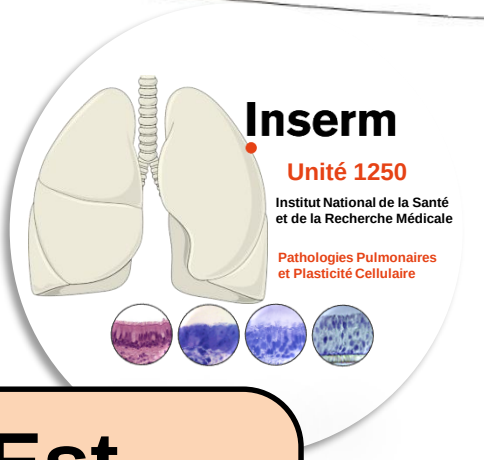
CANCÉROPÔLE Est
Régions Bourgogne-Franche-Comté / Grand Est

Inserm

Dormoy Valérian, PhD, HDR
MCU, chaire Inserm



WORKSHOP Cancéropôle Est

Systèmes modèles précliniques en cancérologie

Modèles *in vivo*

Intraductal xenograft models in breast cancer research

15 Novembre 2019

Centre Paul Strauss, Strasbourg

Université de Reims Champagne-Ardenne

Environmental context

ISREC
Institut Suisse de Recherche Expérimentale sur le Cancer
Schweizerisches Institut für Experimentelle Krebsforschung
Swiss Institute for Experimental Cancer Research

EPFL

BRISKEN LAB UPBRI

Research Activities | Publications | Team | Funding | Students projects

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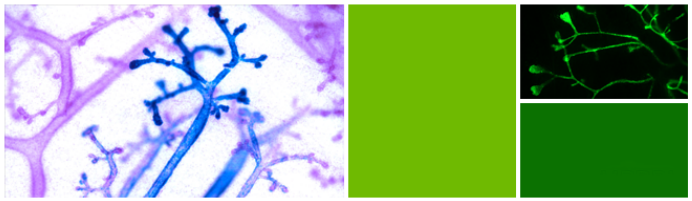


Genetic dissection of signaling pathways important in breast development and breast cancer

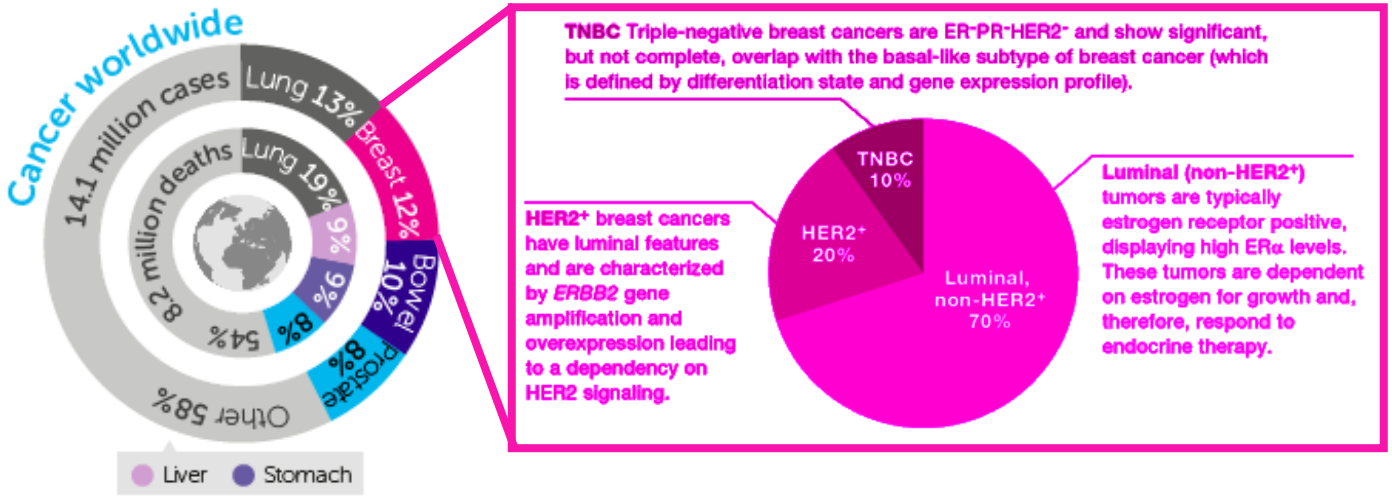
Breast cancer strikes one out of eight women in Switzerland. A woman's risk to get breast cancer is linked to her reproductive history. While early pregnancies have a protective effect, cancer risk increases with the number of menstrual cycles a woman experiences prior to her first pregnancy. Although it is well established that the female sex hormones estrogen, progesterone and prolactin control breast development and have an important role in breast carcinogenesis, the mechanisms by which they exert their effects are poorly understood. Our goal is to understand how hormones interact with developmental signaling pathways in the breast to control growth and differentiation. More...

Prof. Cathrin Brisken

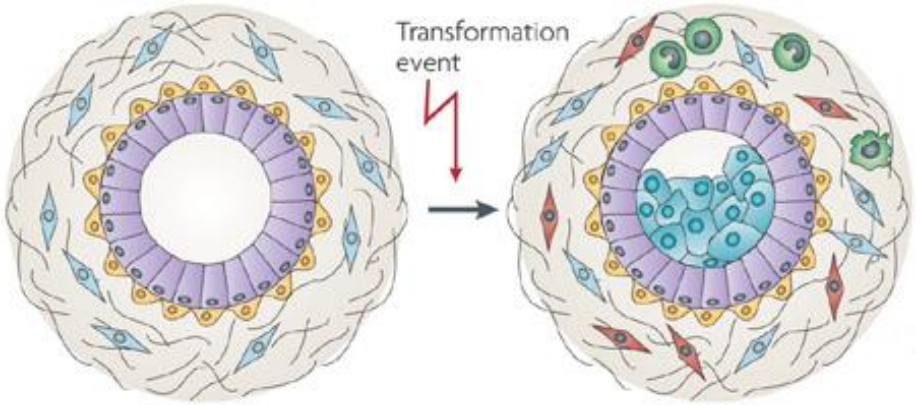
- **Role of Epithelial-Mesenchymal Activating Transcription Factors in invasion and metastasis of ER+ Breast Cancer**
- **The role of the hormone receptors/androgen receptor in breast cancer**
- **Role of ER/PR Receptor and Androgen Receptor in the Human Breast Epithelium *in vivo***

Scientific background

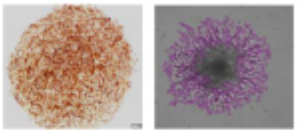
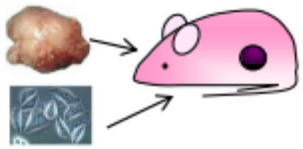
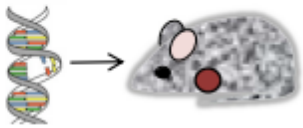
- Many therapeutic options but invasion remains the principal challenge



- From basal state to cancer



Experimental models

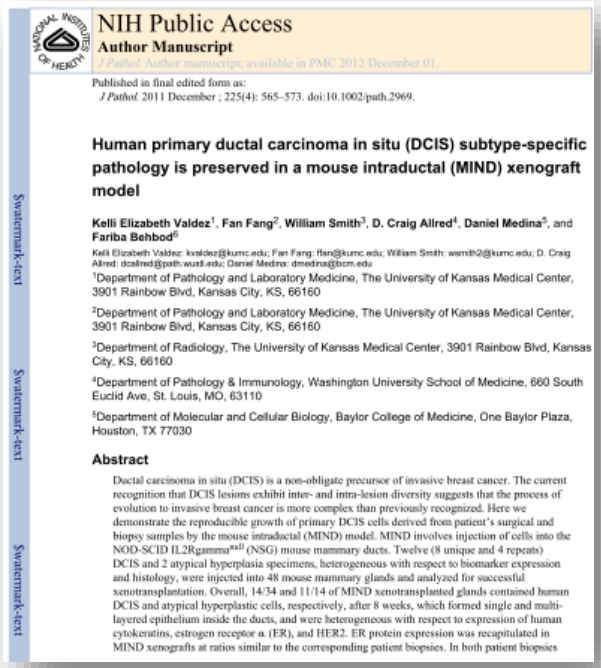
	TYPE OF MODEL	ADVANTAGES	DISADVANTAGES	IMPROVEMENTS
IN VITRO	2D monolayer 	• Standardised format • Widely used, simple • Suitable for cell panels • Suitable for proliferation, signalling pathways, genetic manipulation	• No ECM/stromal cells • Non-physiological • Static conditions • High oxygen/nutrients • Long-established lines • Homogeneous	• ECM substrates • Host cell co-culture • Flow conditions • Hypoxic conditions • Primary cell cultures
IN VITRO	3D spheroid suspension or matrix 	• Multiple assay platforms • ECM &/or stromal cells • Suitable for clonogenicity, migration, invasion etc • Polarity & architecture • Nutrient & O_2 gradients	• More complex/ expensive • Lower throughput • Some assays require imaging capability • Static conditions	• Tag cells for tracking in heterotypic cultures • Host cell co-cultures • CSC assays • Primary cell cultures
IN VIVO	Human tumour xenotransplants 	• S.c is standard model • Simple quantitation • Tissue environment, blood supply, host cells • Suitable for drug trials	• Ectopic growth site • No immune responses • Mouse physiology • Relatively expensive • Cannot study cancer initiation/prevention	• Orthotopic site (mfp) • 'Humanised' hosts • Metastatic models • Primary human cancer transplants (PDX)
IN VIVO	Genetically-modified mice (GEM) 	• Clinically-relevant genes • Anatomically correct • Natural development • Immunocompetent host • Can study initiation, prevention and therapy	• Difficult/expensive to run • Tumours sporadic/ slow • Limited heterogeneity • Mouse tumours and physiology • Seldom metastasise	• Primary transplants to increase reproducibility • Additional mutations to increase malignancy

→ "tumor environment"

→ "mouse tumor"

Towards novel animal models in BC?

♦ Improvement of the xenograft



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Human primary ductal carcinoma in situ (DCIS) subtype-specific pathology is preserved in a mouse intraductal (MIND) xenograft model

Kelli Elizabeth Valdez[1], **Fan Fang**[2], **William Smith**[3], **D. Craig Allred**[4], **Daniel Medina**[5], and **Fariba Behbod**[6]

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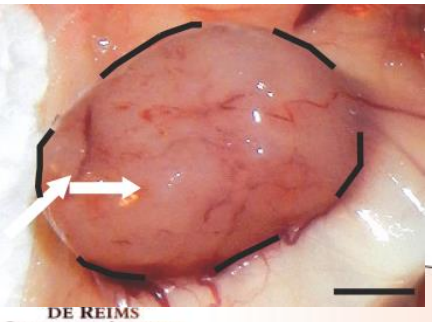
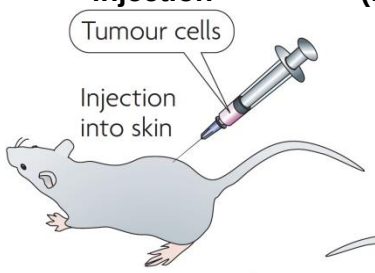
Abstract

Ductal carcinoma in situ (DCIS) is a non-obligate precursor of invasive breast cancer. The current recognition that DCIS lesions exhibit inter- and intra-lesion diversity suggests that the process of evolution to invasive breast cancer is more complex than previously recognized. Here we demonstrate the reproducible growth of primary DCIS cells derived from patient's surgical and biopsy samples by the mouse intraductal (MIND) model. MIND involves injection of cells into the NOD-SCID IL2Rgamma^{null} (NSG) mouse mammary ducts. Twelve (8 unique and 4 repeats) DCIS and 2 atypical hyperplasia specimens, heterogeneous with respect to biomarker expression and histology, were injected into 48 mouse mammary glands and analyzed for successful xenotransplantation. Overall, 14/34 and 11/14 of MIND xenotransplanted glands contained human DCIS and atypical hyperplastic cells, respectively, after 8 weeks, which formed single and multi-layered epithelium inside the ducts, and were heterogeneous with respect to expression of human cytokeratins, estrogen receptor α (ER), and HER2. ER protein expression was recapitulated in MIND xenografts at ratios similar to the corresponding patient biopsies. In both patient biopsies

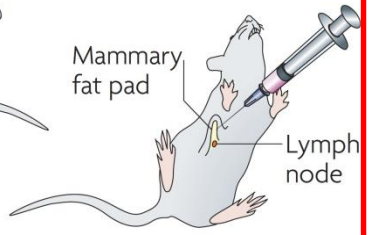
- **Only one type of BC: DCIS → recapitulate pathologic histology (ER/PR/HER2/Ki67)**
- **Hormones supplementation : + estradiol / + progesterone**
- **Only H&E**
- **No follow-up of tumor cells establishment and growth**
- **Not enough material to initiate pre-clinical studies**

Subcutaneous injection

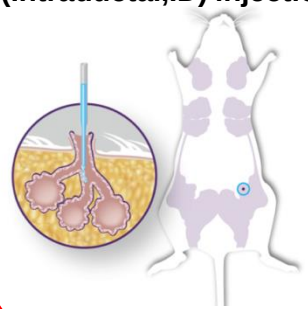


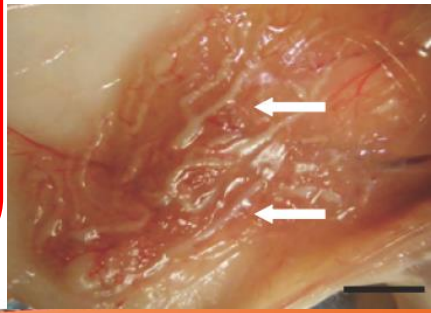


Orthotopic (fat pad,FP) injection

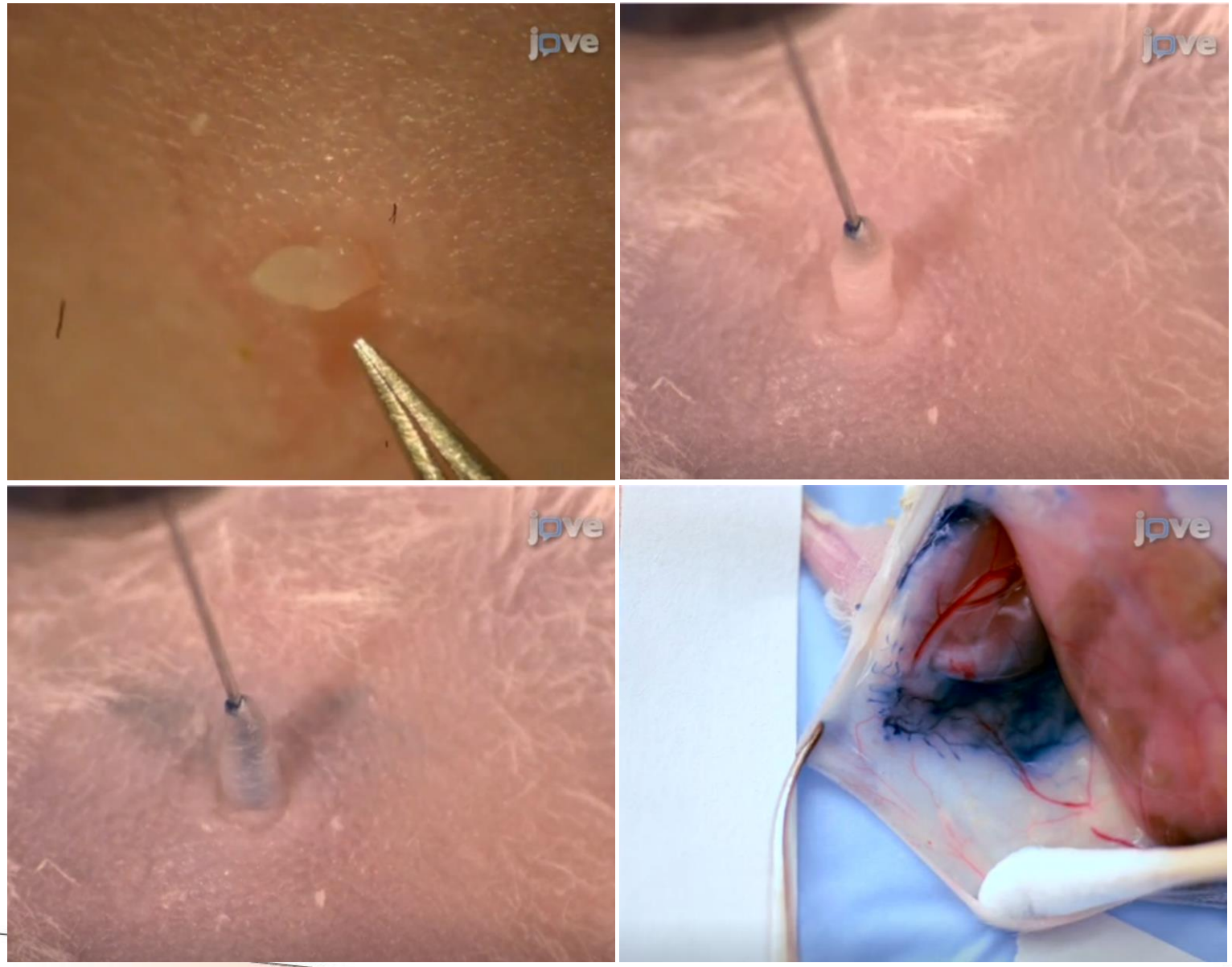


Orthotopic (Intraductal,ID) injection





Mouse intraductal model (MIND)



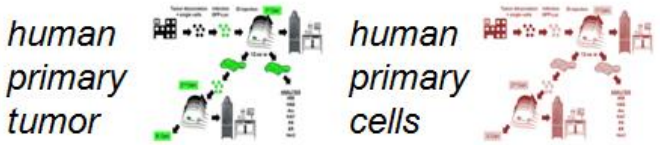
Krause et al., JOVE, 2013

Experimental approach

♦ **Our aim: characterization and validation of the establishment of MIND to study human breast cancers**

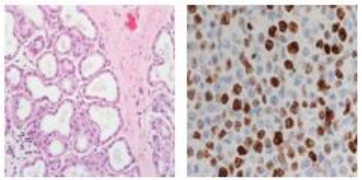
♦ **Overview:**

Intraductal injection method validation



Human primary tumor cells vs human primary cells

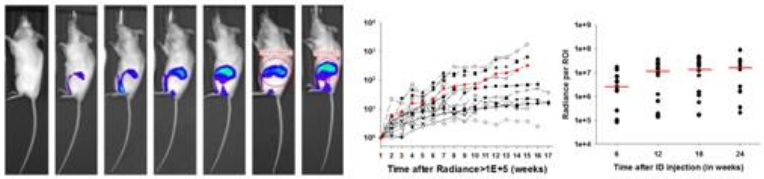
Histopathological recapitulation



H&E Ki67 PR ER Her2…

Human vs mice

Take rates / Growth patterns comparisons



Clinical impact

Treatment of HR+ tumors
Treatment of Her2 tumors

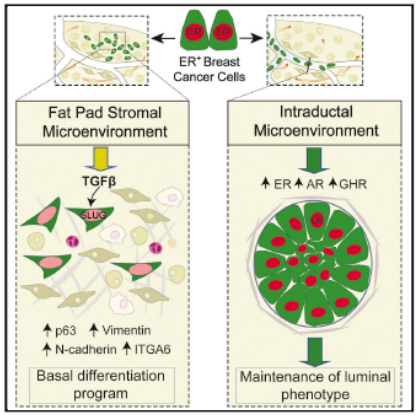
Cancer cell lines & microenvironment + validation of MIND-PDXs

Article

Cancer Cell

A Preclinical Model for ERα-Positive Breast Cancer Points to the Epithelial Microenvironment as Determinant of Luminal Phenotype and Hormone Response

Graphical Abstract



Authors

George Sflomos, Valerian Dormoy, Tauno Metsalu, ..., Jaak Vilo, Ayyakkannu Ayyanan, Cathrin Brisken

Correspondence

cathrin.brisken@epfl.ch

In Brief

Sflomos et al. show that engrafting human estrogen receptor α-positive breast tumors into mouse milk ducts, in contrast to mammary fat pads, efficiently generates retransplantable xenografts that mimic the original tumors. They identify differential induction of SLUG by these microenvironments as a key factor.

Highlights

- Tissue microenvironment is critical for the growth of ER+ breast cancer cells
- Mammary stroma induces TGFβ/SLUG signaling and basal differentiation in MCF7 cells
- Mouse milk ducts enable physiological growth of ER+ breast cancer cells
- Mouse intraductal ER+ PDXs are robust, retransplantable, and predictive

Accession Numbers

GSE68694
GSE74608

Sflomos et al., 2016, Cancer Cell 29, 407–422
March 14, 2016 ©2016 Elsevier Inc.
http://dx.doi.org/10.1016/j.ccell.2016.02.002

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BRIEF DEFINITIVE REPORT

Intraductal patient-derived xenografts of estrogen receptor α–positive breast cancer recapitulate the histopathological spectrum and metastatic potential of human lesions

Maryse Fiche[1†], Valentina Scabia[2†], Patrick Aouad[2], Laura Battista[2], Assia Treboux[3], Athina Stravodimou[3], Khalil Zaman[3], RLS[4], Valerian Dormoy[5], Ayyakkannu Ayyanan[2], George Sflomos[2] and Cathrin Brisken[2*]

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†These authors contributed equally.

Abstract

Estrogen receptor α–positive (ER–positive) or 'luminal' breast cancers were notoriously difficult to establish as patient–derived xenografts (PDXs). We and others recently demonstrated that the microenvironment is critical for ER–positive tumor cells; when grafted as single cells into milk ducts of NOD Scid gamma females, >90% of ER–positive tumors can be established as xenografts and recapitulate many features of the human disease *in vivo*. This intraductal approach holds promise for personalized medicine, yet human and murine stroma are organized differently and this and other species specificities may limit the value of this model. Here, we analyzed 21 ER–positive intraductal PDXs histopathologically. We found that intraductal PDXs vary in extent and define four histopathological patterns: flat, lobular, *in situ* and invasive, which occur in pure and combined forms. The intraductal PDXs replicate earlier stages of tumor development than their clinical counterparts. Micrometastases are already detected when lesions appear *in situ*. Tumor extent, histopathological patterns and micrometastatic load correlate with biological properties of their tumors of origin. Our findings add evidence to the validity of the intraductal model for *in vivo* studies of ER–positive breast cancer and raise the intriguing possibility that tumor cell dissemination may occur earlier than currently thought.

Keywords: intraductal xenografts; luminal breast cancer; preclinical model; patient-derived xenografts; ductal carcinoma *in situ*; micrometastasis

Received 7 August 2018; Revised 5 November 2018; Accepted 8 November 2018

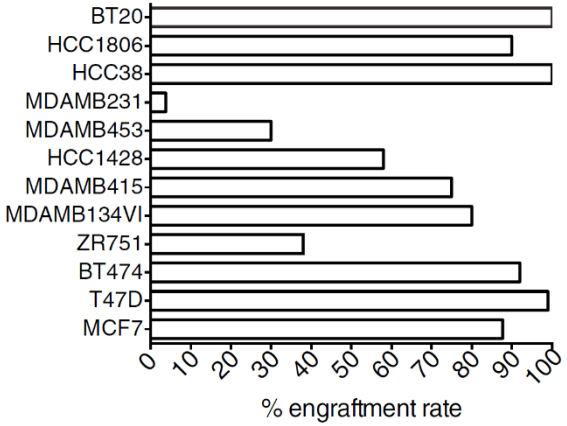
No conflicts of interest were declared.

Introduction

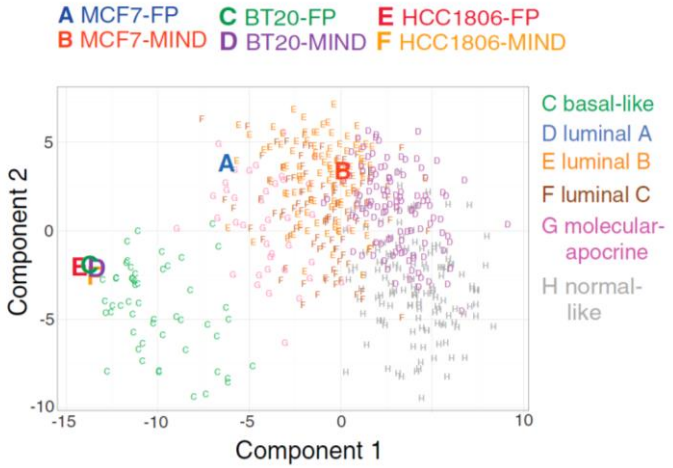
Breast cancer (BC) is a frequent disease worldwide [1]. Over 75% of BCs express estrogen receptor (ER) in >1% of the tumor cells by immunohistochemistry (IHC) [2] and overlap with luminal A and B subtypes defined by global gene expression [3,4] exhibiting low versus high proliferative indices and distant recurrence rates [5]. Twenty percent of patients experience distant recurrence and cancer-related death [6]. Overtreatment of early disease and endocrine resistance are additional problems concerning this subgroup [7]. A lack of preclinical models hampered progress in understanding the biology of luminal tumors and the development of new therapies. Genetically engineered mouse models mostly develop ER-negative tumors; few ER-positive BC cell lines grow *in vivo* requiring non-physiological estrogen supplements [8]. Patient-derived xenografts (PDXs) are increasingly used but difficult to establish from ER-positive tumors [8]. We and others showed that the microenvironment is a major determinant of luminal BC cells and that take rates increase dramatically when luminal BC cells are grafted to mouse milk ducts [9]. They grow without estrogen supplementation, recapitulating many features of their clinical counterpart [9,10]. Yet, mammary stroma and endocrine milieu differ between women and mice. To assess the impact of the mouse host on the biology of the engrafted human cells, we analyzed 21 intraductal PDXs histopathologically.

Cancer cell lines & microenvironment

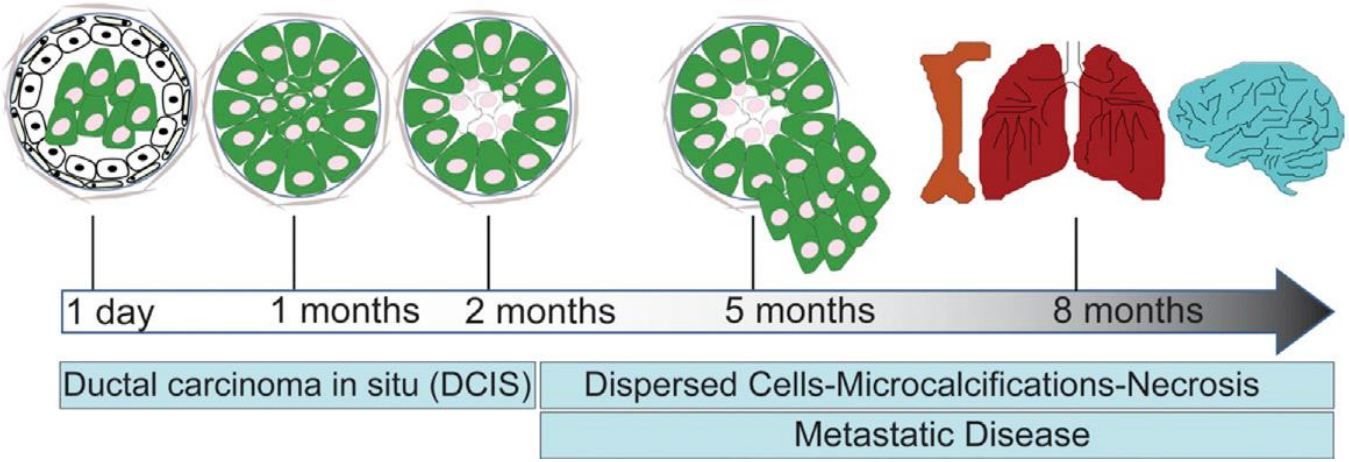
- **All cell lines grow intraductally (except MDAMB231)**



- **PCA and PAM50 to classify 48 breast cancer cell lines: MCF-MIND clustered with luminal**

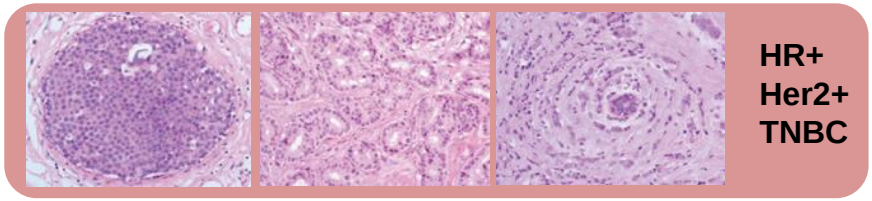


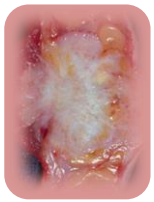
- **MIND recapitulate tumor progression from DCIS to invasion**



PDXs-MIND

♦ **Our aim:** characterize and validate the establishment of Mice IntraDuctally (MIND) injected to study human breast cancers

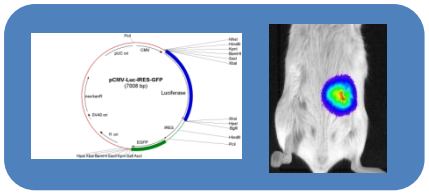




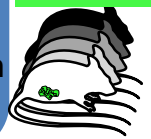
Tumor dissociation
+ single cells

Infection
GFP-Luc

ID injection



1st Gen MIND



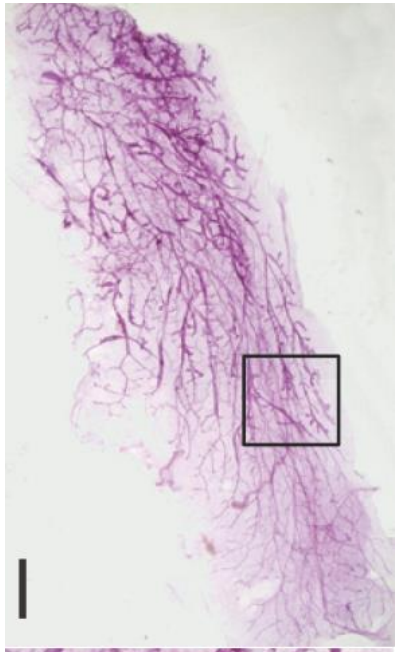
IVIS

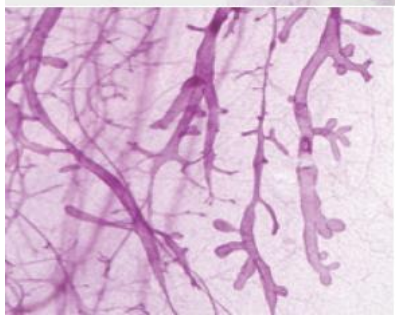
2nd Gen

X Gen

PRE-CLINICAL STUDIES

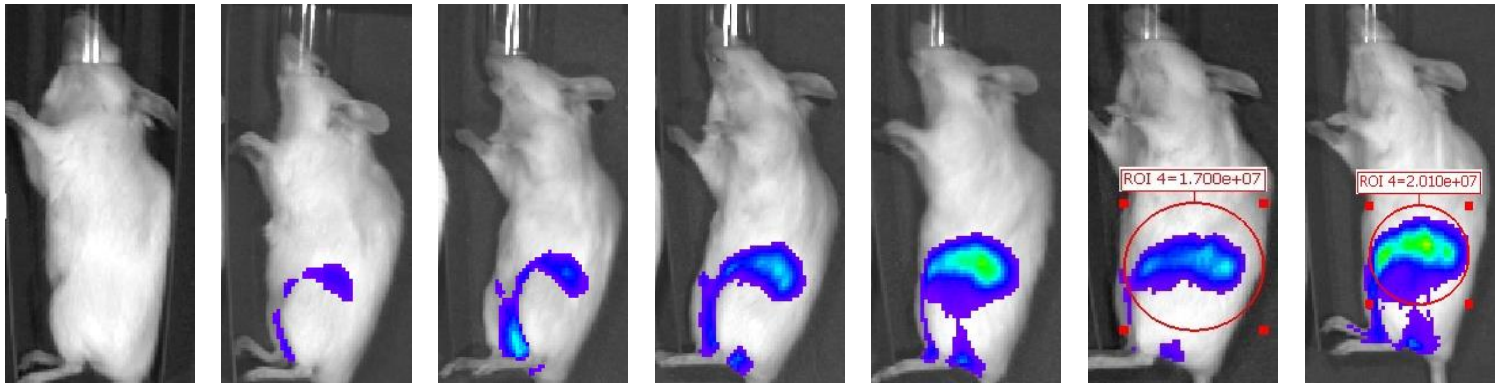
ANALYSIS
WM
H&E
Alu
IHC markers





In vivo monitoring of cancer cells

- ***In vivo* Imaging System (IVIS) measurements**
  - **Case n° T4, M#3/4L**

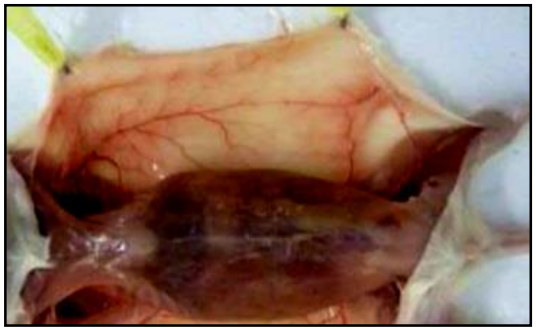


Radiance	<1^{E}+05	2.7^{E}+05	8.6^{E}+05	4.8^{E}+06	1^{E}+07	1.7^{E}+07	2^{E}+07
Weeks after injection	1-3	3	6	9	12	15	18

  - **Case n° T10, M#5/3R**

Radiance	<1^{E}+05	3,90^{E}+05	8,10^{E}+06	2,60^{E}+07	7,70^{E}+07	9,00^{E}+07	1,50^{E}+09
Weeks after injection	1	2	3	4	5	6	12

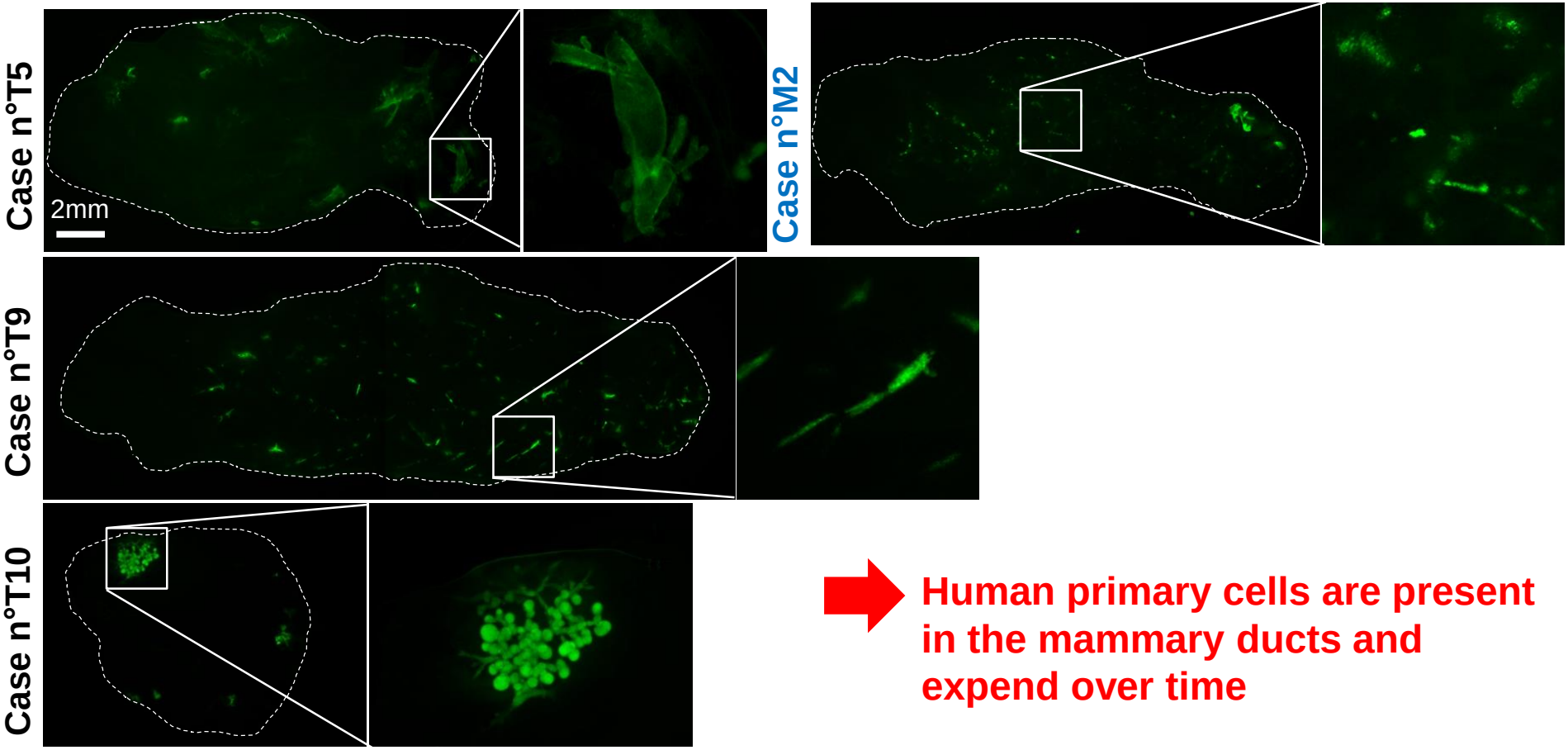
- **No visible/palpable tumors**



What are we monitoring *in vivo*?

- **Distribution of the human cells in the mammary glands**

1. Fluorescence of GFP-positive cells

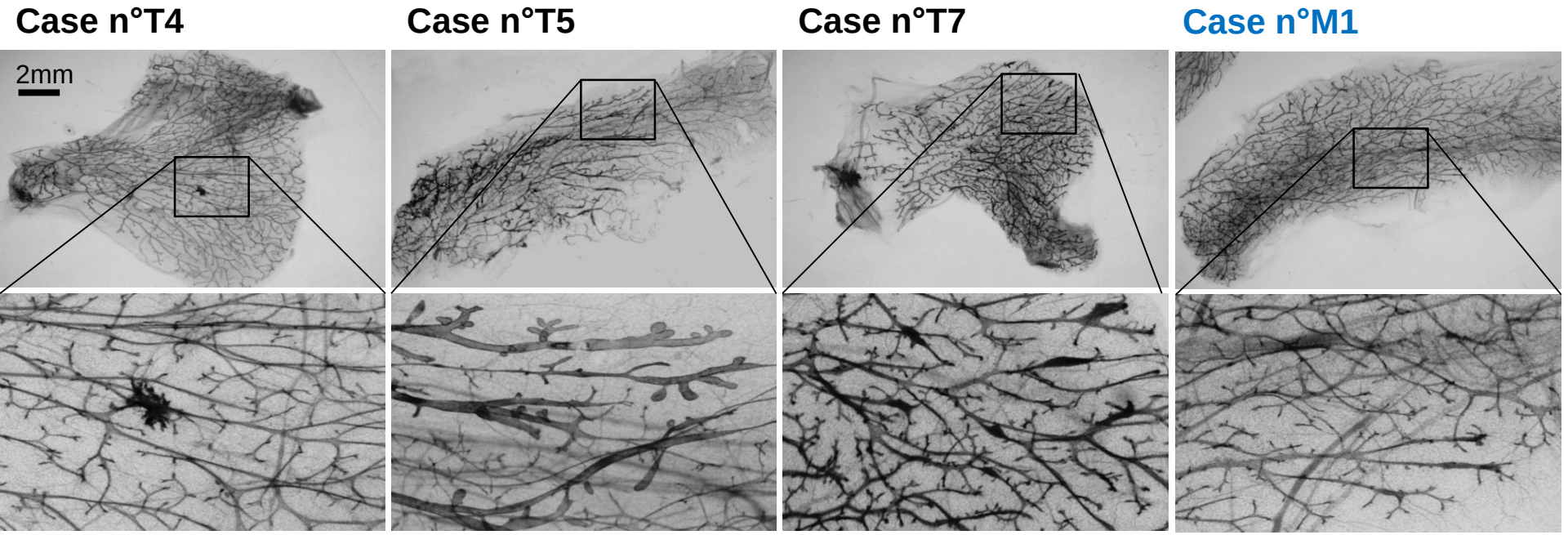


Human primary cells are present in the mammary ducts and expend over time

What are we monitoring *in vivo*?

- **Distribution of the human cells in the mammary glands**

2. Wholemount Carmine

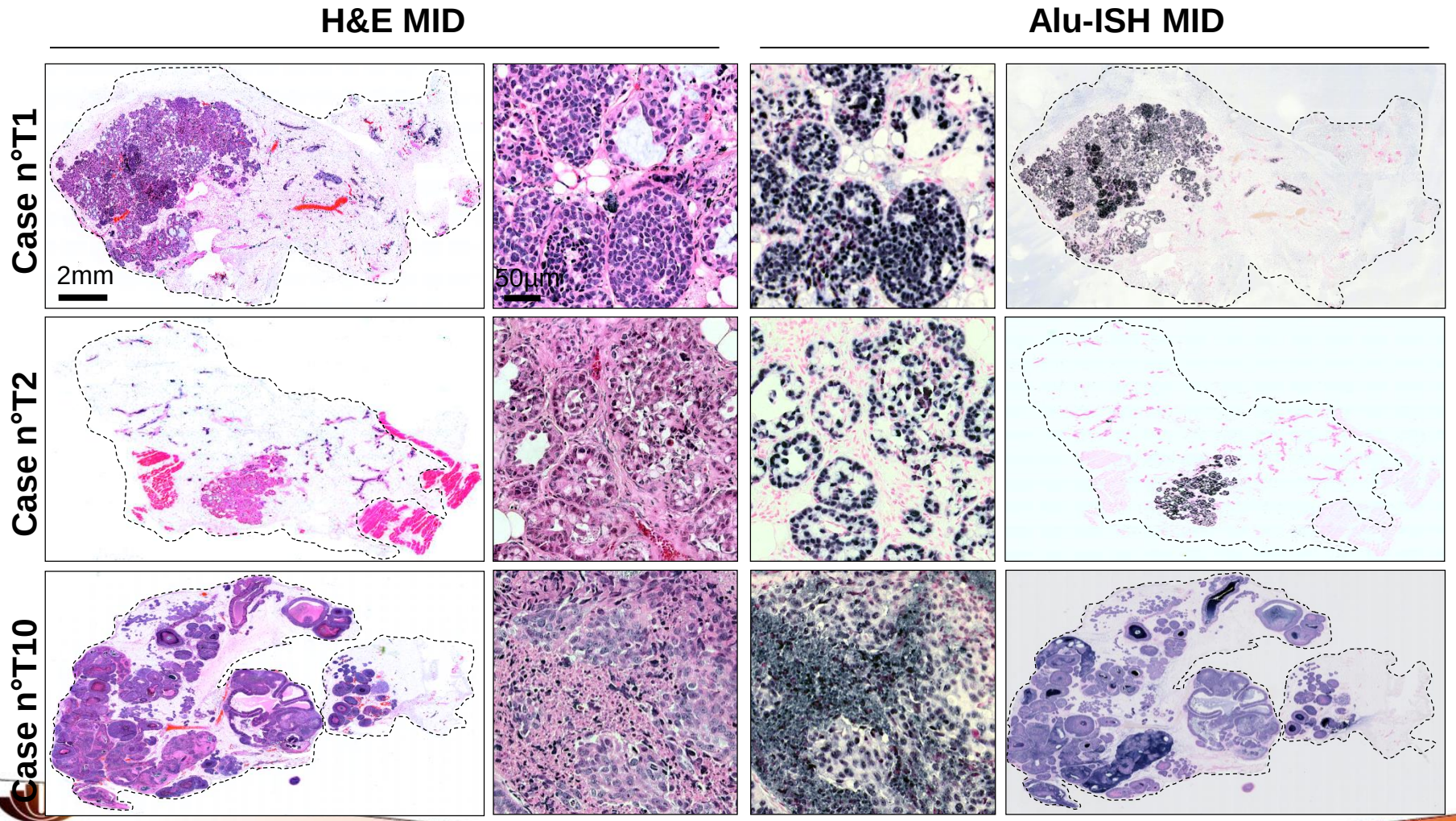


The pattern of localization and the expansion of the human primary cells vary from one PDX to another

What are we monitoring *in vivo*?

- **Distribution of the human cells in the mammary glands**

3. Alu-ISH

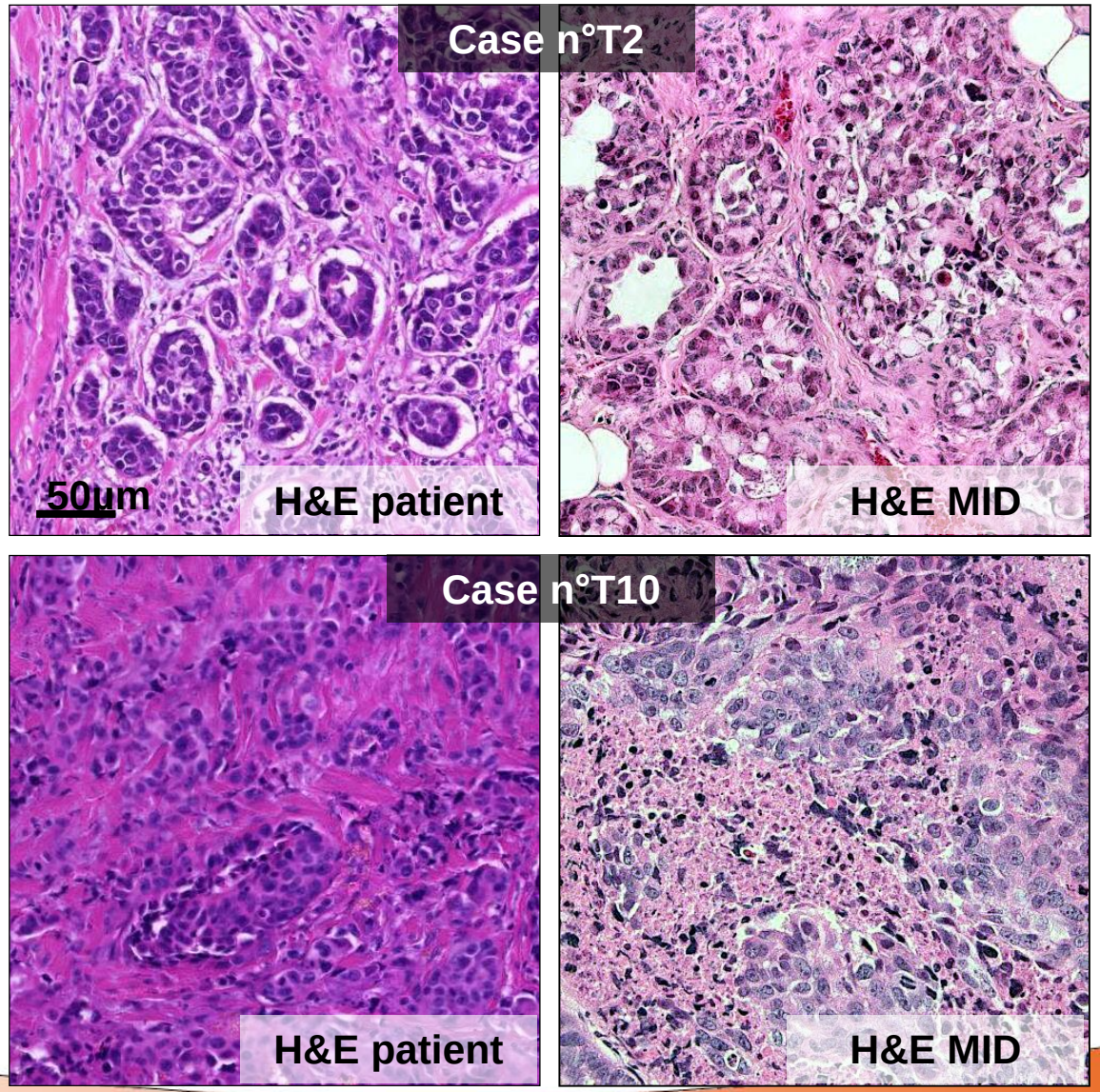


What are we monitoring *in vivo*?

♦ **Recapitulation of the histopathological features**

- **3 types:**
  - **(1)** morphology (size, mitosis…)
  - **(2)** type (ID, IDC…)
  - **(3)** histological markers (ER, PR, Ki67, Her2…)

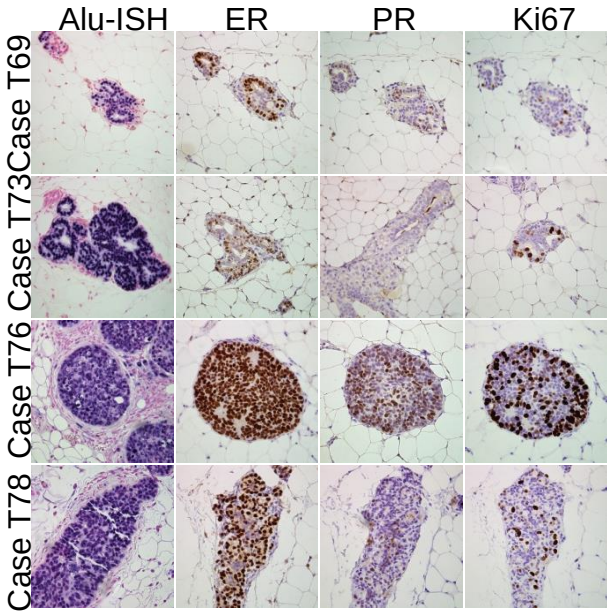
Morphology and types are recapitulated by the MIND

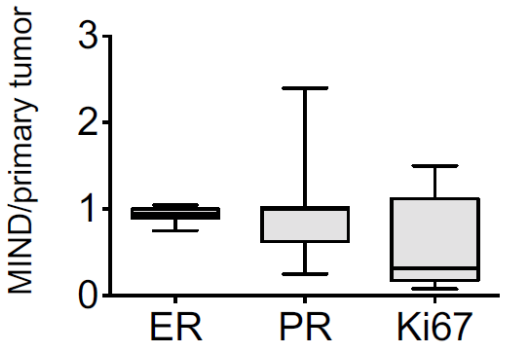


MIND conserved HR/proliferative signature

- **Recapitulation of the histopathological features**

Patient tumor				PDX-MIND		
patient	ER	PR	Ki67	ER	PR	Ki67
1	100	0	90	95	0	30
2	100	10	25	90	0-100	5
3	100	90	17	100	95	5
4	100	5	29	100	12	35
5	95	30	20	100	28	30
6	100	100	16	92	25	5
7	100	0	26	75	0	2
8	100	60	80	90	40	60
9	100	80	10	95	40	1
10	0	0	>90	0	0	98



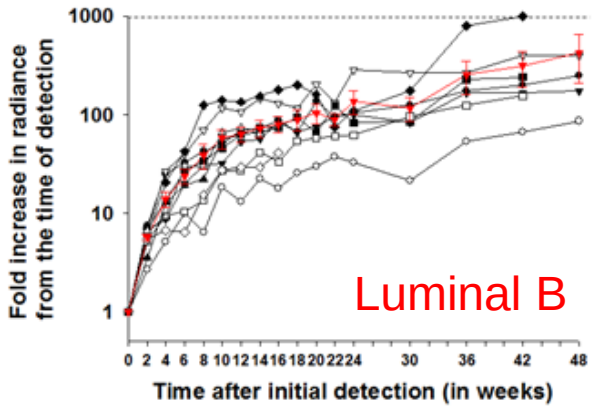


ER/PR status are conserved & proliferative status is similar

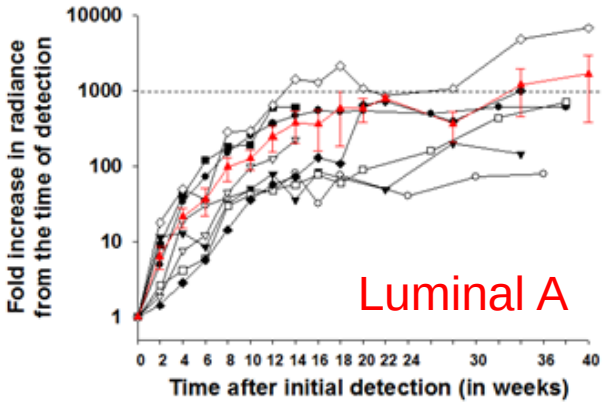
Input acquired with *in vivo* follow-up

♦ Growth curves for each patients: tumors

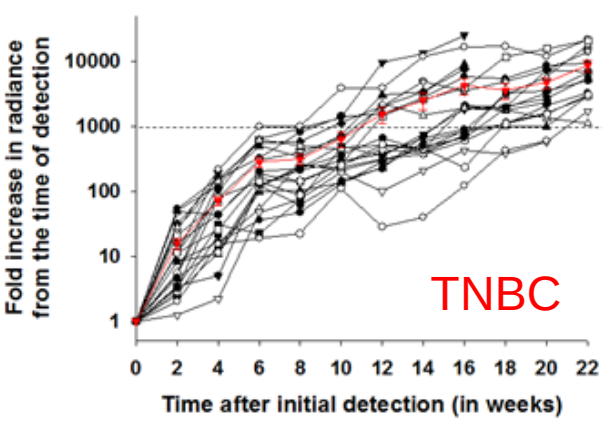
Case n°T4

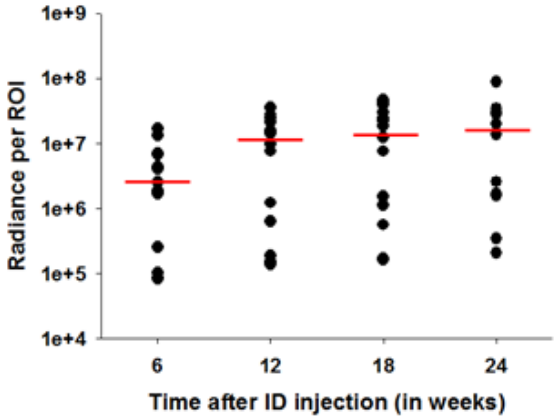


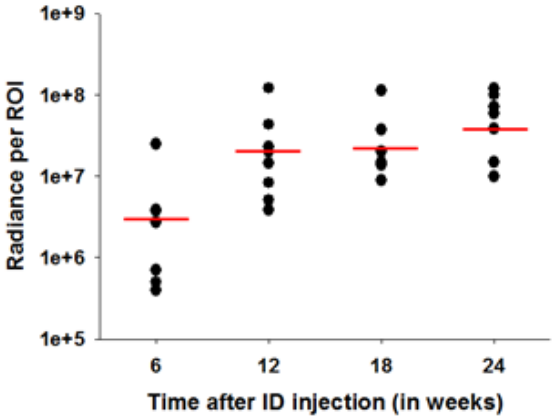
Case n°T5

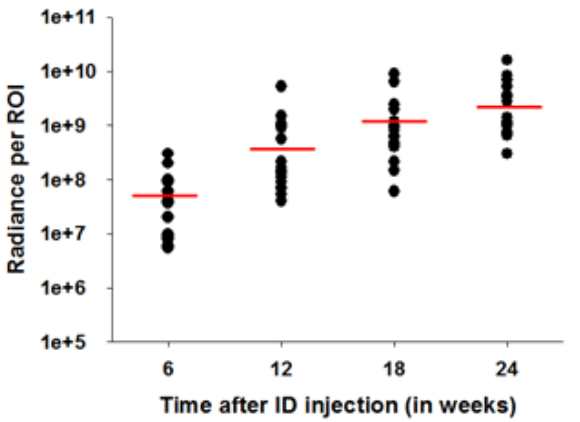


Case n°T10







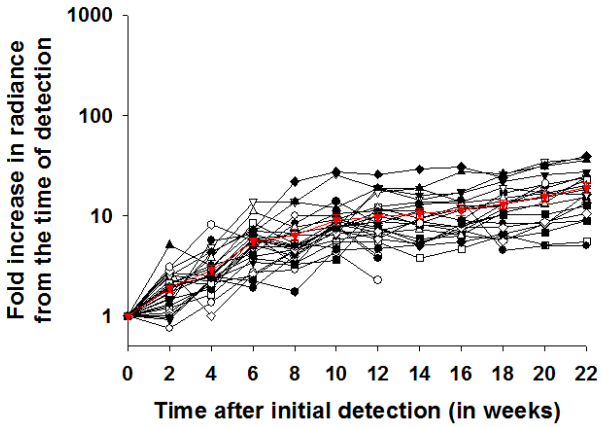


All human primary tumor specimen established in MIND PDXs

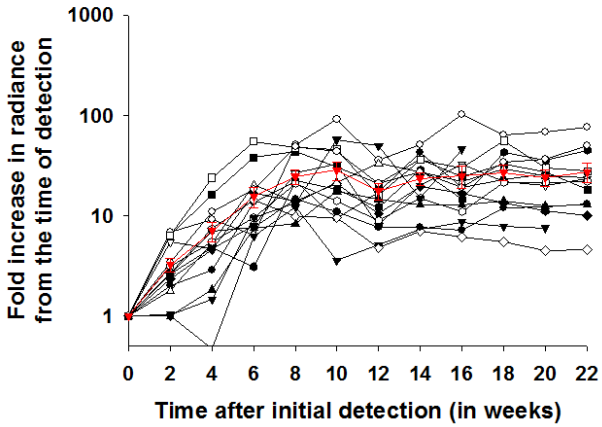
Input acquired with *in vivo* follow-up

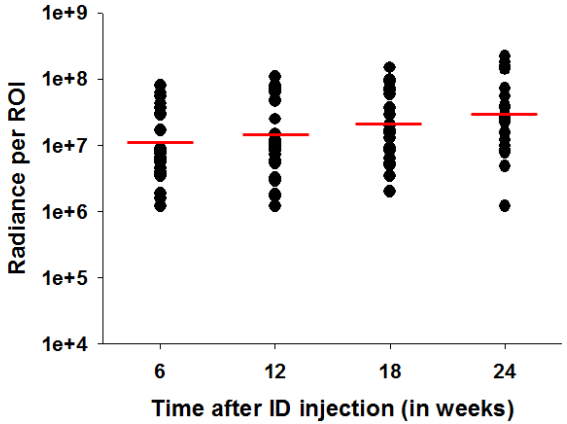
Growth curves for each patients: mammoplasty

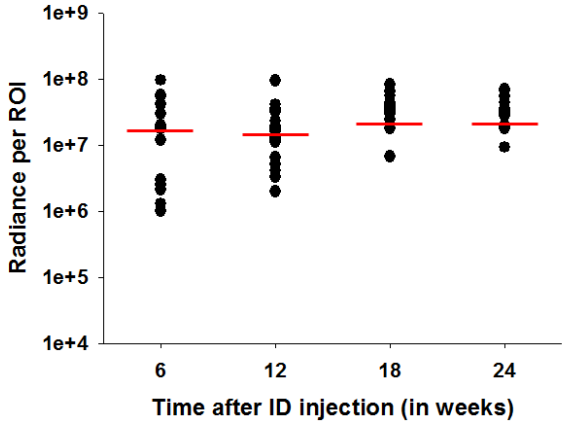
Case n°M2



Case n°M3





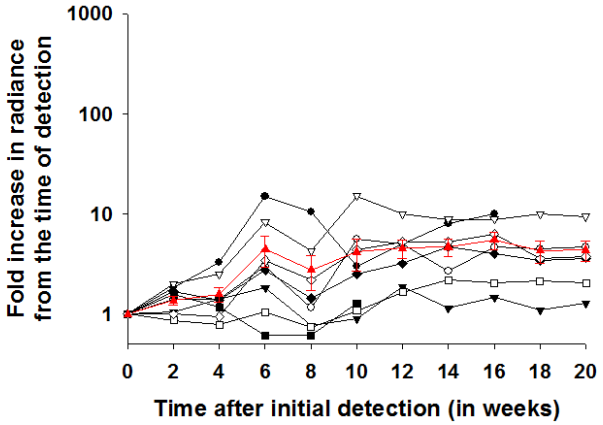


Human primary cells established but do NOT form tumors

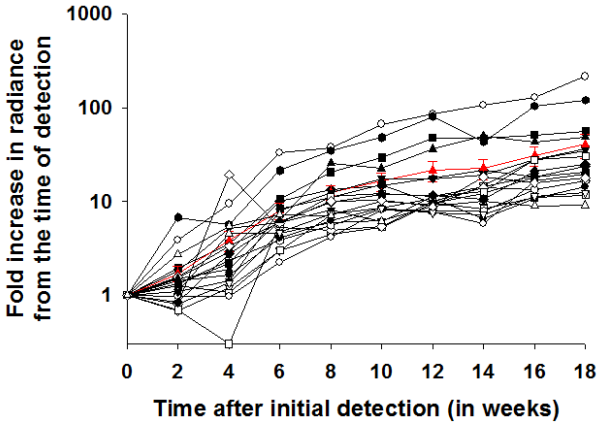
Input acquired with *in vivo* follow-up

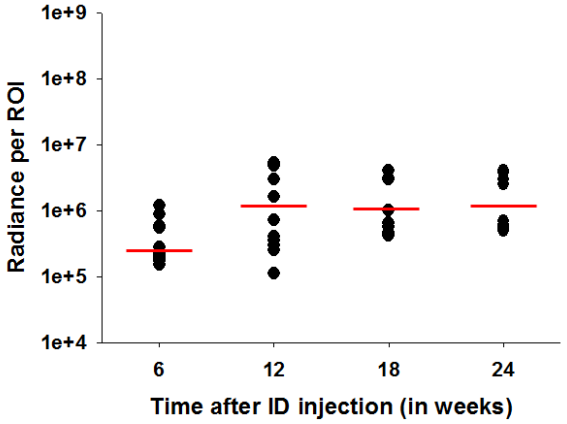
- **Growth curves for each patients: BRCA mutants**

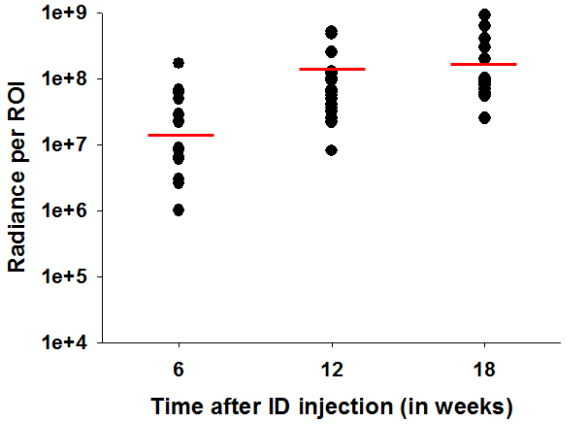
Case n°T11



Case n°T12

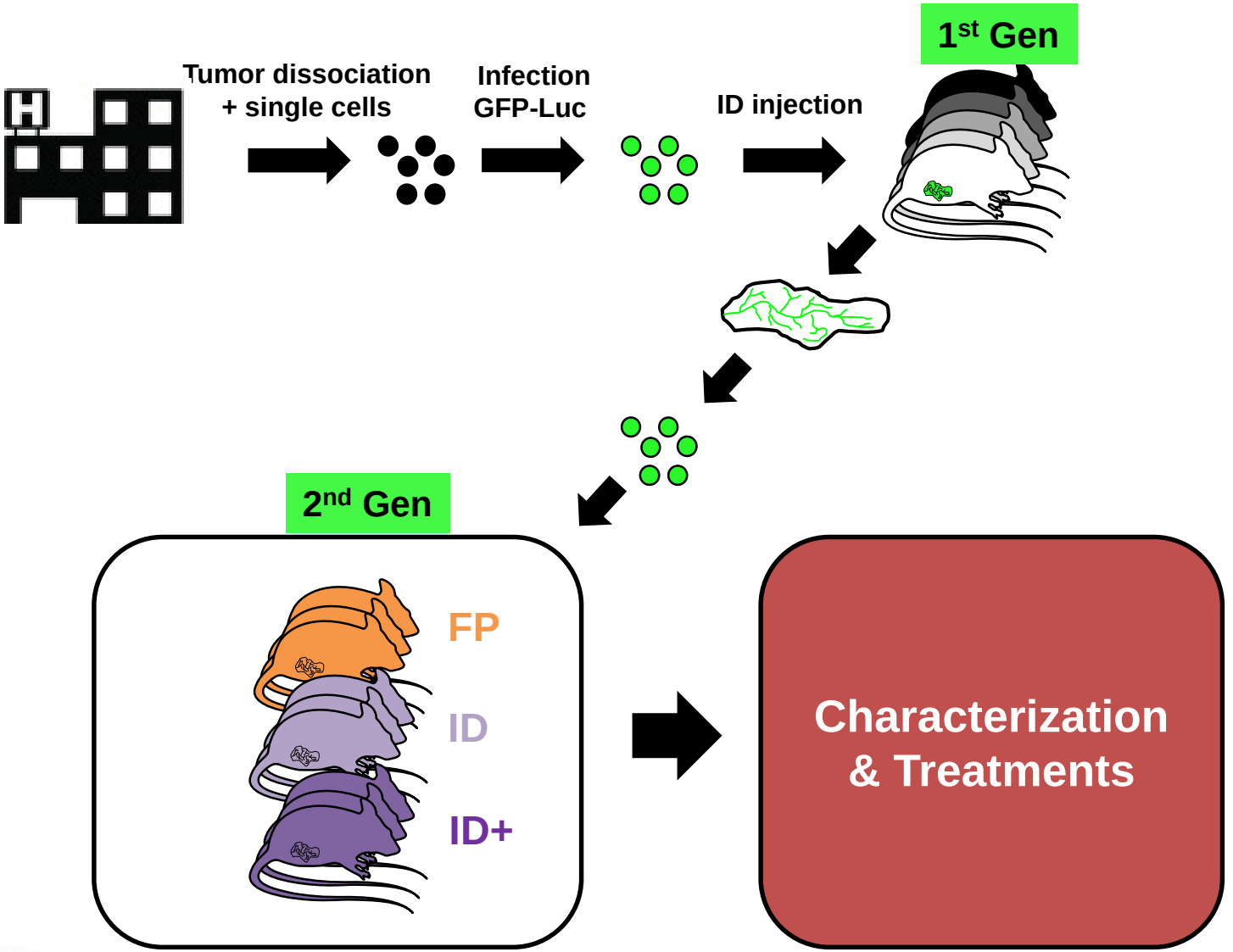






Possible investigation of the roles of mutations in the MIND PDXs

2nd generations for pre-clinical studies

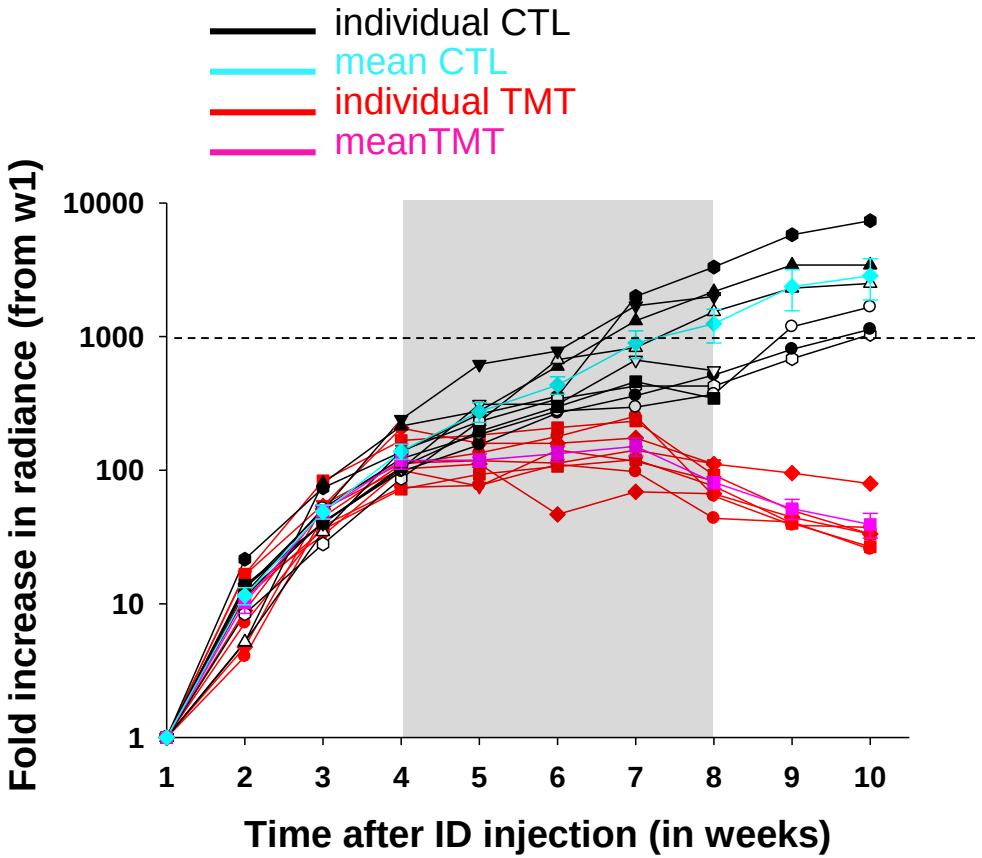


MIND-therapies to test patient's response?

♦ **Treatment of MIND ER-/Her2-**

Doxorubicine 3mg/kg/day i.Tu (50µg/mouse/week i.p.)

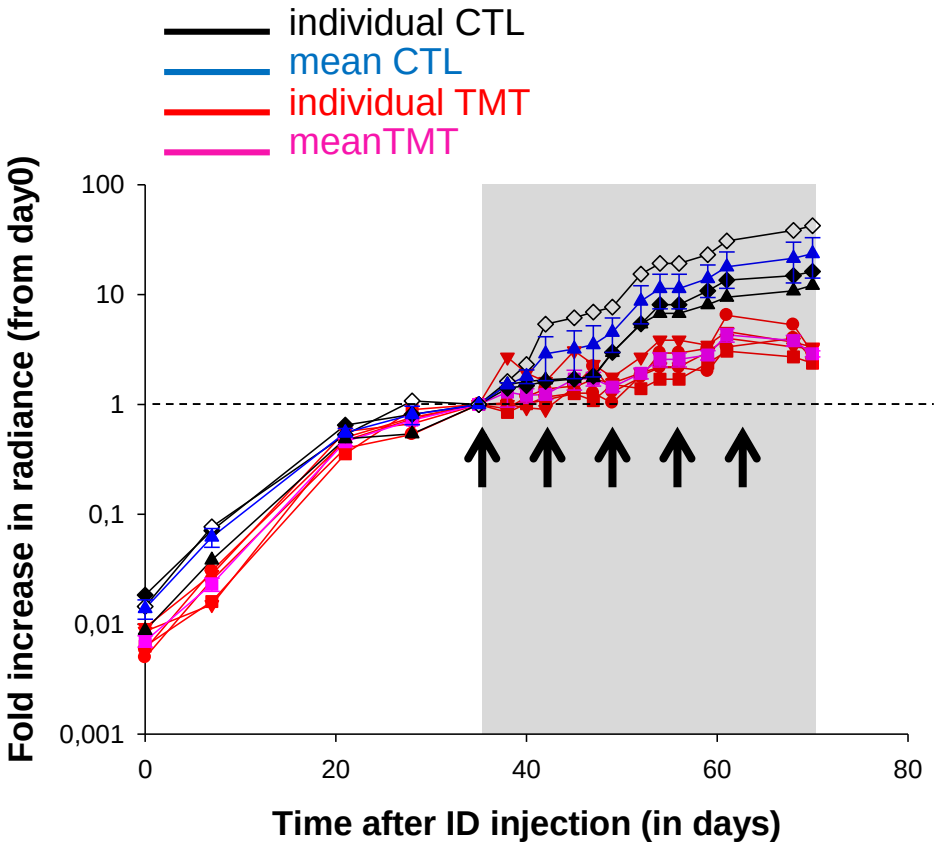
Cyclophosphamide 10mg/kg in drinking water



MIND-therapies to test patient's response?

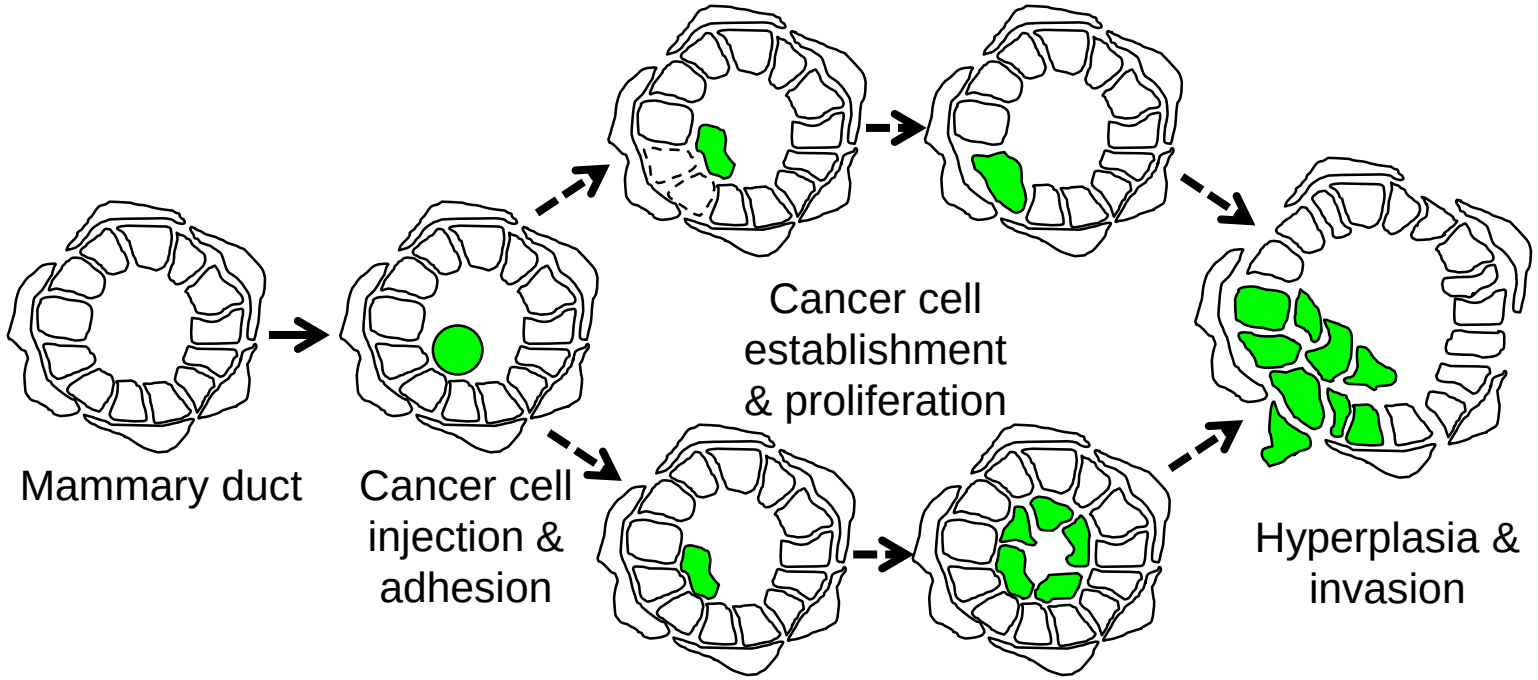
♦ **Treatment of MIND ER+**

Faslodex (Fulvestrant)
25mg/mouse/week s.c.



Understanding cancer cells establishment, growth and invasion

- **Modelisation of cancer cell establishment: insertion? growth? differentiation?**
- **Factors necessary/sufficient for cancer cell establishment: adhesion proteins? hormone signaling?**
- **Factors necessary/sufficient for cancer cell invasion/migration**



> Understand & characterize MIND model

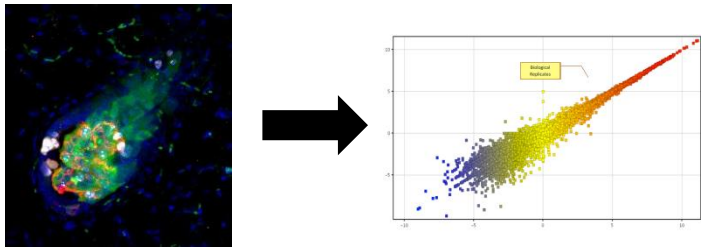
> Identify new targets/markers for pre-clinical & clinical studies

Understanding cancer cells establishment, growth and invasion

♦ **3 approaches:**

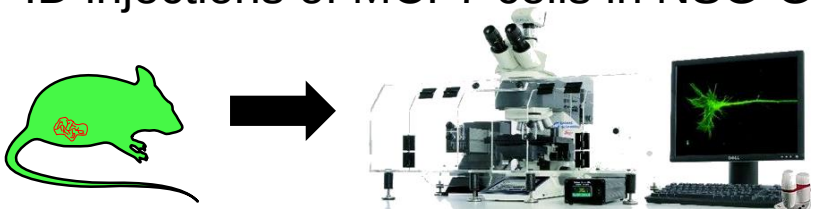
1. **RNAseq analysis:**

   MCF7 at the time of injection vs ID for 1/2/3/5 days
   >identification of targets to prevent establishment of the cancer cells



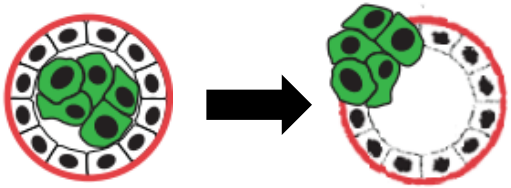
2. ***In vivo* follow-up of cancer cell establishment**

   ID injections of MCF7 cells in NSG-GFP mice and 2-photons live imaging



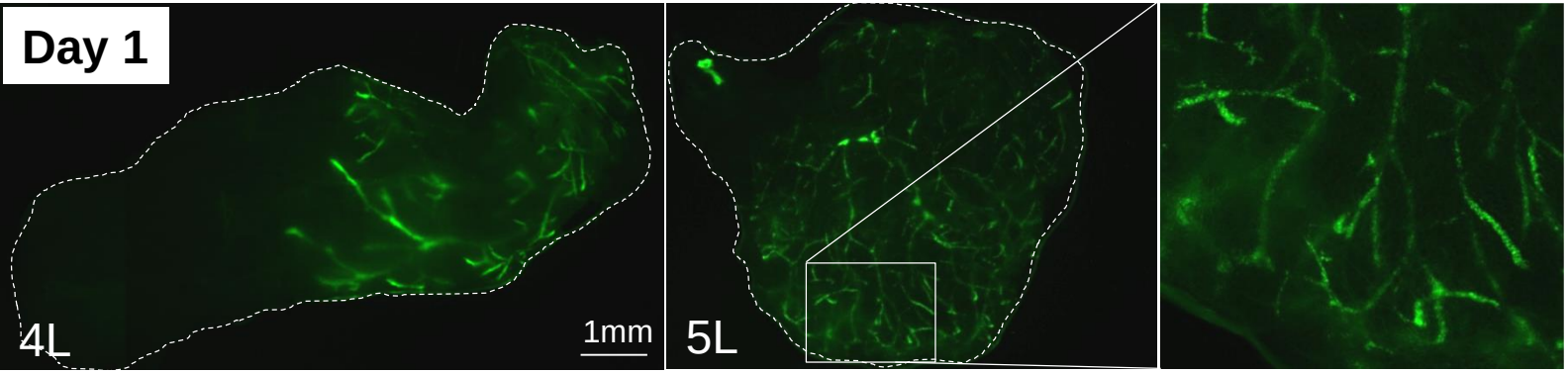
3. **3D culture of mammary epithelial cells and microinjection of cancer cells to follow *in vivo* establishment**

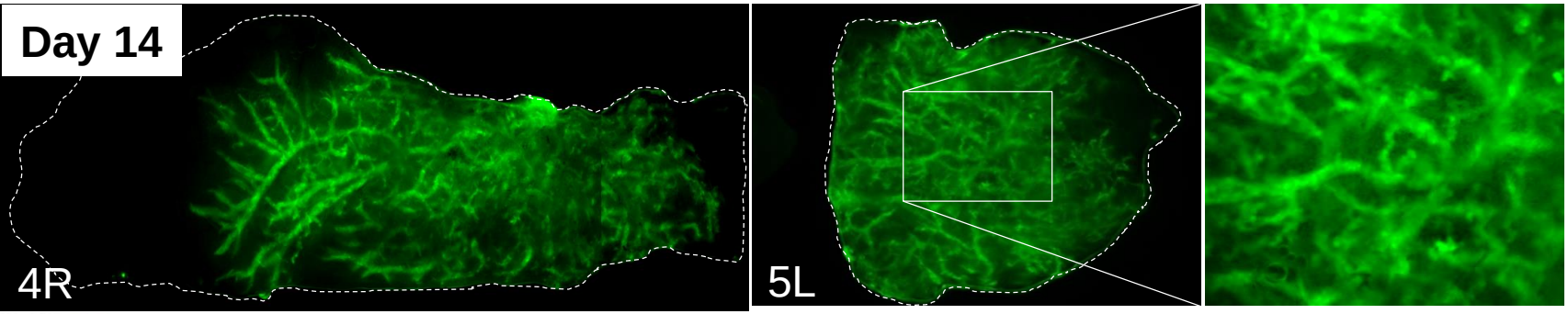
   >assay for drug testing & identify molecular actors in cancer cell establishment, growth and invasion



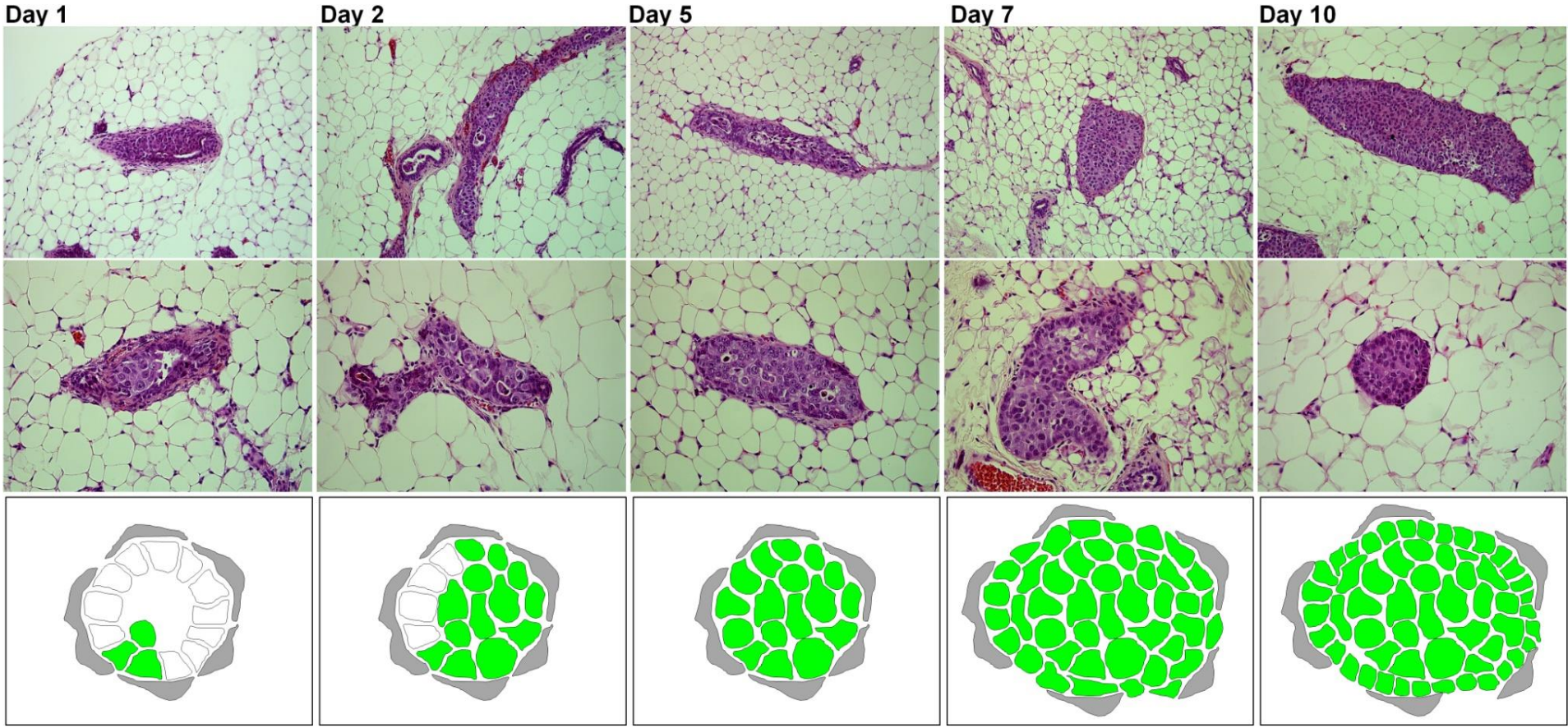
Characterizing tumor patterns in MIND

> Insertion of the cancer cells and proliferation





Characterizing tumor patterns in MIND

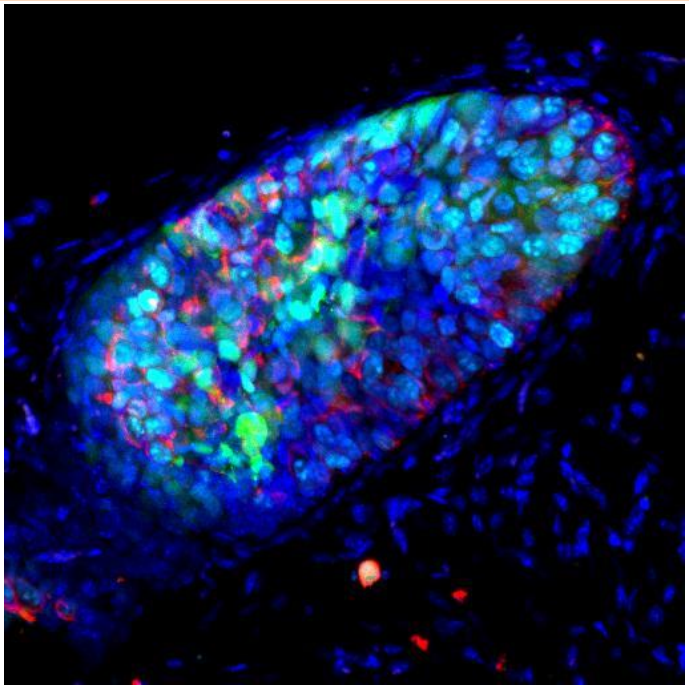


In less than 3 days, human cancer cells replace endogenous epithelium

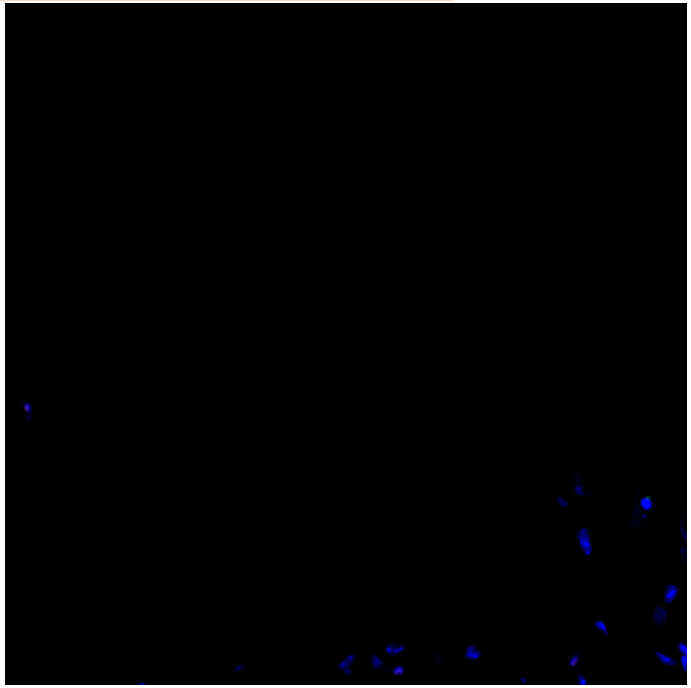
Characterizing tumor patterns in MIND

- DAY 5
- Vibratome sections: 250 µm
- LSM700
- Z-Stack 50µm
- 40X Oil

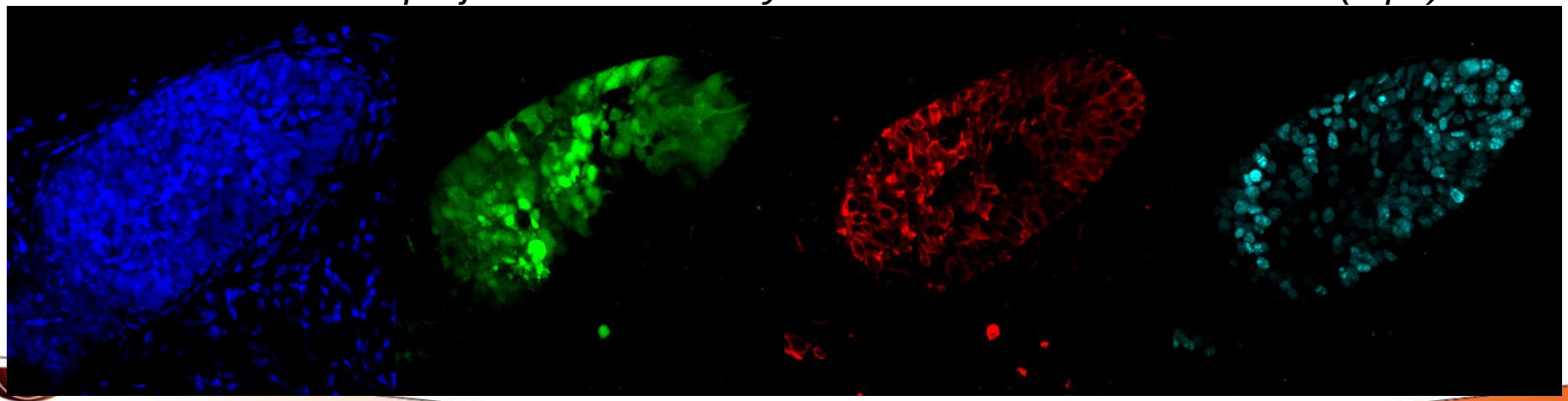
GFP
DNA
K8
Ki67



Z-project MAX intensity



Movie z-stack (2fps)

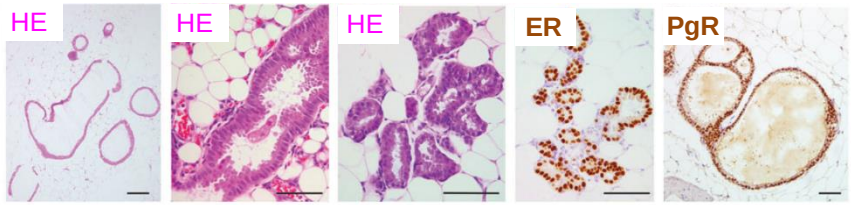


Montage on Z-Project

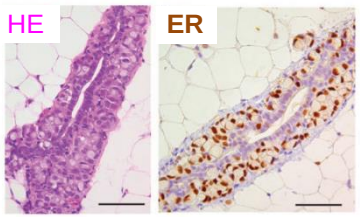
Characterizing tumor patterns of HR+ tumors in MIND

♦ **Characterization of 4 different patterns in pairs primary tumor/PDX: flat (T), lobular (LOB), in situ (IS), invasive (INV)**

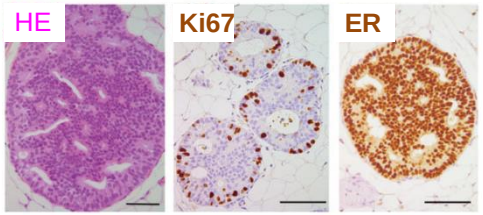
FLAT



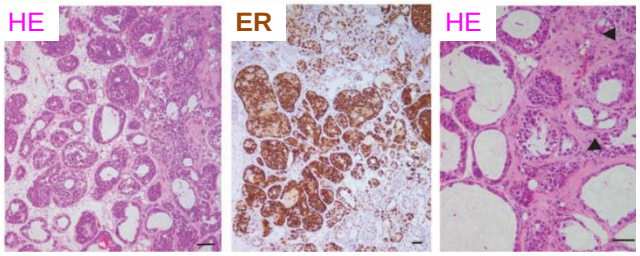
LOBULAR

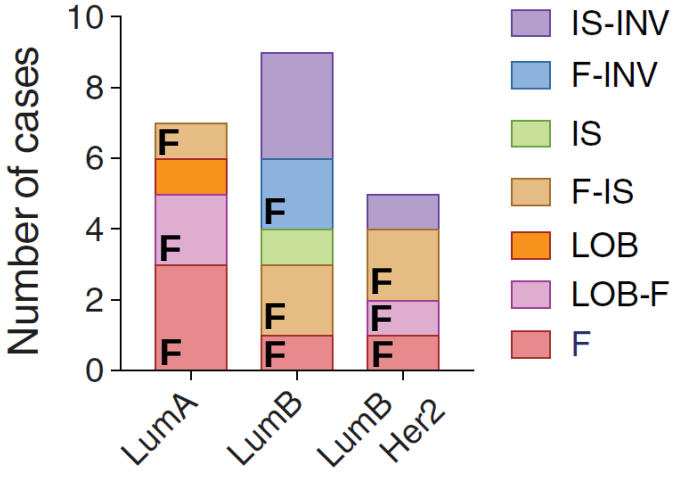


IN SITU



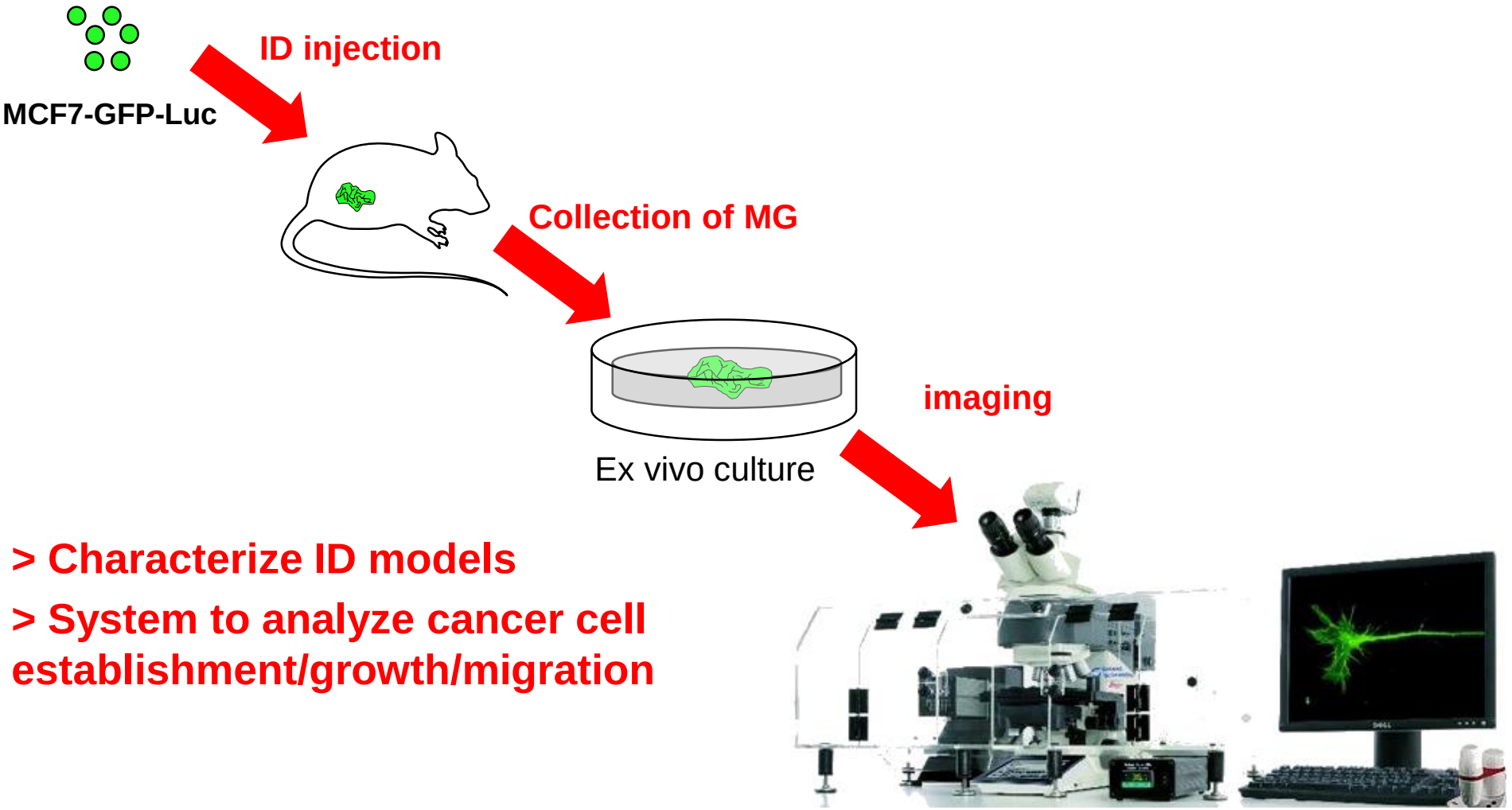
INVASIVE





Identifying cellular events during the first steps of cancerogenesis

♦ **Live imaging of ID injected cells**

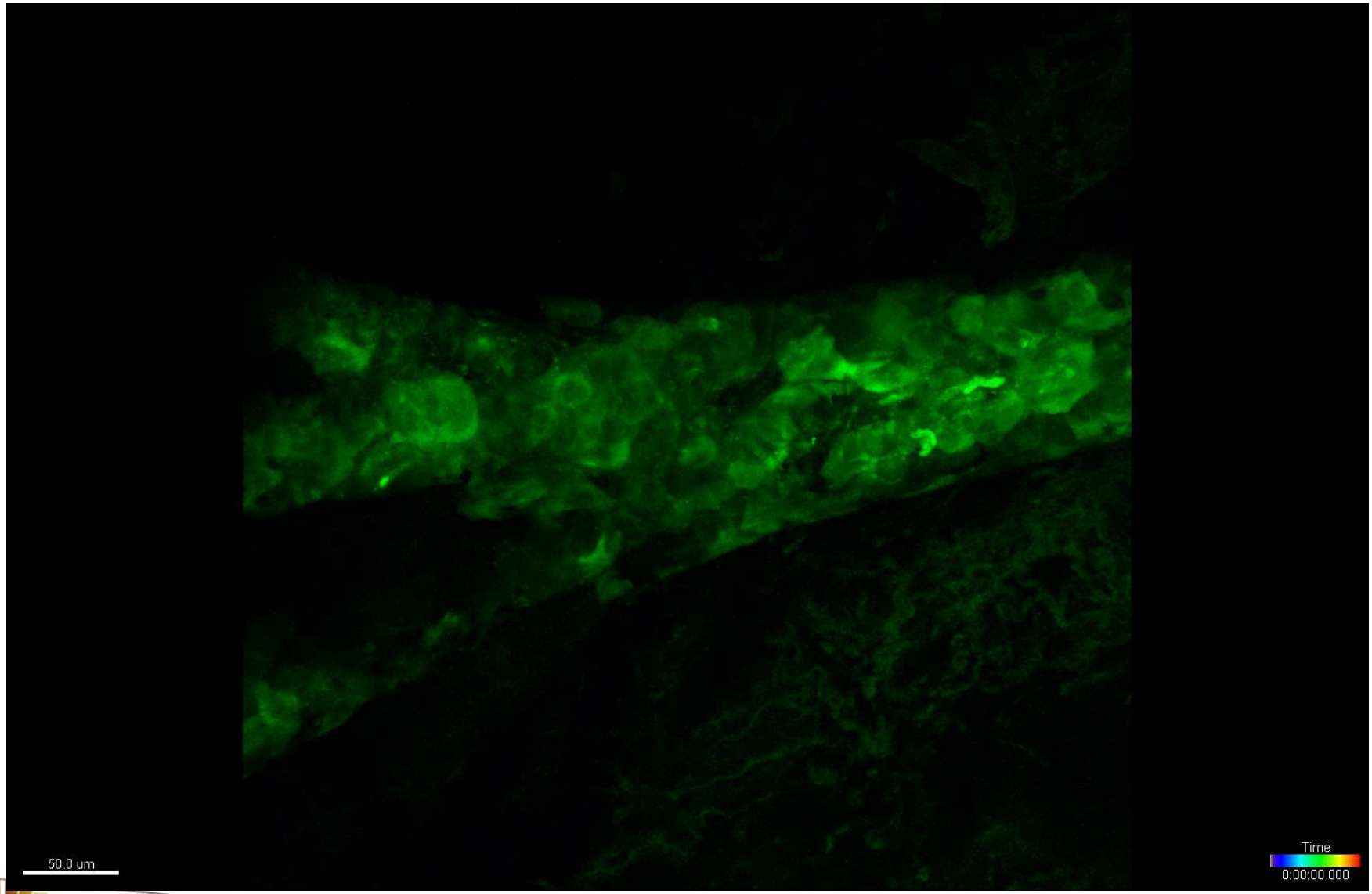


> Characterize ID models

> System to analyze cancer cell establishment/growth/migration

Leica SP5 (2-photons)

Identifying cellular events during the first steps of cancerogenesis



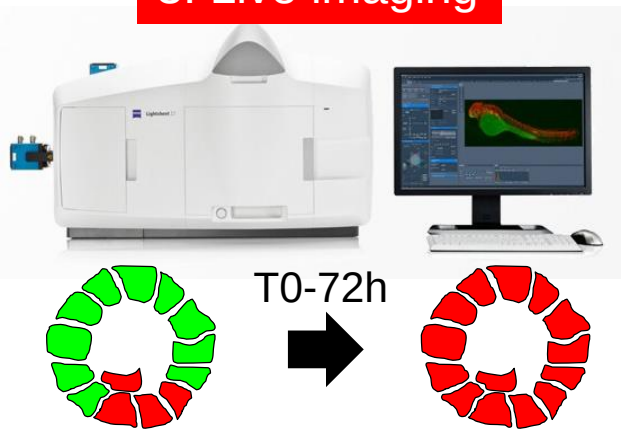
Identifying cellular events during the first steps of cancerogenesis

3D culture of mammary epithelial cells and microinjection of cancer cells to follow *in vivo* establishment

1. FACSorting and cell culture

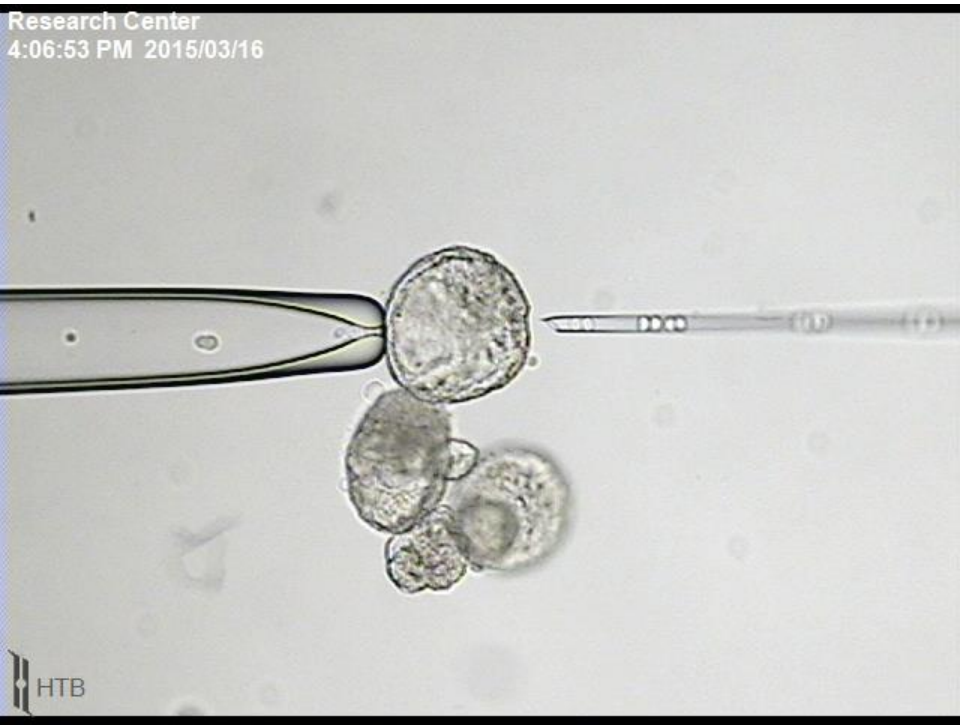
2. Microinjection

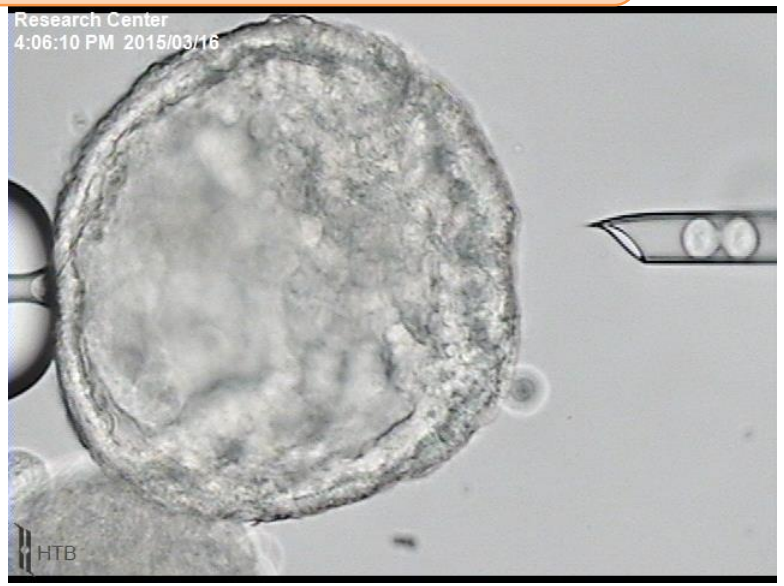
3. Live imaging

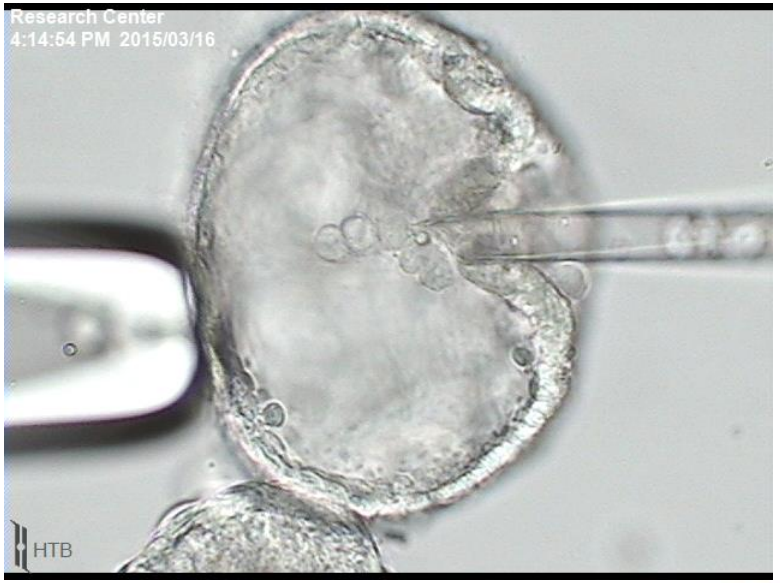


Identifying cellular events during the first steps of cancerogenesis

> Microinjection of mammary epithelial spheroids with breast cancer cells







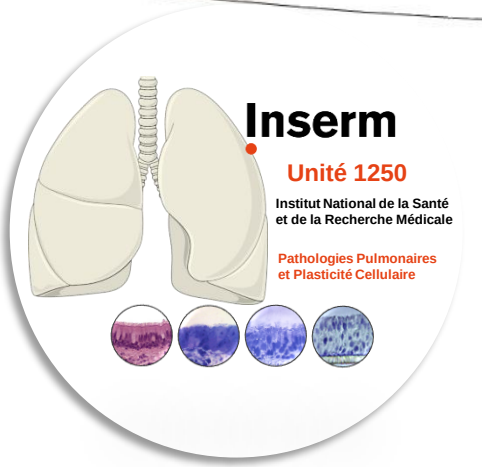
MIND-PDX in breast cancer research

- MIND established with cell lines to manipulate environment & experimental conditions
- MIND-PDXs recapitulate facefully histopathological features
- MIND-PDXs to understand cellular and molecular events of cancerogenesis?
- MIND-PDXs for drug testing?

Inserm

EPFL

ISREC
Institut Suisse de Recherche Expérimentale sur le Cancer
Schweizerisches Institut für Experimentelle Krebsforschung
Swiss Institute for Experimental Cancer Research



Thank you for your attention

UNIVERSITÉ DE REIMS CHAMPAGNE-ARDENNE