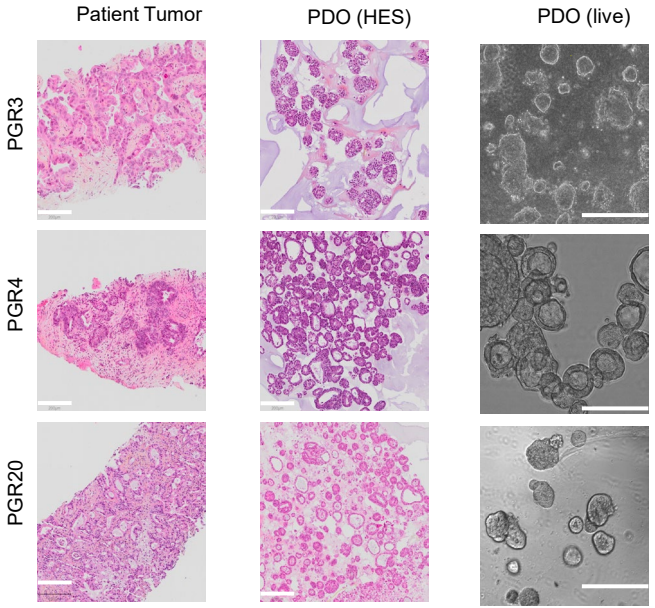


PDO from CRC patient in microwells over 4days (Jaulin lab, Gontran/Cartry/Bedja)

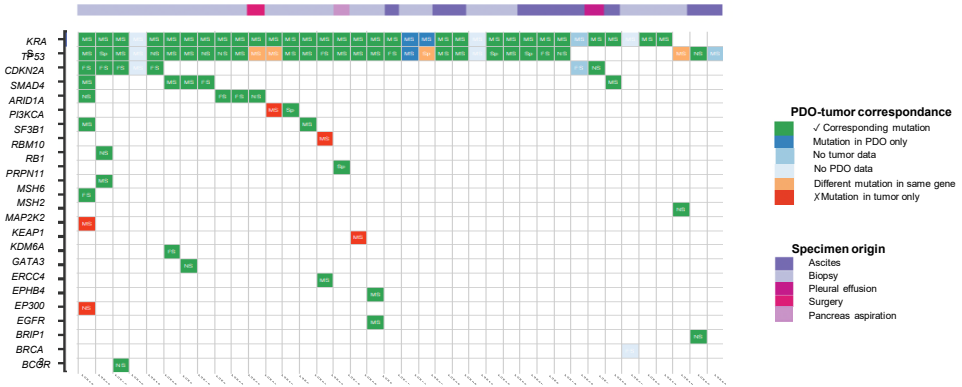
Biological concordance between PDOs & patient tumors

2

Histological concordance
(cell autonomous multicellular organisation)



Genomic concordance (93%)
(Early passages, MSS stable)



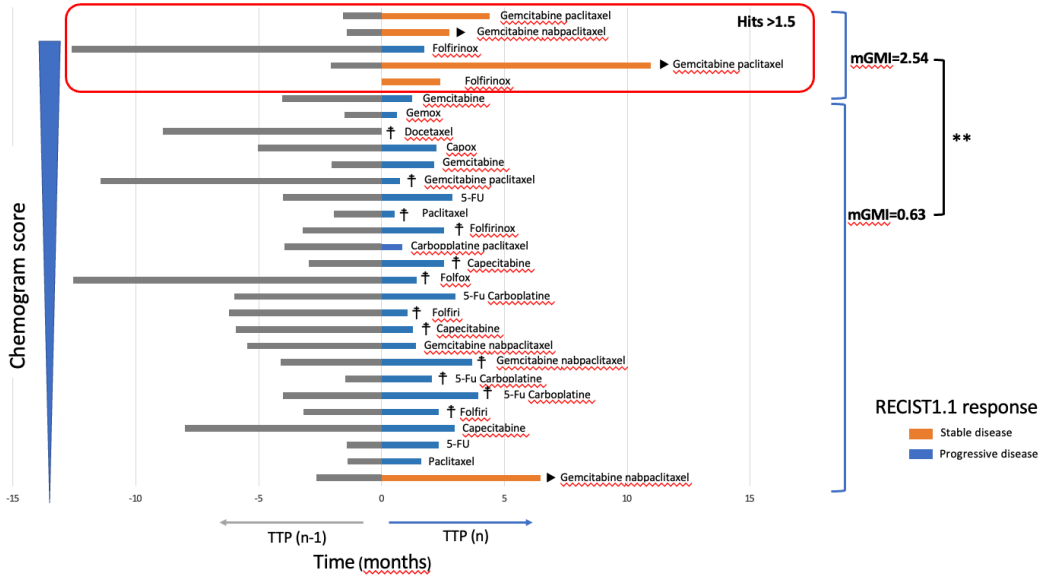
Exome sequencing from 40 tumors and associated organoids from pancreatic cancer patients

Panoramic observational study on PDAC ,Boileve et al. submitted

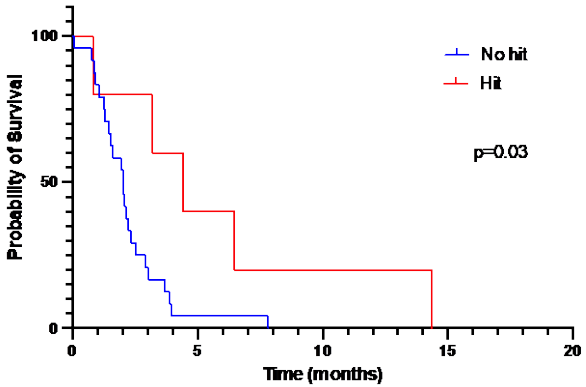
Clinical concordance

2

PDOs predict patient response
(34 assessable patients)



...& correlate with patient survival



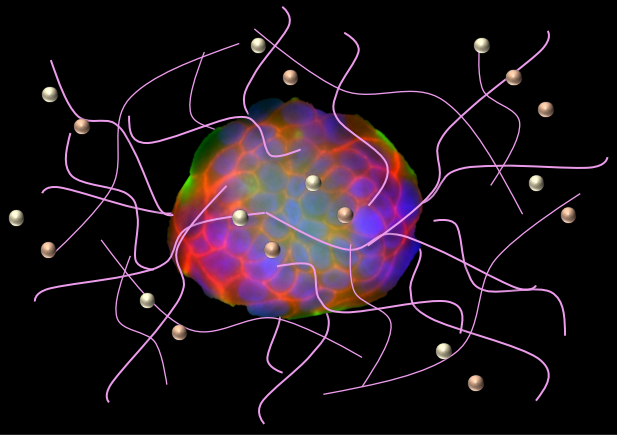
Panoramic observational study on PDAC ,Boileve et al. submitted

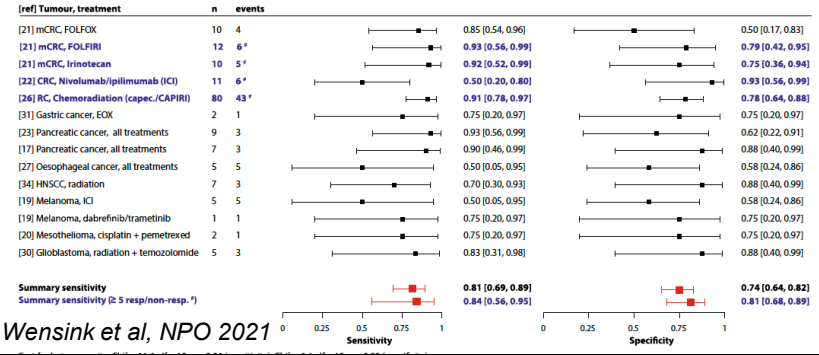
Clinical concordance

2

	PDAC (34 patients)	CRC (8 patients)
Sensitivity	**83.3%**	**75%**
Specificity	**92.9%**	**75%**
Positive pred. Value	**71.4%**	**75%**
Negative pred. Value	**91.3%**	**75%**

=> “Simple” PDO/matrigel culture predicts patient drug response to chemo & targeted therapies in clinical setting





Wensink et al, NPO 2021

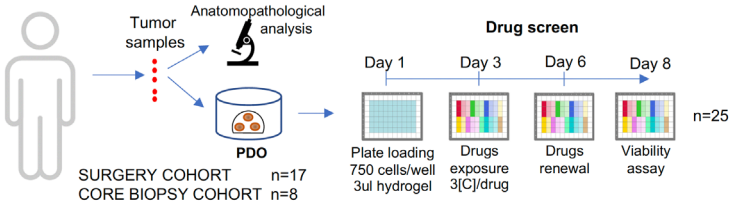
« Culture media composition influences patient-derived organoid ability to predict therapeutic responses in gastrointestinal cancers » *Hogenson et al, JCI insight 2022*

ORGANOTREAT Clinical trial

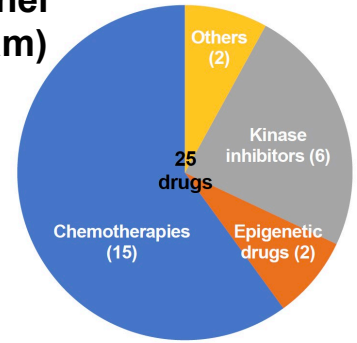
3

Interventional clinical trial aiming to orient patient treatment

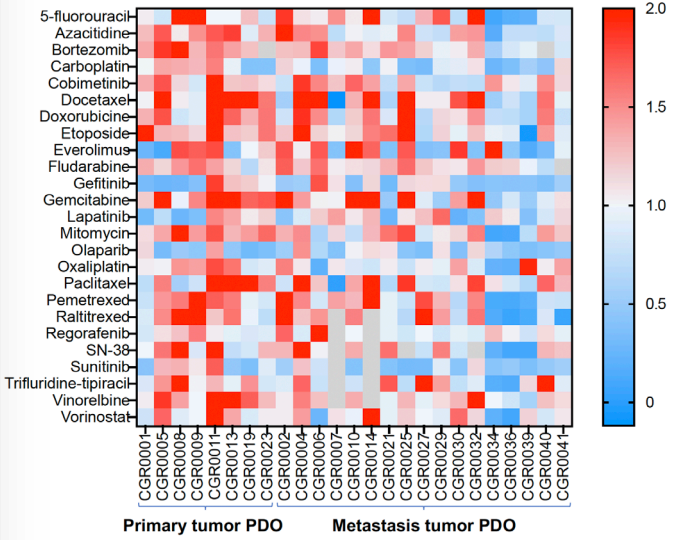
Chemogram

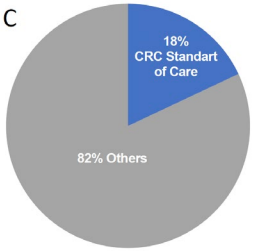


25-drug panel (Chemogram)



Drug response landscape in 25 patient pilot study





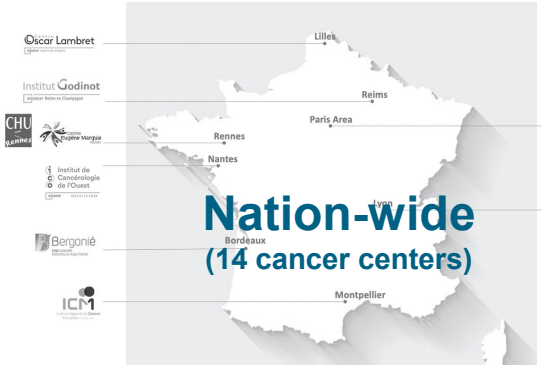
Could offer unexpected therapeutic options to patients

Cartry et al. under revision

ORGANOTREAT Clinical trial

3

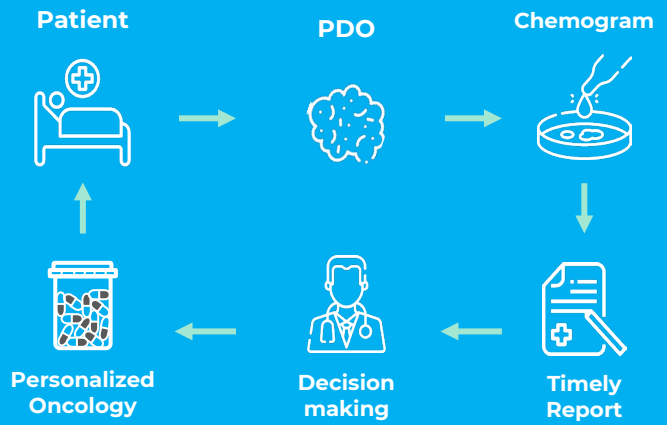
Prospective multicenter study evaluating the feasibility and efficacy of tumor **organoid-based precision medicine** in patients with advanced refractory **solid tumor**



	Phase :	Organ :	Patients
ORGANOTREAT-01	I/II	CRC	60 (GMI)
ORGANOTREAT-02R	III	PDAC	314
ORGANOTREAT-02R	III	CRC	582
ORGANOTREAT-02	II	Other	...

PI/Clinic: Pr Michel Ducreux, Alice Boileve

ALL solid tumors, >1000 patients



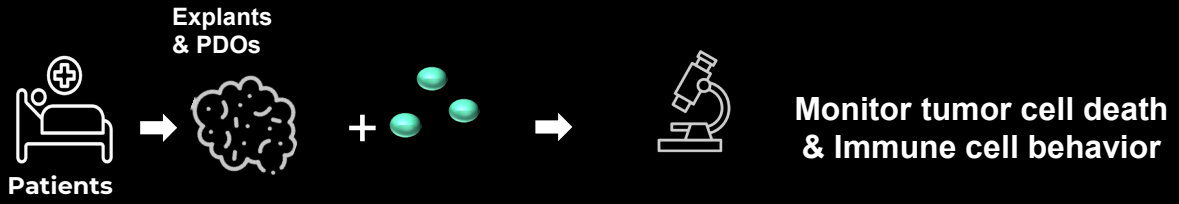
Interventional trial

Jerome Cartry and Sabrina Bedja

Leverage the pharmacotyping technology for all drugs

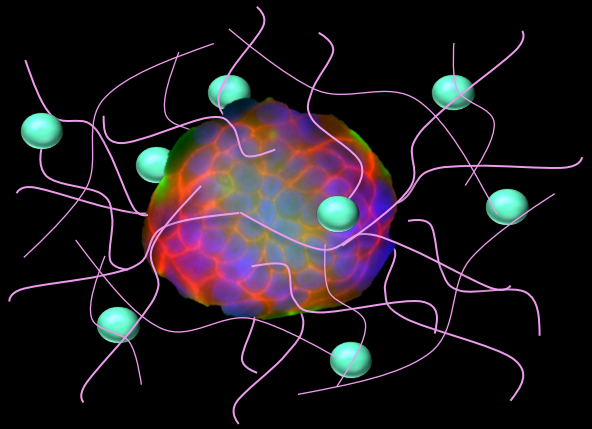
4

From chemograms to immunograms



1/ Engineered Car-T cells (Her-2/CD19)

2/ Autologous Immune cells (+/- ICB)



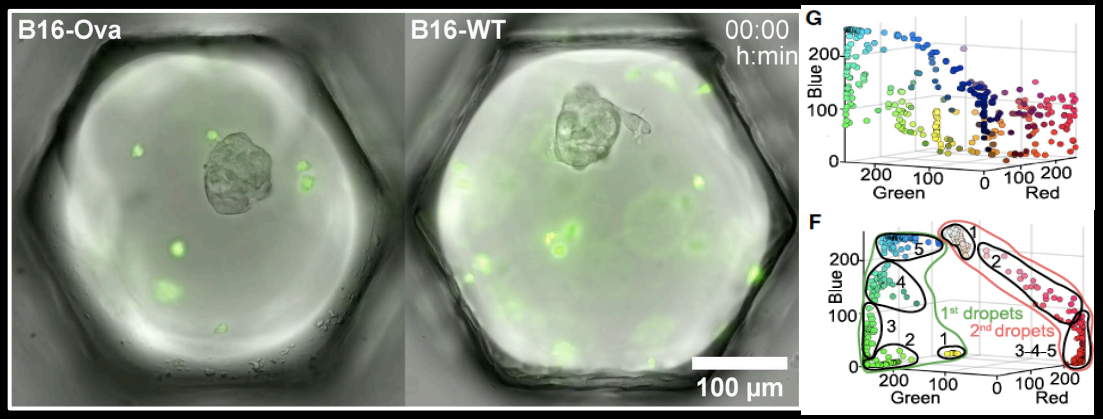
IMMUNOGRAM

Capture tumor immunogenicity & predict response to Immune Checkpoint Blockers (ICB)

Leverage the pharmacotyping technology for all drugs

4

From chemograms to immunograms



Ronteix et al. 2022

Charles Baroud, PhD
Group leader, Professor at Polytechnique
Physic-based approaches to droplet microfluidic

Philippe Bousso, PhD
Dir. Immunology department
Dynamic of immune response

« All Models are wrong but some are useful »

Depend on:

- The question you ask
- The angle: holistic (relevance) or reductionist (mechanism, causality)
- The mission (cognitive, clinical application)
- Practical considerations (scalability, cost…)

We chose the patient-derived organoids as *ex vivo* tumor models (and happy about it!)

BUT, missing a lot of the tumor microenvironment
Adding specific cellular component will only **partially recover tissue complexity**
Restrict our understanding of disease, but will enable progresses

ACKNOWLEDGEMENTS

J. Cartry
Project leader

Patients!
(My) Dream Team !

U1279
Comité pathologies digestives
Département d'anatomopathologie
Radiologie interventionnelle
Pharmacie
PFEP, PFIC, PETRA
SEAT (Karelia Lipson/Negaar Goudarzi)

Collaborateurs

Matthieu Piel, IPGG, Paris
Raphael Voituriez, Sorbonne, Paris
Mathieu Coppey, Curie, Paris
Christophe Desterke, Paul Brousse
Vito Conte, UTE, Endhoven, Netherlands
Valeria Barresi, Italy
Julie Pannequin, IGF, Montpellier

Financements

MINISTÈRE DES SOLIDARITÉS ET DE LA SANTÉ
Liberté Égalité Fraternité

INVESTIR L'AVENIR

anr agence nationale de la recherche

FRM FONDATION RECHERCHE MÉDICALE

ANR AGENCE NATIONALE DE LA RECHERCHE

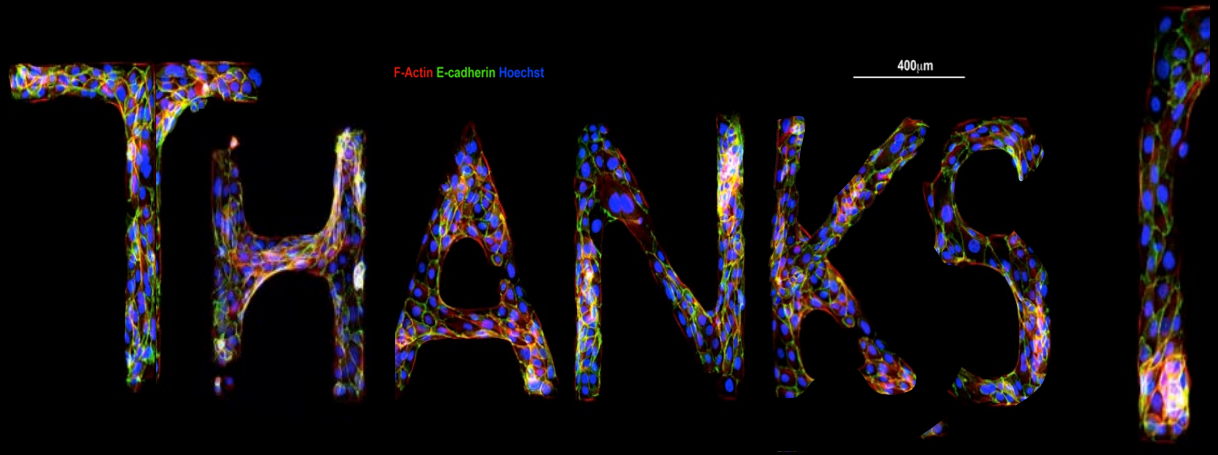
Inserm

INSTITUT NATIONAL DU CANCER

MARS bleu
AIDEZ LES CHERCHEURS DE GUSTAVE ROUSSY À COMBATTRE LE CANCER COLORECTAL !

FONDATION PHILANTHROPIA
LOMBARD ODIER

AstraZeneca

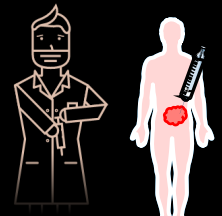


Jobs opportunities for Organoids fans!

- Postdoctoral position / RHU Organomic
- Senior Organoid Scientist / Orakl-oncology
- Head of Lab / Orakl-oncology

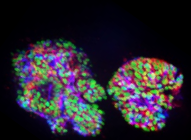
Send applications to fanny.Jaulin@gustaveroussy.fr

Functional personalized medicine



Starting last line of treatment

Core needle biopsies



PDO establishment

70% Take-on rate

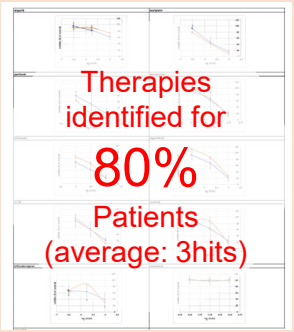
5.3 weeks turnaround time

CHEMOGRAMs

PDO drug test

25 FDA approved drugs

PDO response Analysis & report



Therapies identified for **80%** Patients (average: 3hits)

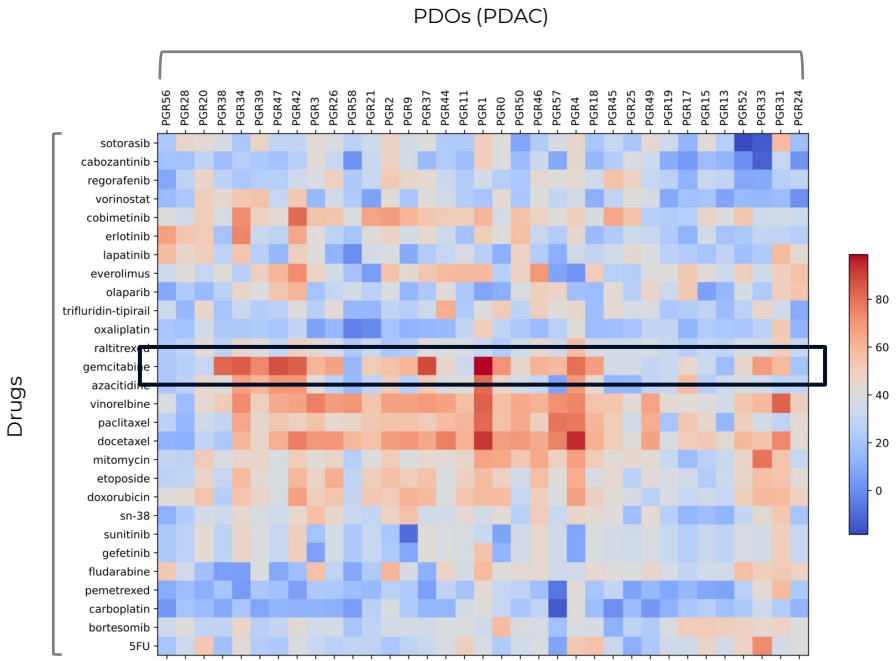
Orientation of Patient treatment

Chemogram tumor board

Personalized oncology

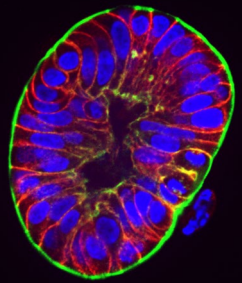
PANORAMIC: PDO drug response landscape

PDO drug response reflects **individual diversity**
& known sensitivity at the **population scale**



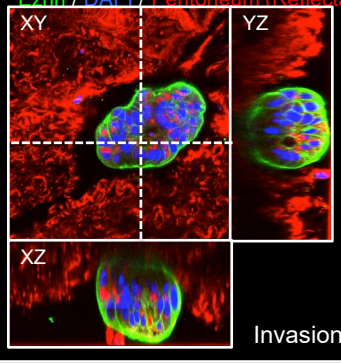
Collective migration without traction/adhesion???

TSIP (Tumor Spheres with Inverted Polarity)



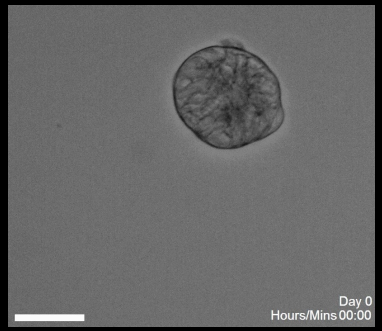
ITGB1/ Actin/ DAPI

Ezrin / DAPI / Peritoneum (Reflectance)



Invasion into peritoneum explants

No protrusion? No leader?



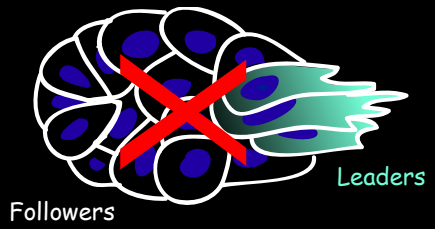
Migration into Collagen-I gels

Cell-autonomous

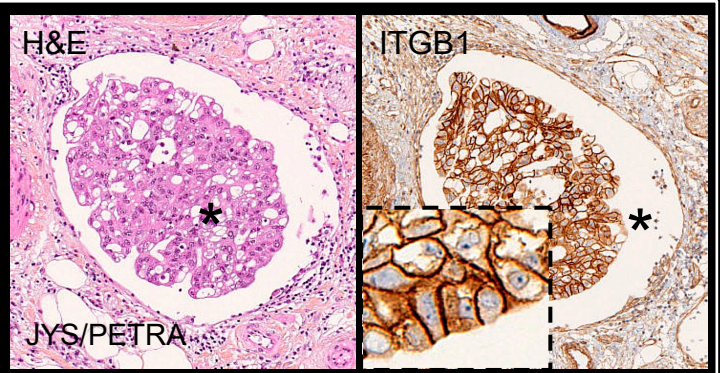
Zajac et al. 2018
Canet-Jourdan 2022

Collective

"Traction"
Adhesion/front



Mismatch between tumor cell adhesion repertoire & local ECM in the dissemination route



CRC tumour embolies into lymphatic vessels

Non Cell-autonomous

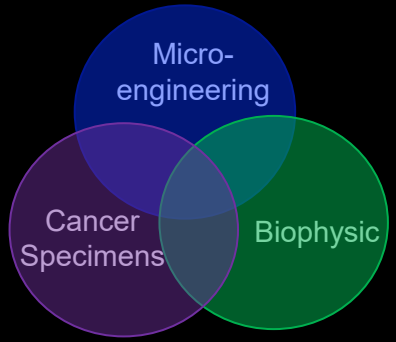
Uncovering collective amoeboid migration

Matthieu Piel
IPGG/curie

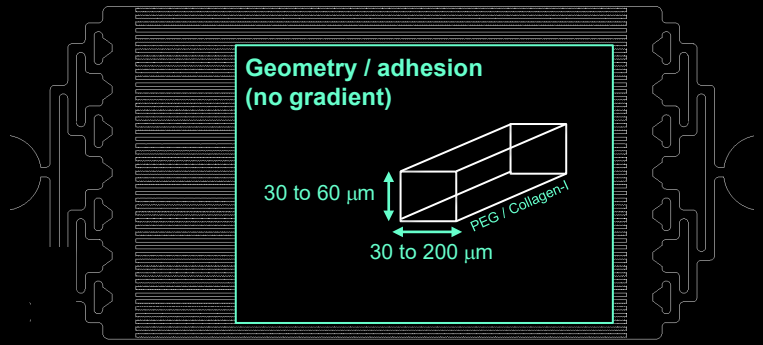
Raphael Voituriez
Sorbonne University

Mathieu Coppey
Curie Institute

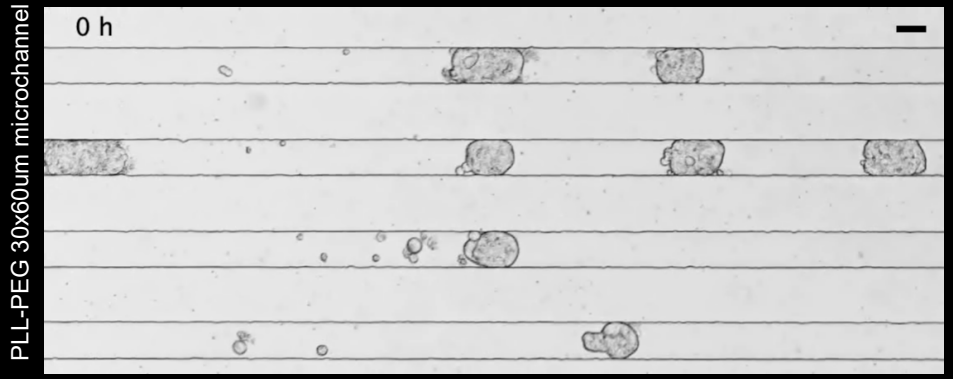
… from physicians to physicists!

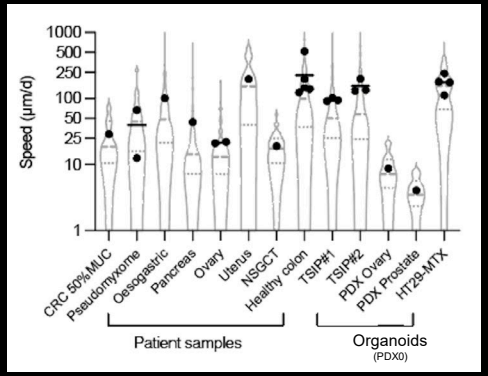


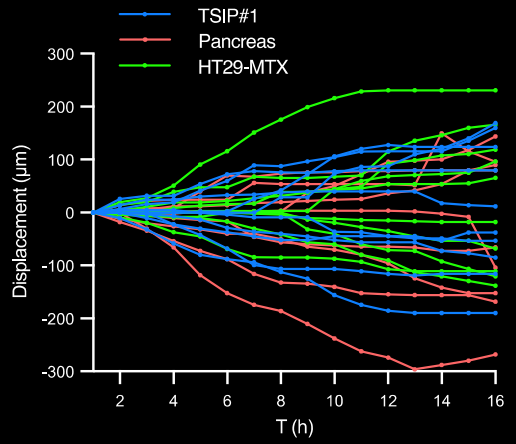
Microfluidics

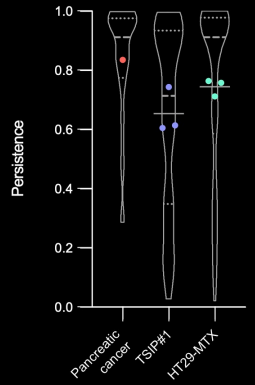


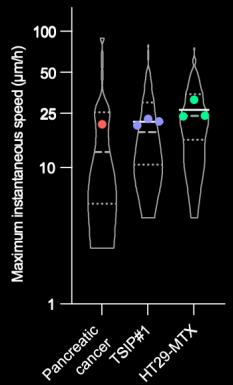
Most cancer cell clusters migrate into non-adhesive microchannels







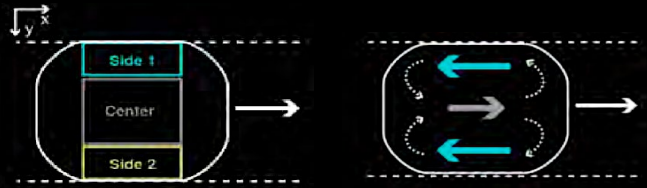




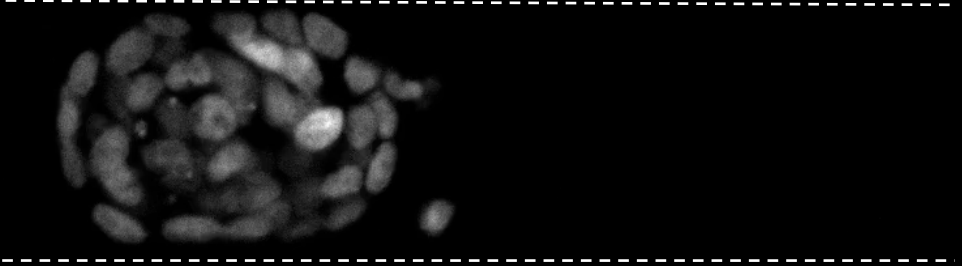
- No contribution of focal adhesion & traction forces
- Integrins participate in migration by exerting friction forces
(In line with Berget et al. NCB 2015)

Cluster migration does not involve persistent retrograde flows of cells

How are propulsive forces generated?



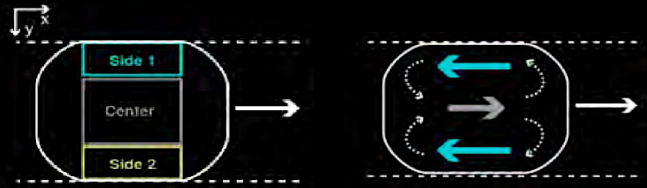
No neighbors exchange, clusters move as solids



HT-29 Cherry-H2B30x60 μm PLL-PEG

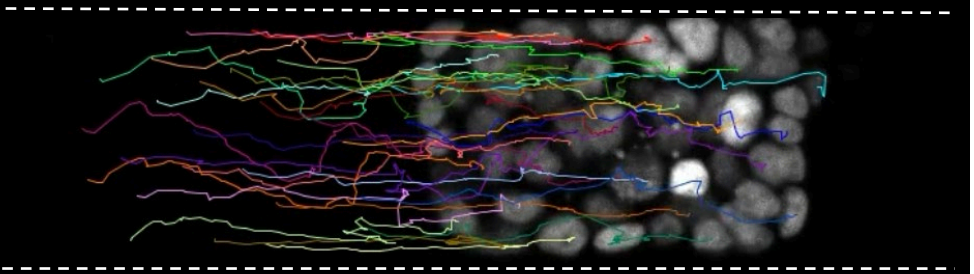
Cluster migration does not involve persistent retrograde flows of cells

How are propulsive forces generated?



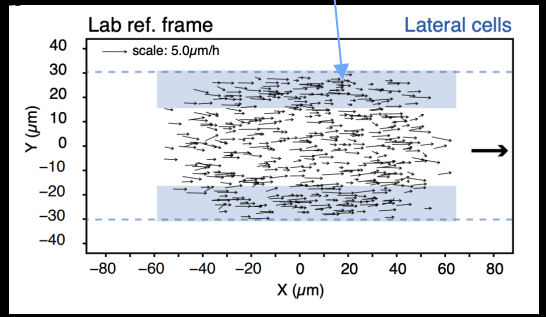
=> No cell Treadmilling !!

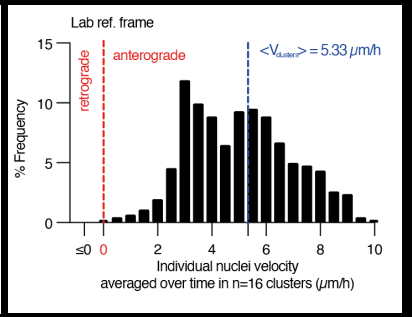
No neighbors exchange, clusters move as solids



HT-29 Cherry-H2B30x60 µm PLL-PEG

Anterograde flows

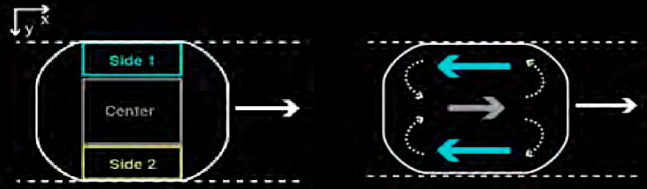




Average over time & clusters (n=16)

Cluster migration does not involve persistent retrograde flows

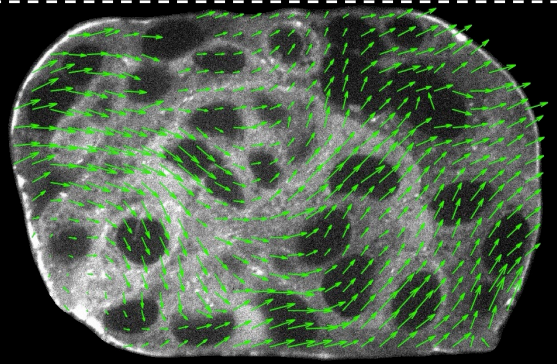
How are propulsive forces generated?



=> No persistent acto-myosin retrograde

0 min

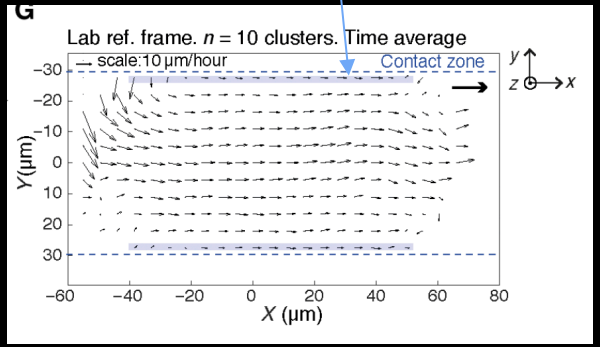
Particle Image Velocimetry

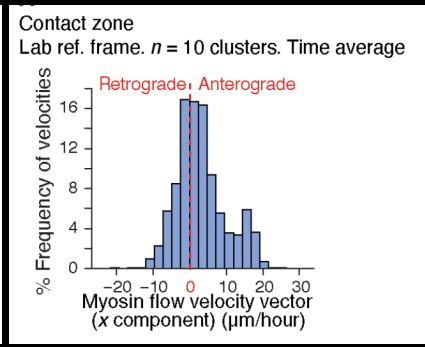


HT-29 mTurquoise MLC
30x60 µm PLL-PEG

20 µm

Anterograde flows

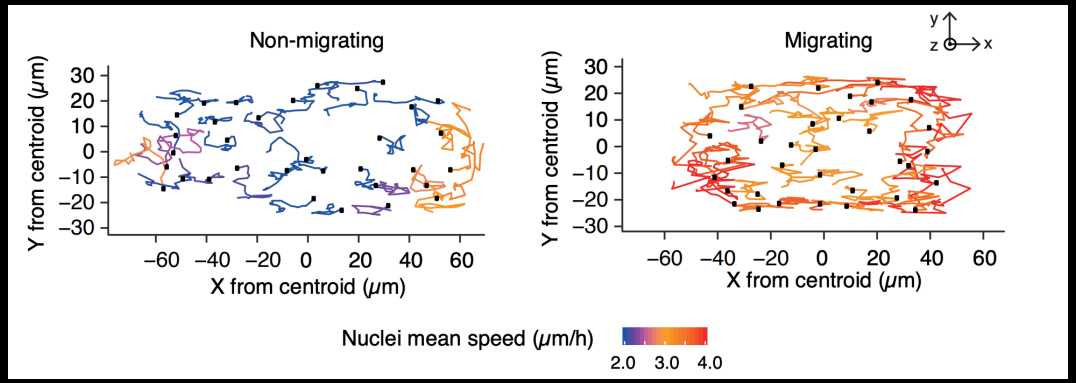


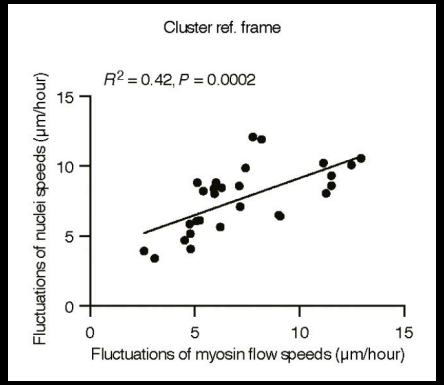


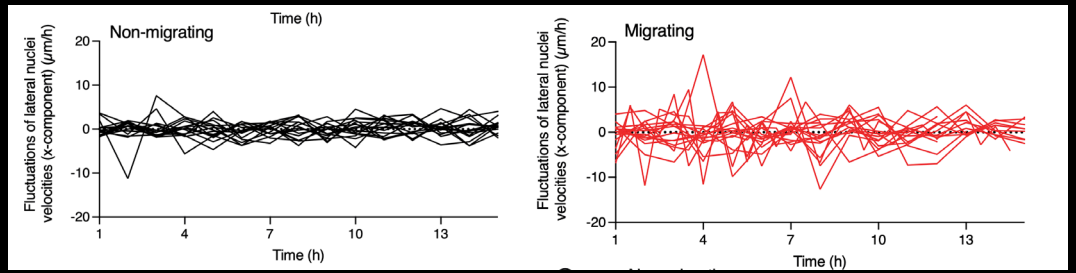
Average over clusters (n=10) & time (hours)

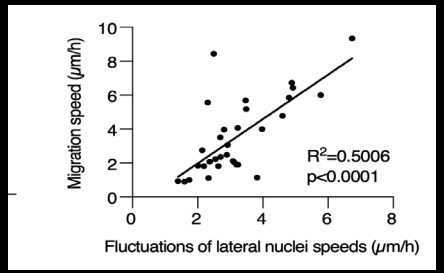
Fluctuation of myosin flow associated to cell « Jiggling »

Fluctuation of nuclei displacements

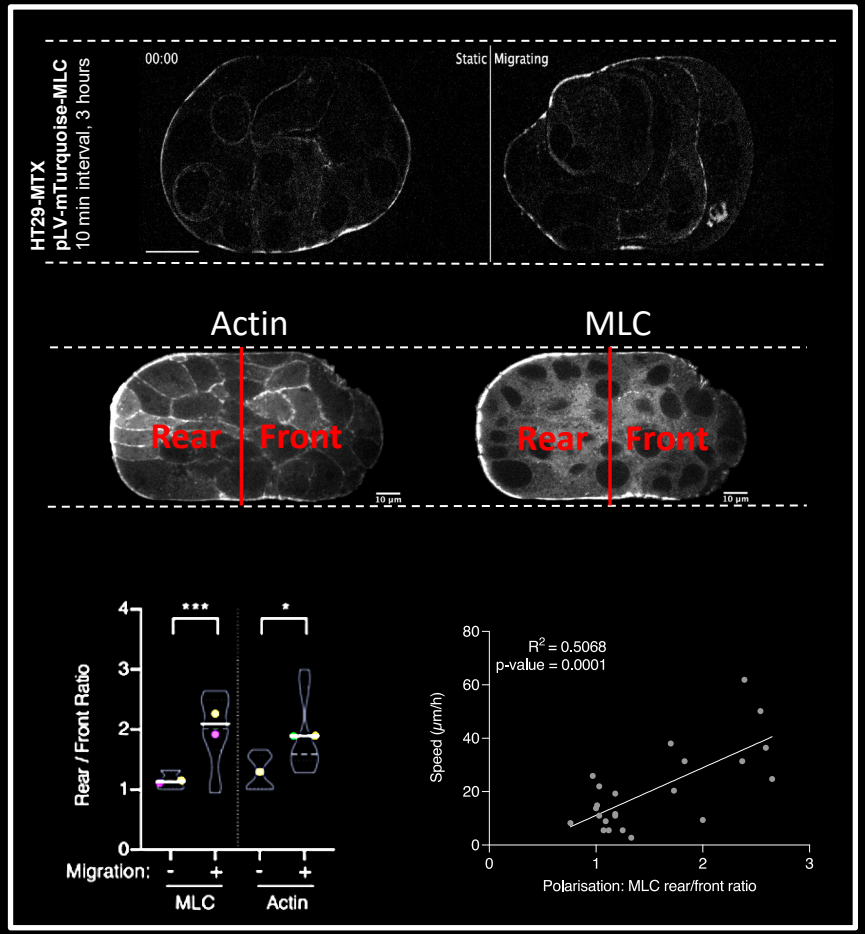


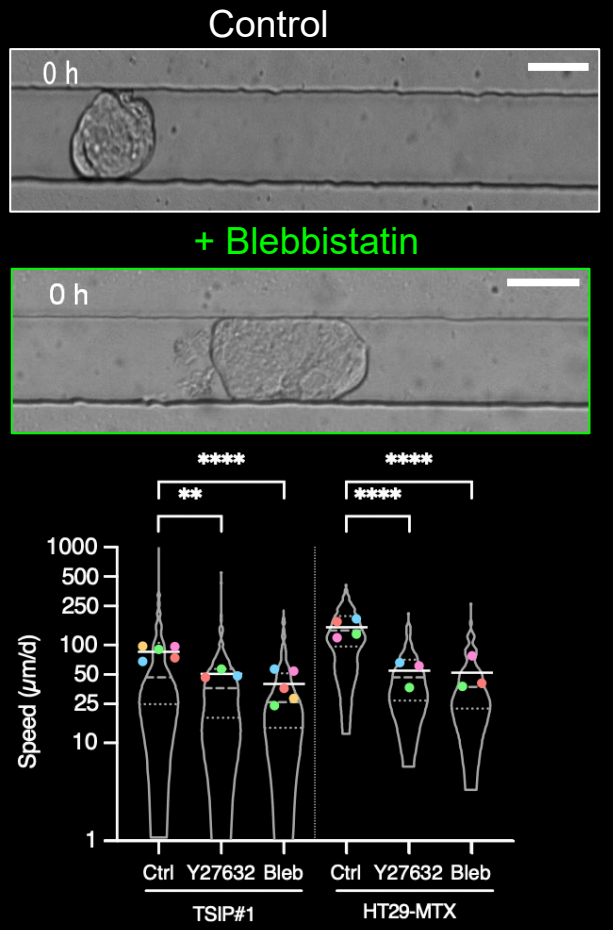






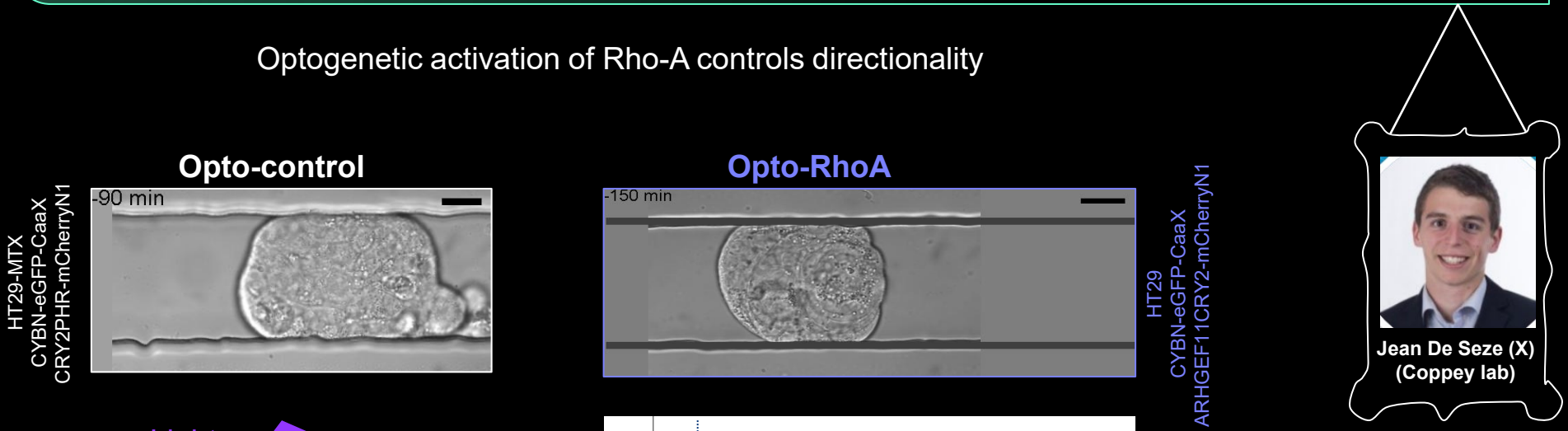
The Front/back polarization of the cortical acto-myosin cortex drives cluster migration





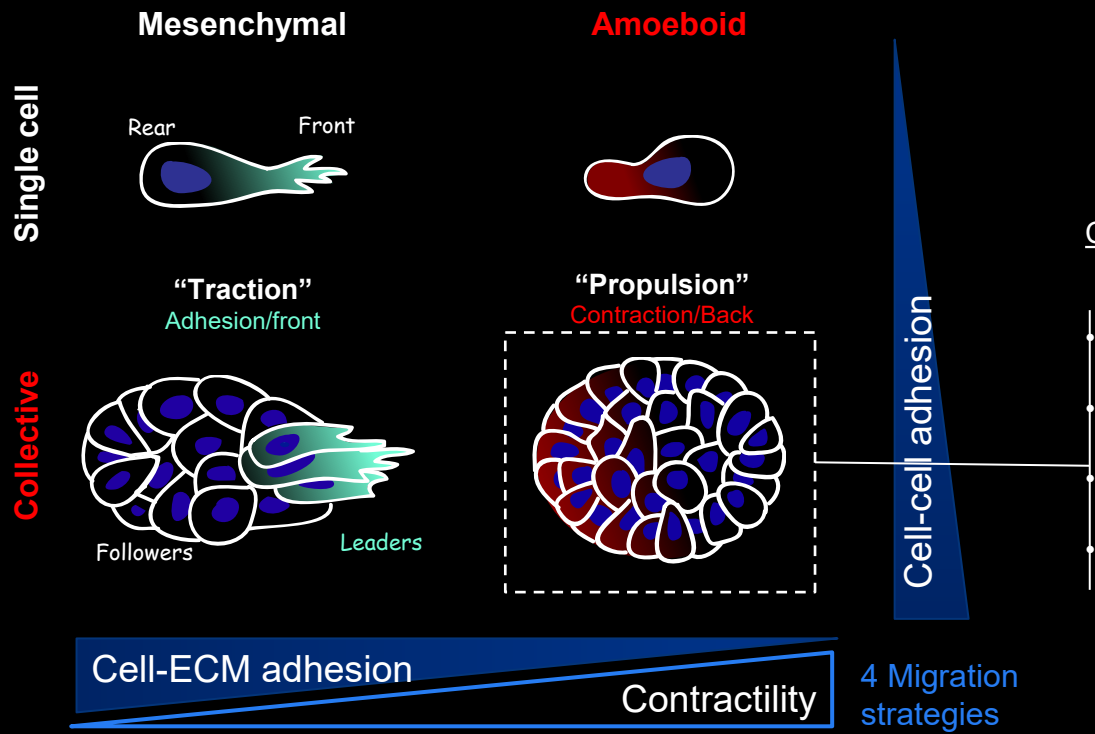
The Front/back polarization of the cortical acto-myosin cortex drives cluster migration

Optogenetic activation of Rho-A controls directionality



Jean De Seze (X)
(Coppey lab)

There is a 4th mode of cell migration, not powered by persistent retrograde flows



Collective Amoeboid Migration:

- Independent of focal adhesion
- Driven by a polarised supracellular acto-myosin cortex
- Fueled by stochastic myosin flows (unique!)
- Enabled by cell autonomous or non-autonomous factors

Pages et al. Science Advances, 2022