



Response of cancer-associated fibroblasts (CAF) to targeted radionuclide therapy (TRT) of pancreatic cancer microenvironment

Laura ORDAS – 2nd year PhD Student

Director: Jean-Pierre Pouget – **Co-director:** Sophie Poty

JP Pouget's team - Radiobiology for Targeted and Personalized Radiotherapy

March, 16th 2021 – Workshop RIV - Montpellier

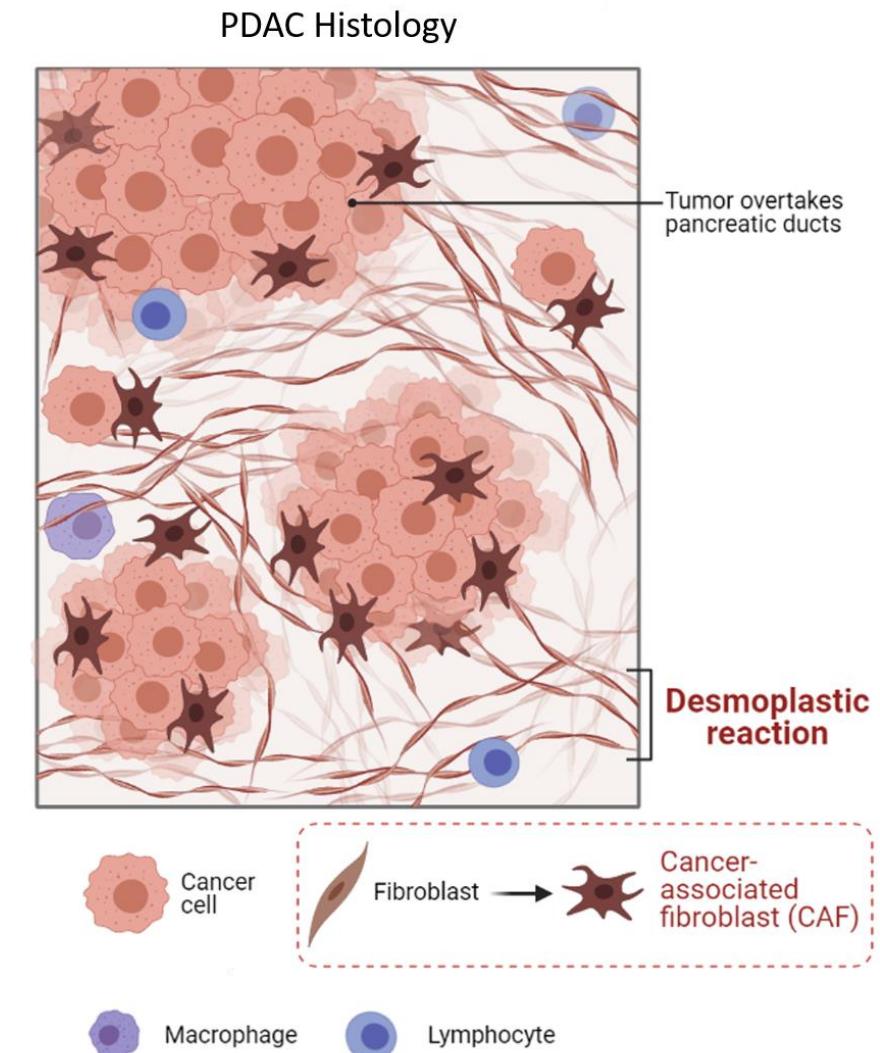
Pancreatic Cancer: Pancreatic ductal adenocarcinoma PDAC

- One of the most lethal cancer (4th leading cause of cancer-associated mortality)
- 5-year survival for patients is around 10%
- Late detection / asymptomatic cancer (metastasis)

Treatments available:

- Surgical resection of the tumors (< 20% of the patients)
- Chemotherapies: **FOLFIRINOX** (fluorouracil (FU) + leucovorin (LV) + irinotecan + oxaliplatin) Or **Gemcitabine**.
- Conventional Radiotherapy

PDAC = High stroma compartment (up to 80-90%)
→ Main cause of treatment failure

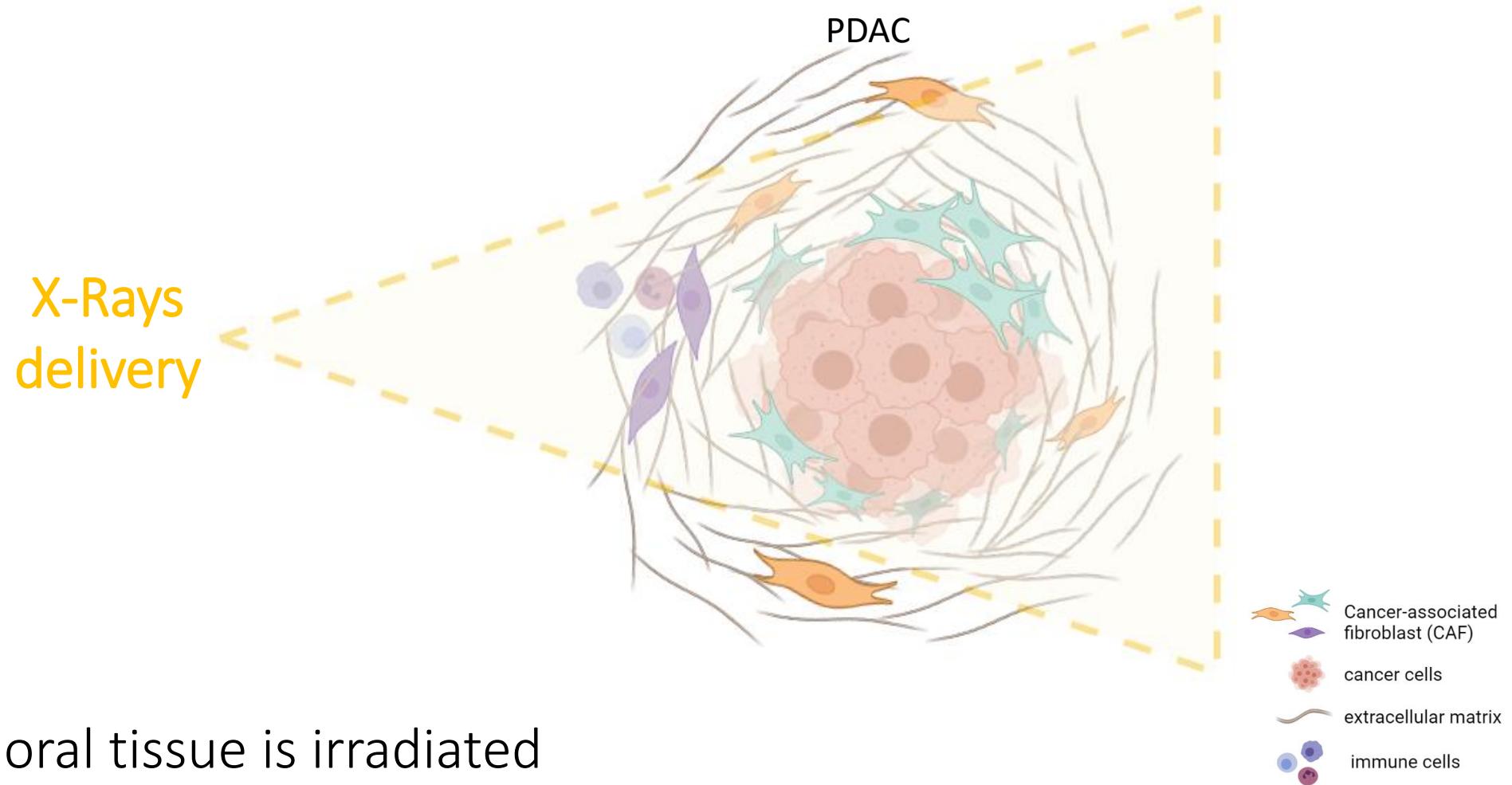


Targeting the pancreatic tumor microenvironment?

CAFs response to Conventional Radiotherapy (X-RT)



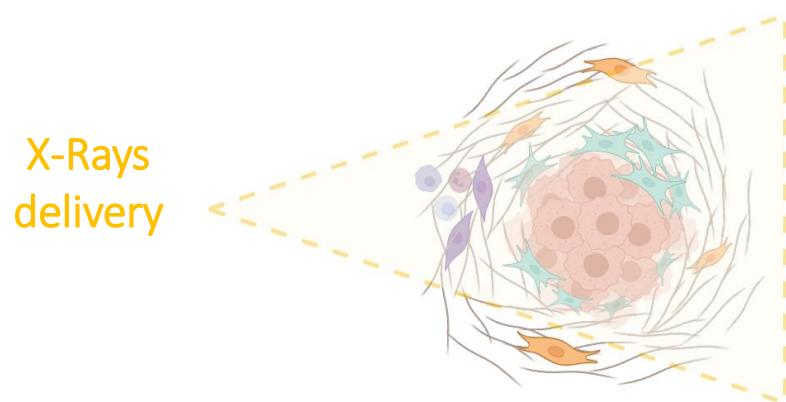
X-Rays
delivery



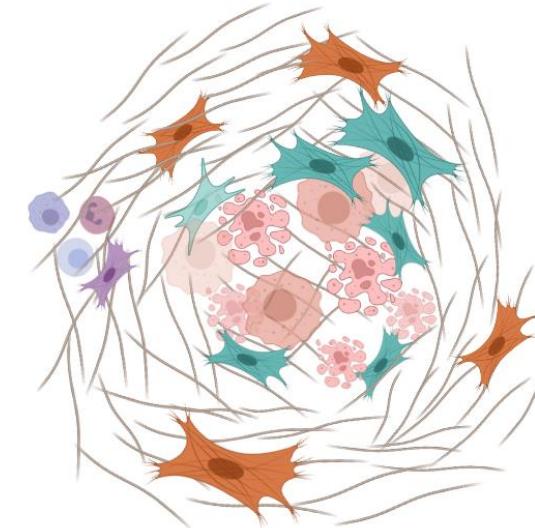
- Whole tumoral tissue is irradiated

CAFs response to Conventional Radiotherapy (X-RT): Senescence

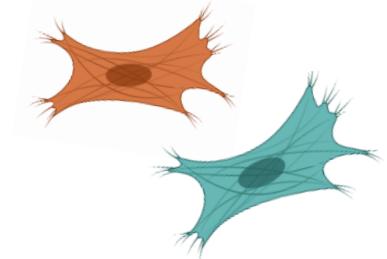
X-RT



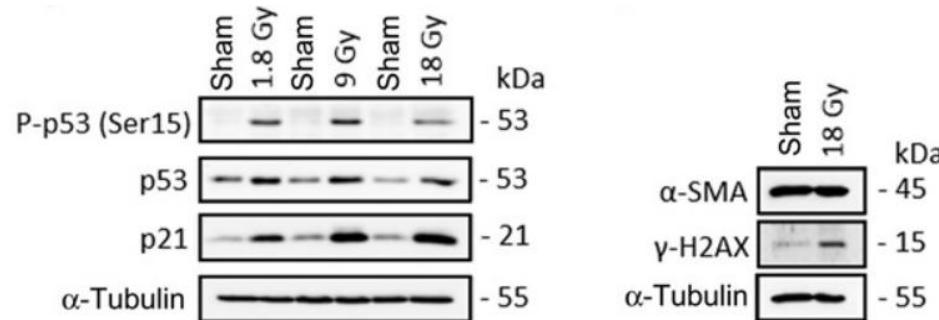
After X-RT



Senescent CAF

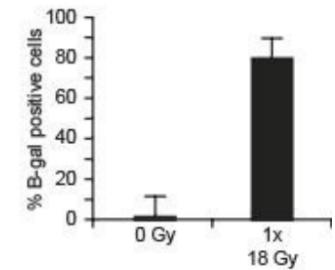
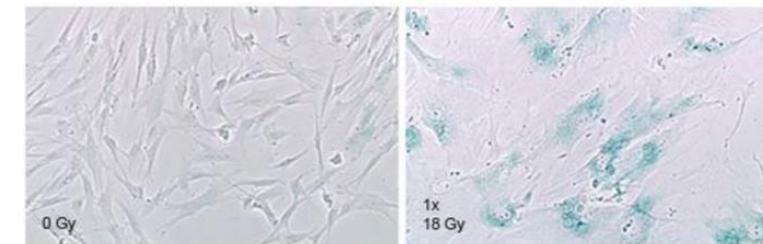


- Irradiation activates DDR signaling pathway



Tommelein. J et al., Cancer Res 2017

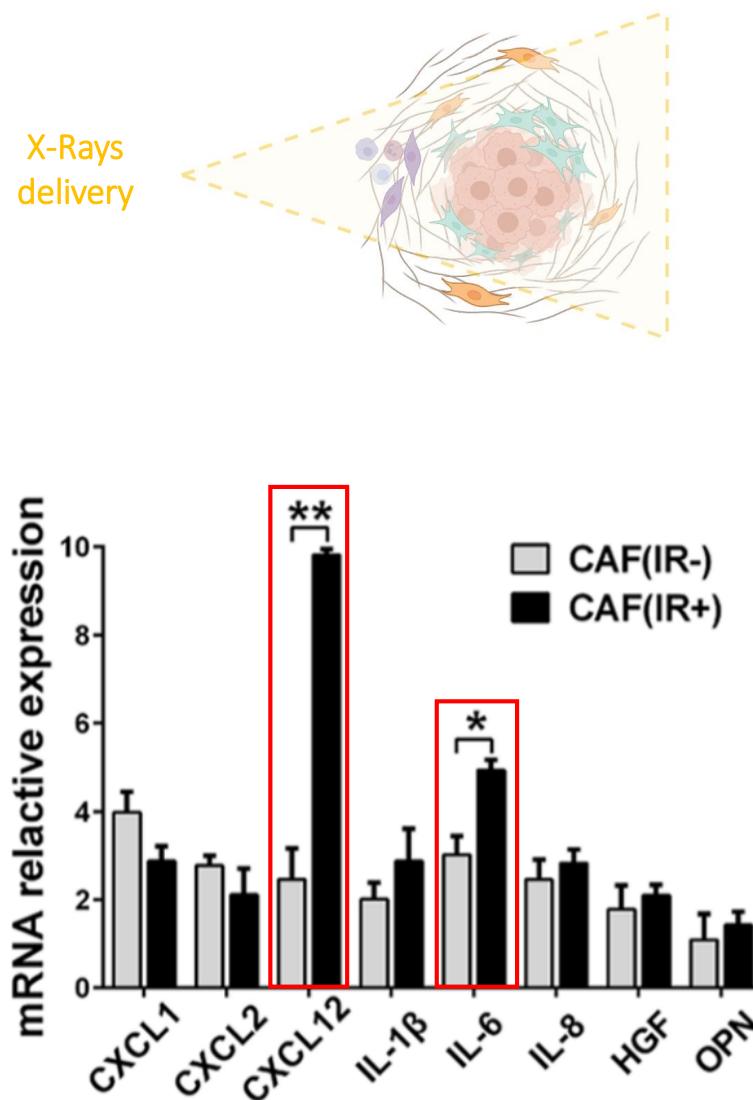
- Irradiation increases SA- β -Galactosidase activity



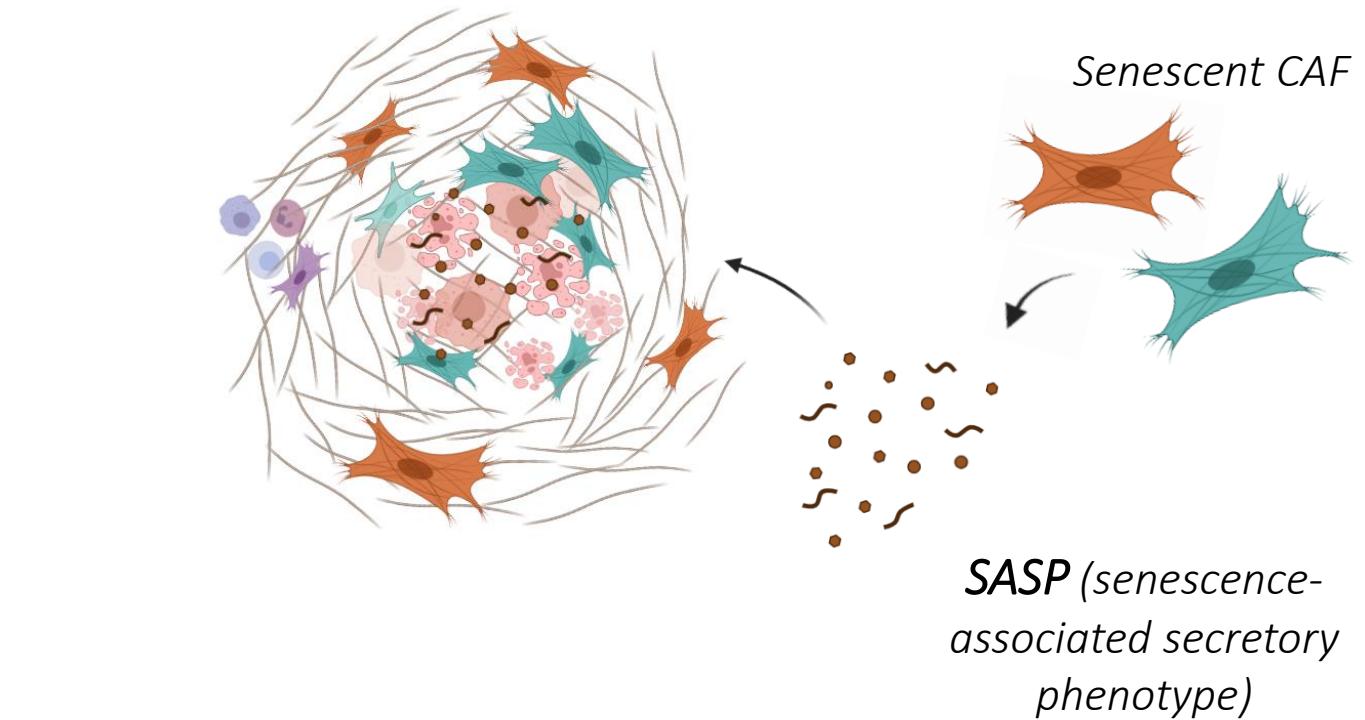
Hellevik et al. Radiation Oncology 2012

CAFs response to Conventional Radiotherapy (X-RT): SASP

Before X-RT



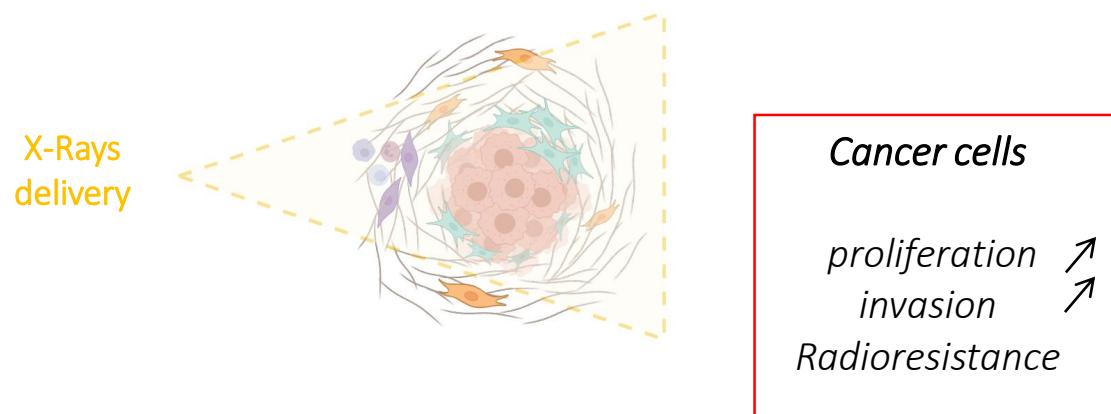
After X-RT



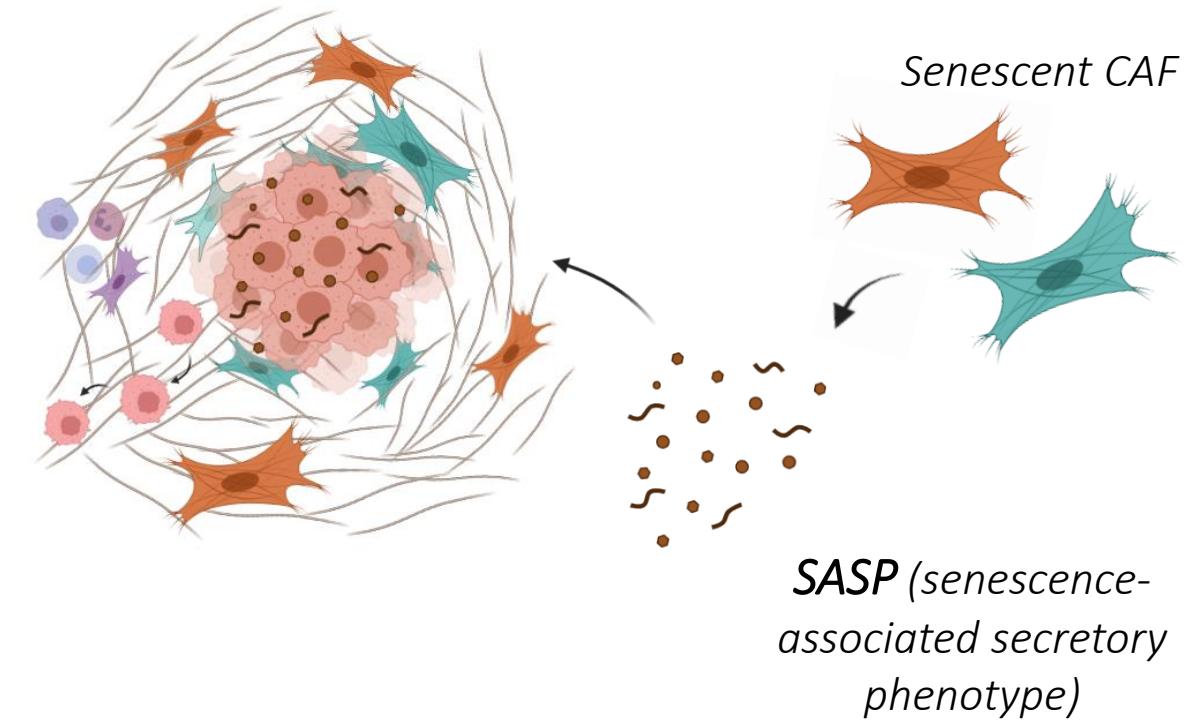
SASP: composed of cytokines, chemokines, proteins and components of extracellular matrix

CAFs response to Conventional Radiotherapy (X-RT): SASP

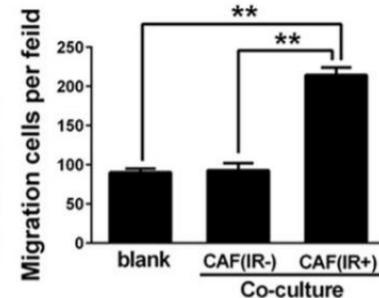
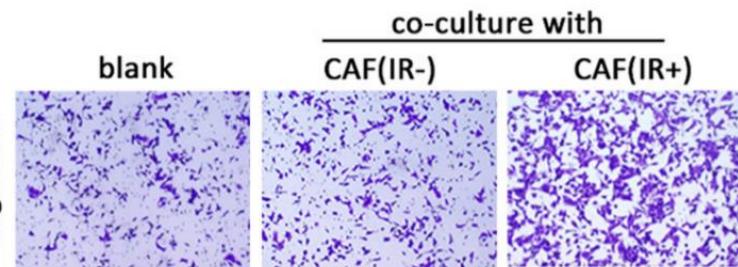
Before X-RT



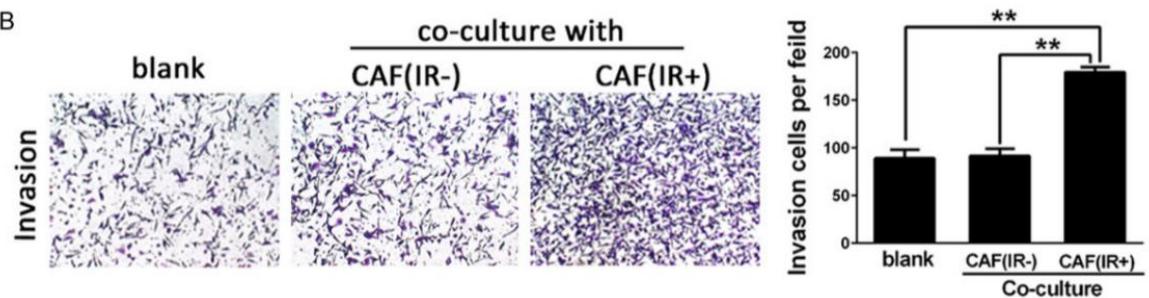
After X-RT



A

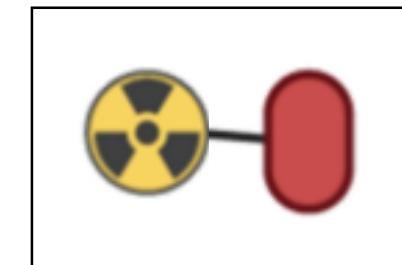
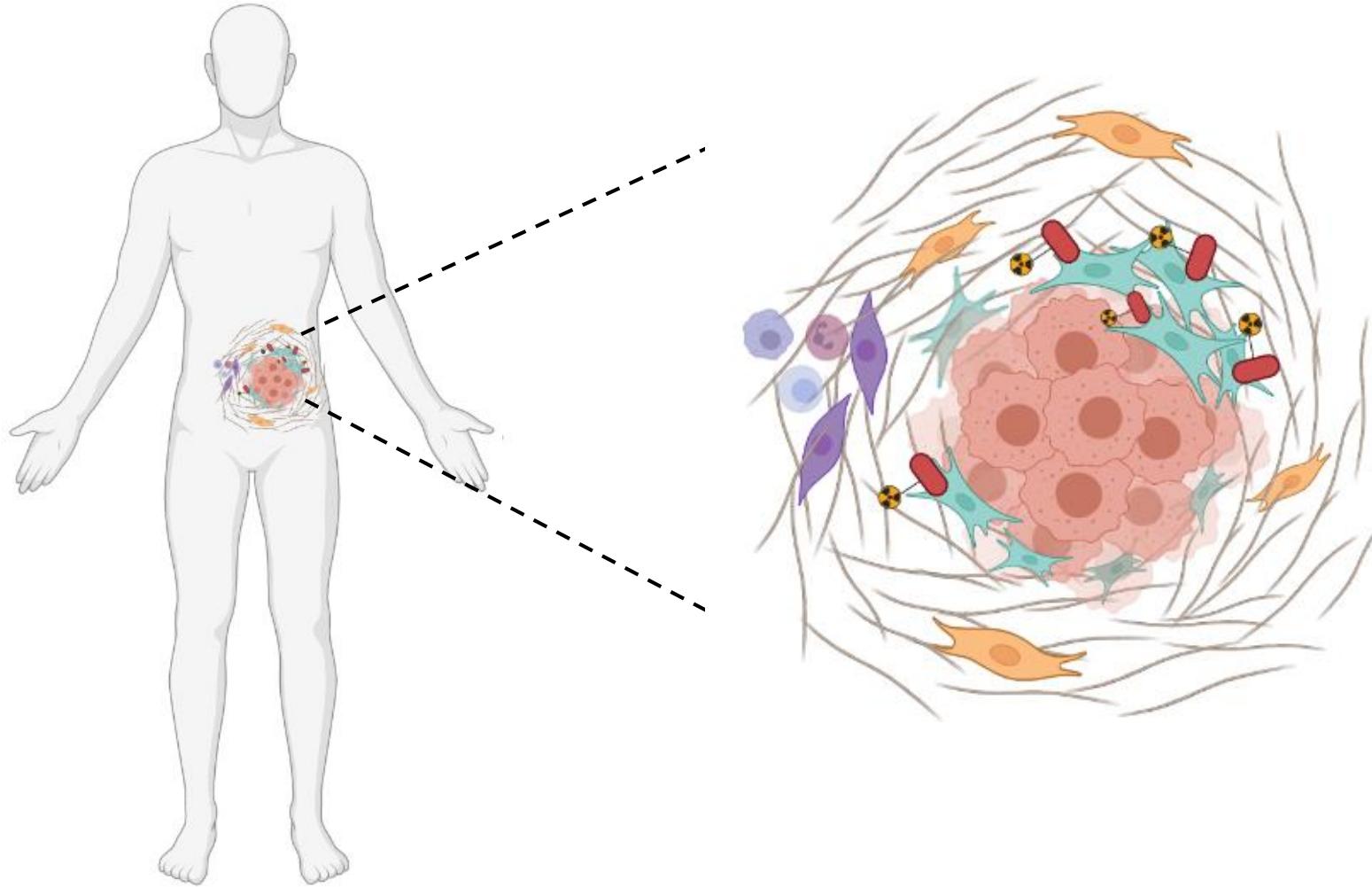


B



Targeted Radionuclide Therapy (TRT) vs Conventional Radiotherapy (X-RT)

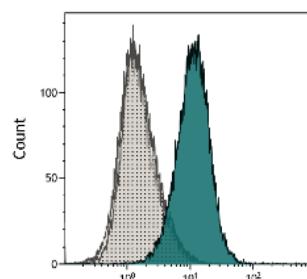
Objective: Evaluating CAFs response to TRT



Method:

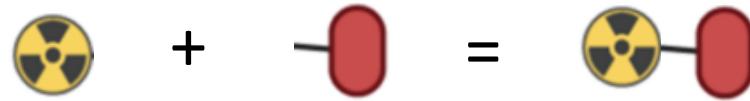
iCAF136 give by C. Bousquet (CRCT Toulouse) / C. Larbouret (IRCM)

Immortalized PDAC CAF: *iCAF136 cells*



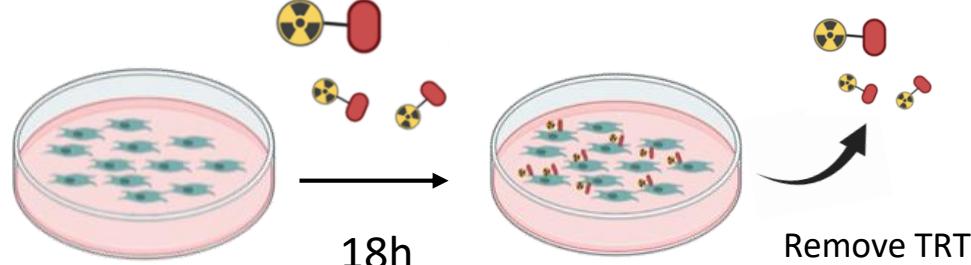
TRT

Radiolabelling : ^{177}Lu Antibody fragment **^{177}Lu anti-CAF Ab**



Treatment: Different volumic activities:

- Untreated
- 2 MBq/mL
- 4 MBq/mL
- 8 MBq/mL

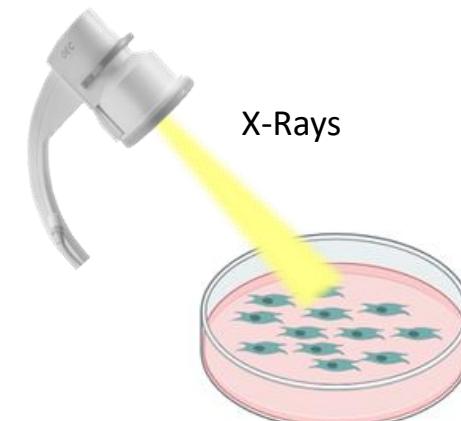


X-RT

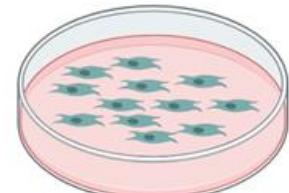
Tiphany Gouveia
Laura Bourillon

Treatment:

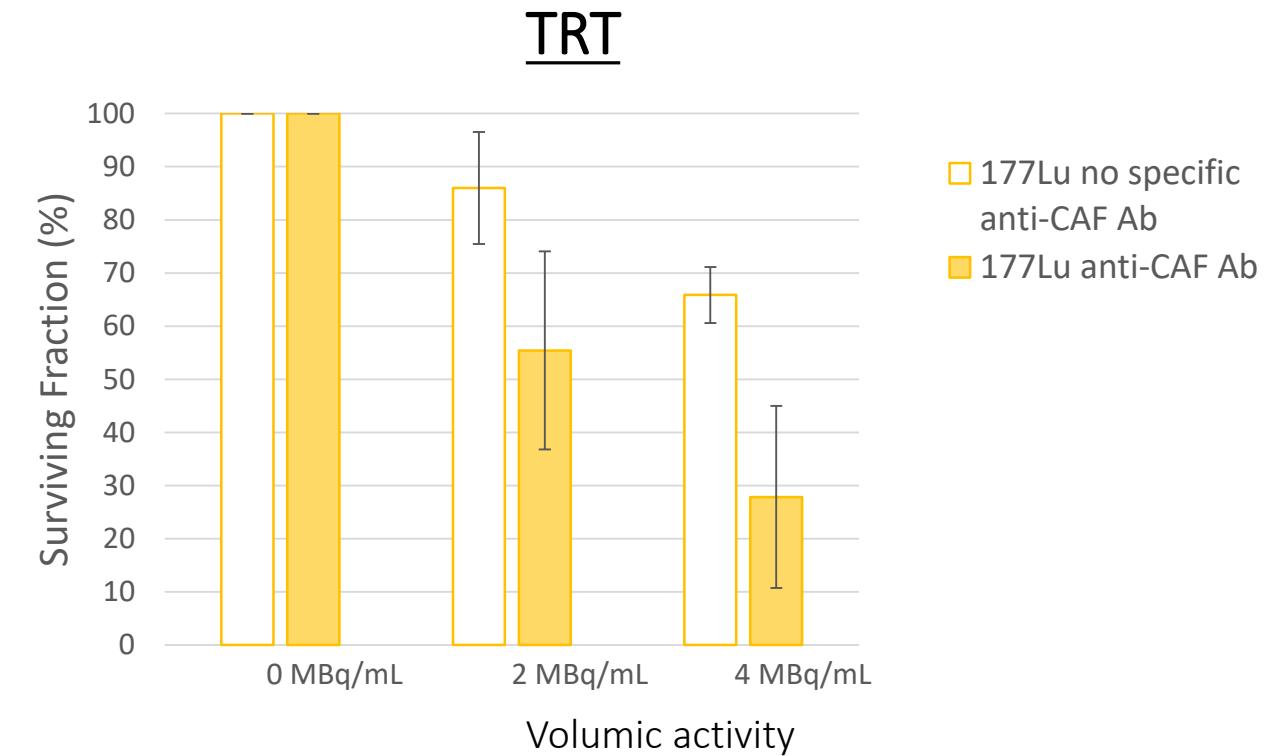
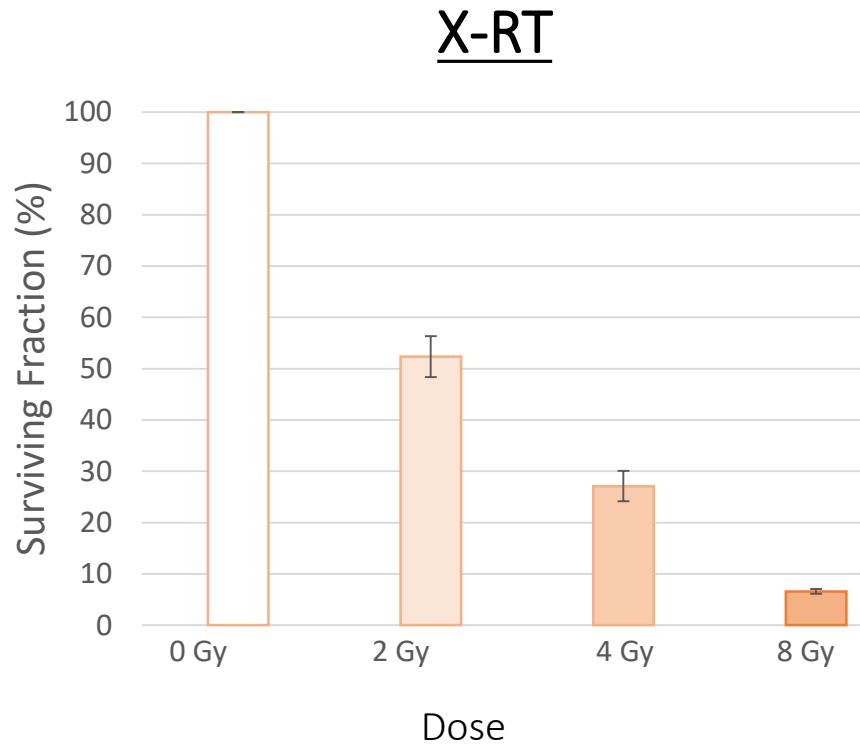
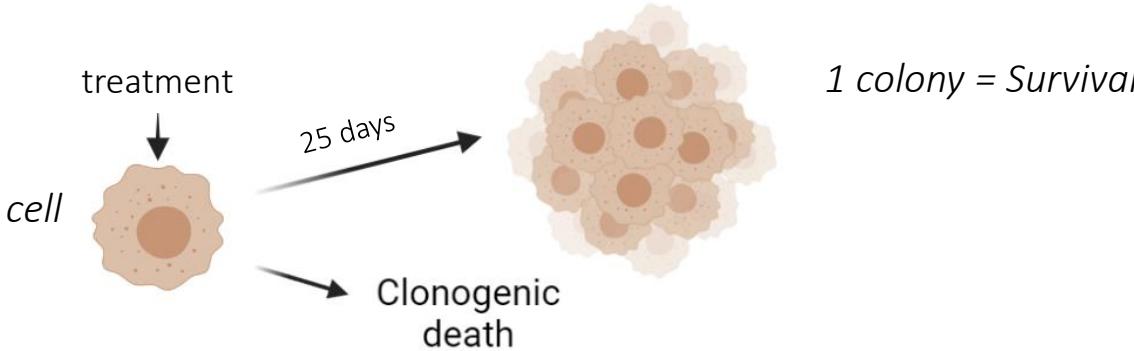
Irradiator XenX



Dose Delivery:
Untreated
2 Gy
8 Gy

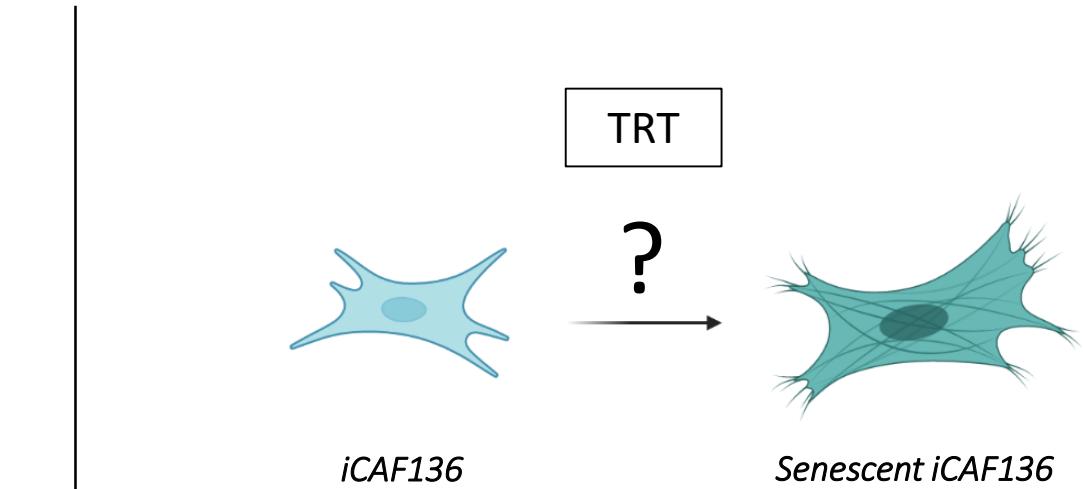
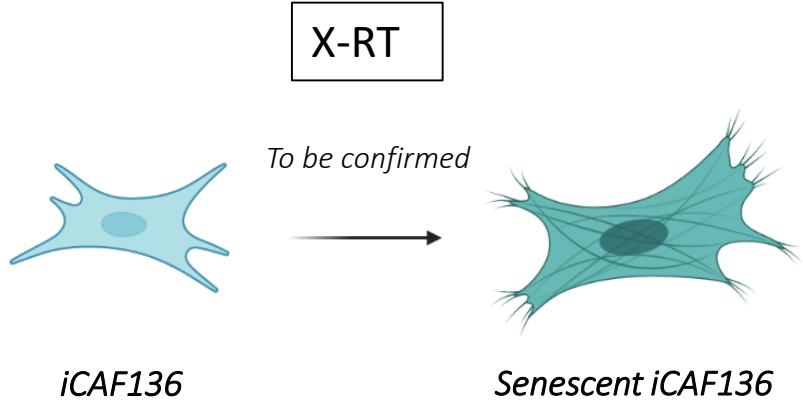
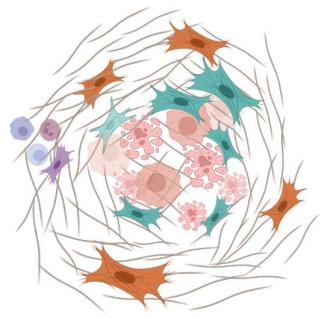


Does TRT induce cytotoxicity ? Clonogenic assay



→ iCAF136 cells respond to X-RT & to TRT

- 177Lu no specific anti-CAF Ab
- 177Lu anti-CAF Ab



Hallmarks of Senescence:

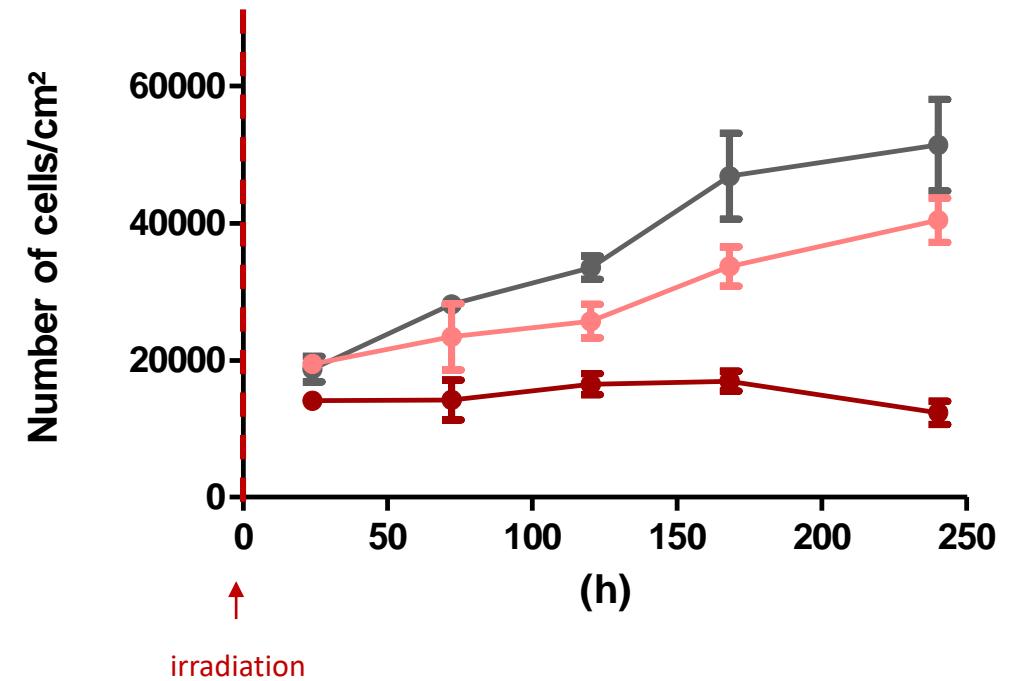
- Proliferation
- Cell cycle progression
- Persistent DNA Damage (foci γH2AX)
- SA-β-Galactosidase activity
- Lamin B1 expression

Hallmarks of Senescence:

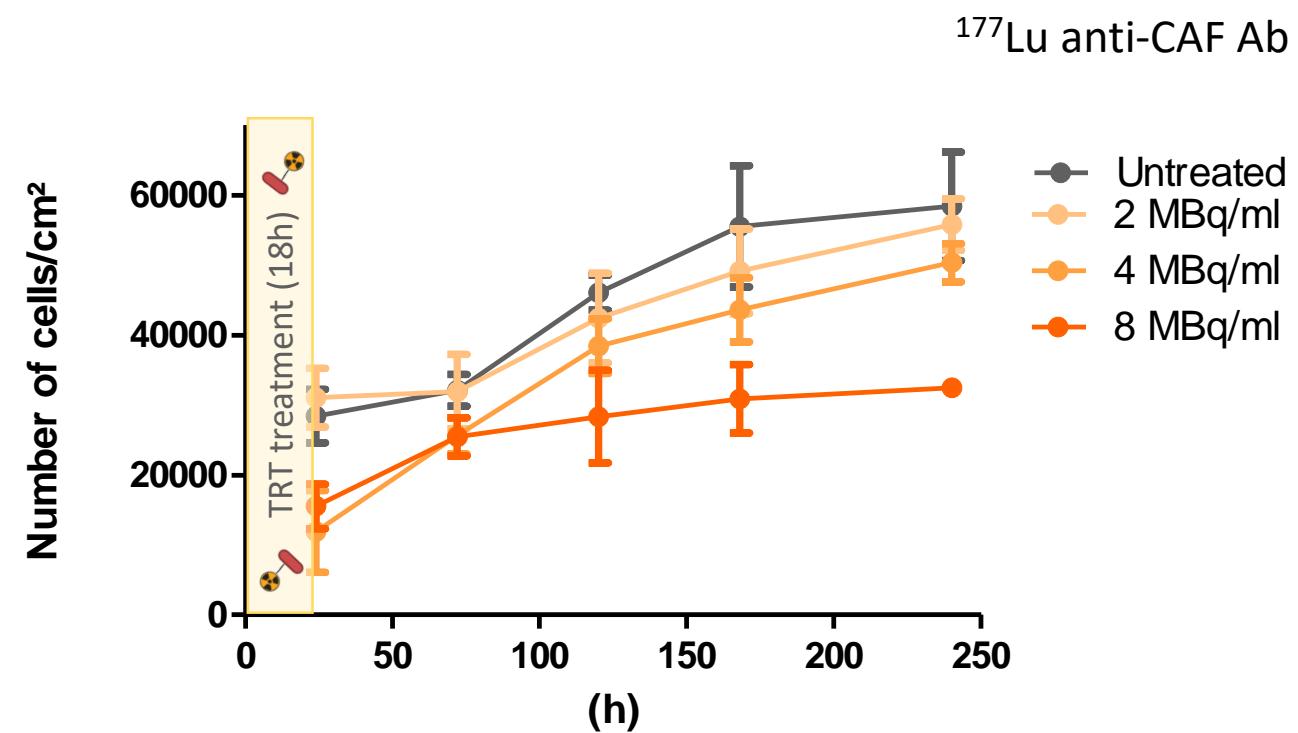
- Proliferation
- Cell cycle progression
- Persistent DNA Damage (foci γH2AX)
- SA-β-Galactosidase activity
- Lamin B1 expression

CAF proliferation after exposure to X-RT or TRT

X-RT

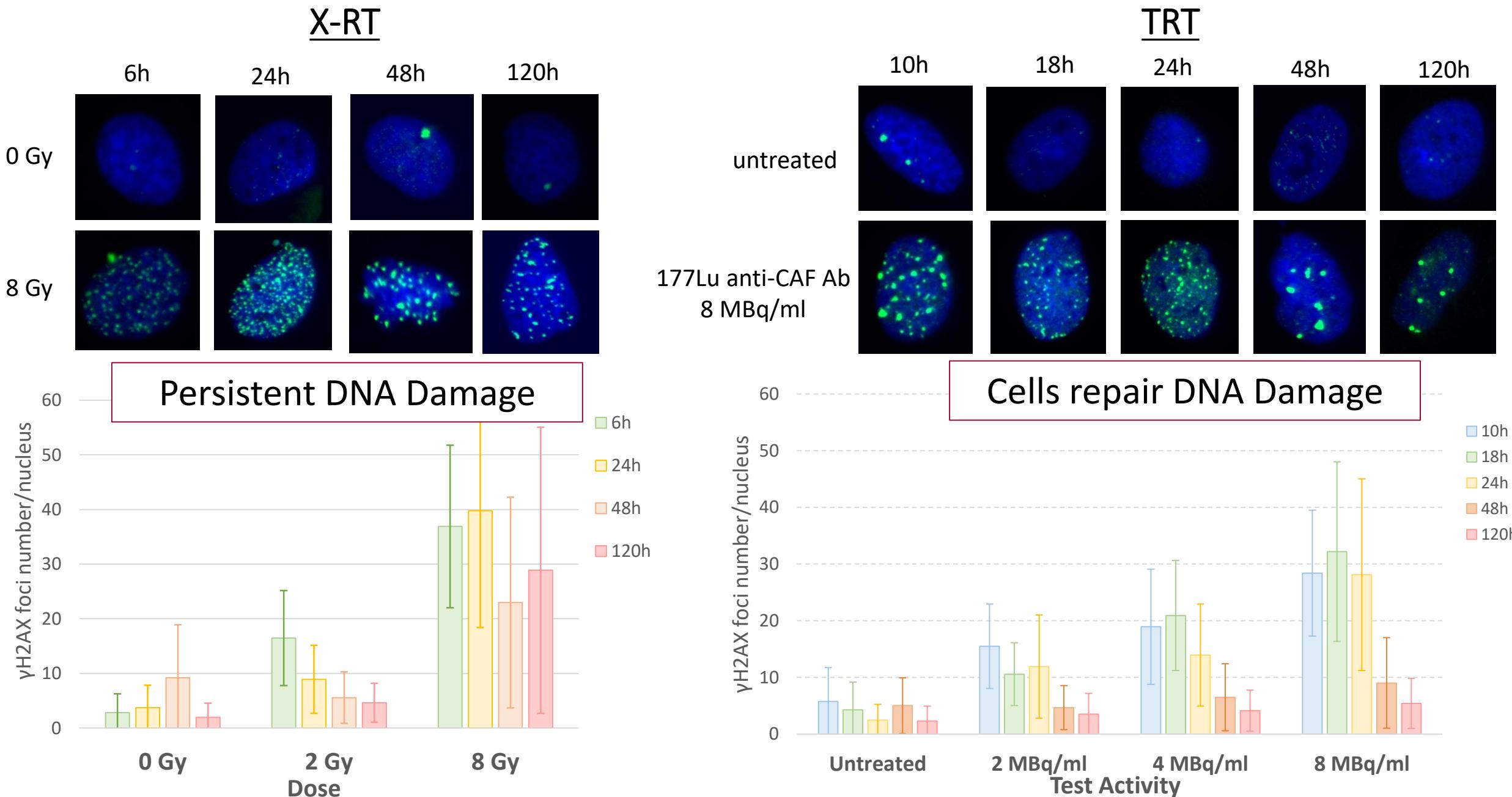


TRT



→ X-RT but not TRT stops iCAF136 cells proliferation

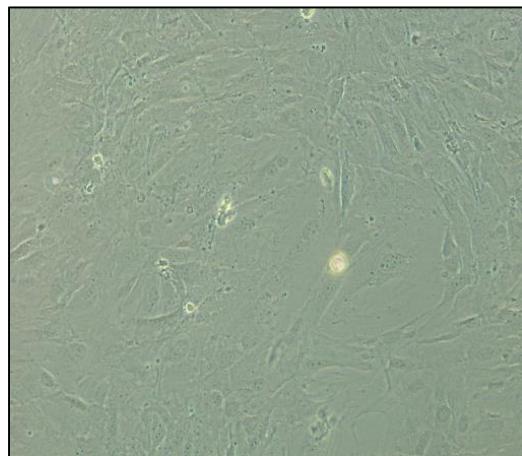
Persistent DNA Damage are observed after X-rays but not TRT



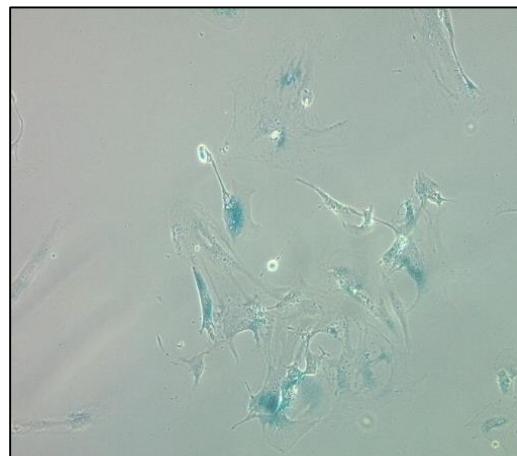
SA- β -Galactosidase activity is observed after X-RT but not TRT

Positive blue cell = senescent cell

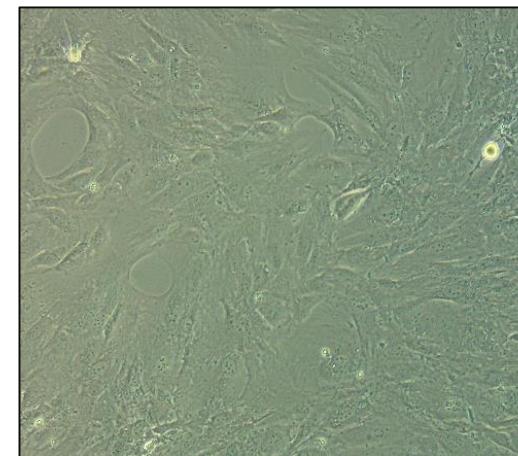
Untreated



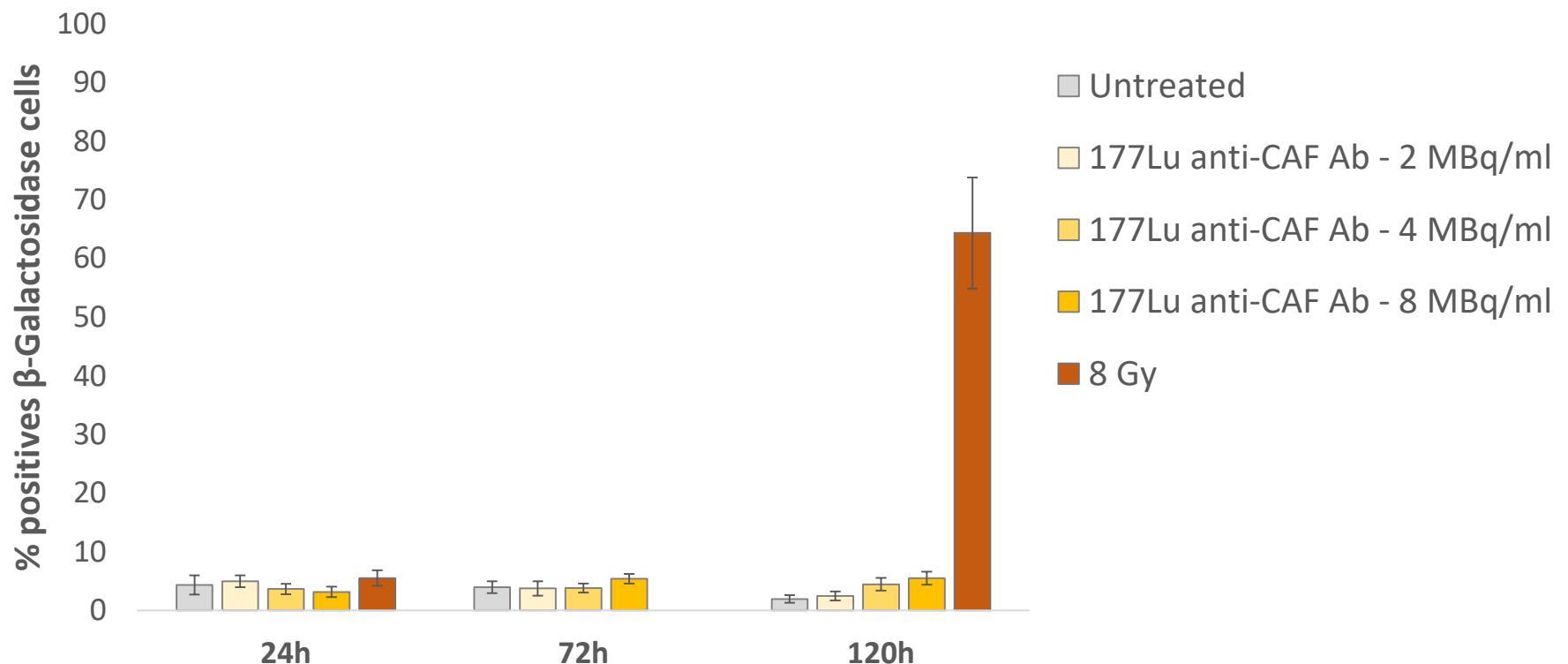
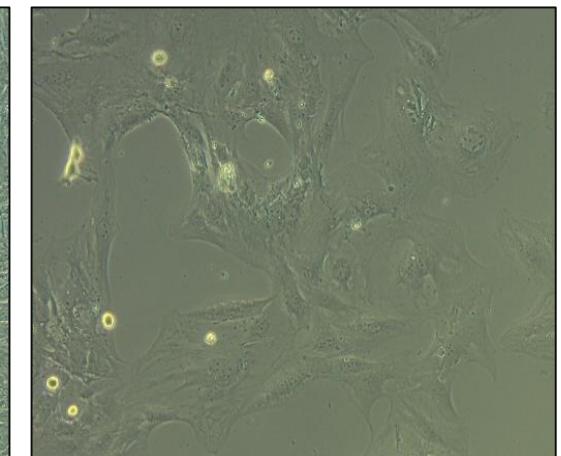
8 Gy



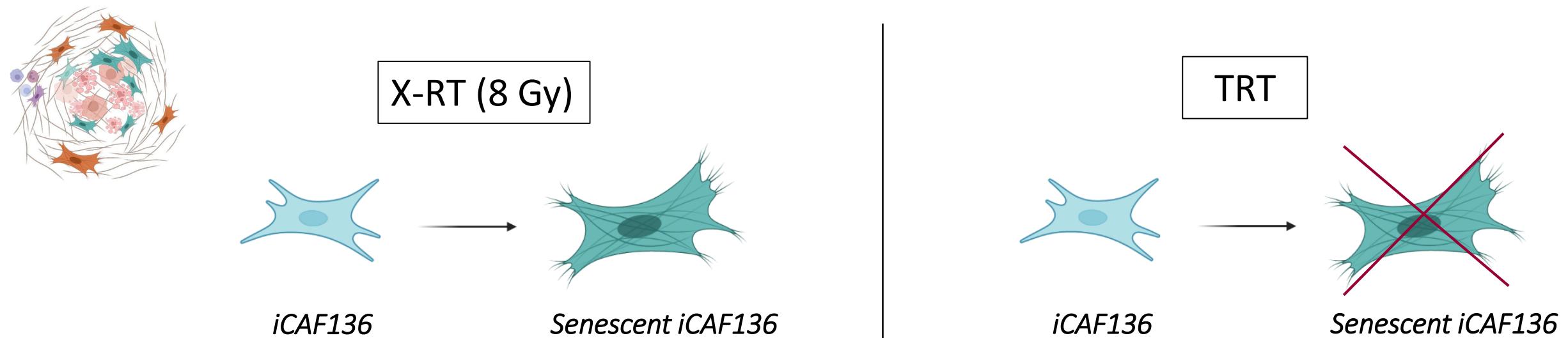
Untreated



^{177}Lu anti-CAF Ab – 8MBq/ml



To sum up



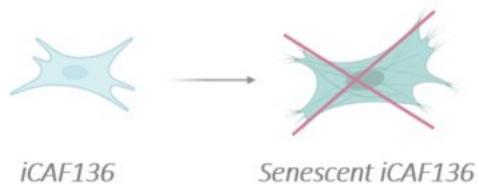
Hallmarks of Senescence:

- Impaired Proliferation
- Cell cycle arrest
- Persistent DNA Damage (foci γH2AX)
- SA-β-Galactosidase activity
- Lamin B1 expression decreased

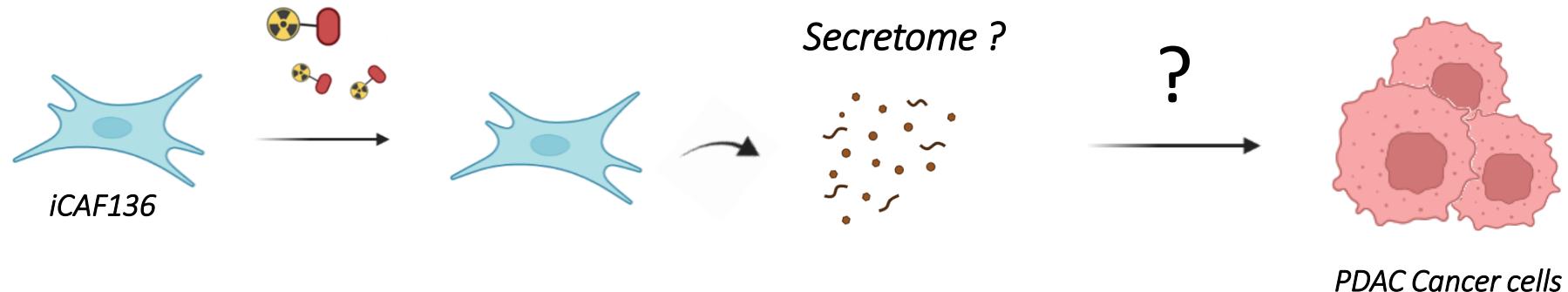
Hallmarks of Senescence:

- Proliferation is slowing down
- Cell cycle is transiently arrested
- DNA Damage are repaired
- No SA-β-Galactosidase activity
- No decrease in Lamin B1 expression

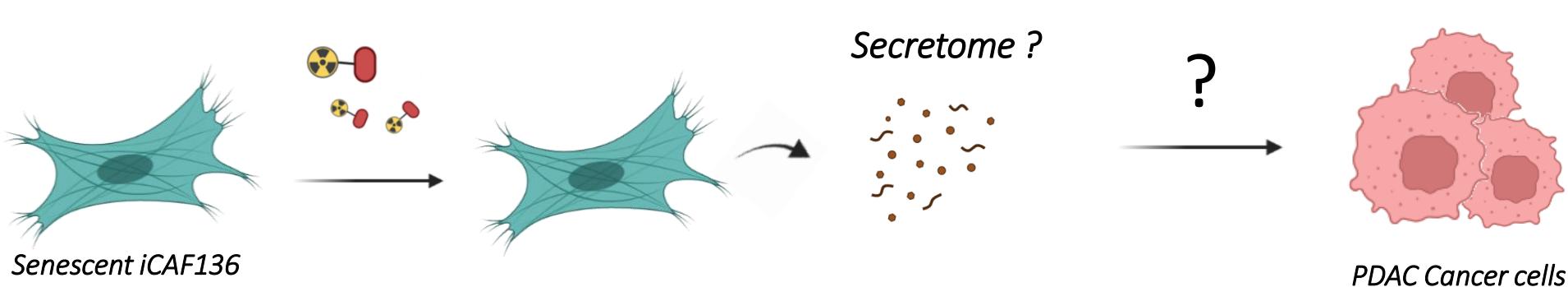
Bystander effects ?

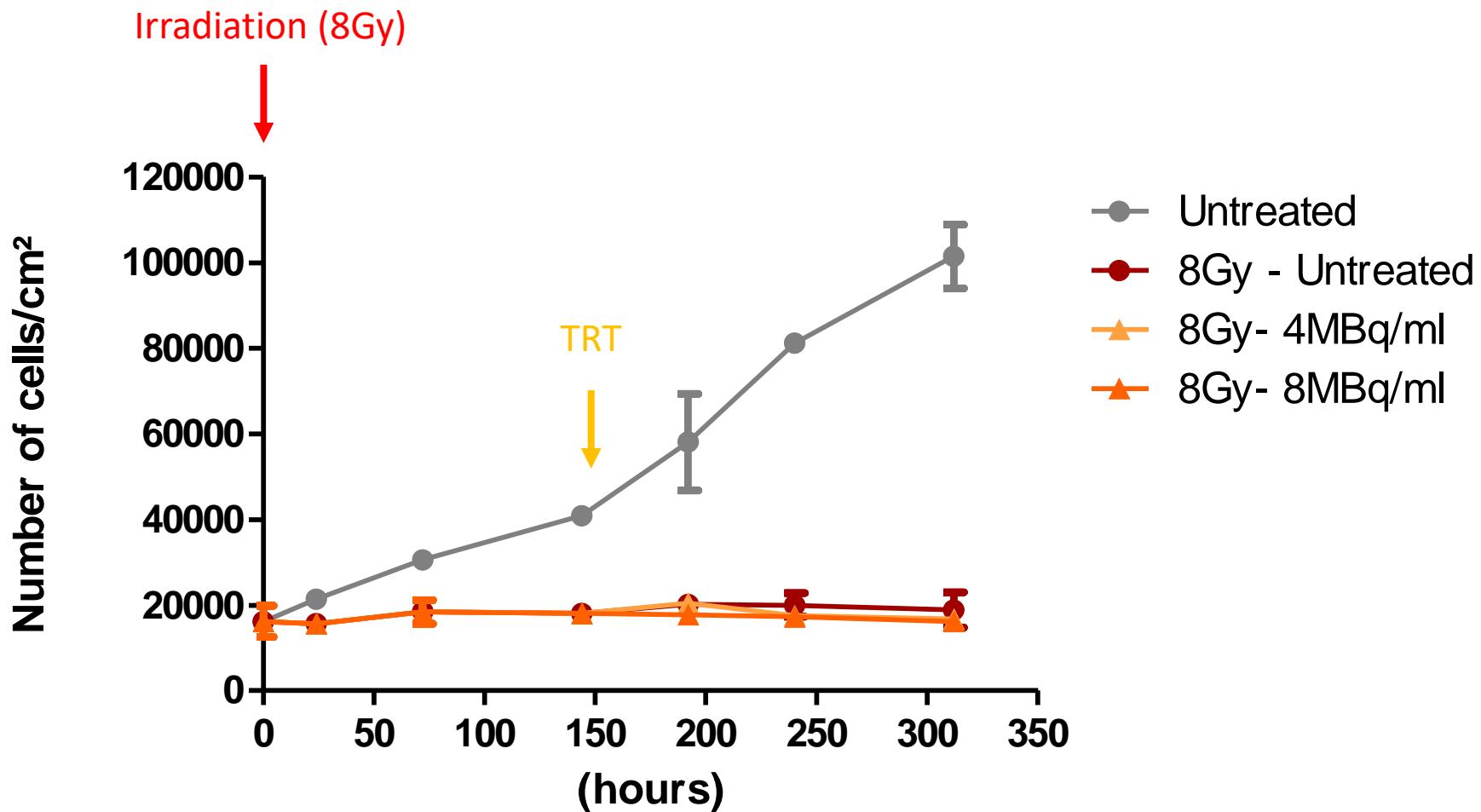
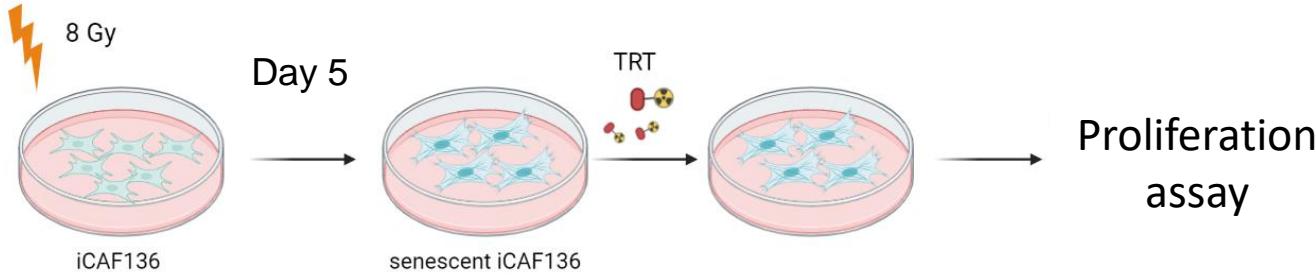


- 1st line of treatment :

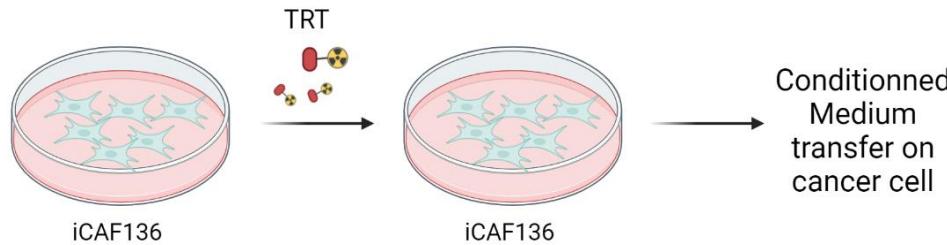


- 2nd or 3th line of treatment :

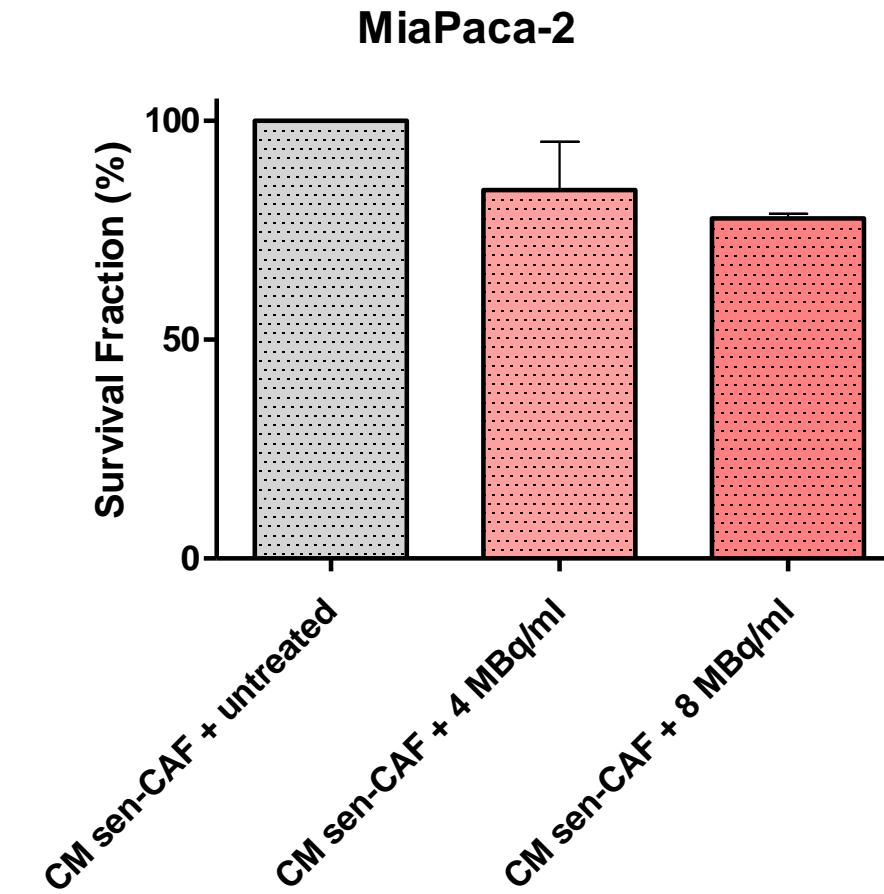
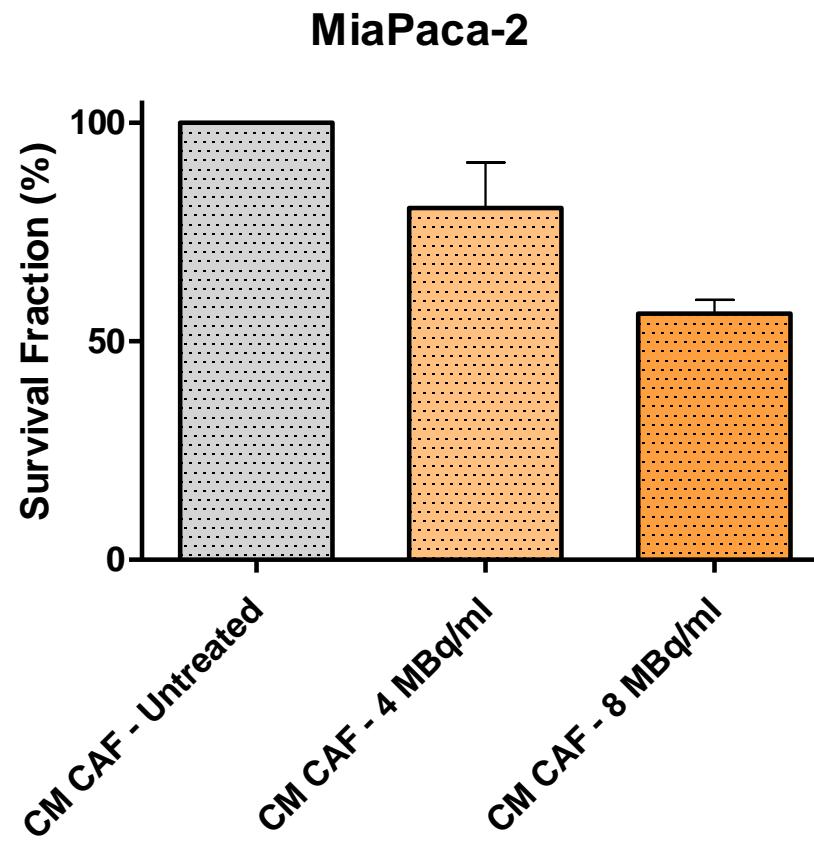
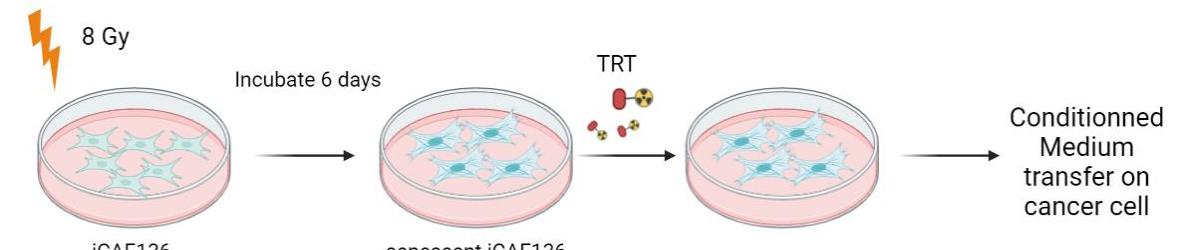




- 1st line of treatment :



- 2nd or 3rd line of treatment :





Institut de Recherche en Cancérologie de Montpellier

TEAM

“Radiobiology for Targeted and Personalized Radiotherapy”

Jean-Pierre POUGET
Sophie POTY
David AZRIA
Manuel BARDIES
Malick BIO IDRISOU
Vincent BOUDOUSQ
Julie CONSTANZO
Emmanuel DESHAYES
Clara DÍAZ GARCÍA-PRADA
Julien DUBOIS
Jihad KARAM
Pierre-Olivier KOTZKI
Marion LARROQUE
Laura ORDAS
Gaël OURDANE
Ali PARACH
Marion TARDIEU
Soumaya TURPAULT

Plateforme Irradiation Expérimentale

Muriel BRENGUES
Laura BOURILLON
Tiphany GOUVEIA

Centre de Recherche en Cancérologie de Toulouse

Corinne BOUSQUET

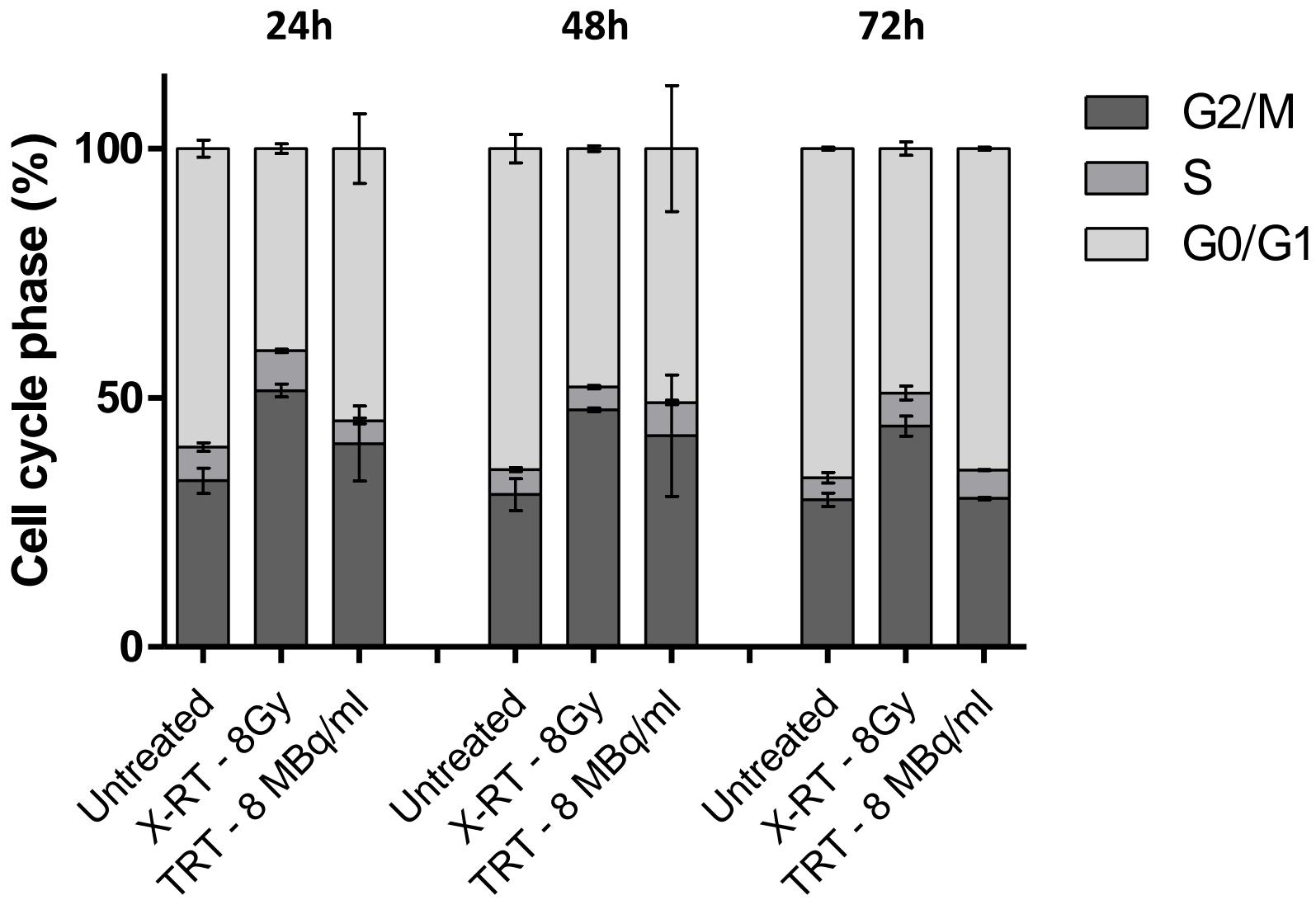
Gongora's team
Christel LARBOURET

ICM

Pierre-Emmanuel Colombo

**Thank You For
Your Attention!**

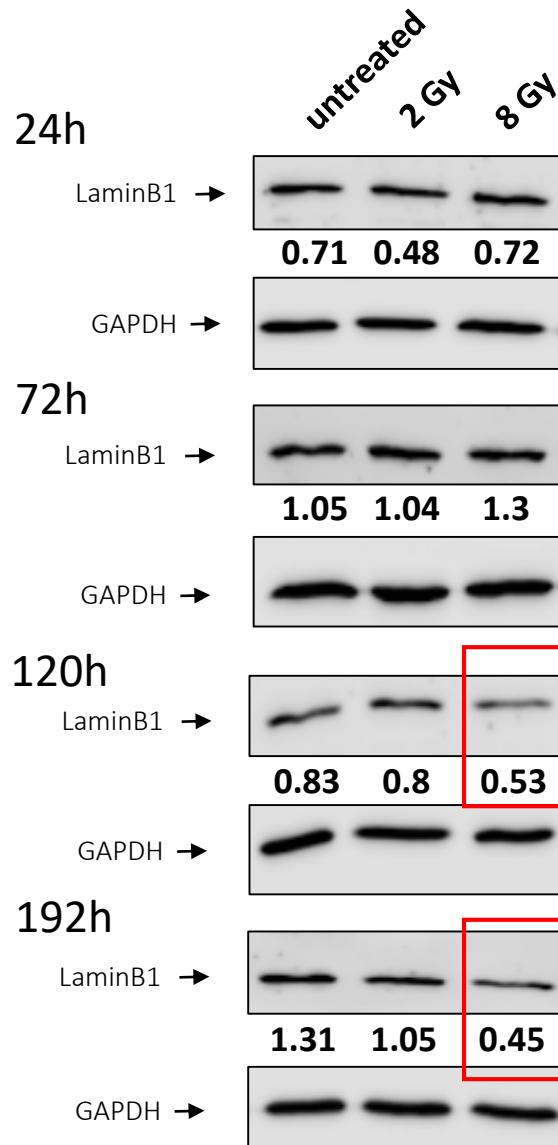
Cell cycle progression on CAF exposed to X-RT or TRT



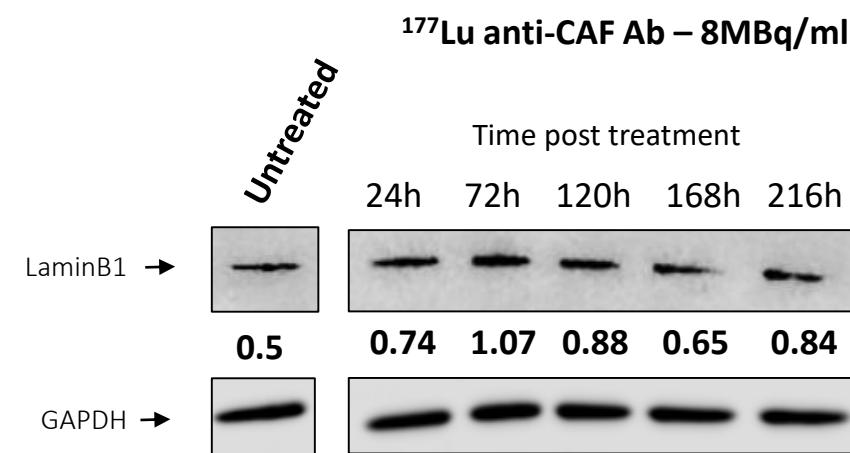
→ After TRT, cells are not blocked in cell cycle

Loss of laminB1 is observed after X-RT but not TRT

X-RT



TRT



From 120h, lamin B1 decreases in cells exposed to X-RT but not in TRT