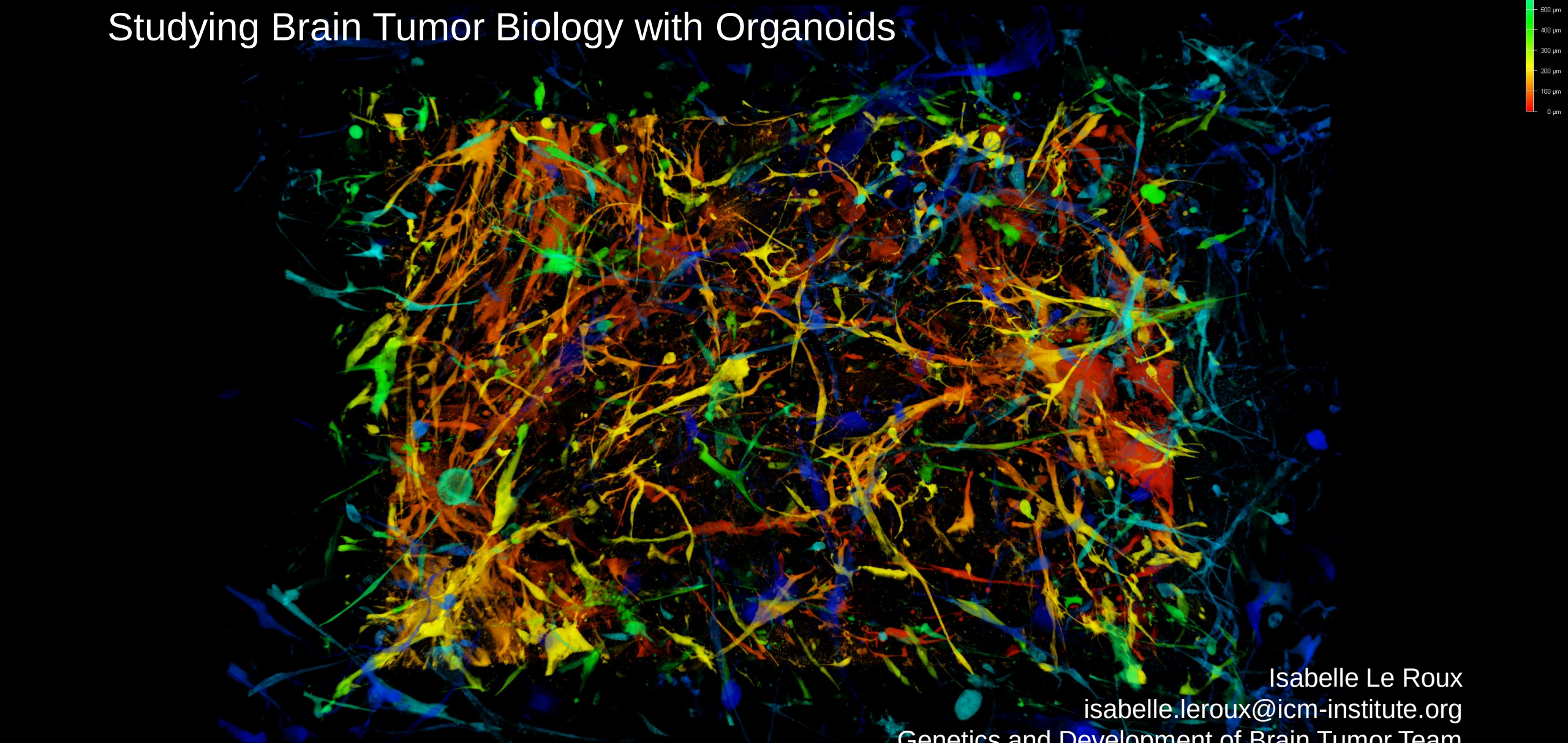
Studying Brain Tumor Biology with Organoids



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Co directed by Emmanuelle Huillard and Marc Sanson

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Cancer model systems

Table 1. Properties of cancer model systems.
GEMM, genetically engineered mouse model; MDO, murine-derived organoid; MDOX, murine-derived organoid transplantation; CLs, cell lines; PDX, patient-derived xenograft; iPS, inducible pluripotential stem cell; PDO, patient-derived organoid; PDOX, patient-derived organoid transplantation.

	GEMM	MDO	MDOX	CLs	PDX	iPS	PDO	PDOX
Wild-type cell culture	+	+	+	–	–	+	+	–
Preinvasive cancer models	+	+	+	–	–	+	+	+
Invasive cancer models	+	+	+	+	+	+	+	+
Metastatic cancer models	+	+	+	+	+	+	+	+
Cost	$$$$	$$	$$$	$	$$	$$	$$	$$$
Time	++++	+	++	+	++++	+++	++	+++
Success rate	high	med	med	med	med	low	med	med
Throughput therapies	low	med	low	high	low	high	med	low

+ denotes 1 month or less; ++, 1–2 months; +++, 1–6 months; ++++, oftentimes more than several months.

Tuveson *et al.*, *Science* **364**, 952–955 (2019) 7 June 2019

- Need to choose the model according to the cancer type and to the scientific question to be addressed

Gliomas

- Gliomas are the most common primary malignant brain tumors in adults
- Glioblastoma (GBM, grade IV astrocytoma) is the most aggressive glioma and its incidence has significantly risen in the last two decades across all ages
- GBM remains resistant to treatment and disease progression is fatal with a median survival below 15 months
- Distinct factors may account for current treatments' failure
    - ✓ invasiveness
    - ✓ immunosuppressive microenvironment
    - ✓ Inter and intra-tumoral heterogeneity

Gliomas derived-organoids

	(A) Embedded tumor cells or minced tissus in ECM	(B) Cerebral organoids (1) grafted with tumor cells (2) de novo genetically modified	(C) Minced tissus w/o dissociation
References	Hubert et al., 2016	Da Silva et al., 2018 (1) Linkous et al., 2019 (1) Bian et al., 2018 (2) Ogawa et al., 2018 (2)	Jacob et al., 2020
Materials	Patient-derived organoids / Mouse-derived organoids	Patient hiPSCs or hESCs : organoids Patient derived cells : graft	Patient derived
Properties	Low success rate (30%) High Success rate (>90%) Slow growing Short growing No microenvironment Microenvironment* Histological features of parental tumor Heterogeneity of parental tumor	High success rate Short growing No microenvironment	High success rate (91 %IDHwt ; 66% IDHmut) Short growing Microenvironment* Histological features of parental tumor Heterogeneity of parental tumor
Applications	Treatments MultiOmics Xenograft	Invasion Understanding a specific mutation Virus infection (ZIKA)	Treatments Drug screening Xenograft CAR T cell immunotherapy

* lack of immune cell expansion over time

(A) Embedded tumor cells in ECM : Patient-derived organoids

Harvest

chopping with scalpels

papain digestion @37°C , 5-10 min

ovomucoid + medium Filtration 70 µM, 30 µM

spin 300g @4°C , 10 min

pellet resuspended in HBSS 20% Hepes

if myelin Debris removal (Miltenyi)

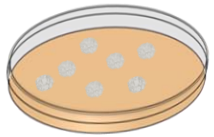
if red blood cells lysis buffer (Roche)

resuspension of cells 1 vol NB medium 4 vol of Geltrex

Put 20 µL drops (=8 000 cells) in parafilm molds

solidification @37°C, 1h

Remove the drops from the molds gently with medium*



3 days @37°C, 5% CO_2 still

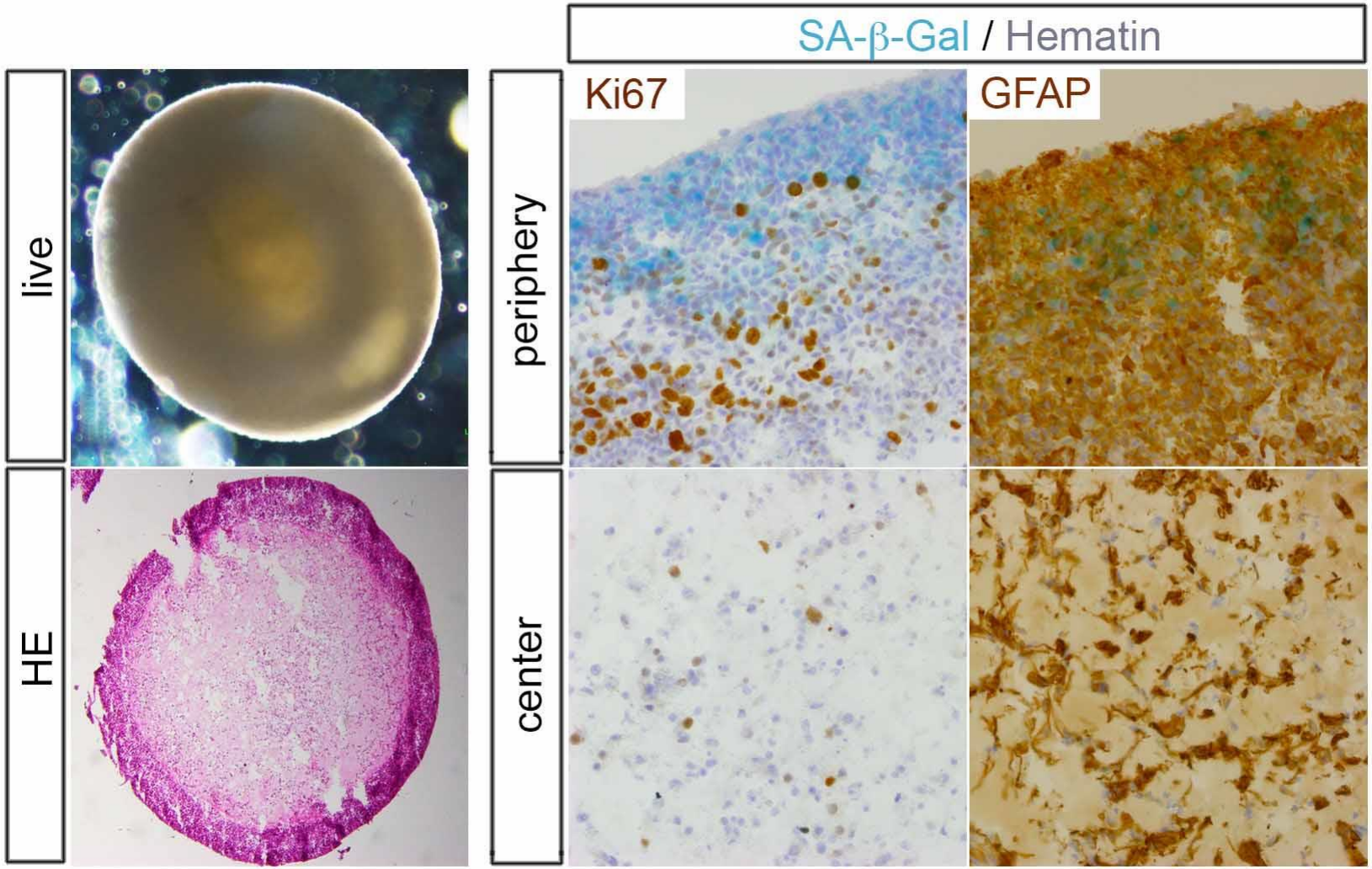
On shaker, 80 rpm

½ medium changed every 3-4 days

* medium: Neurobasal - GlutaMax - PS - B27 w/o Vitamin A - FGF - EGF

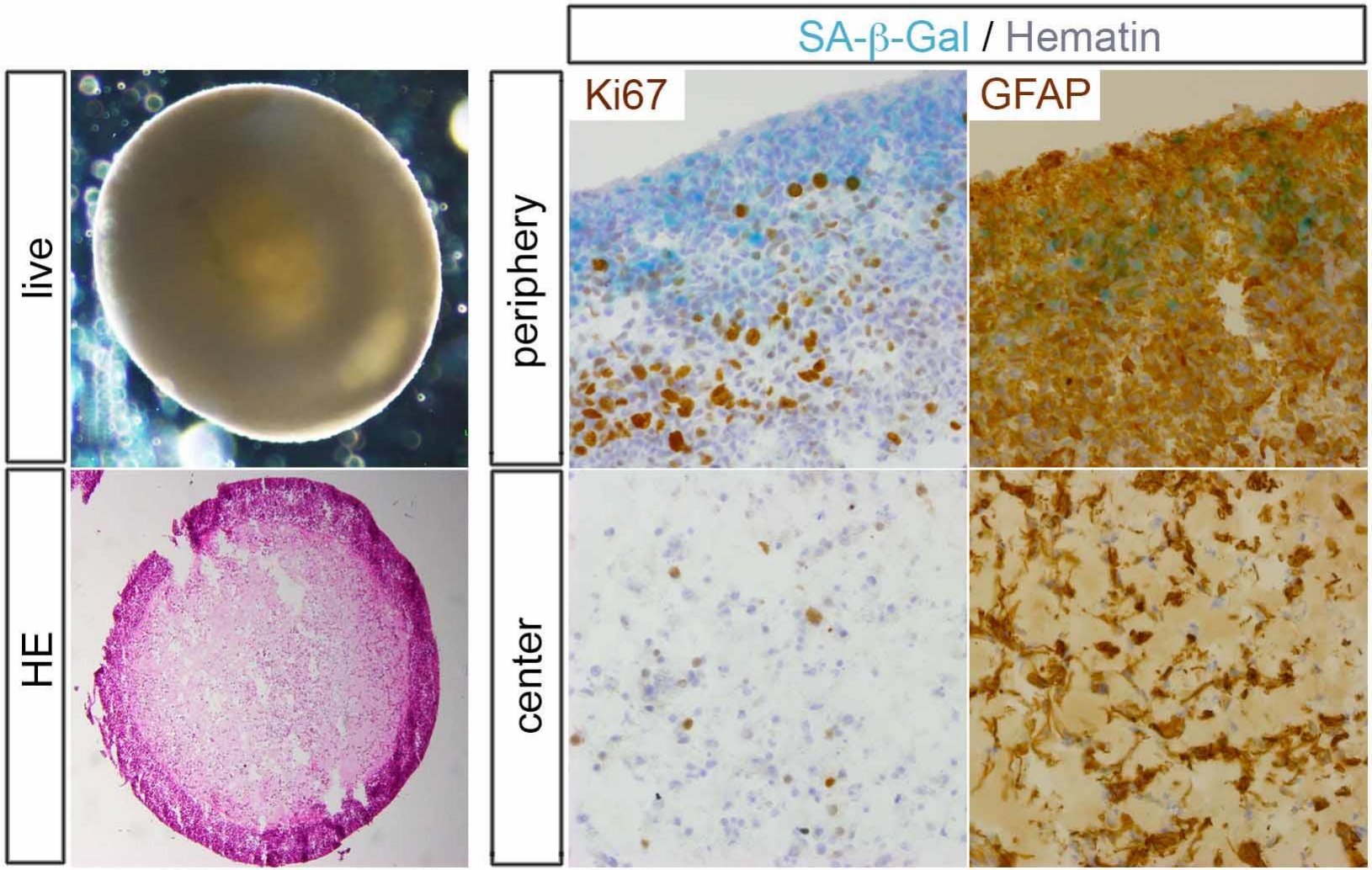
Patient gliomas are provided by Frank Bielle, OncoNeuroTek, Hôpital Pitié-Salpétrière

(A) Embedded tumor cells in ECM : Patient-derived organoids



105 days *in vitro*

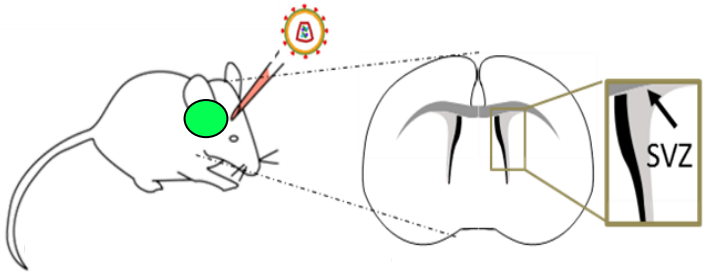
(A) Embedded tumor cells in ECM : Patient-derived organoids



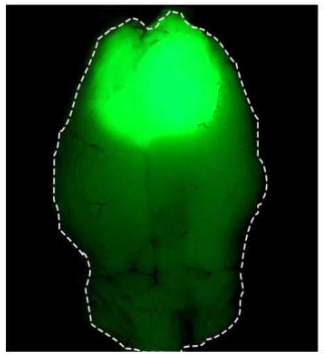
105 days *in vitro*

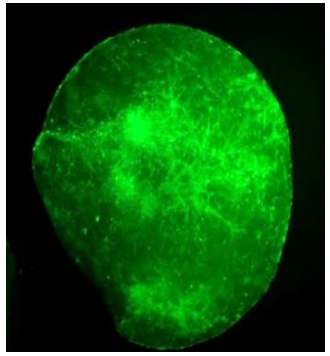
- about 30% success rate on fresh or DMSO-frozen tumors
- number of cells per sample varies from $4.5.10^6$ to 10^5 cells
- massive cell death the 2nd week of culture; only malignant cells remained
- slow growth
- no growth for Low Grade Glioma

(A) Embedded tumor cells in ECM : Mouse-derived organoids



Mouse : *Glast*$^{Cre\text{-}RT2/+}$*; Pten*$^{fl/fl}$*; p16-3MR/+*
Lentivirus : *HRasV12-eGFP-shp53*





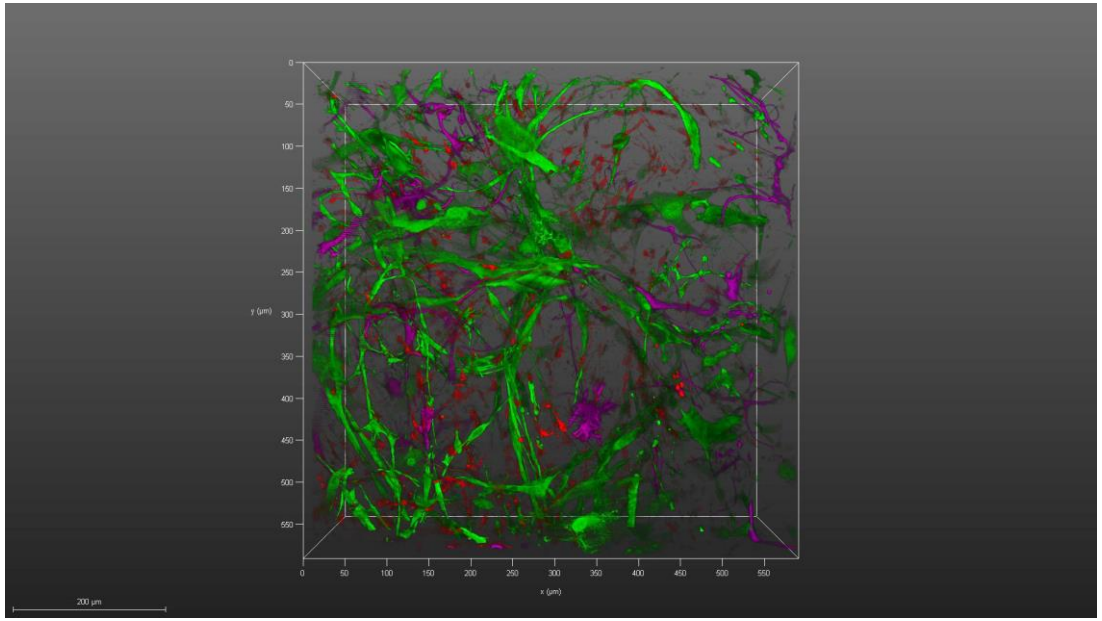
GFP/IBA1/GFAP

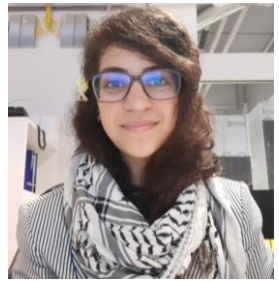
7 days in culture

no clearing;
250µM thickness

Leica SP8 Dive

icmQUANT





Rana Salam

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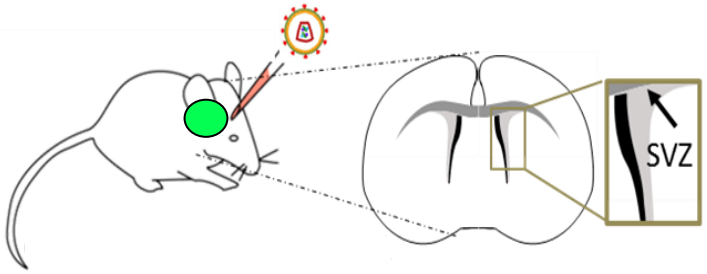
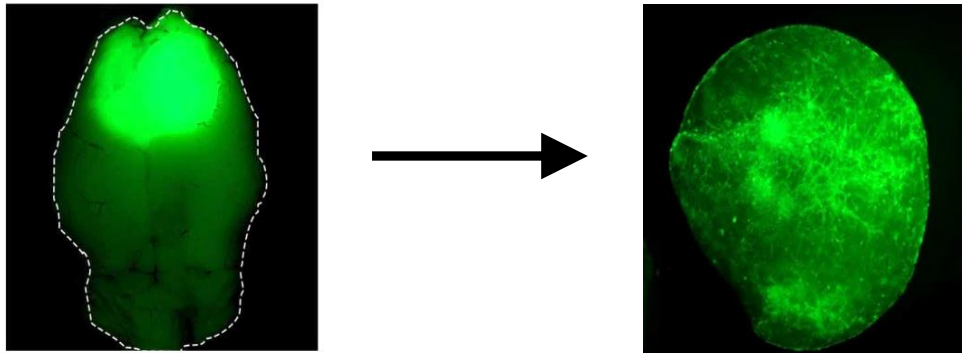
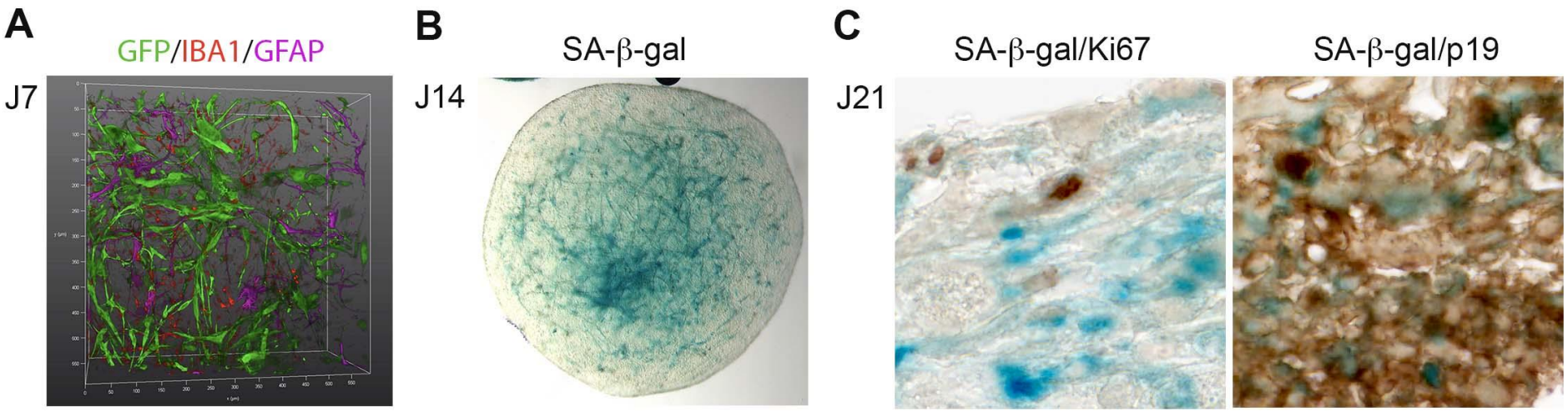
(A) Embedded tumor cells in ECM : Mouse-derived organoids



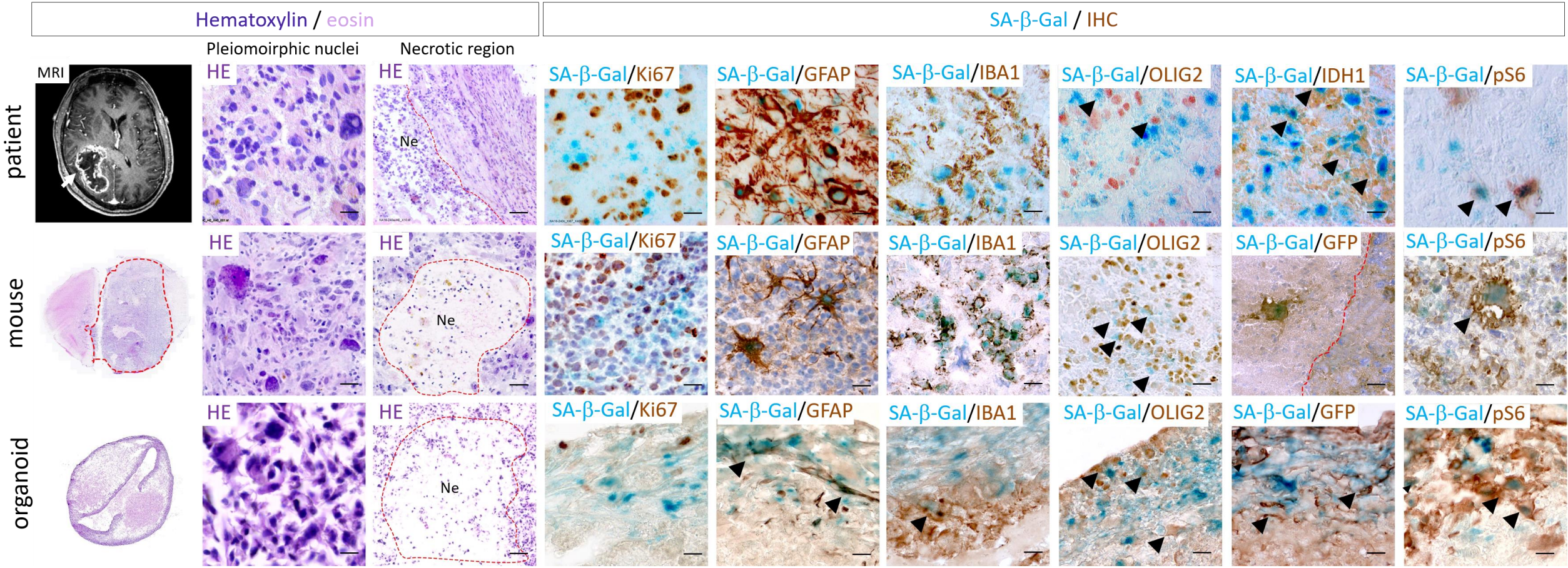
Mouse : *Glast*$^{Cre\text{-}RT2/+}$; *Pten*$^{fl/fl}$; *p16-3MR/+*
Lentivirus : *HRasV12-eGFP-shp53*





- 1 to 2.10^6 cells/tumor at end points = 125 to 250 organoids/tumor

(A) Embedded tumor cells in ECM : Mouse-derived organoids



- Mouse GBM-derived organoids recapitulate some features of patient and mouse GBMs

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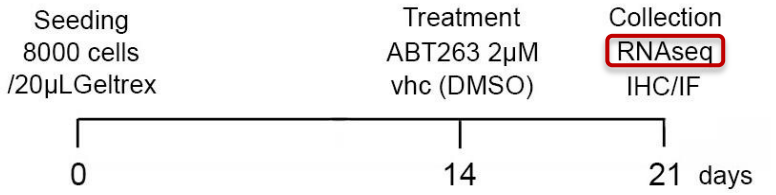
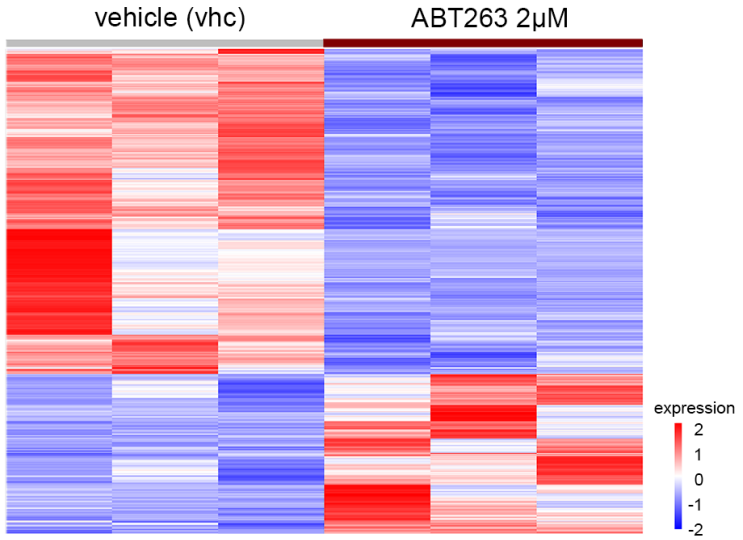
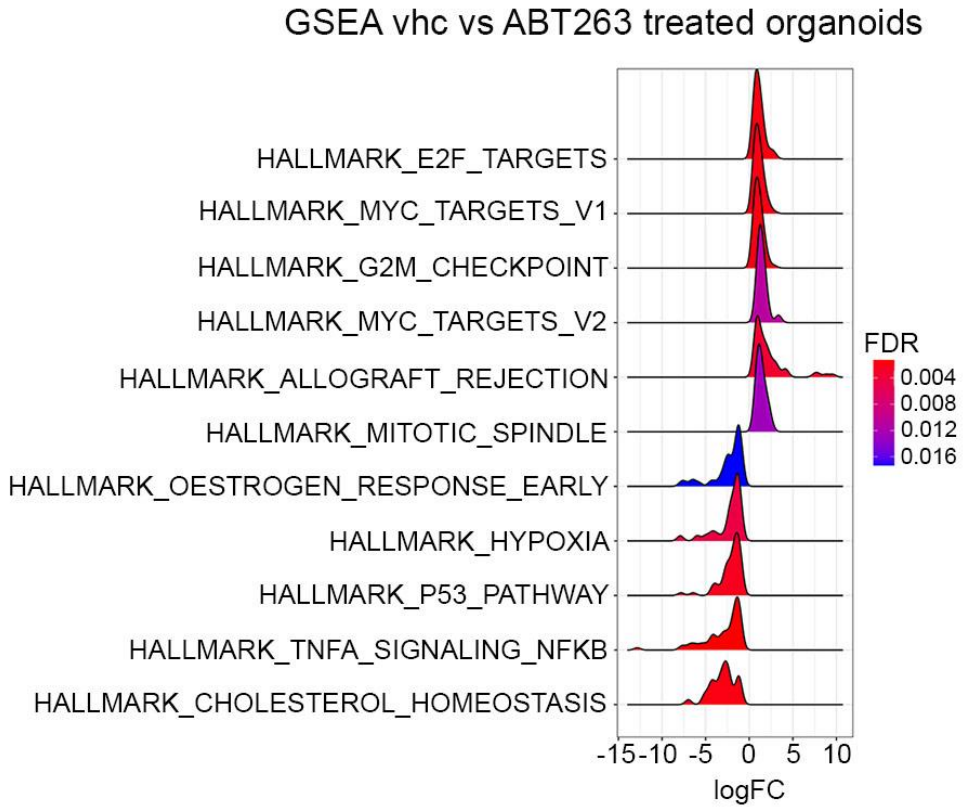
(A) Embedded tumor cells in ECM : Mouse-derived organoids

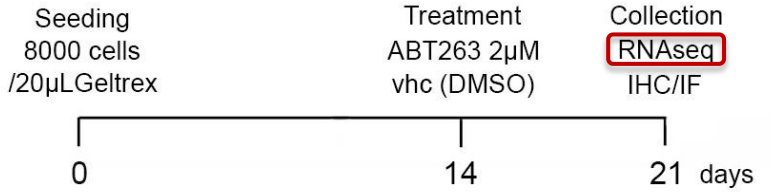


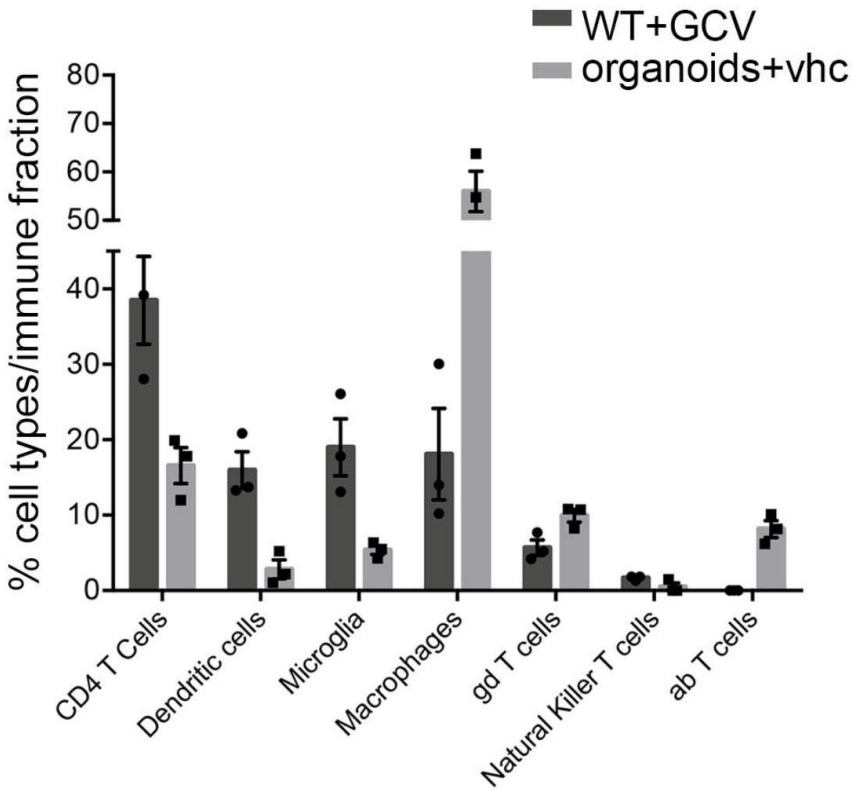




- All the dysregulated Hallmark pathways upon senolytic treatments are similarly up or down regulated in the organoids and in the *in vivo* model

(A) Embedded tumor cells in ECM : Mouse-derived organoids



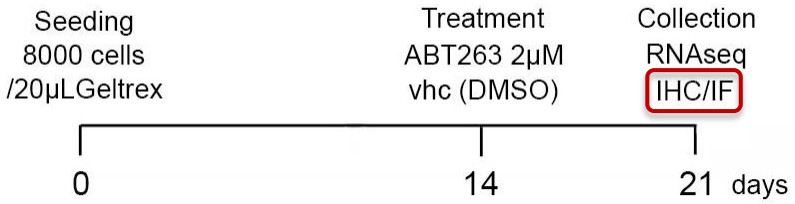


CIBERSORT analysis
from data set GSE124829

- The abundance of immune cell types is different in fresh tumors compared to tumor-derived organoids

Mathilde Bertrand, Iconics ICM Platform

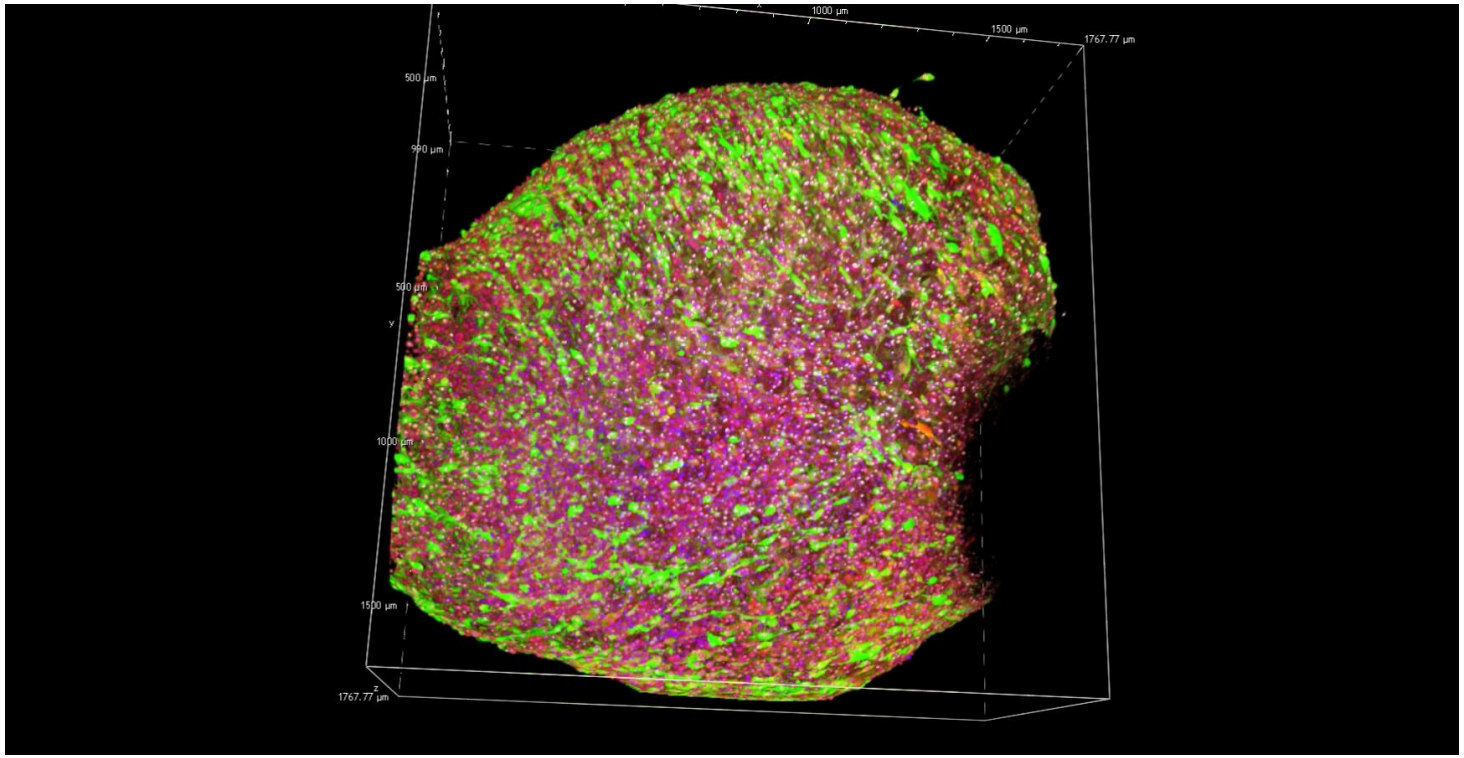
(A) Embedded tumor cells in ECM : Mouse-derived organoids



- Nikon confoncal A1 R HD25

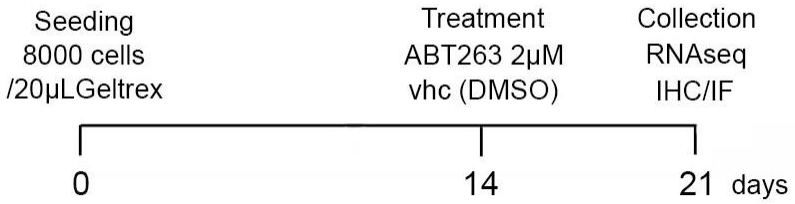
Objectif 10X CFI plan apo; NA 0.50; WD 2.2mm

- Thickness around 1000µM; step 2µM; galvo scanner
- Mounted in Rapiclear



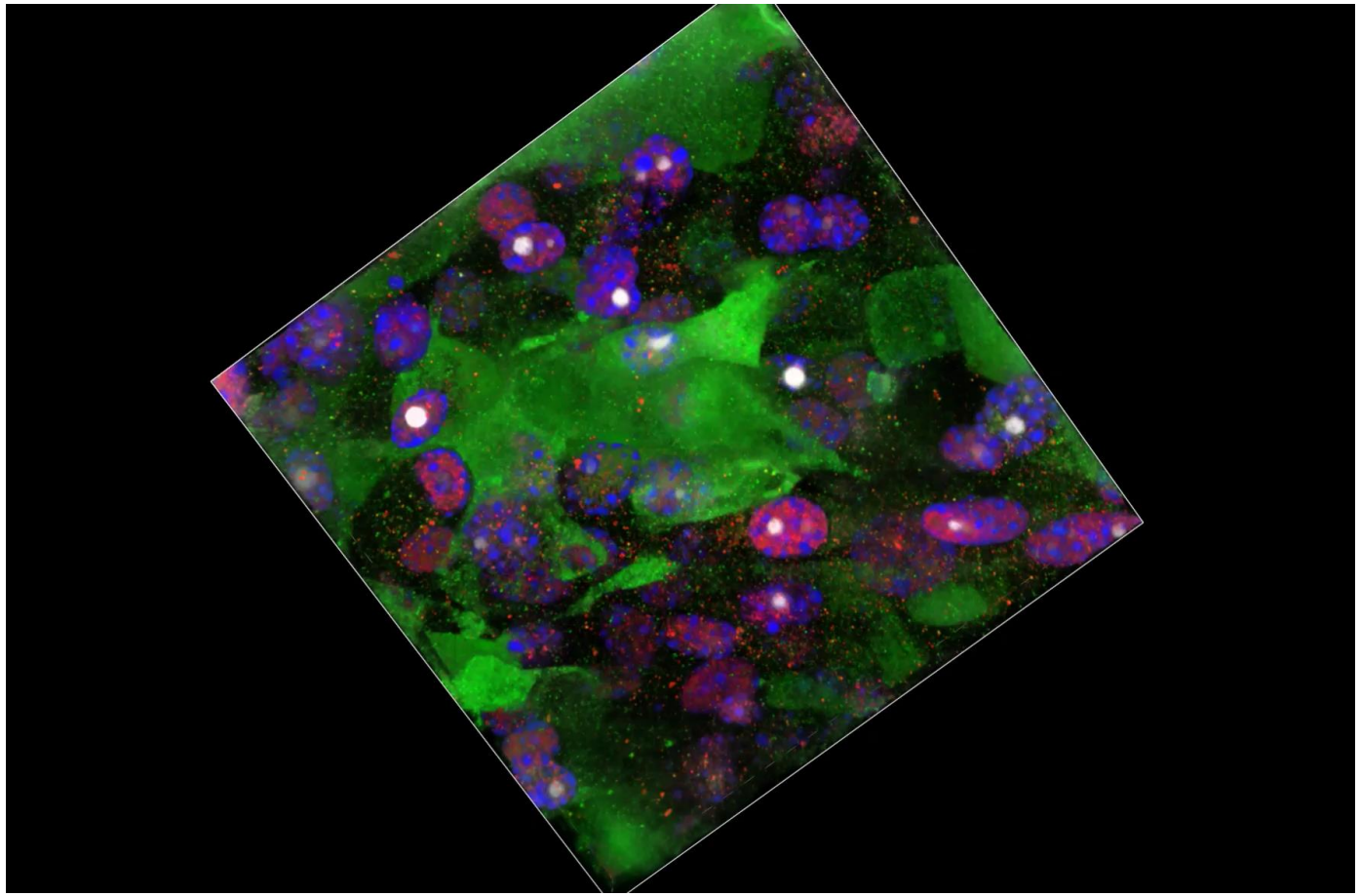
David Akbar, Celis ICM platform

(A) Embedded tumor cells in ECM : Mouse-derived organoids



- Nikon confoncal A1 R HD25
- Objectif 60X CFI plan apo; NA 1.40; WD 0.13mm

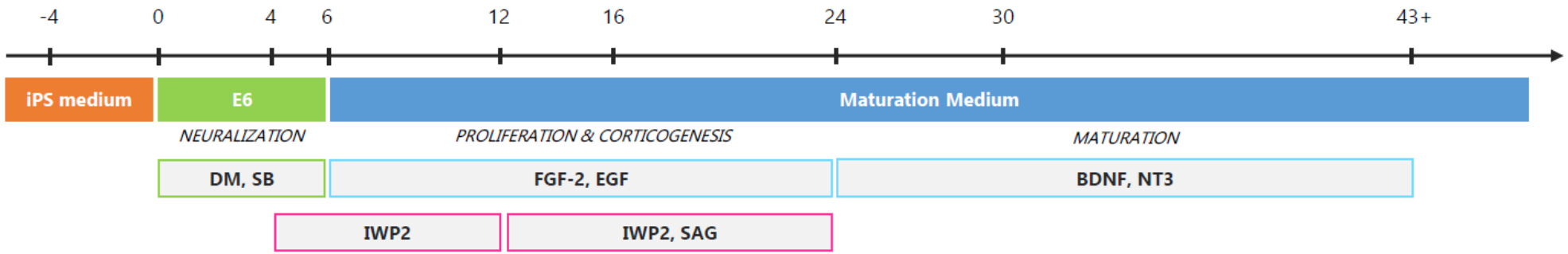
- Mounted in Rapiclear



David Akbar, Celis ICM platform

(B) Cerebral organoids grafted with PDCL

(1) Ventral Forebrain Organoid
Differentiation protocol based on Sloan et al., 2018



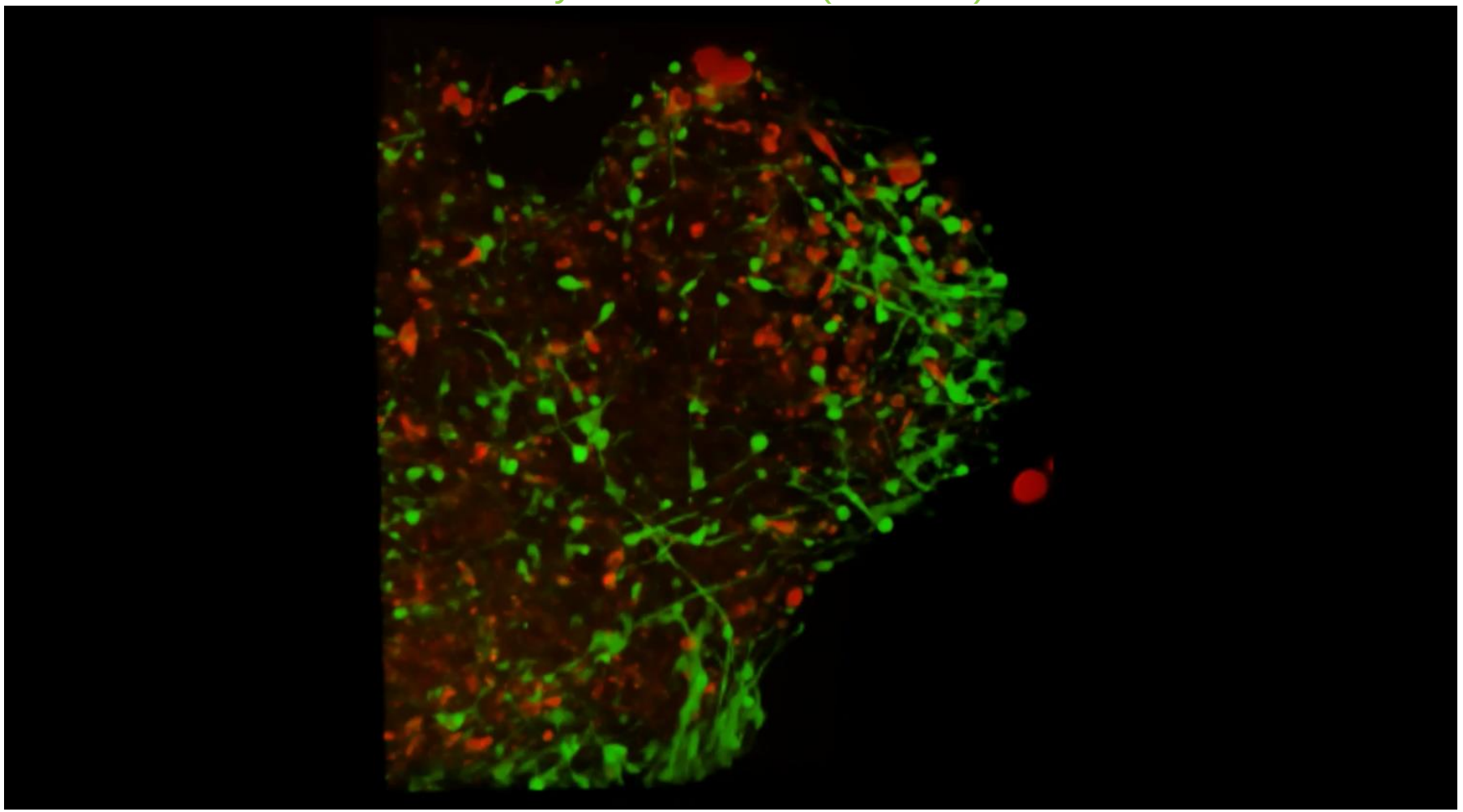
Benjamin Galet and Phillipe Ravassard ; O. Corti and J.C. Corvol ‘s lab (ICM)

(2) Incubation with Patient Derived-Cell Lines (PDCL)

Maité Verreault, Emie Quissac and Ahmed Idbaih (Gliotex team, ICM)

(B) Cerebral organoids grafted with PDCLs

GFP : synuclein+ cells (neurons) / Red : PDCL



- 10 000 PDCL
on organoid (120 days *in vitro*)

- Live imaging 24hrs after the initiation of the co-culture

- Acquisition during 15hrs every 15 min; 300µM thickness

- Objectif 20X immersion glycerol; NA 0.75; WD 0.51mm

- Nikon confocal A1 R HD25
Imaris 3D reconstruction

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David Akbar, Celis ICM platform

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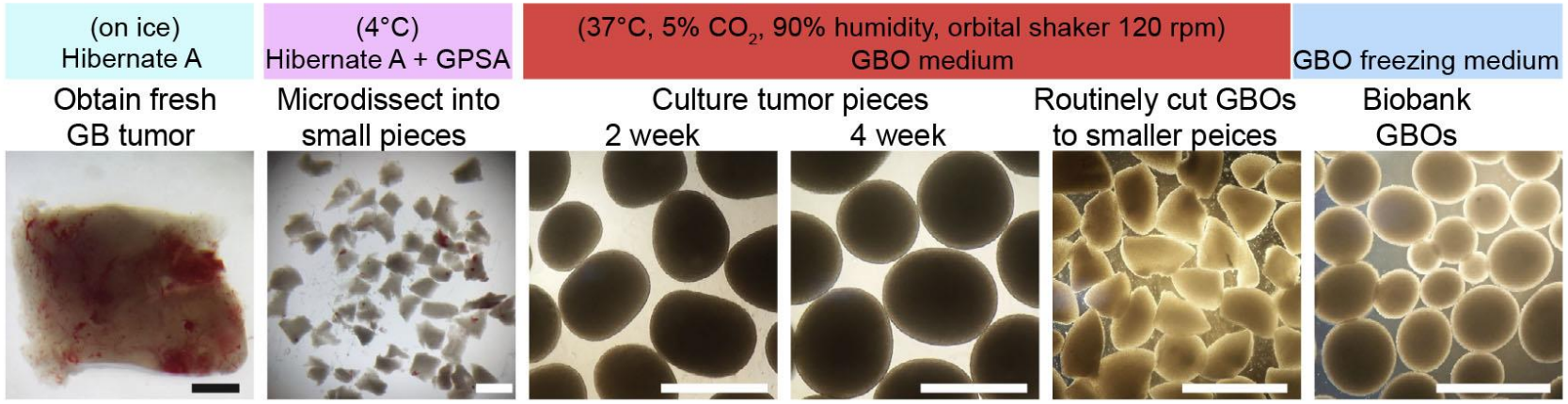
(C) Minced tissus w/o dissociation



GBO medium : 50% DMEM:F12 + 50% Neurobasal - GlutaMax - NEAAs - PS - N2 - N2 - B27 w/o Vitamin A - 2-mercaptoethanol - human insulin

(C) Minced tissus w/o dissociation
Jacob et al., 2020
Patient derived
High success rate Short growing Microenvironment Histological features of parental tumor Heterogeneity preserved of parental tumor
Treatments Drug screening Xenograft CAR T cell immunotherapy

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GBM derived-organoids

	(A) Embedded tumor cells or minced tissus in ECM	(B) Cerebral organoids (1) grafted with tumor cells (2) de novo genetically modified	(C) Minced tissus w/o dissociation
References	Hubert et al., 2016	Da Silva et al., 2018 (1) Linkous et al., 2019 (1) Bian et al., 2018 (2) Ogawa et al., 2018 (2)	Jacob et al., 2020
Materials	Patient-derived organoids / Mouse-derived organoids	Patient hiPSCs or hESCs : organoids Patient derived cells : graft	Patient derived
Properties	Low success rate — High Success rate Slow growing — Short growing No microenvironment — Microenvironment Histological features of parental tumor Heterogeneity of parental tumor	High success rate Short growing No microenvironment	High success rate Short growing Microenvironment Histological features of parental tumor Heterogeneity of parental tumor
Applications	Treatments MultiOmics Xenograft	Invasion Understanding a specific mutation Virus infection (ZIKA)	Treatments Drug screening Xenograft CAR T cell immunotherapy
Perspectives	Drug screening Include factors to mimic/maintain the microenvironment	Inclusion of endothelial cells and/or immune cells within the cerebral organoid	Model to design personalized therapy?

Genetics and Development of Brain Tumors

ICM platforms

iCONICS – Justine Guégan, Mathilde Bertrand
CELIS – David Akbar
HISTOMICS – Histology
iGenSeq – Sequencing
PHENO ICMice – Animal facility

ICM

Benjamin Galet - Phillippe Ravassard

Hôpital Pitié-Salpêtrière

OncoNeuroTek
Cyto-ICAN
Centre d'expérimentation fonctionnelle -105

Collaborators

Judith Campisi, Buck Institute for Aging
Christophe Antoniewski, Léa Bellenger Institut de Biologie Paris Seine

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