Bioprinting to modelize response to radiation therapy

New model to decipher radiobiological responses

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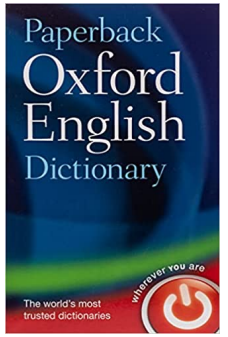
Models : definition



Model : representation of an object, person or system. The term originally derived via from Latin *modulus*, a measure.

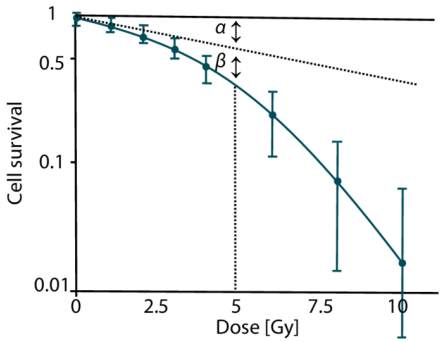
Biological (physical) model : a copy of something, usually smaller than the original object

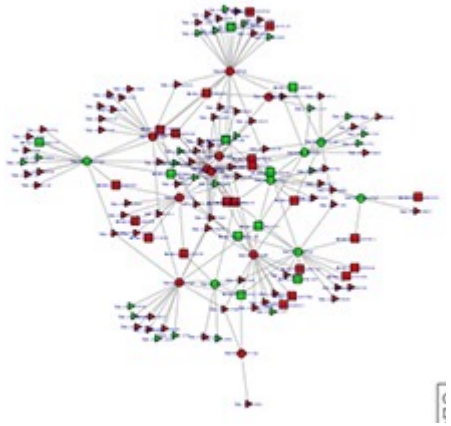
Mathematic (abstract) model : a simple description of a system, used for explaining how something works or calculating what might happen

Models : Exemples in radiobiology

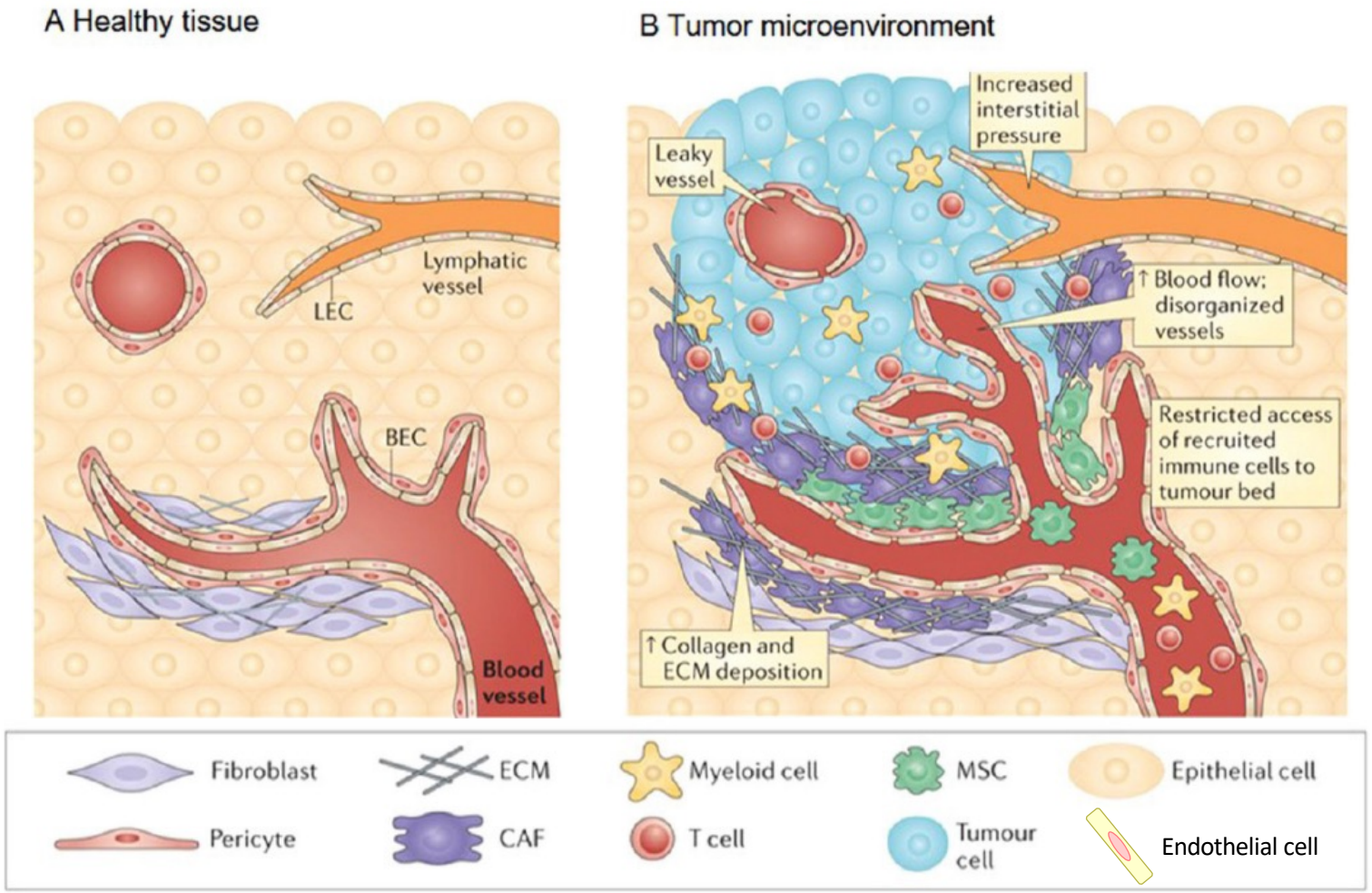
Biological model :

Mathematic model :





Why needs for new radiobiological models ?

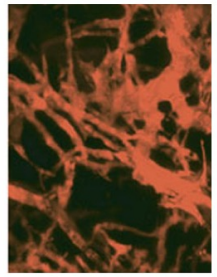


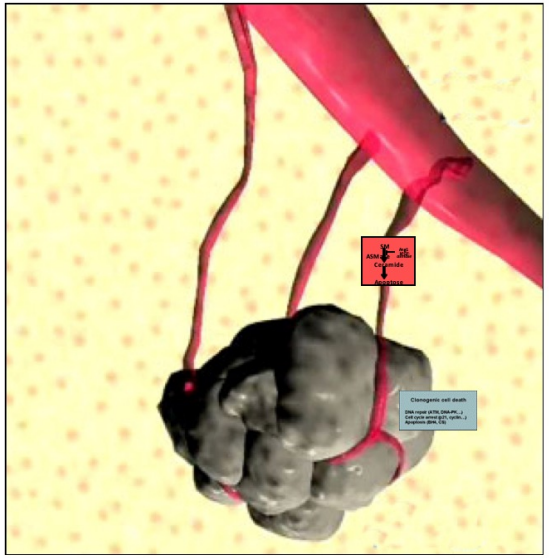
- Growing evidences in the last 20 years that **microenvironment influences treatment response** (Soysal et al, 2015)

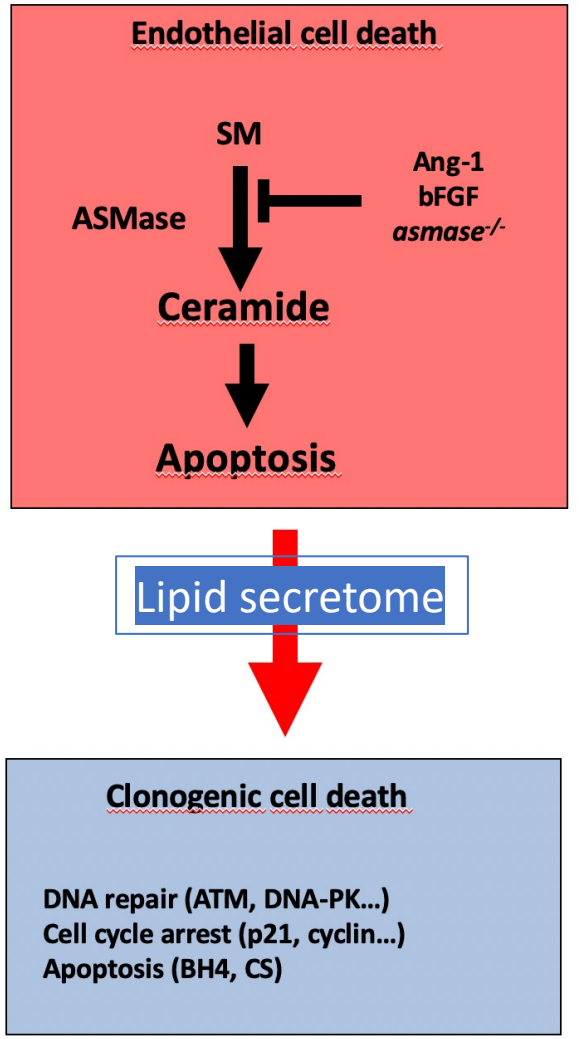
➔ can we **improve treatment efficacy** by **understanding the µenvironment** interaction with the tumor?

Adapted from Zhang et al, 2016

Involvement of endothelium in tumor response to RT







Garcia-Barros et al. Science 2003
Bonnaud et al. Cancer Res. 2010

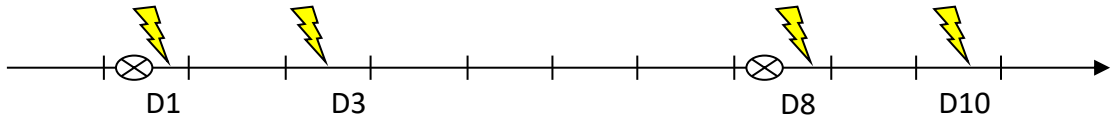
Sphingolipidomic as biomarkers of RT efficacy

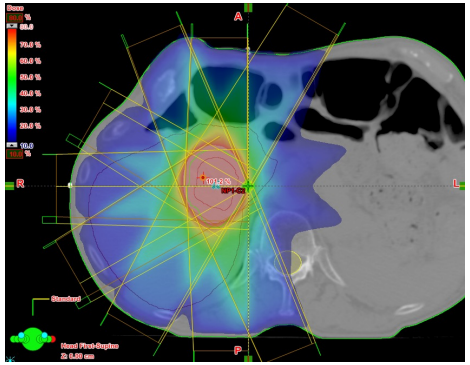
Phase II multricentric clinical study using SBRT with irinotecan against unoperable hepatic and lung metastases from colorectal cancers (35 patients).

- **Treatment**

Irinotecan 40mg/m² at D1 & D8

RT 10 Gy at D1, D3, D8 & D10



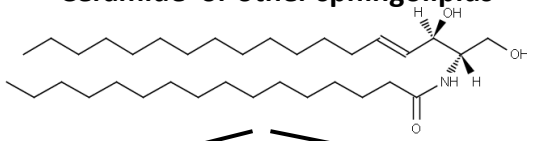


		Location	
		lung	liver
Patient (number)	Male	8	21
	Femelle	1	5
Age (year)	Median	65	66.5
	Youngest	32	33
	Oldest	77	84
Tumor Diameter (cm)	Median	13	36
	smallest	4	11
	largest	26	100

Sphingolipid extraction & correlation to clinical response

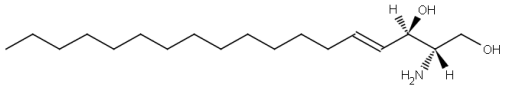
Analysis by UPLC/MS/MS; TRIPLE QUAD (TQD)

Ceramide or other sphingolipids



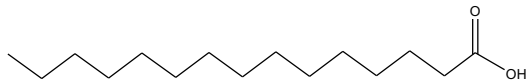
Sphingoidic body

Sphingosine Sph: C18:1



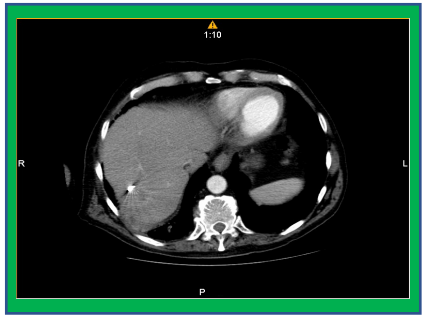
Faty acid

Carbon chain: C14 to C24



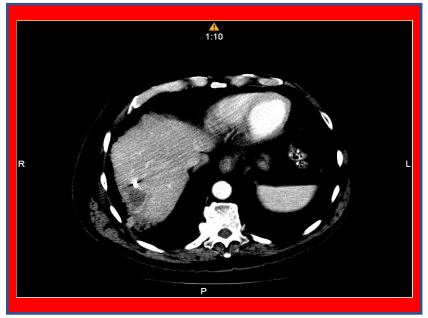
CT Scan over the year

Responder



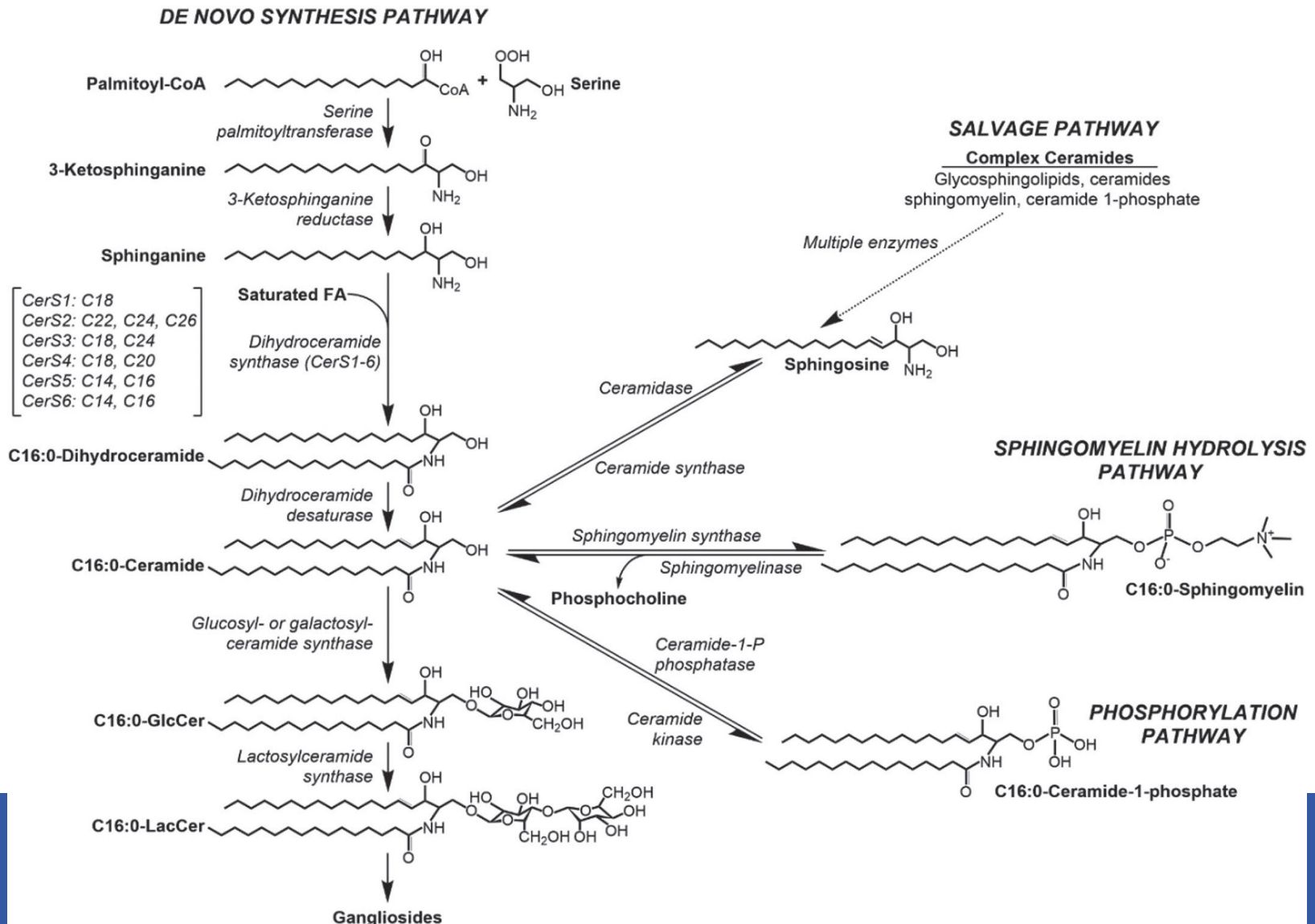
Complete
Partial (tumor volume decreases by 30%)

Non responder

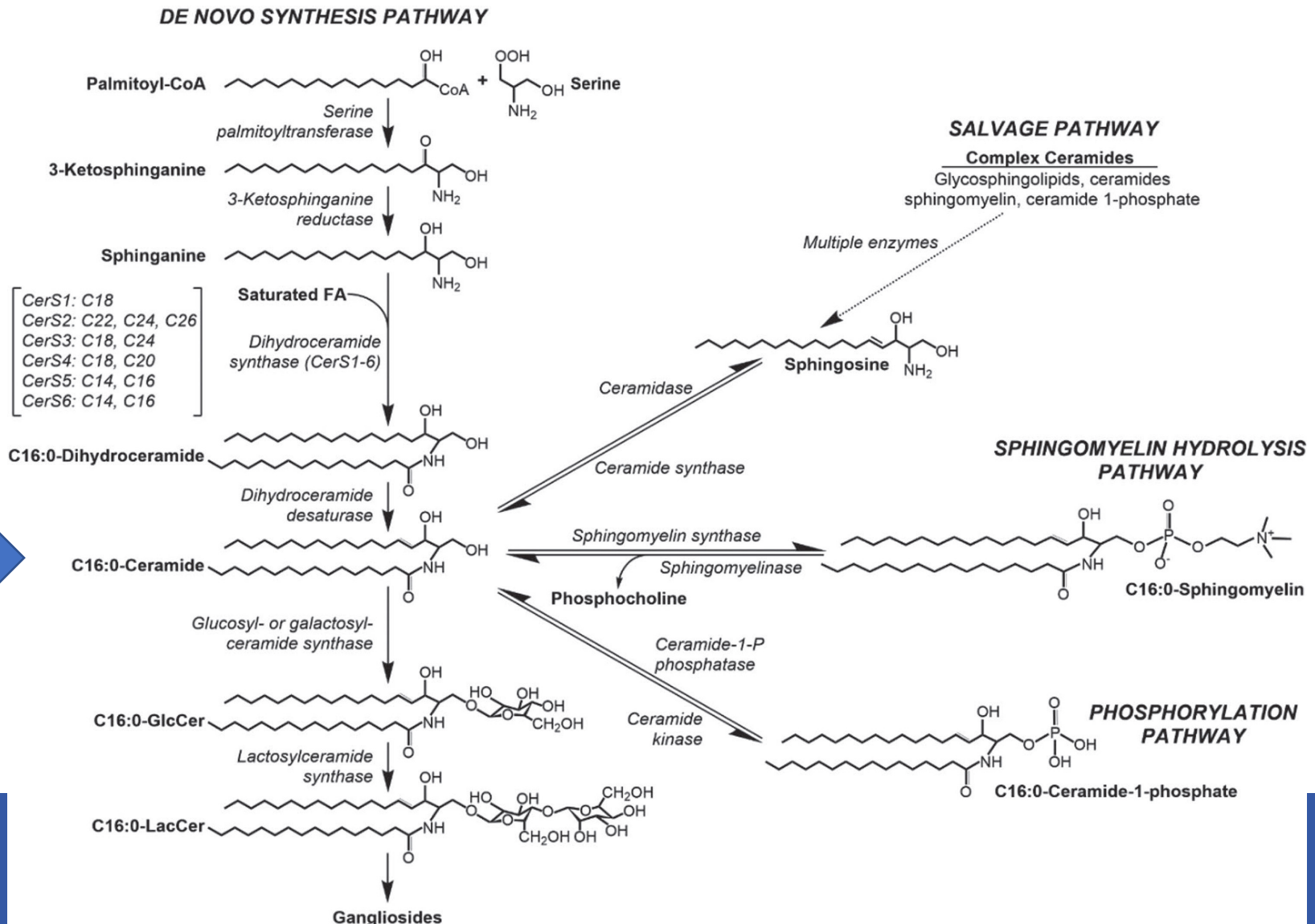


Stable
Tumor volume increases by 30%

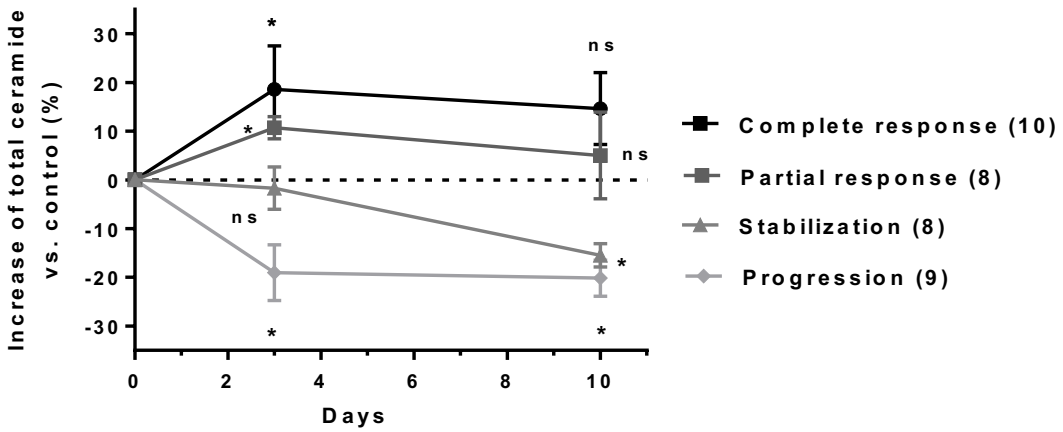
Sphingolipid pathway

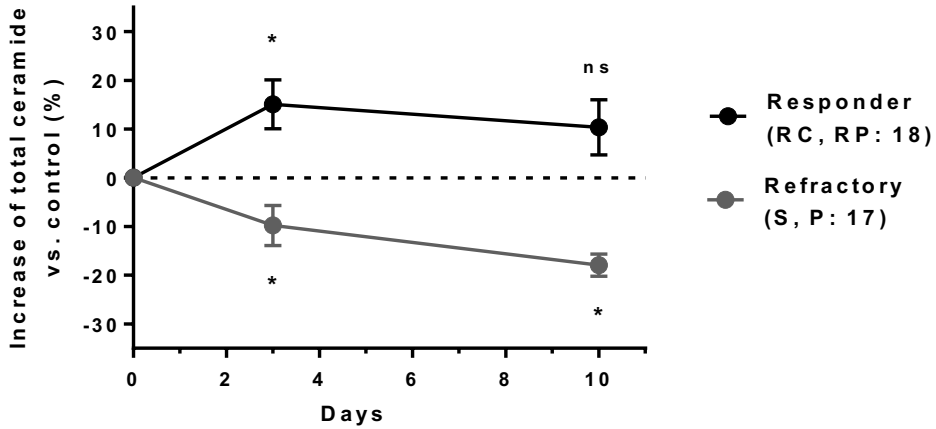


Sphingolipid pathway



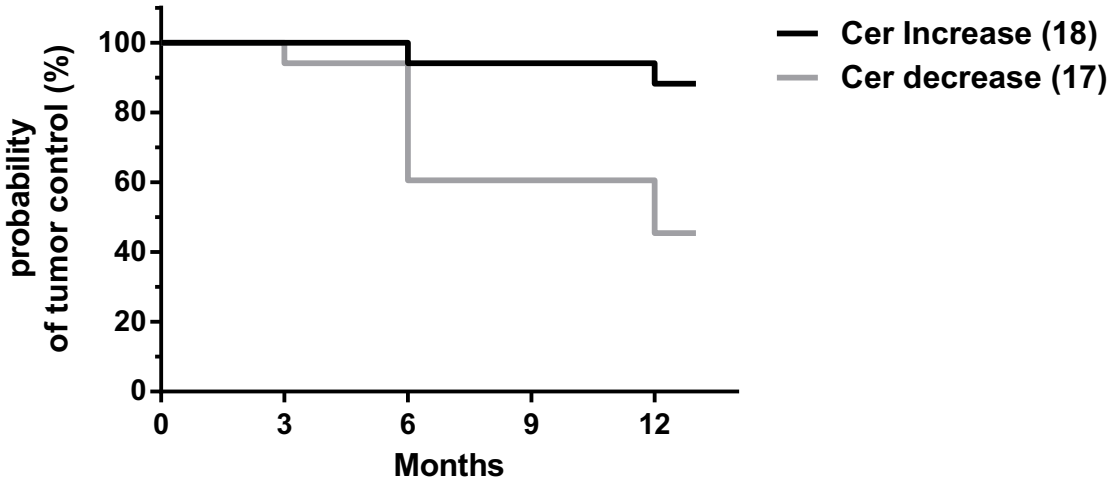
Plasma ceramide level is correlated with tumor response during SBRT



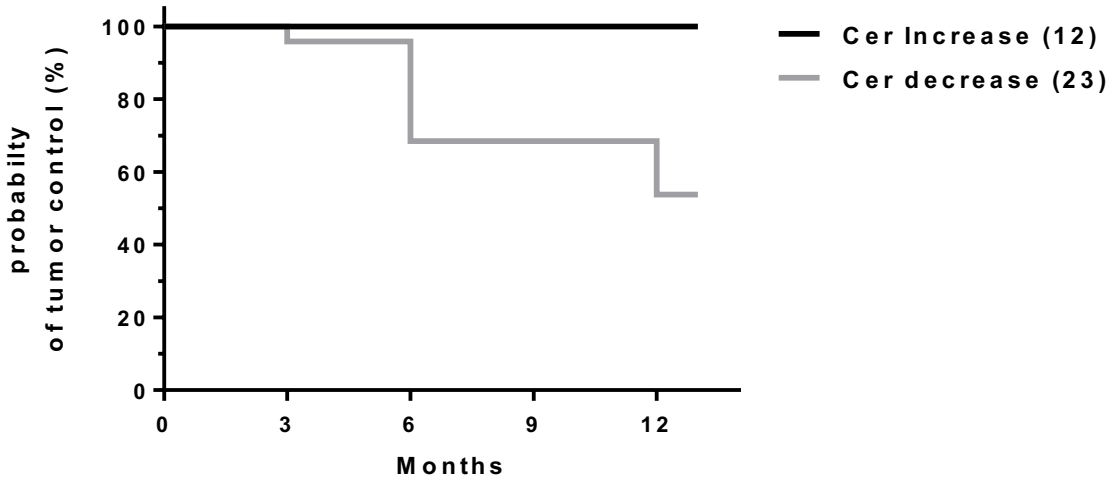


Tumor control over the year is correlated with early plasma ceramide increase

D3 vs. D0

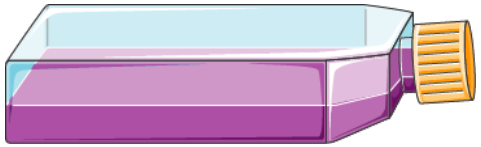


D10 vs. D0

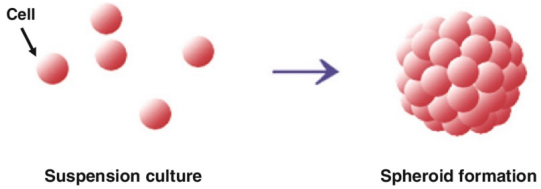


Dubois et al Rad Onc 2016

Experimental Models



Classical “2D” cell culture



Spheroids

In vivo

- **High throughput**
- Easy to tune
- Allow study of **precise molecular mechanisms**

- **3D organization**
- Possible multicellular organoids

- **High physiological relevance**
- **Systemic conditions**

- **Low physiological relevance**
- Limited co-culture possibilities
- 2D organization

- Rely on self organization = **no spatial control**
- **Limited complexity**

- **Non-human** environment (even in humanized model)
- **“Black box”**
- Ethical concern

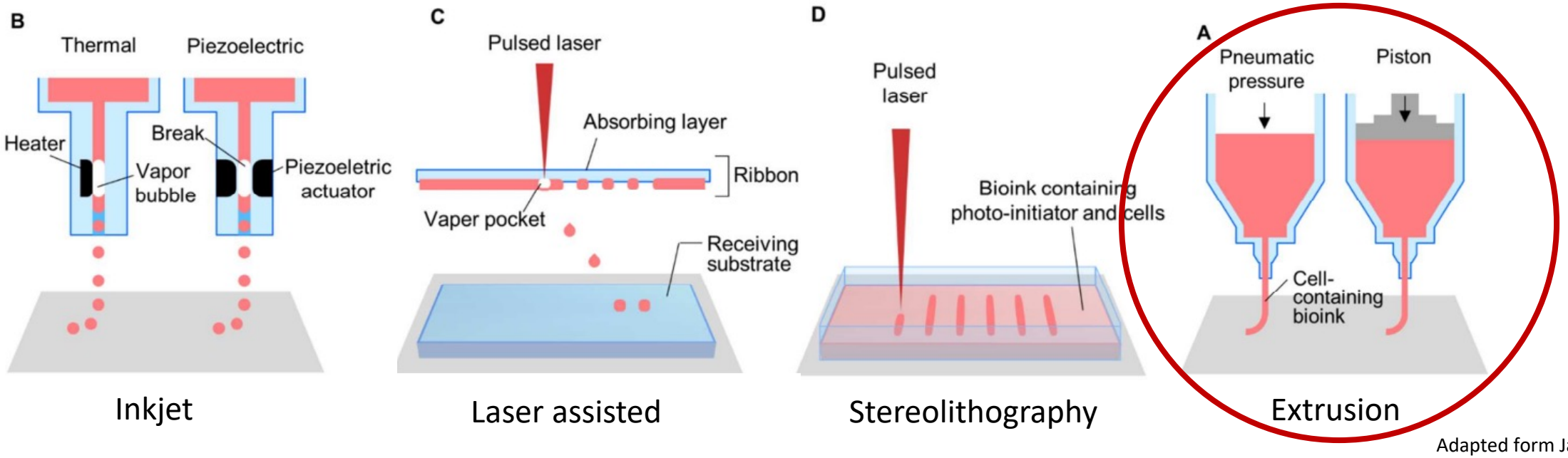
➔ **Models are still a huge limitation for research on the tumor microenvironment**

Biofabrication

*'the fabrication of **hierarchical constructs** with a prescribed **2D or 3D organization** through **automated assembly** of pre-formed **cell-containing** fabrication units generated via cell-driven self-organization or through preparation of hybrid cell-material building blocks, typically by applying enabling technologies, including microfabricated molds or microfluidics'*

Groll et al, 2016

BIOPRINTING



Inkjet | Laser assisted | Stereolithography | Extrusion

Adapted form Jang et al, 2016

The different objectives of 3D bioprinted models

I. Create a 3D bioprinted tumor cancer model

II. Study the model reaction to radiotherapy

III. Assess cancer-microenvironment communication during radiotherapy

IV. Integrate patient-derived cells to evaluate the model prediction abilities

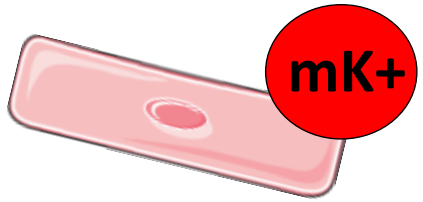
bioprinted cell types

Tumor CANCER

- **U251,**
Aggressive, Glioblastoma. Robust model.
- **MDA-MB-231** (MDA231)
Aggressive, triple negative cell line. Commonly used in publication. Robust model.
- **MCF-7**:
Less aggressive, Hormone responsive cell line. Commonly used in publication. Robust model.

➔ **Cell line are “old” and very different from primary cells physiology**

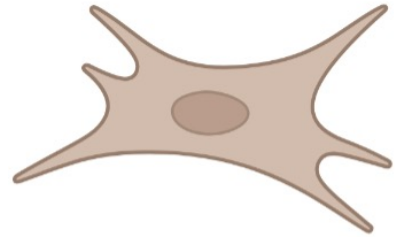
ENDOTHELIAL CELLS



- **Human Umbilical Vein Endothelial cell** (HUVEC)

Primary human cells, **commonly used** for tissue engineering application. Known source.
But **low physiological relevance** toward breast cancer pathology.

FIBROBLASTS



- **Human Skin Fibroblasts (HSF)**

Used in routine in our lab. Good results for HUVEC maturation.
But **low physiological relevance** toward breast cancer pathology
➔ Used it for **model design and optimization**

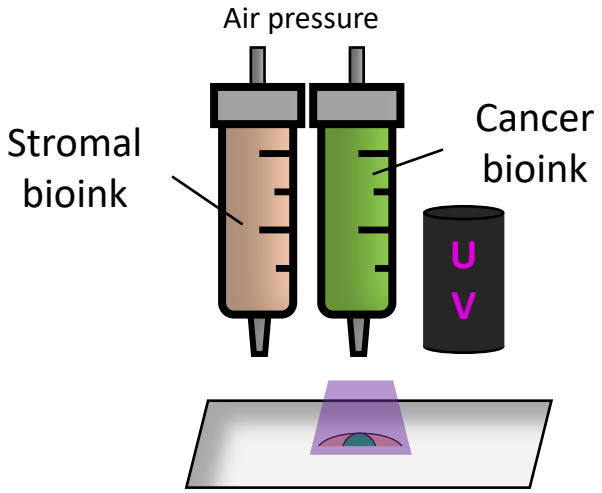
- **Normal Mammary Fibroblasts (NMF)**

Non pathologic **breast primary fibroblasts**, good for comparison with breast cancer associated fibroblasts.

- **Cancer Associated Fibroblasts (CAF)**

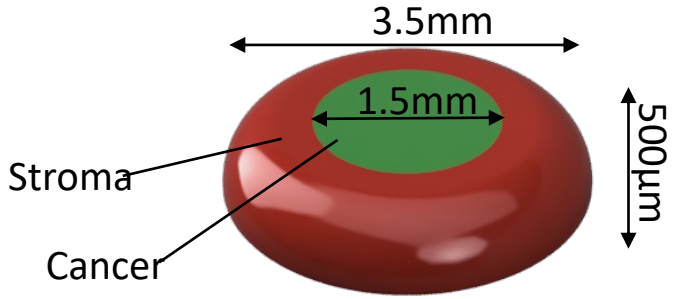
Fibroblasts collected in the **breast cancer stroma**.

3D Bioprinted tumor Model



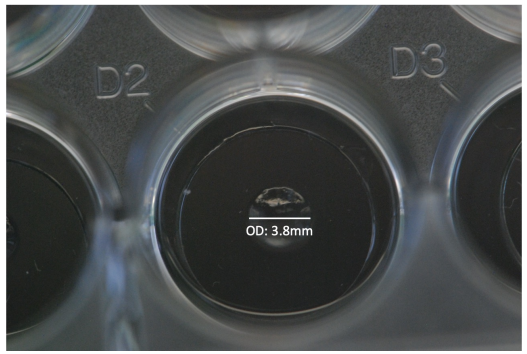
Different Configurations

- Monocultures: tumor cells
- Tricultures: Tumor + HUVECs + Fibroblasts (HSF)
- Bicultures: HUVEC + HSF or tumot + HSF



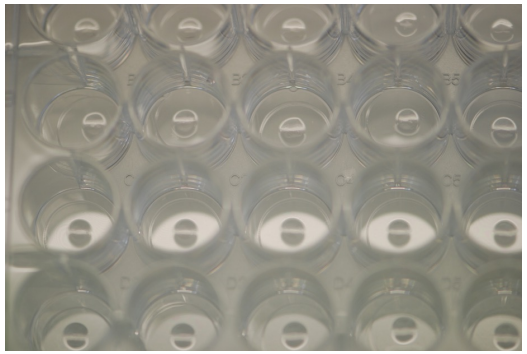
3D representation of the model

Size

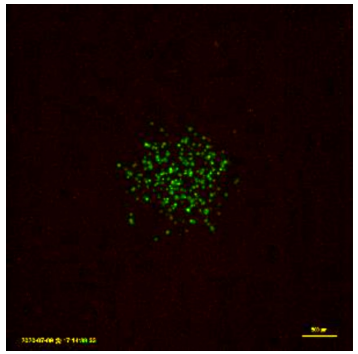


Model height : 330 ± xxµm

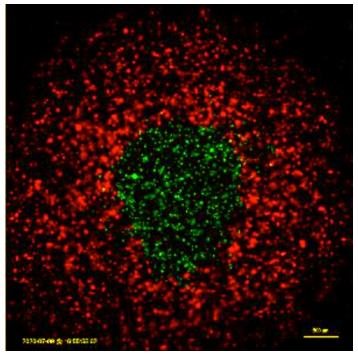
Reproducibility



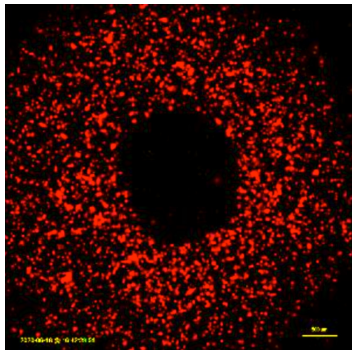
Fluorescent Microscopy



U251 or U251 + HSF

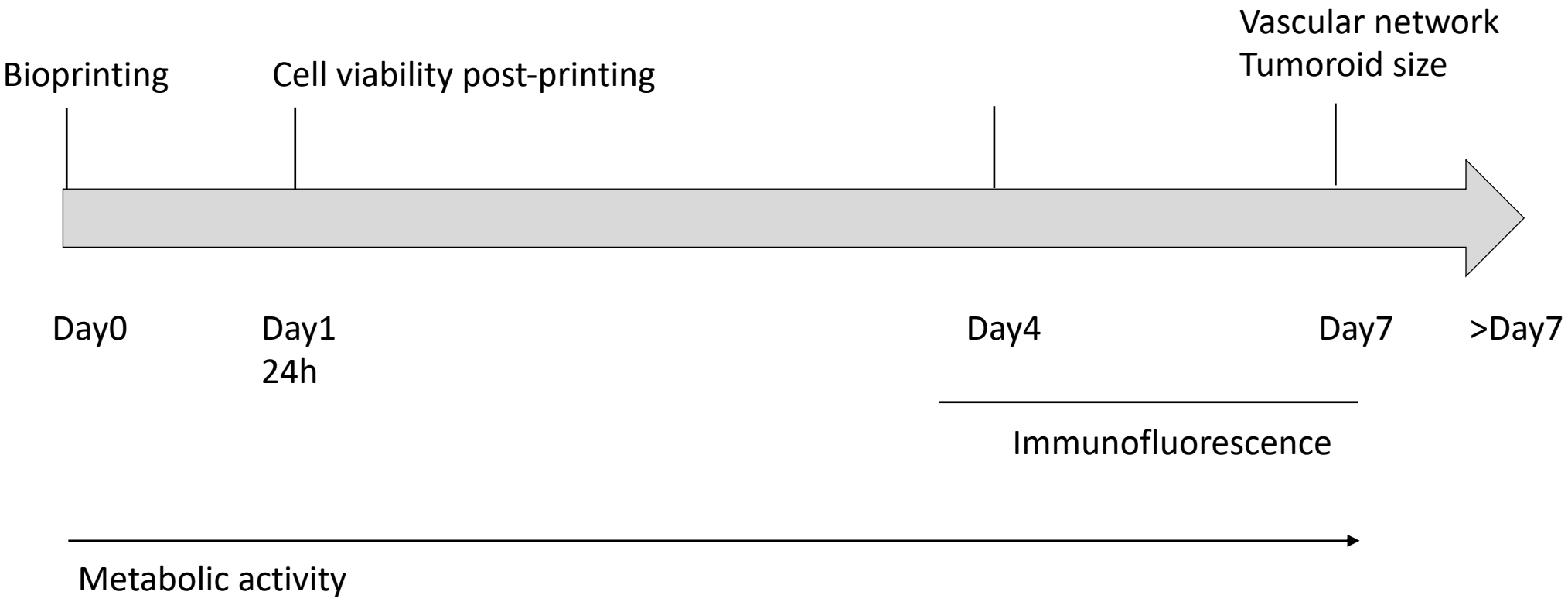


U251 + HUVECs + HSF

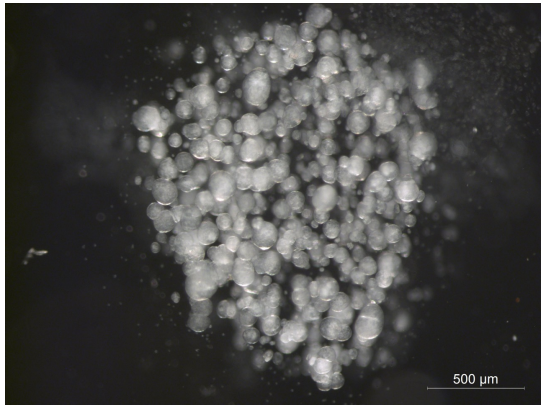


HUVECs + HSF

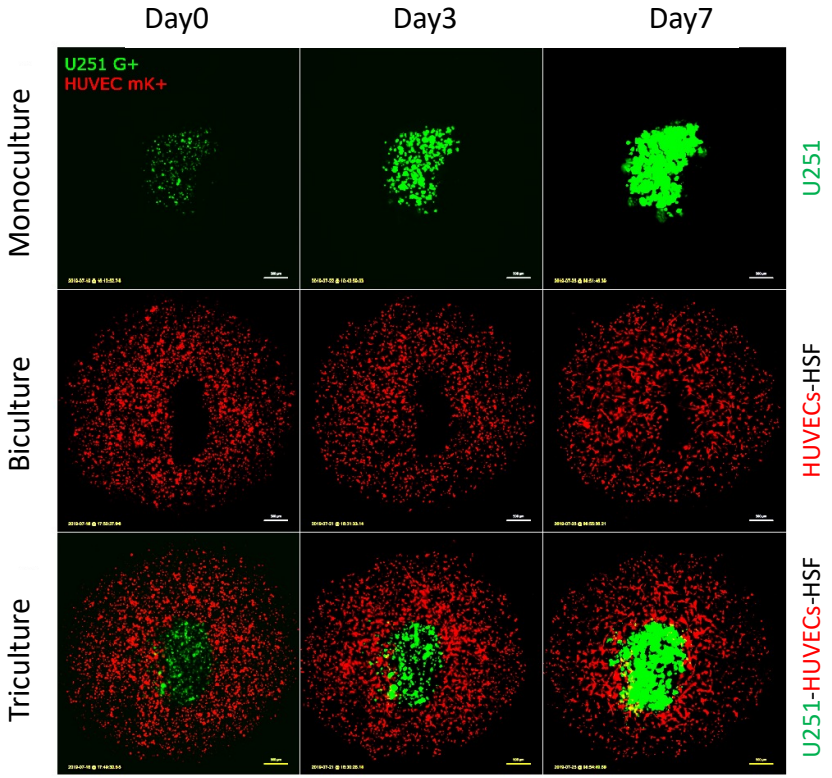
3D tumor Model Characterization

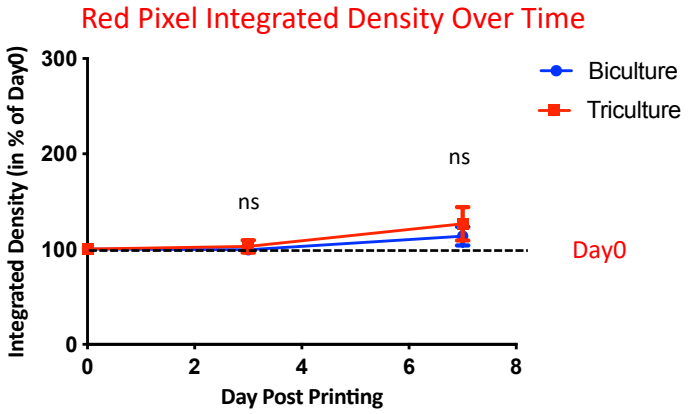


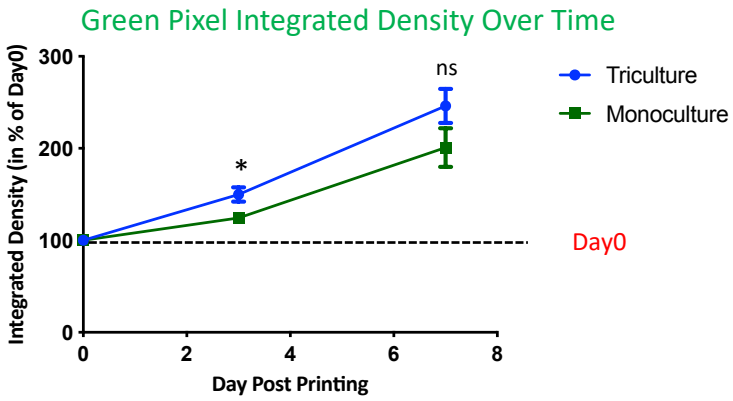
Evolution of 3D GBM Bioprints over time



Tumoroids in monoculture at D14 (bright field, binocular)

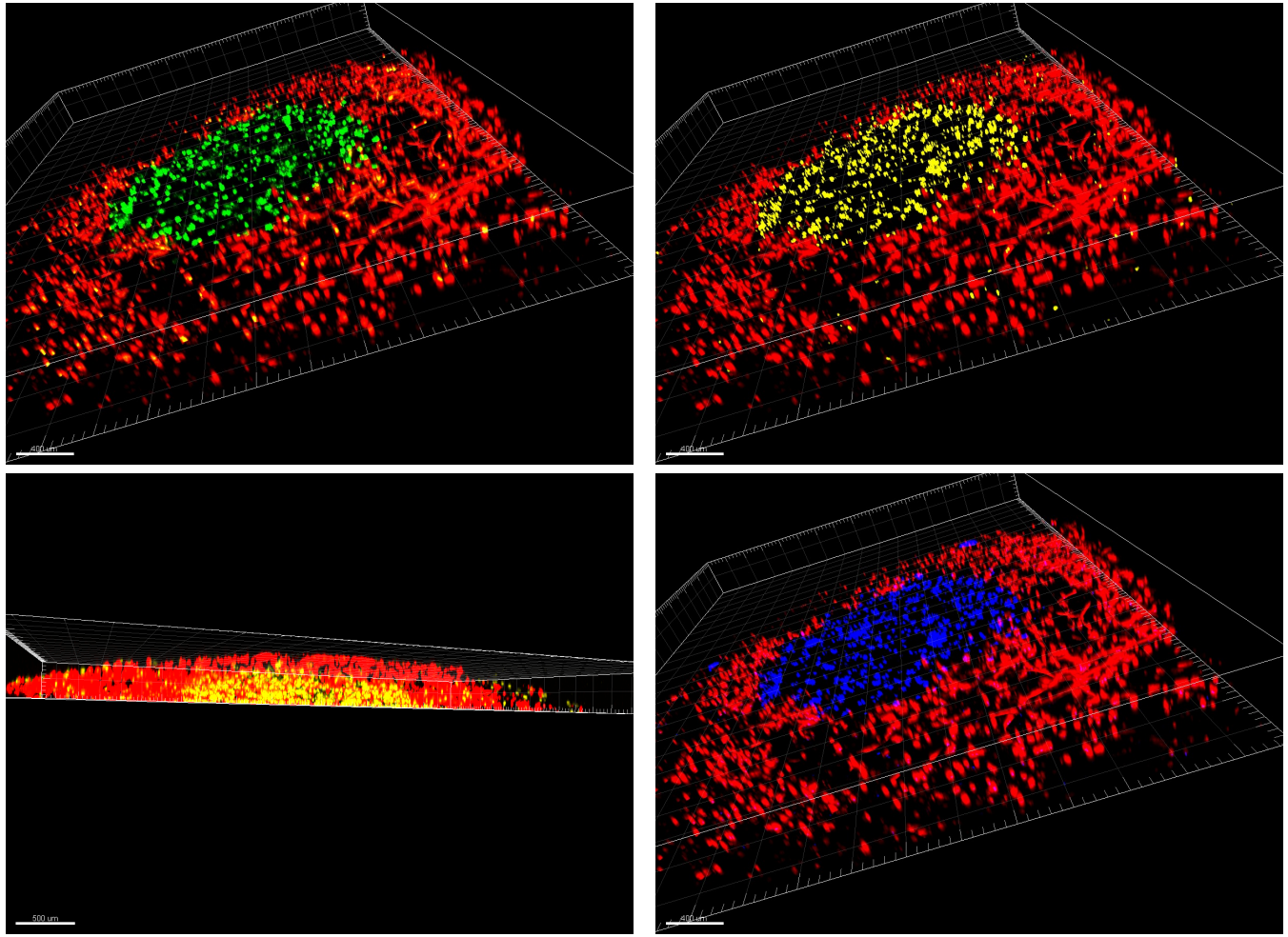




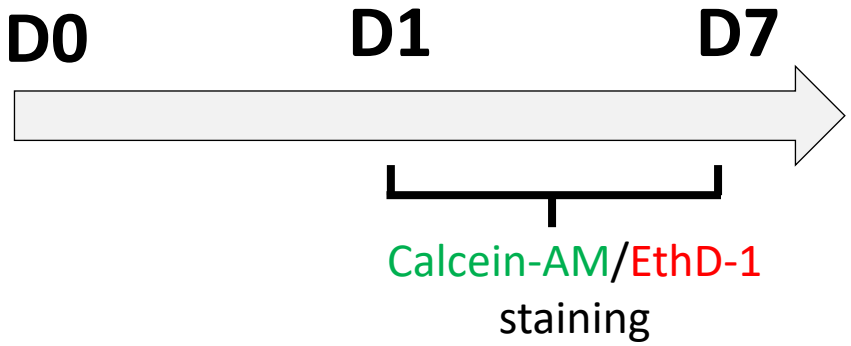


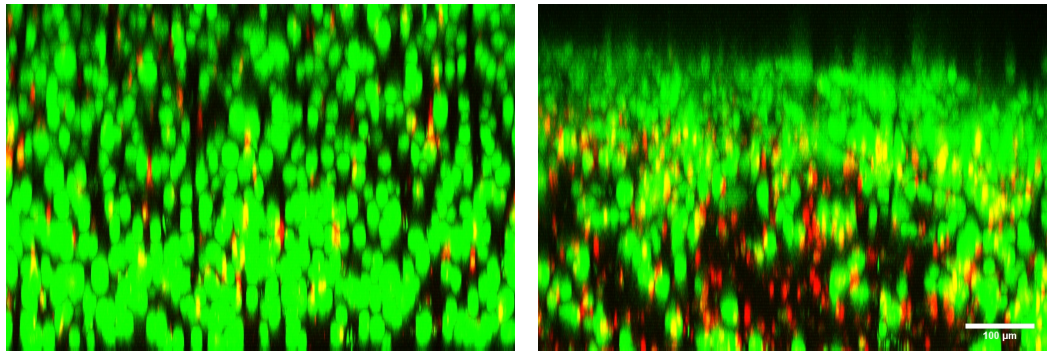
Geographical Localization of EdU+ Proliferative Cells

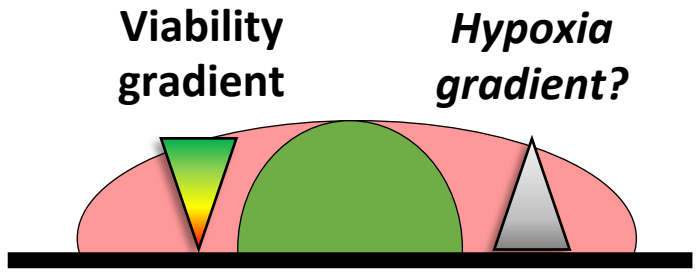
HUVECs-GBM-DAPI-EdU

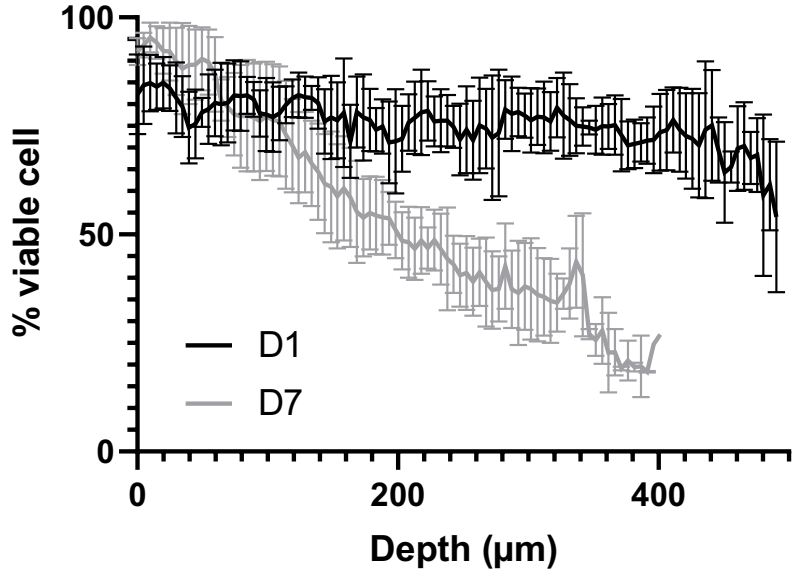


Tumor cell viability into 3D tumor bioprints

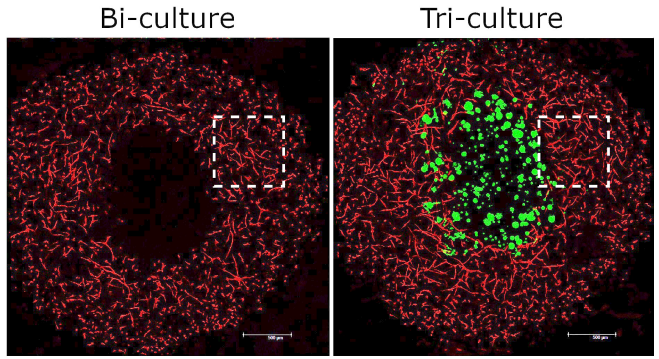




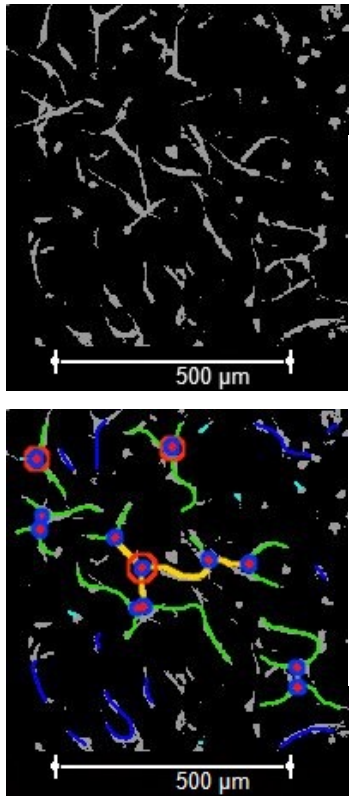


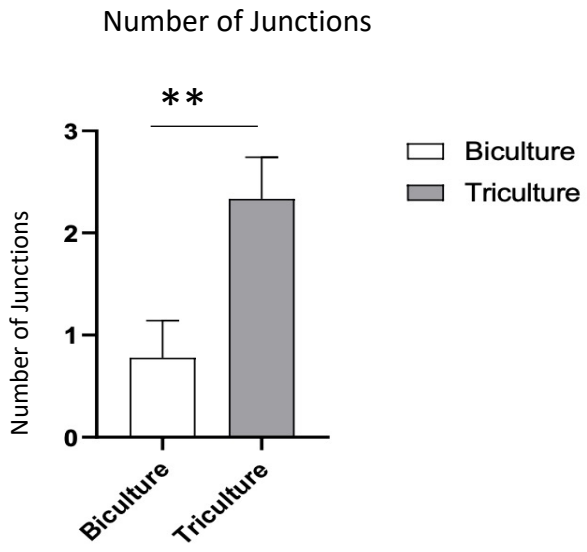


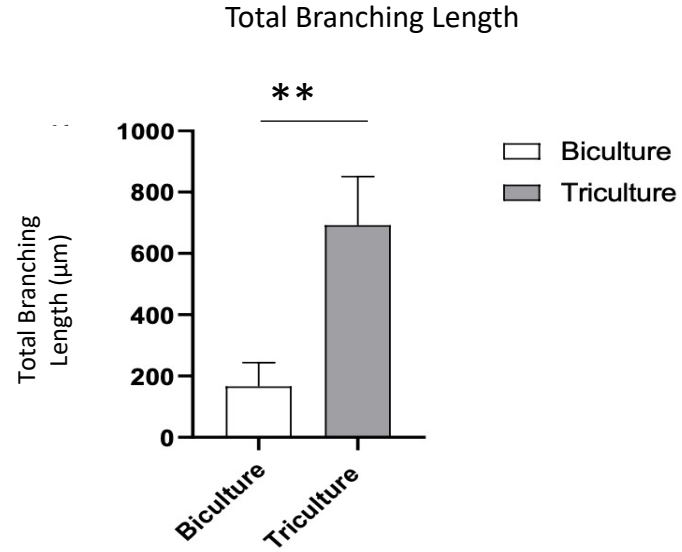
GBM Cells Stimulate Vascular-Like Network Formation



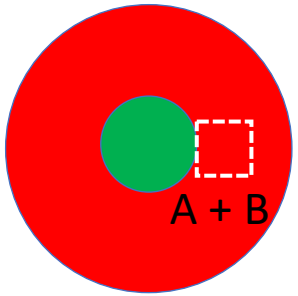
HUVECs + HSF U251 +HUVECs + HSF



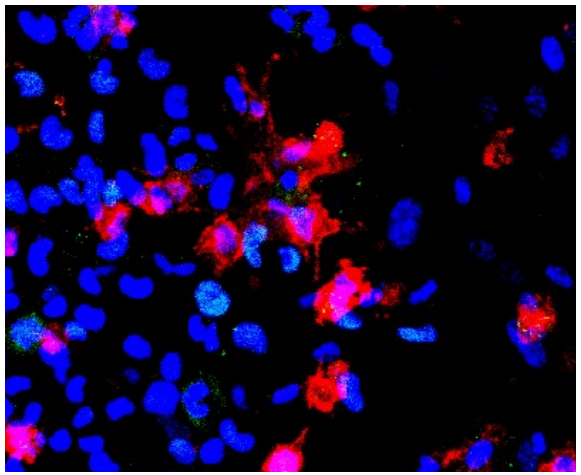




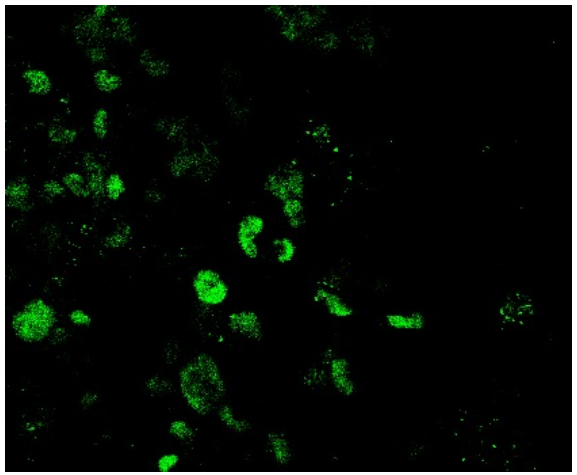
Invasive Proliferative Tumor Cells in the Peripheral Area



A. Sox 2 positive cells migrating from the tumor area on Day6

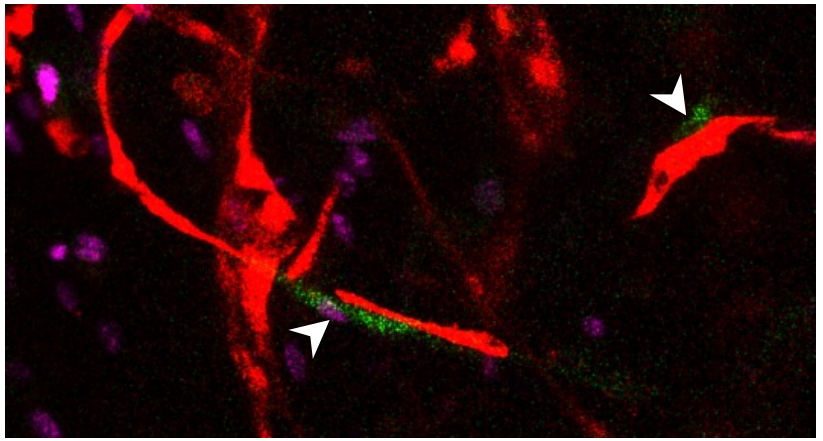


Sox2 + DAPI + CD31



Sox2

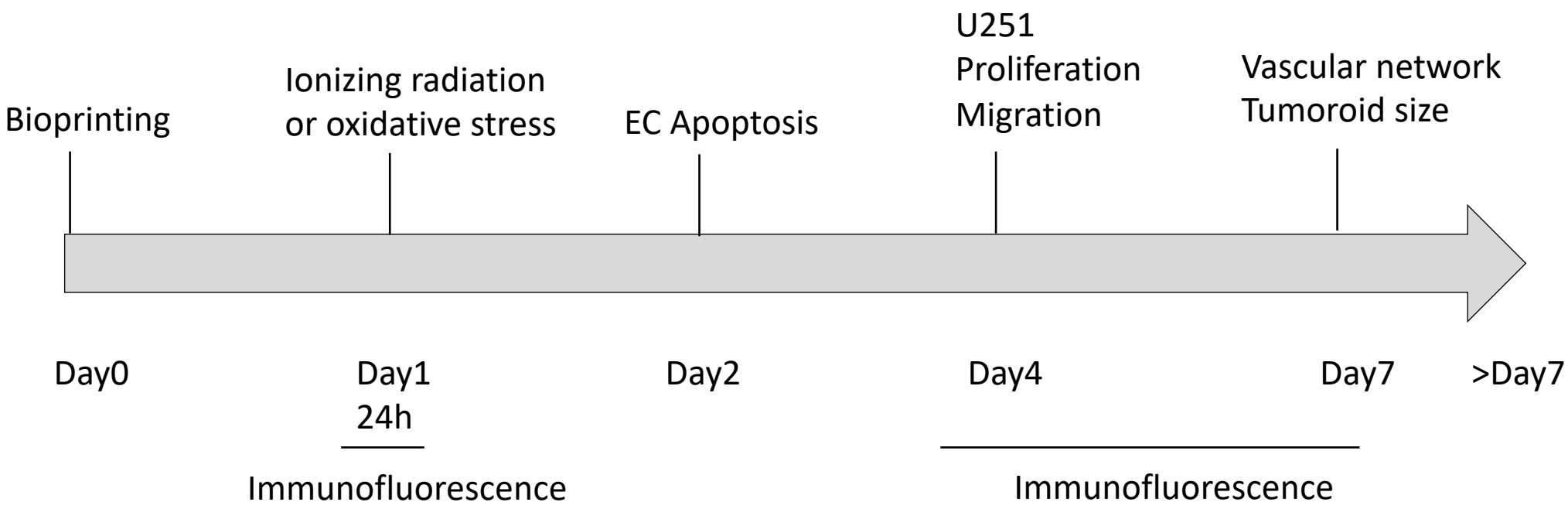
B. Tumor Cells Proliferating Along Vascular-like Structures on Day7



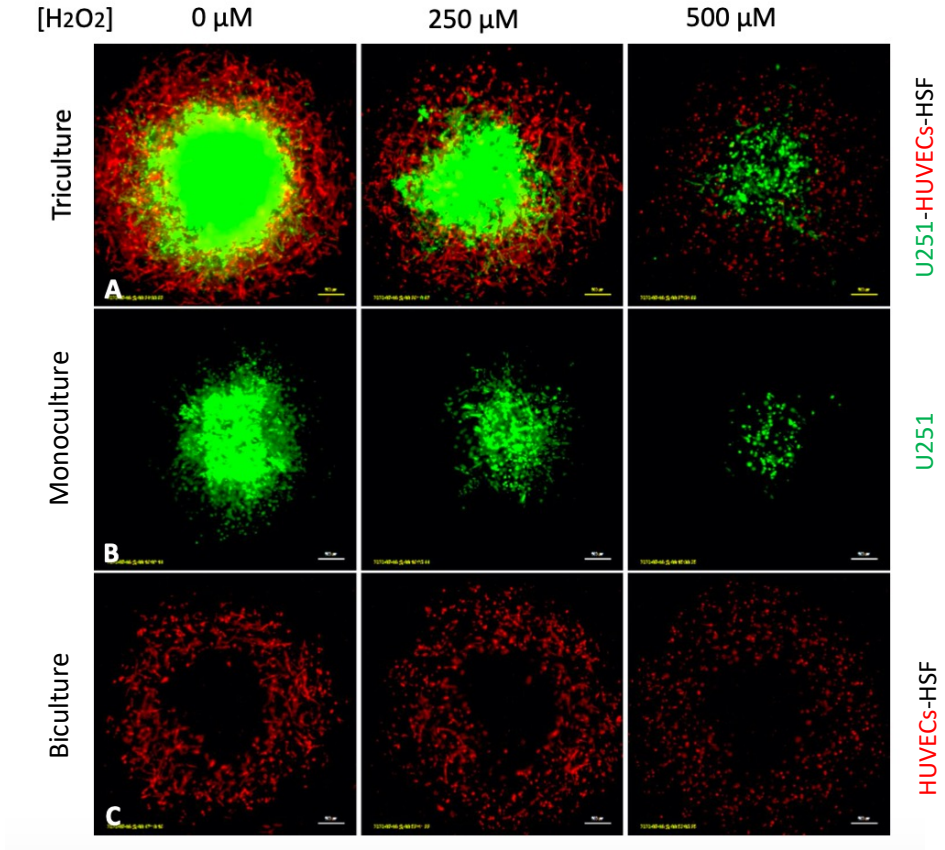
EDU + U251-GFP+ + HUVECs-RFP+

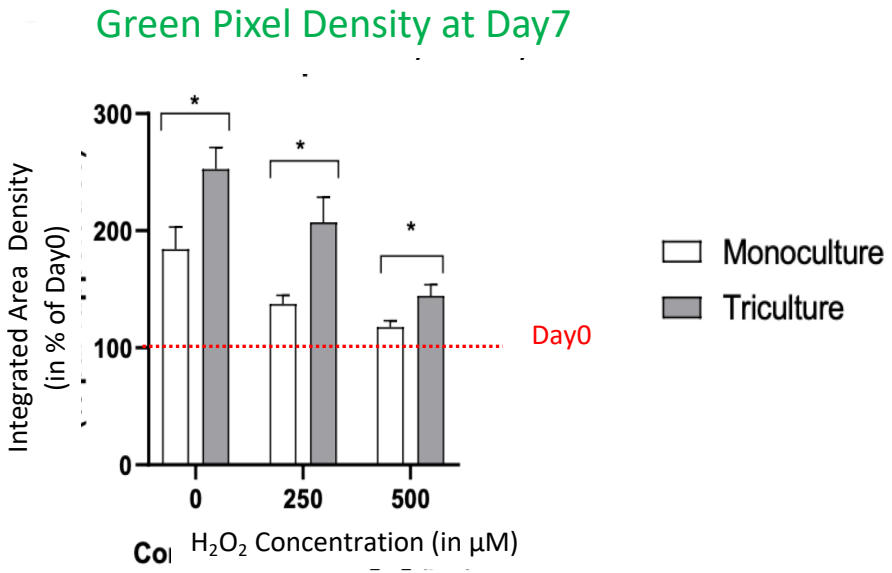
Confocal microscopy

Impact of RT or oxidative stress on 3D GBM bioprints



Dose-Dependence of oxidative stress

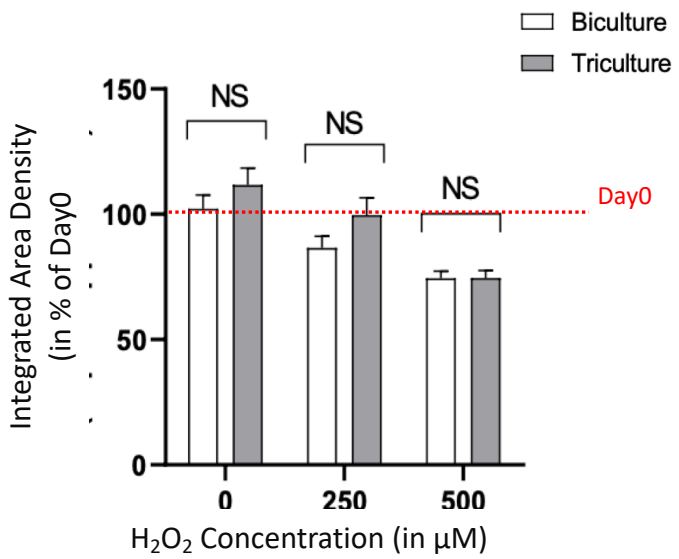


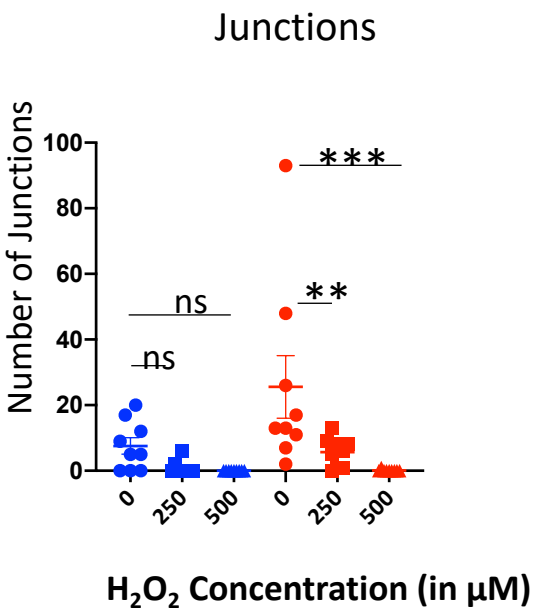


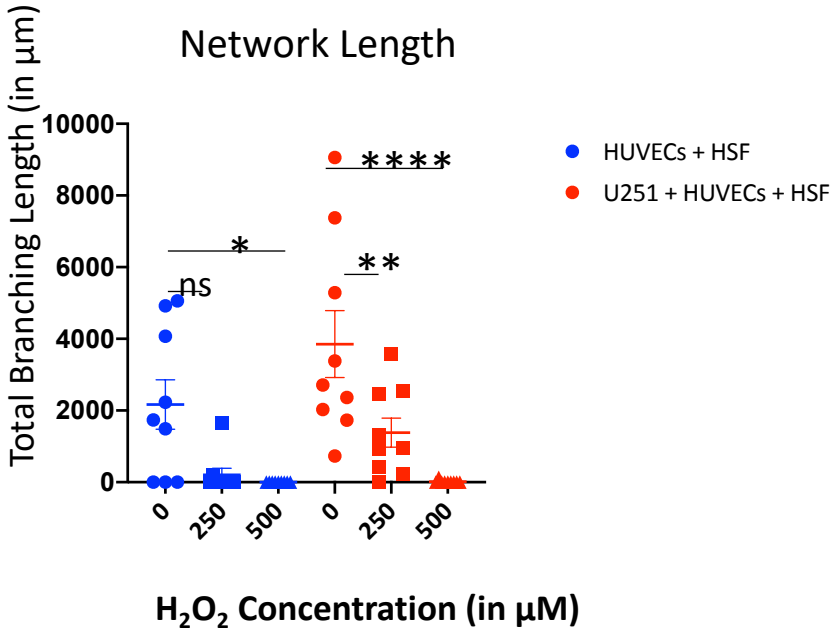
oxidative stress inhibits endothelial maturation

Assessment of the network complexity – Angiogenesis Analyzer- Image J

Red Pixel Density at Day7





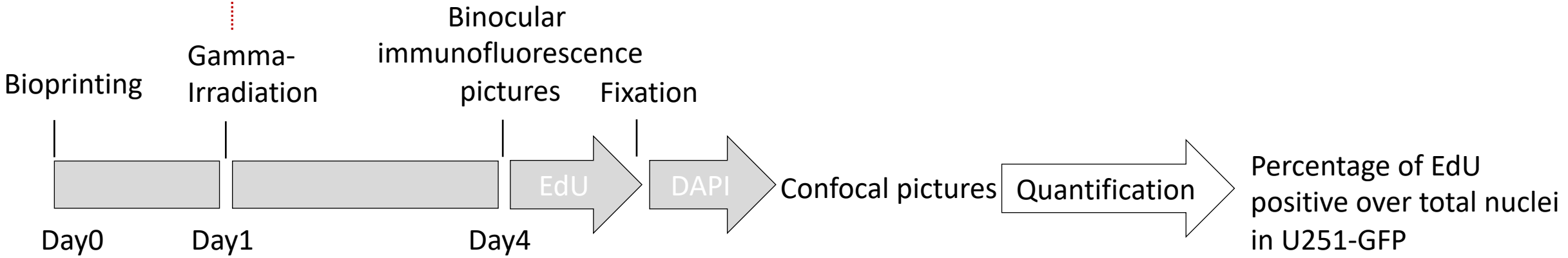


Impact of RT or oxidative microenvironment on GBM proliferation

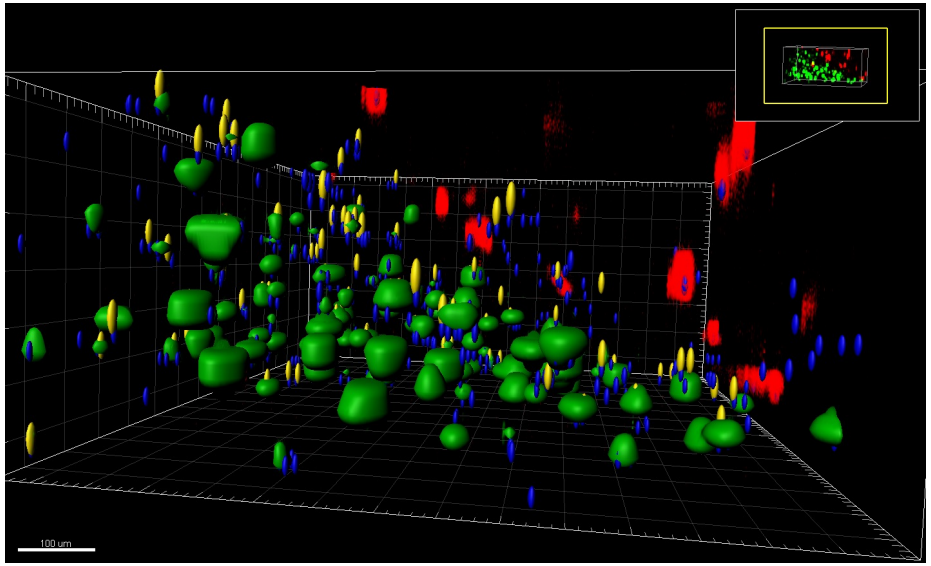
γ-rays, 4.5Gy/min
A single dose: 0, 5, or 10Gy

γ-rays, 3.5Gy/min
A single dose: 0, 3.9, or 7.8Gy.
Or 0, 4, or 8Gy

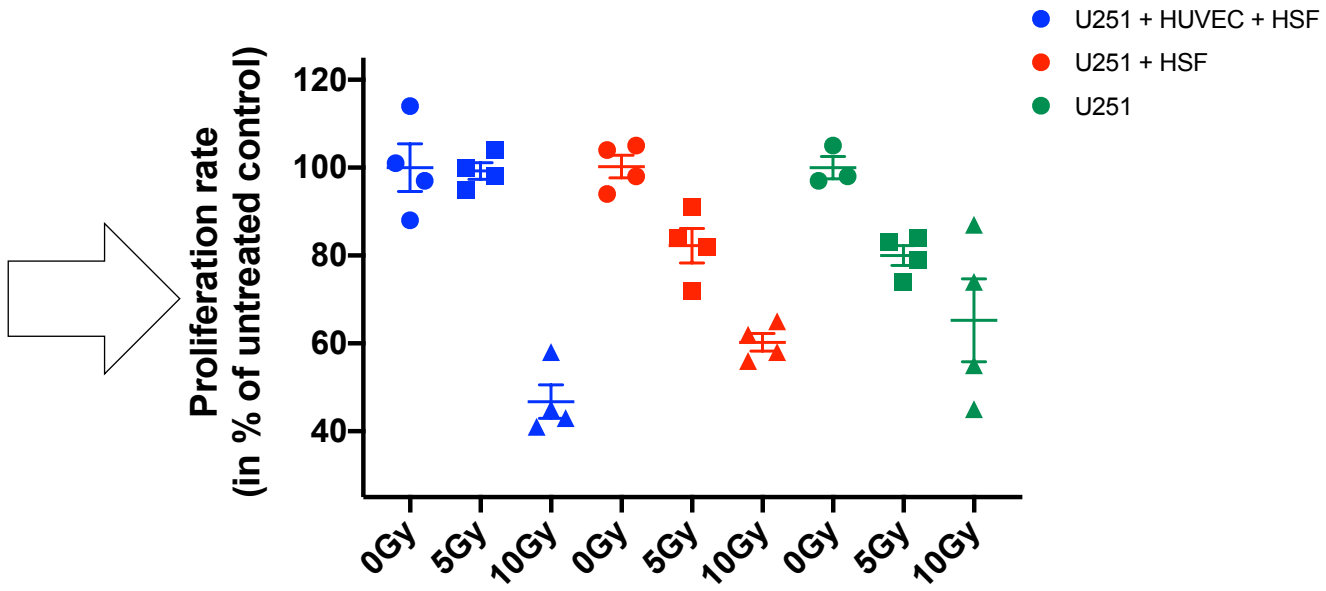
From Dose-Map Record 0ct 11, 2021



RT or oxidative stress inhibit GBM proliferation



Quantification in Imaris

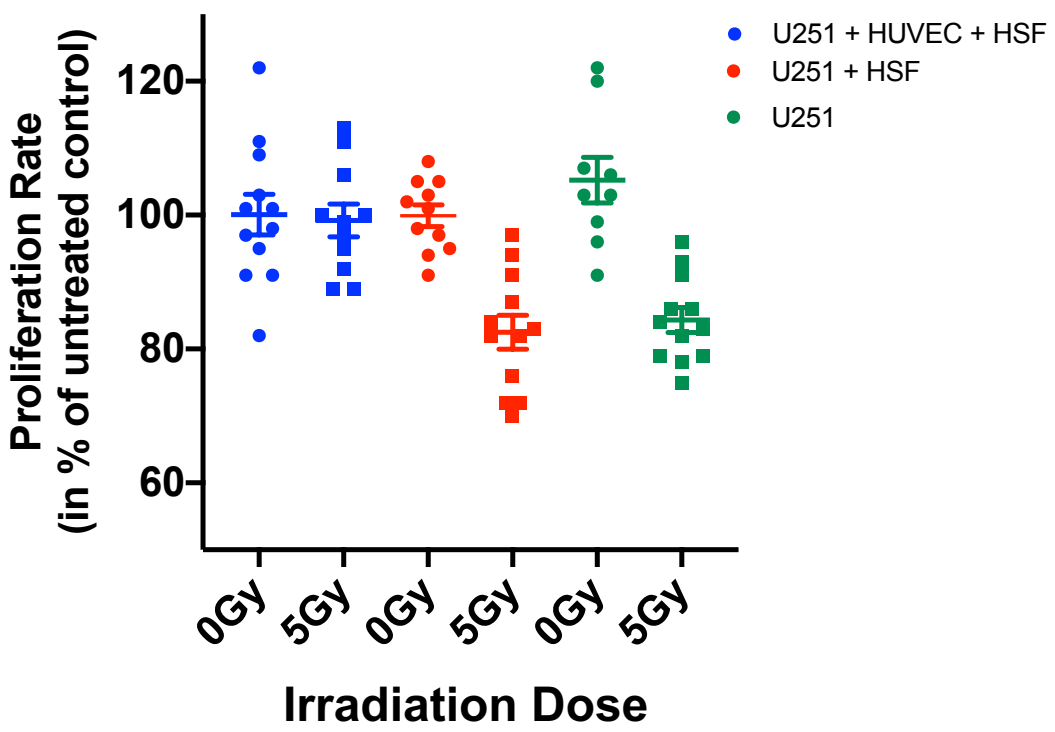


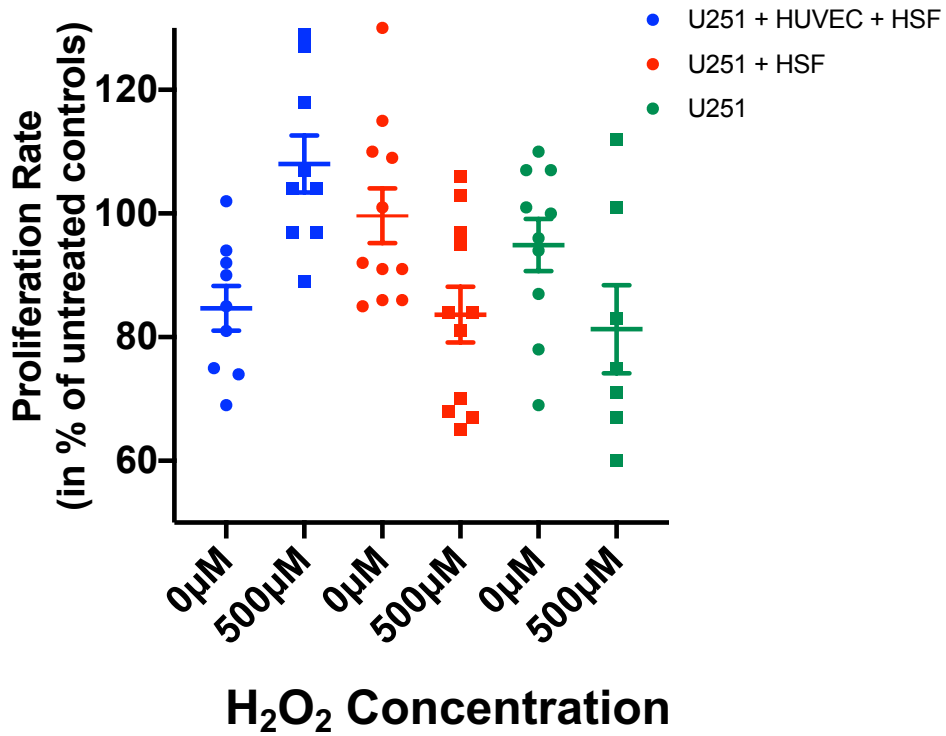
(3 fields per well, 4 wells per condition, Mann-Whitney, two-tailed, * = p<0.05

"Of note, EC apoptosis by the production of ceramide was shown to depend on doses higher than 5Gy"

Ketteler et al. Cell Death and Disease (2020), Baselet et al. Cellular and Molecular Life Sciences (2019)

Impact of RT or oxidative microenvironment on GBM proliferation





Where are we so far?

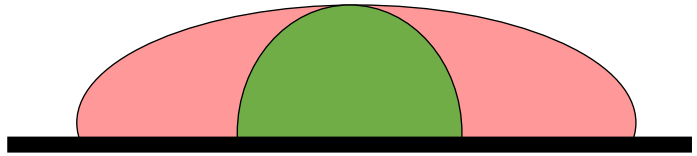
I. Create a 3D bioprinted cancer model

- **Ability to bioprint the model with viable cells** post-printing
- **Necrotic core** that mimics the tumor physiopathological environment
- **Matured micro-vascular like endothelial cells**

II. Study the model reaction to treatment

- The model **responds to oxidative stress and radiotherapy**
- **Impact of stroma cells into tumor cell response**

Where we want to go

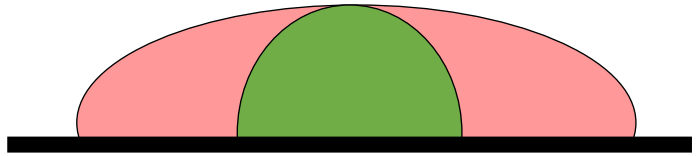


III. Assess cancer-microenvironment communication during treatment

- Specific gene invalidation in one of the compartiment by CRISPR
- Search for secreted factors (proinflammatory cytokines, Ceramide, exosomes….)

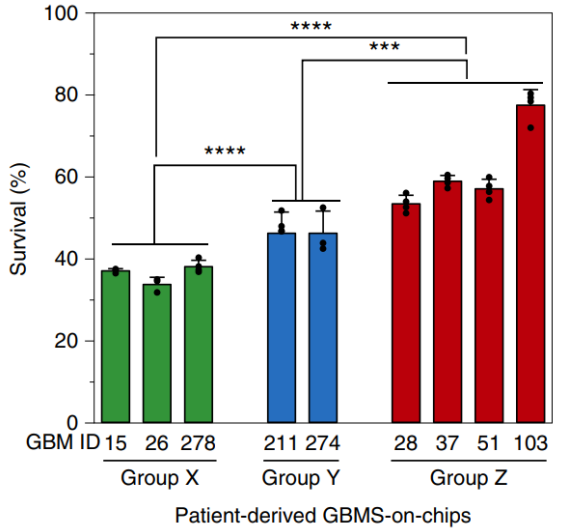
→ Miss a dynamic follow-up into the bioprint

Impact of RT or oxidative microenvironment on GBM proliferation



IV. Integrate patient-derived cells to evaluate the model prediction abilities

- **Confirm our results** in primary tumor cells
- Add other stroma cells (Astrocyte, glial cell, macrophage,…)
- E**valuate our model efficiency to predict patient response**



G-Y Yi et al, 2019

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ART Bioprint

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