



Tumoroïdes et étude des cellules persistantes dans le cancer du poumon

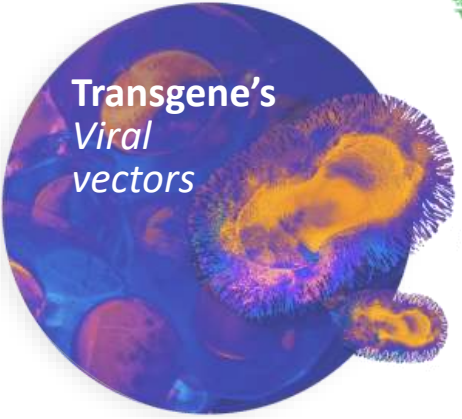
Clara fauveau – PhD candidate

14 juin 2024




Transgene


An expert in viral vector-based immunotherapy




Transgene's
Viral
vectors



Strasbourg



A biotechnology company focused on the development of viral vector-based cancer treatments

THERAPEUTIC VACCINE 

MVA

Stimulate patient's immune response against tumor-associated antigens

ONCOLYTIC VIRUS
Copenhagen strain of the Vaccinia Virus

Virus specifically designed to infect tumor cells and induce their apoptosis

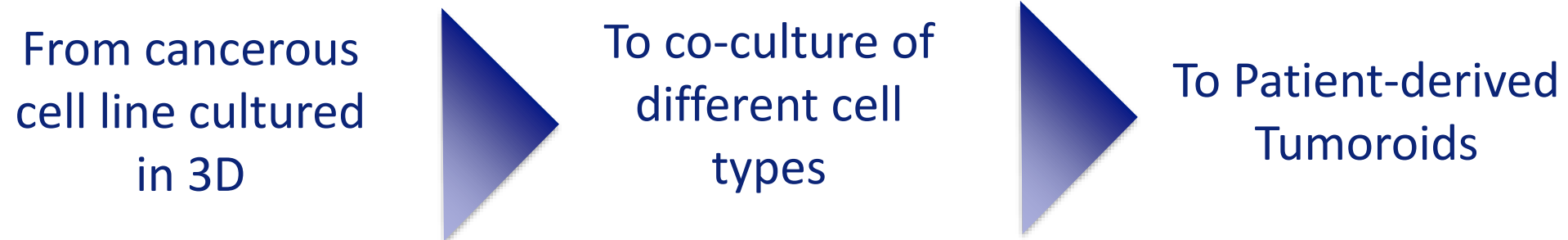
Indirect Immune cells activation through virus oncolytic activity

● Transgene's strategy in the development of new *in vitro* models

- **Current challenges :**

- High attrition rates in drug development
- Current animal or *in vitro* models are not predictive enough
- Ethical concerns (3Rs policy)
- Advancements in personalized medicine

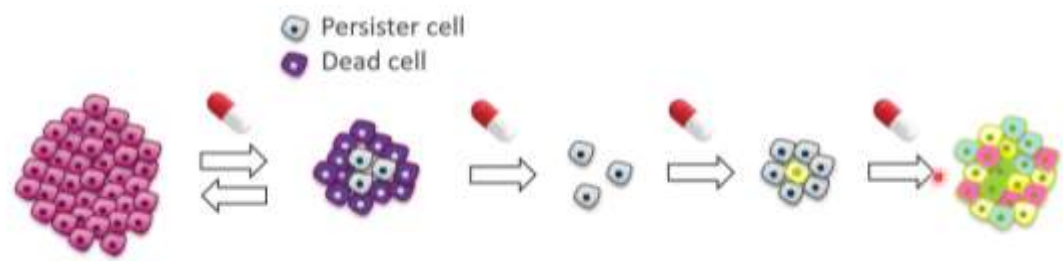
→ **Generation of 3D human NSCLC models with different levels of complexity :**



● An open innovation approach to access new predictive models

- Transgene is a member of  PERSISTSEQ

“Promote development novel human *in vitro* cancer models & the use transcriptomic method to detect and identify drug persistence in cancer”



Hallmarks of persisters:

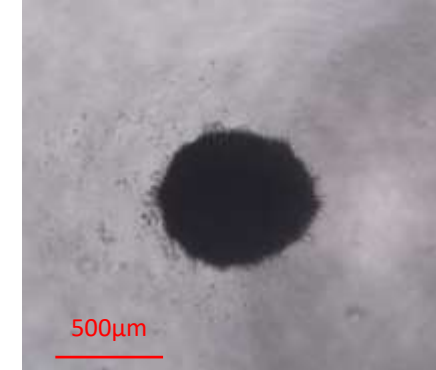
1. Prolonged arrest on treatment
2. Reversibility
3. Not genetic

- **Transgene’s objective :**

- Gain in-depth insights into *in vitro* models within a collaborative environment, allowing to compare and refine our models.
- Investigate whether any of the therapies developed by Transgene have the potential to target and challenge persister cells

● Tumoroids generation and treatment efficacy assessment

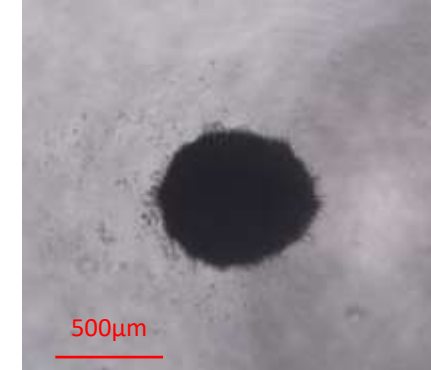
- Tumoroids generated in ULA plates
(ULA = Ultra Low Attachment plate)



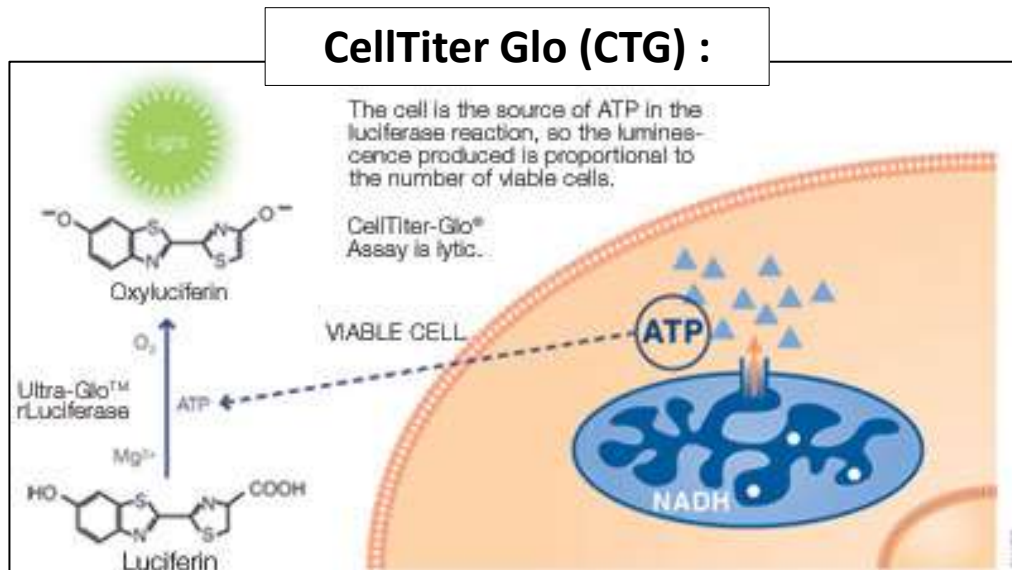
- Advantages :
 - Eliminates the need for hydrogel addition
 - Rapid formation of aggregates
 - Tumoroids with uniform size and shape
 - Simple Handling and Analysis
 - Suitable for high-throughput screening applications

Tumoroids generation and treatment efficacy assessment

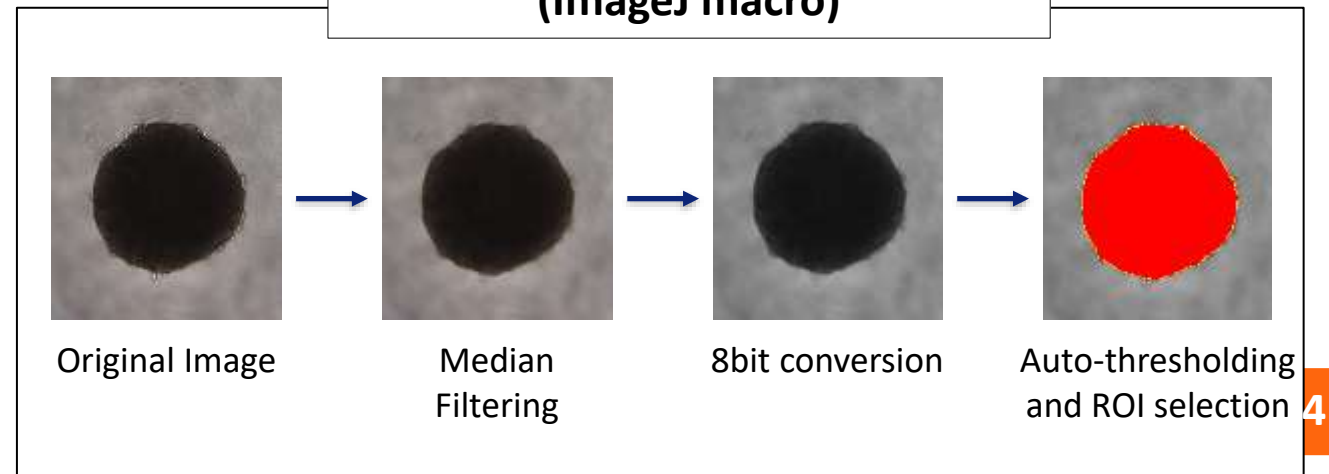
- Tumoroids generated in ULA plates (ULA = Ultra Low Attachment plate)



- Combined with Viability suited for 3D culture :

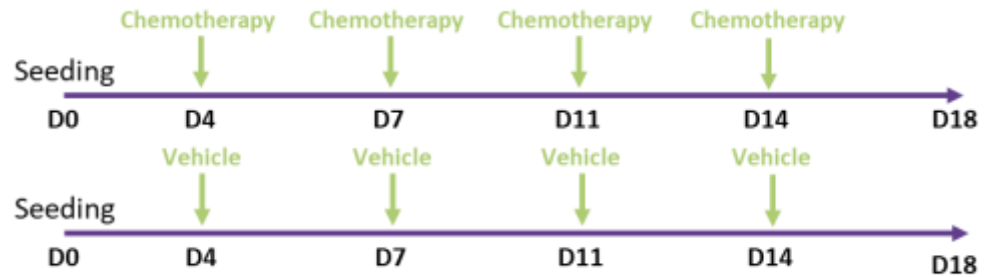


Tumoroid surface measurement (ImageJ macro)



Study of persistence and its impact on virotherapy efficacy

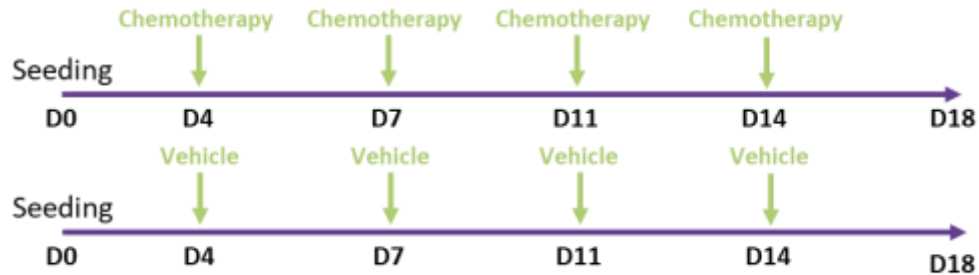
Step 1 : Persistence induction & study



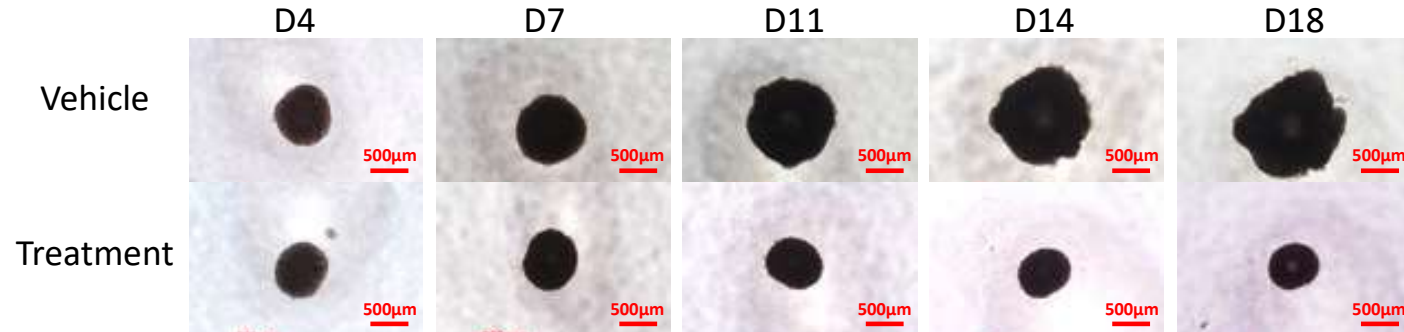
10k A549 (Lung adenocarcinoma cell line) cells per well
Chemotherapy : 10 μ M of cisplatin, 250 μ M of pemetrexed
Vehicle : 0,9% NaCl solution

Study of persistence and its impact on virotherapy efficacy

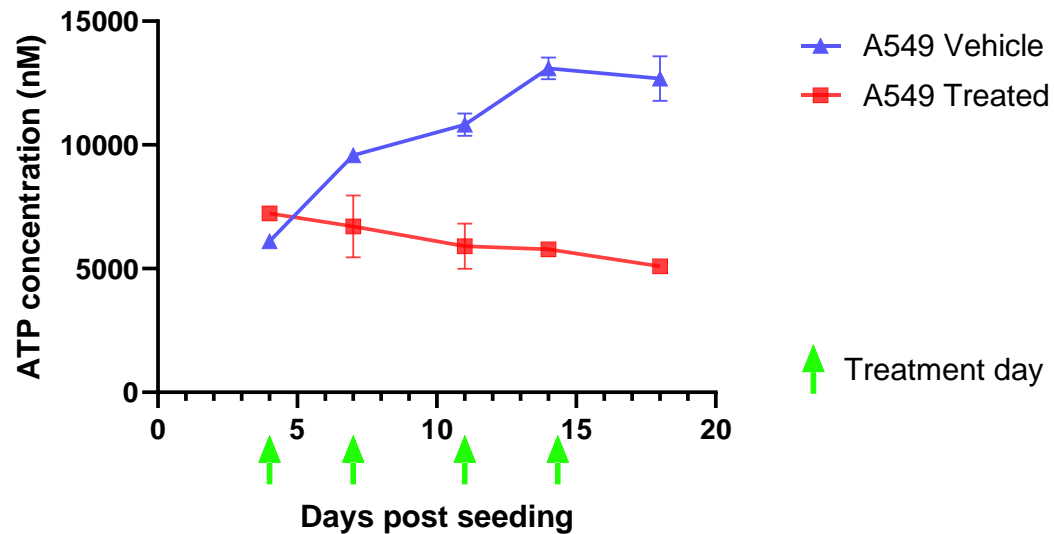
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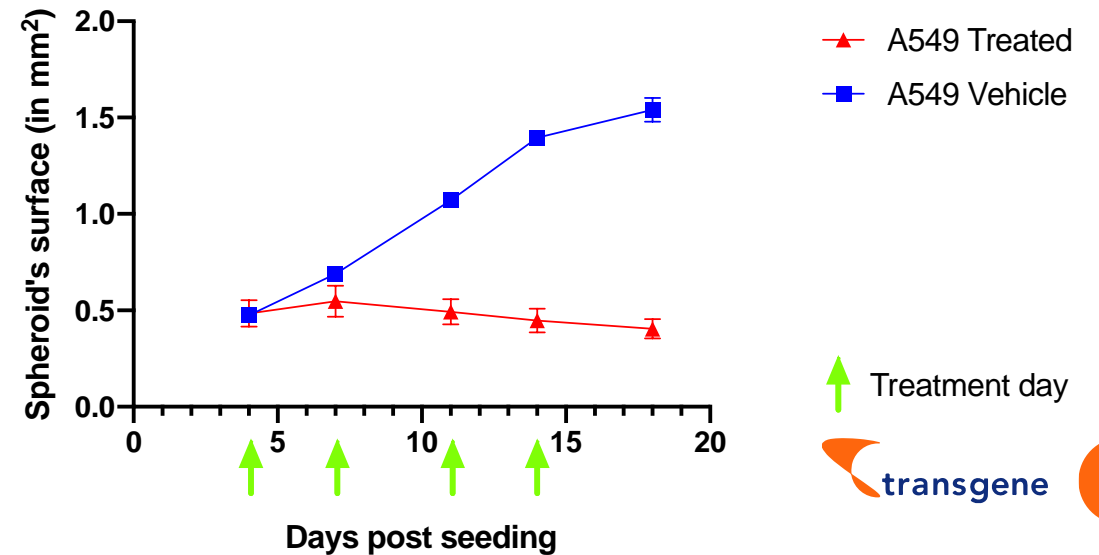
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Tumoroid ATP content evolution



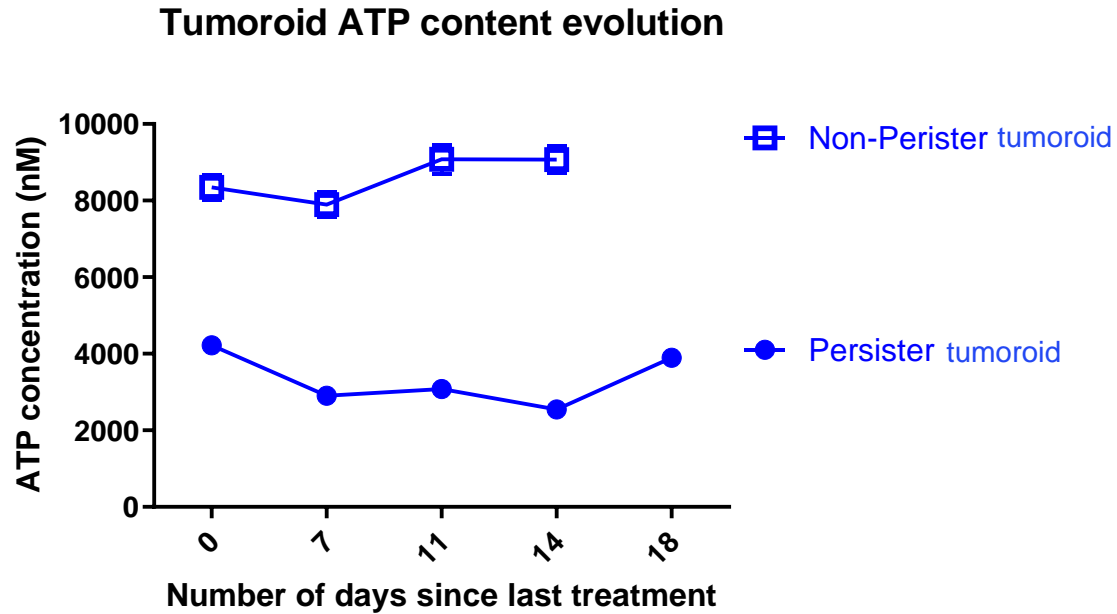
Tumoroid surface evolution



Study of persistence and its impact on virotherapy efficacy

Step 2 : Virus efficacy on persistent tumoroids

Persistent and Non-Persistent tumoroids :

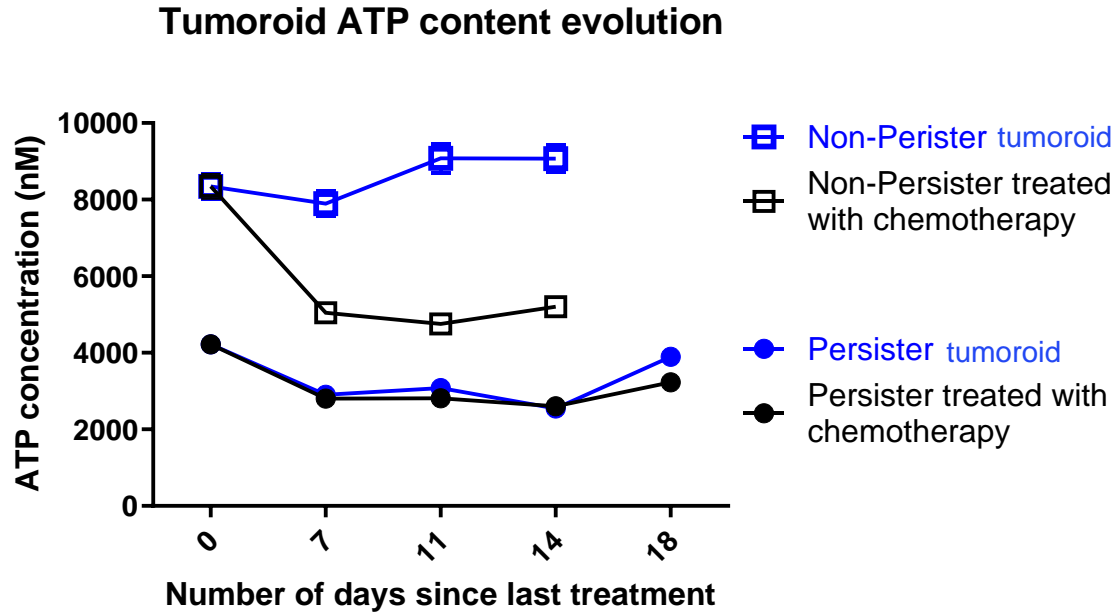


Study of persistence and its impact on virotherapy efficacy

Step 2 : Virus efficacy on persistent tumoroids

Persistent and Non-Persistent tumoroids :

- Treated with chemotherapy (10 μ M of cisplatin, 250 μ M of pemetrexed)



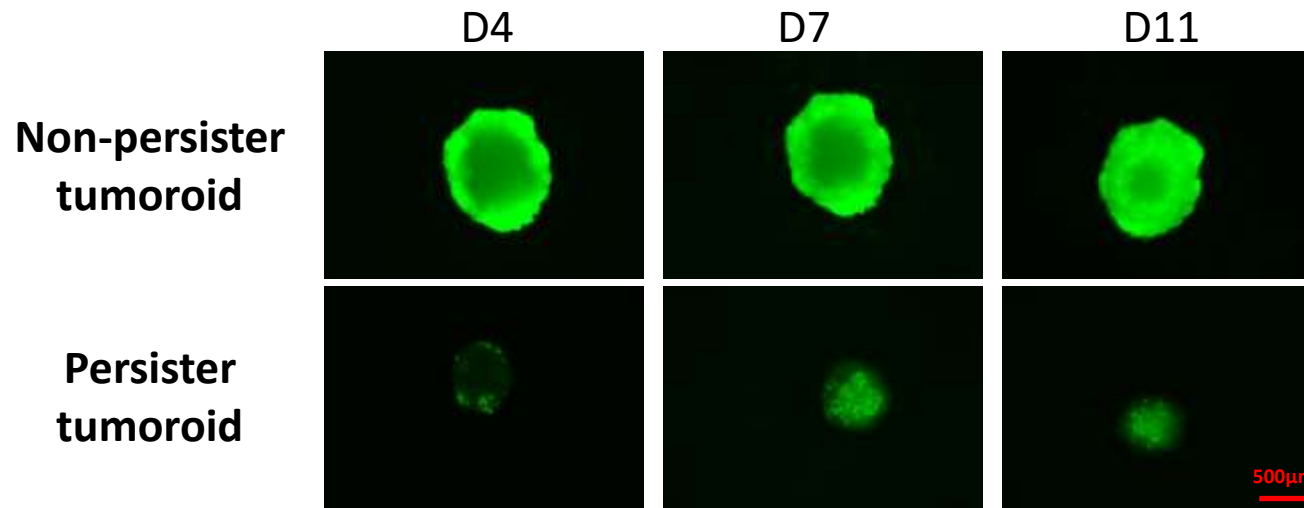
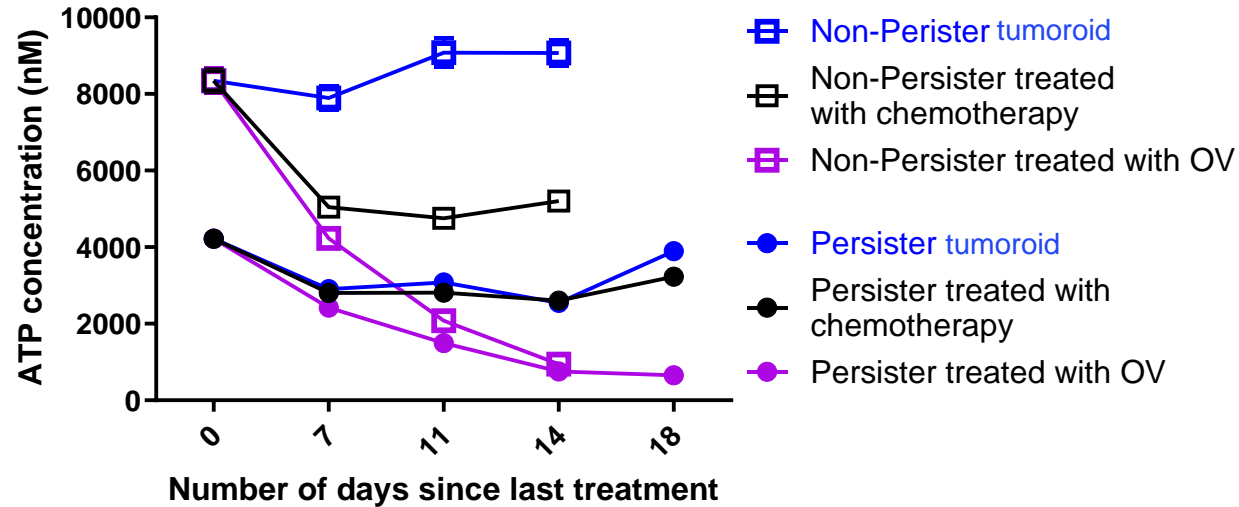
Study of persistence and its impact on virotherapy efficacy

Step 2 : Virus efficacy on persistent tumoroids

Persistent and Non-Persistent tumoroids :

- Treated with chemotherapy (10 μ M of cisplatin, 250 μ M of pemetrexed)
- Treated with an OV expressing GFP (40.10³ PFU/mL)

Tumoroid ATP content evolution



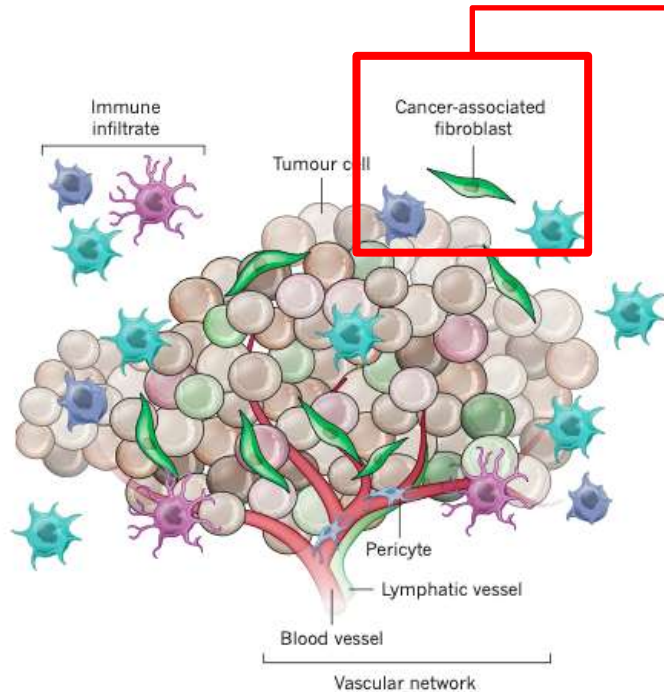
● Study of persistence and its impact on virotherapy efficacy

Results

- **Model generating reproducible results**
- **Treatment efficacy assessed using various methods**
- **Suitable for high-throughput screening applications**

Evaluating CAF impact on persistence and virotherapy thanks to a coculture model

Project

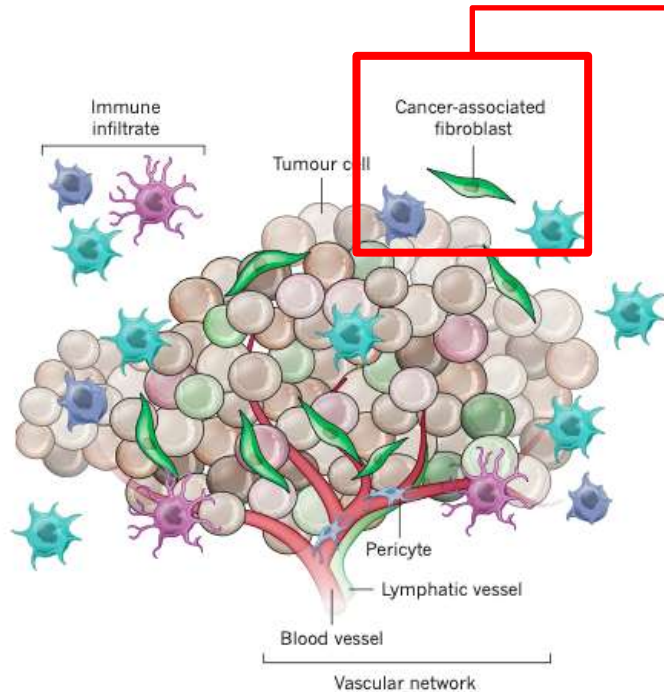


- **Cancer-associated fibroblasts (CAFs)** are non-transformed, heterogeneous populations of cells
 - Tumor-cell-activated
 - Associated with poor prognosis in various cancers
 - Exert a pro-tumoral behaviour (e.g ECM remodelling, angiogenesis or EMT promotion)

CAFs' impact on OV's efficacy and on persistence development remains largely unexplored

Evaluating CAF impact on persistence and virotherapy thanks to a coculture model

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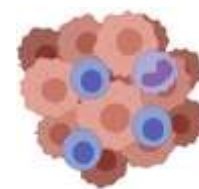


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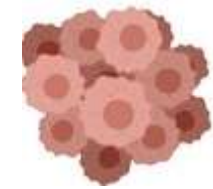
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CAF tumoroids



A549-CAF tumoroids

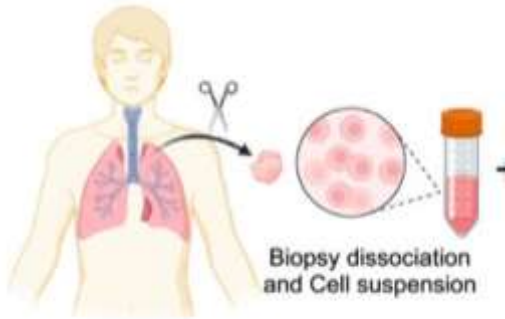


A549 tumoroids

**Treated with
Vehicle, chemotherapy
and/or Ovs**

Evaluating CAF impact on persistence and virotherapy thanks to a coculture model

First Step : CAF isolation

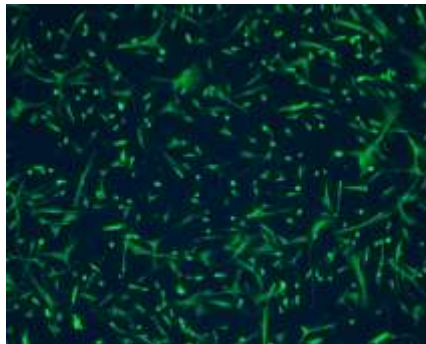


Isolated by culturing NSCLC patient biopsy cells in fibroblast media

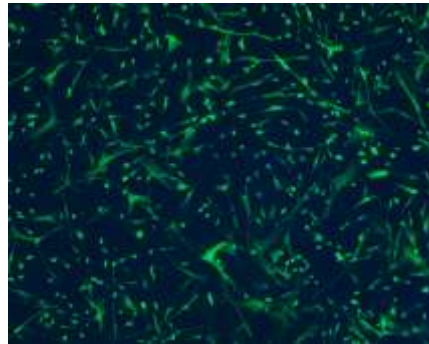


Validation by immunofluorescence :

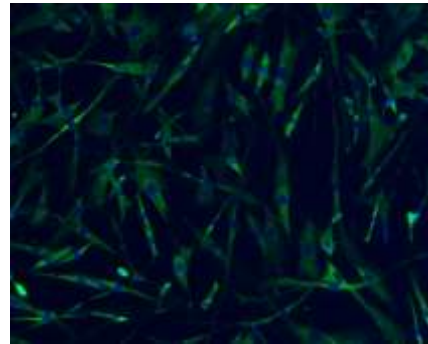
Positive for
CAF markers



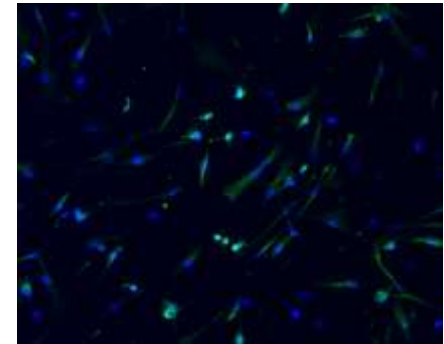
Vimentin



FAP

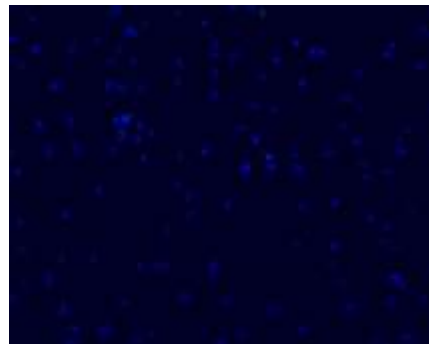


α SMA

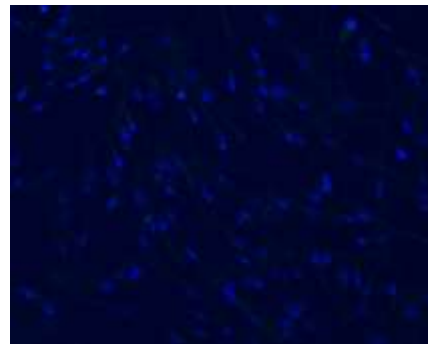


PDGFR α

Negative for Epithelial
and Endothelial
markers



EpCAM

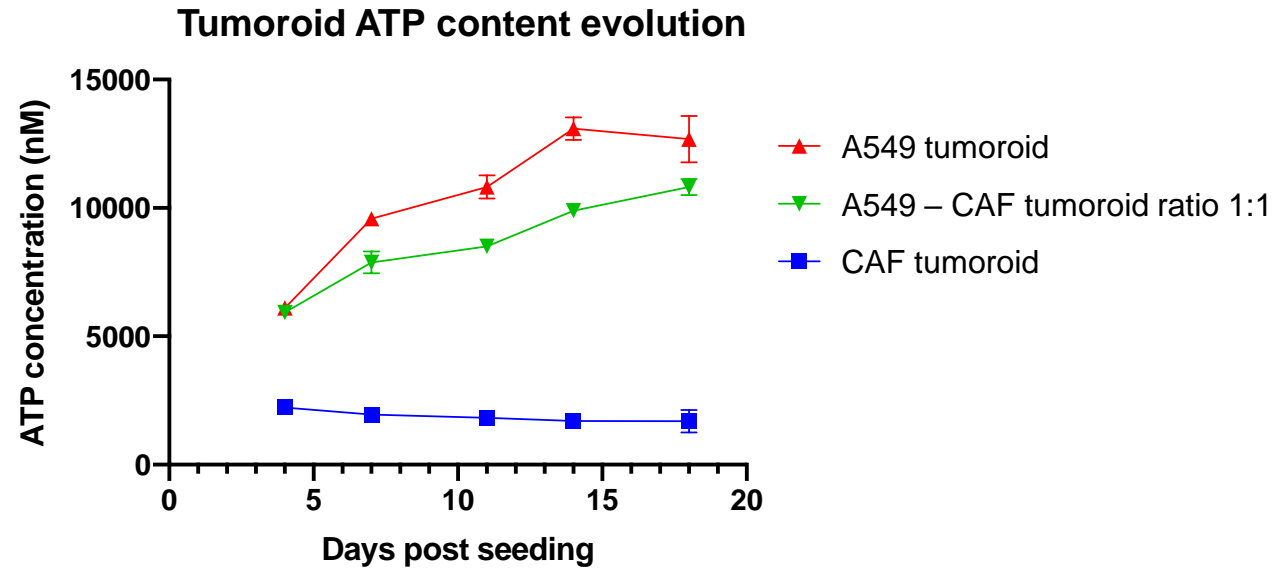


CD31

FAP (Fibroblast activation protein)
 α SMA (Alpha Smooth Muscle Actin)
PDGFR α (Platelet-derived growth factor
receptor alpha)
EpCAM (Epithelial cell adhesion molecule)

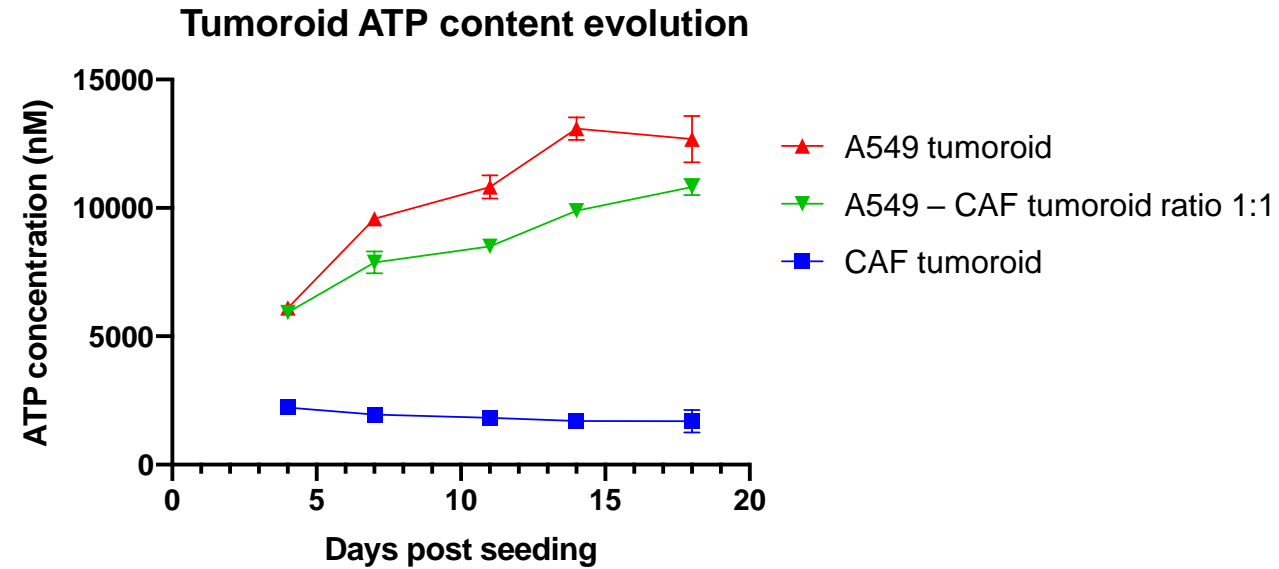
Evaluating CAF impact on persistence and virotherapy thanks to a coculture model

Second Step : Co-culture optimization



Evaluating CAF impact on persistence and virotherapy thanks to a coculture model

Second Step : Co-culture optimization



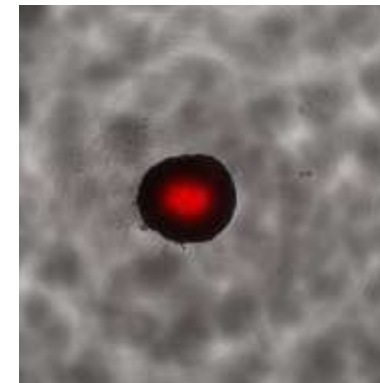
CAF expressing mCherry
cocultured with A549



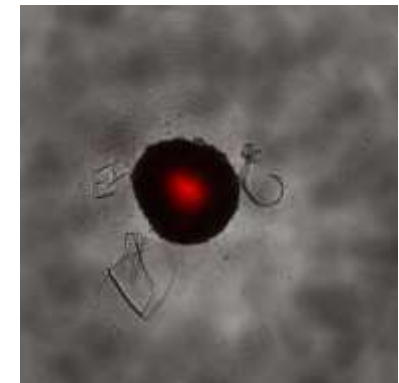
D4



D7

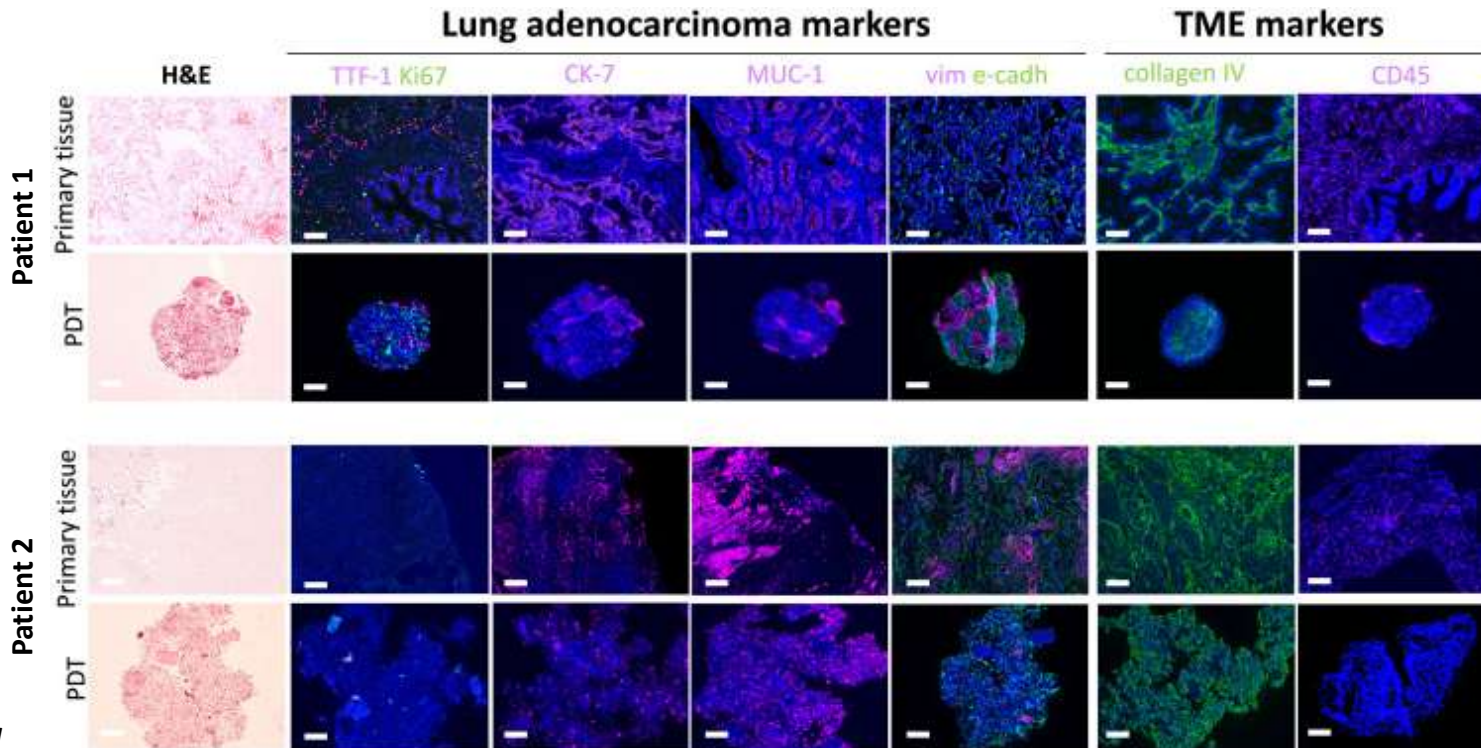
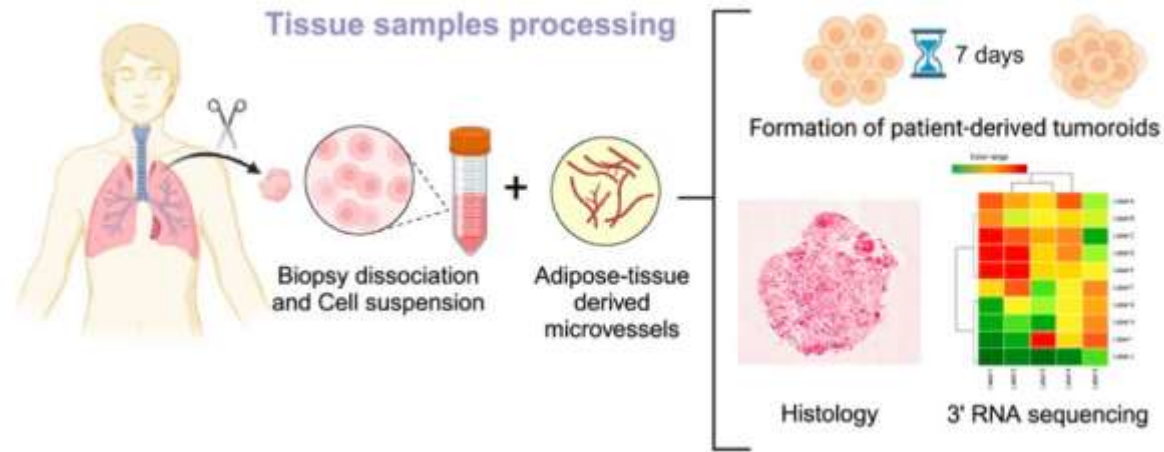


D11

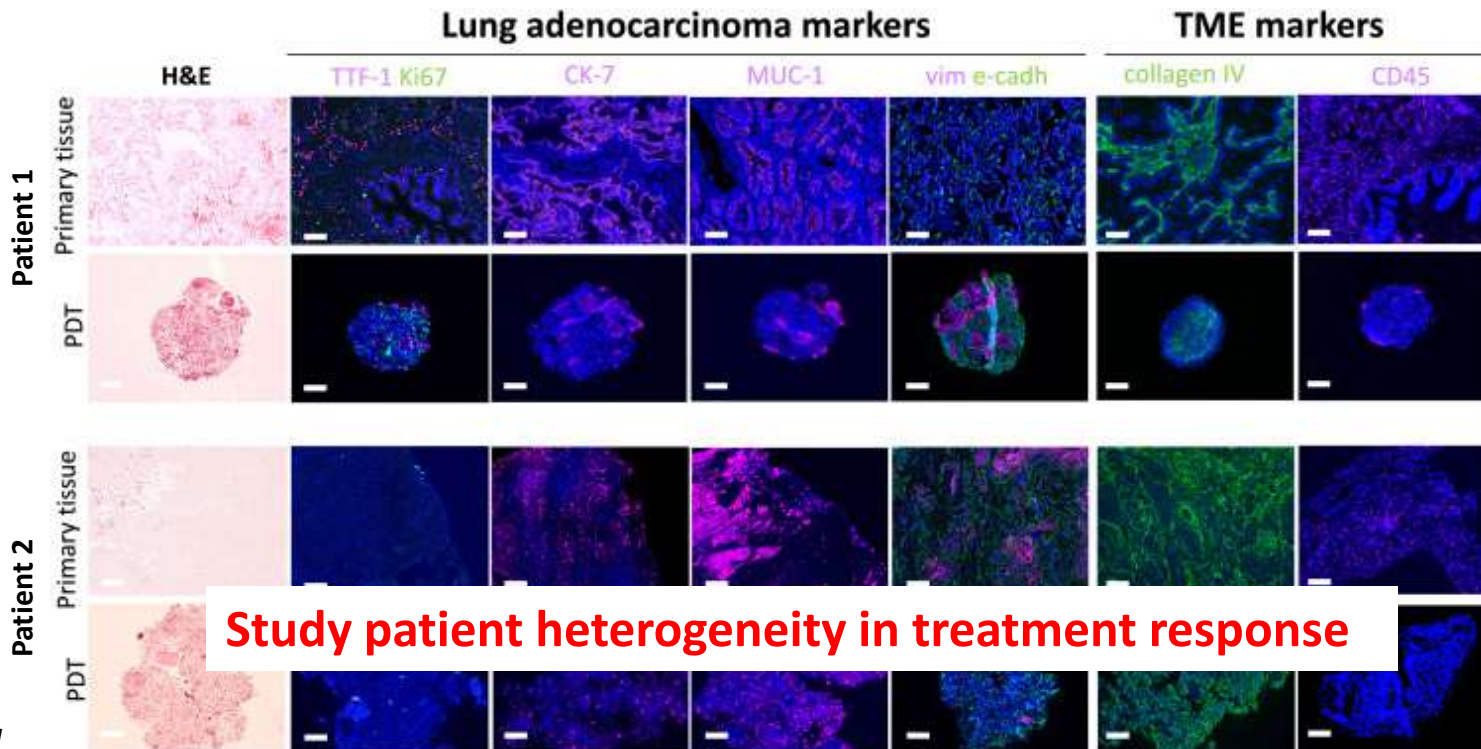
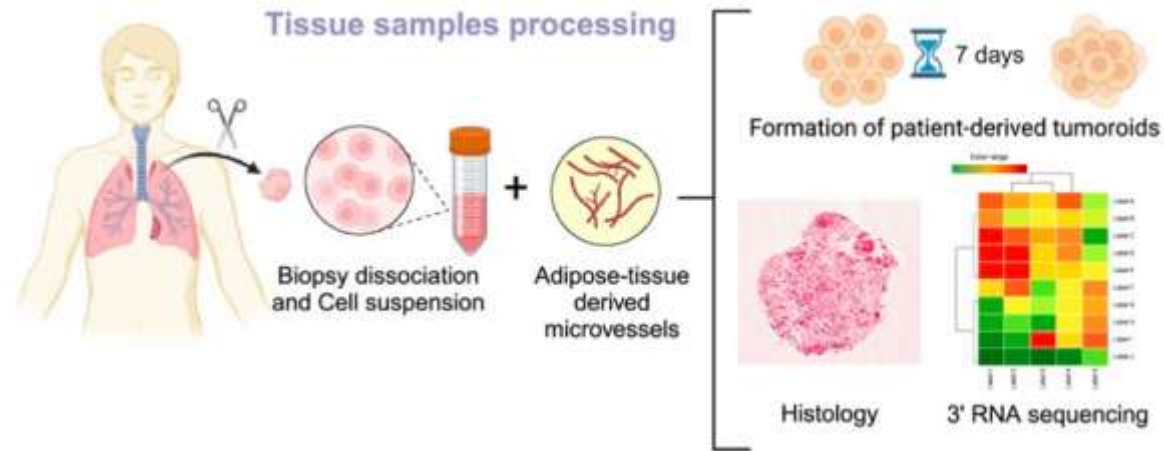


D14

● Patient-derived Tumoroids



● Patient-derived Tumoroids



Study patient heterogeneity in treatment response

Conclusions & perspectives

- **Generation 3D human models enabling the exploration of various questions :**



**Cell line
Tumoroids**

- Simple Handling
 - Uniform tumoroids
 - Applicable to different type of characterized cell lines
- Treatment response screening



**Co-culture
tumoroids**

- In vitro settings enabling separated and controlled conditions
 - Direct contact between cell-types
- deciphering interactions present inside the tumor microenvironment



**Patient-derived
Tumoroids**

- Conservation of Patient tumor heterogeneity
- Study treatment response across patients
- Personalized medicine

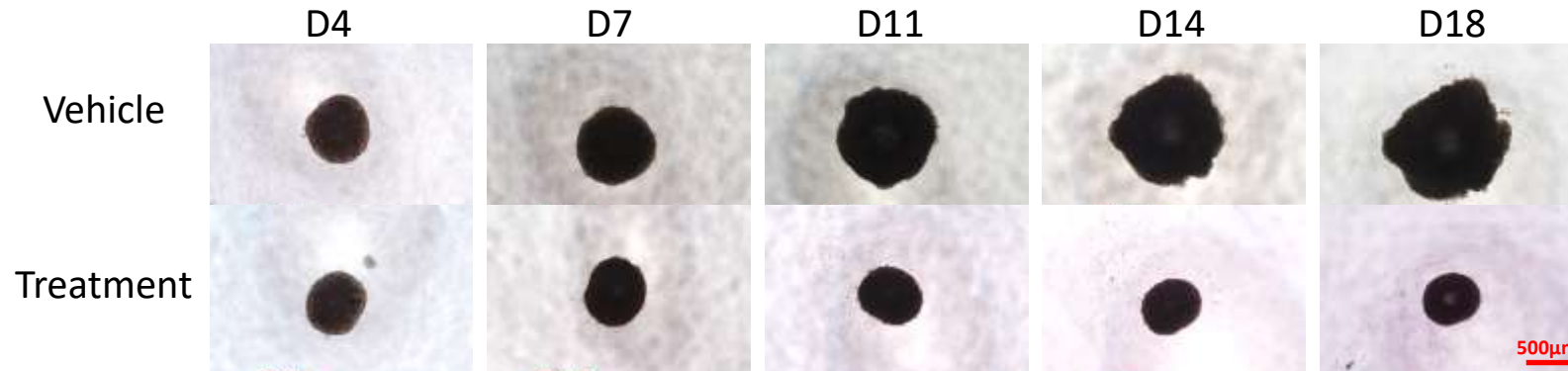


Merci pour votre attention

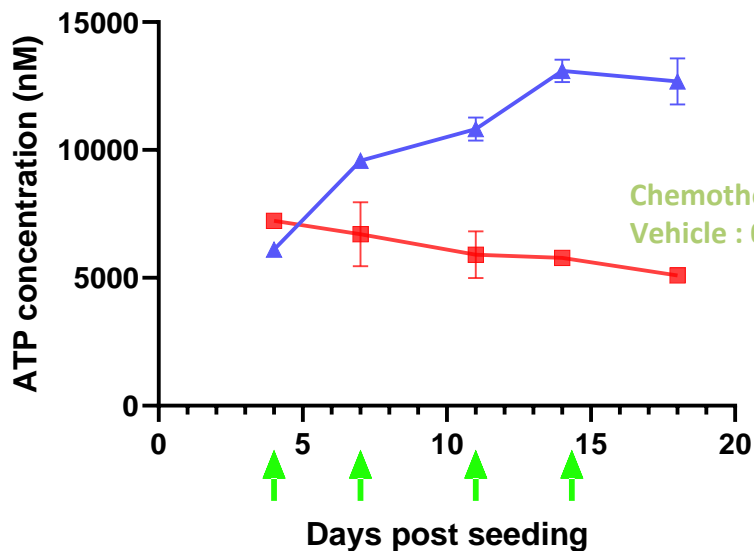


Study of persistence and its impact on virotherapy efficacy on A549 tumoroids

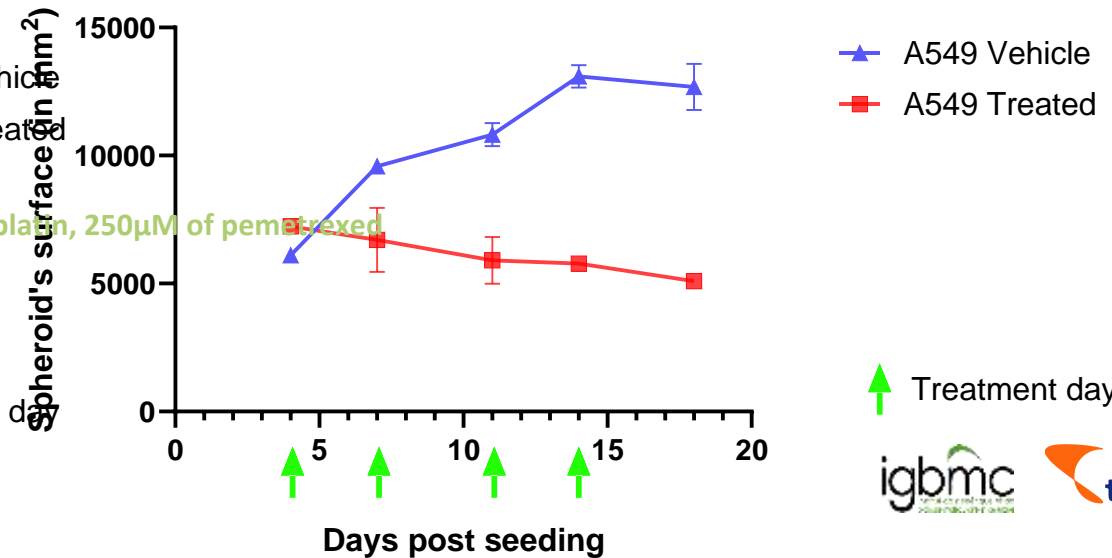
Results



Spheroid ATP content evolution



Spheroid surface evolution

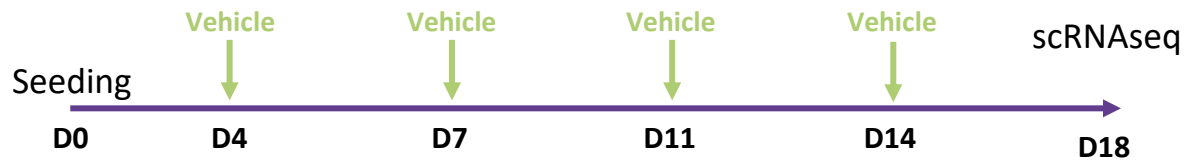
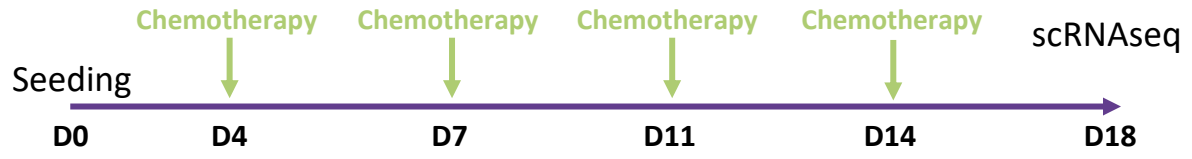


Chemotherapy : 10µM of cisplatin, 250µM of pemetrexed
 Vehicle : 0,9% NaCl solution

↑ Treatment day

↑ Treatment day

● Study of persistence and its impact on virotherapy efficacy on A549 tumoroids



Chemotherapy : 10 μ M of cisplatin, 250 μ M of pemetrexed
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