



DISSECTING THE MIR-x / WILD TYPE P53 / SENESCENCE CROSSTALK TO PREDICT ABSCOPAL EFFECTS IN CANCER PATIENTS UNDERGOING RADIOTHERAPY.

PI IRE

Dr. Gianluca Bossi

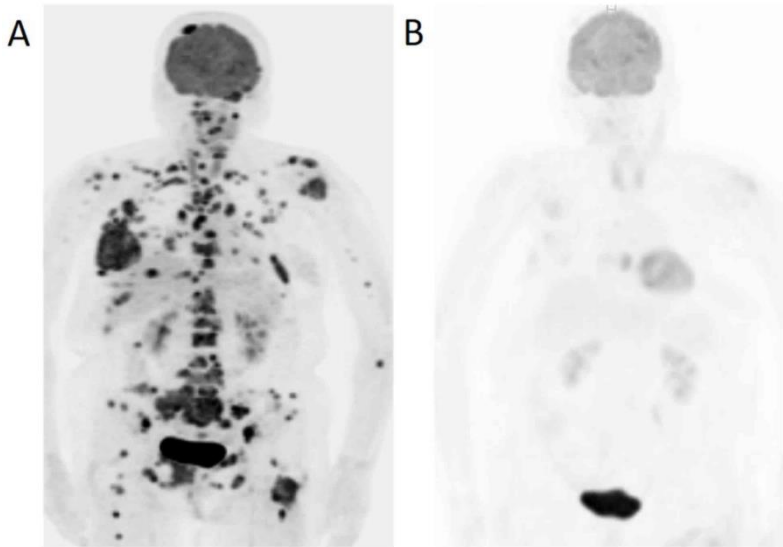
Epigenetic and Oncogenomic Unit

PI WIS (MICC)

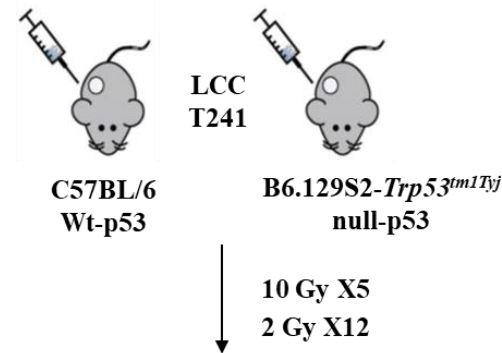
Dr. Valery Krizhanovsky

Department of Molecular Cell Biology

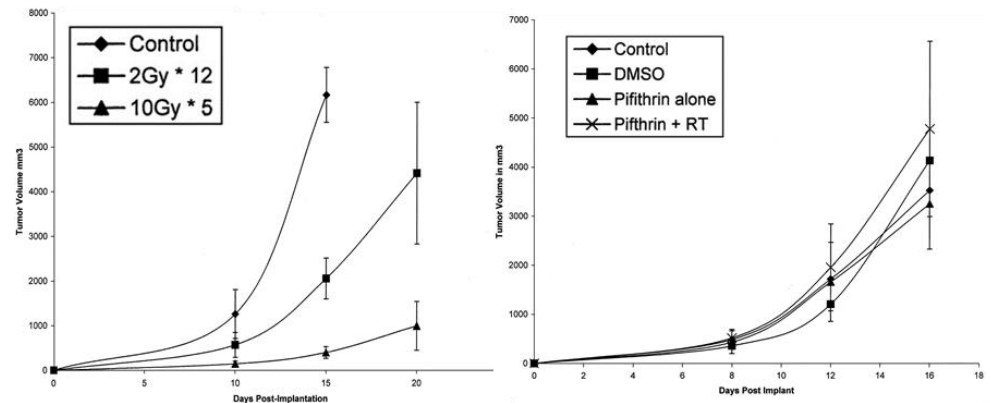
The radiation induced abscopal effect



Radiation abscopal antitumor effect is mediated through p53



Azami A, et al., *Mol Clin Oncol.* 2018 Sep;9(3):283-286. doi: 10.3892/mco.2018.1677.

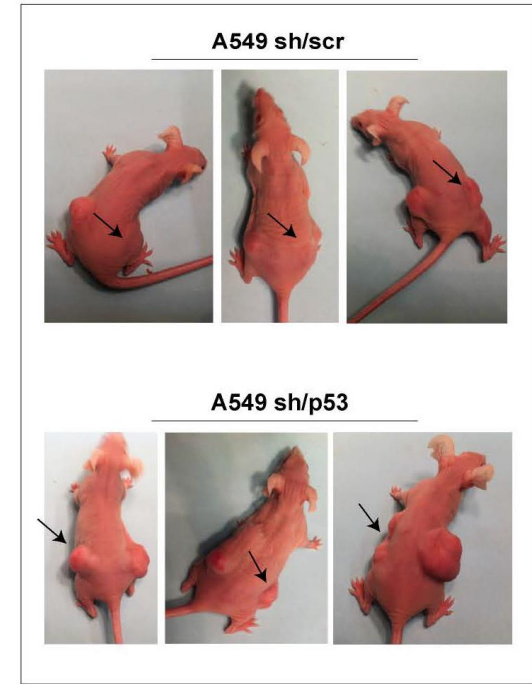
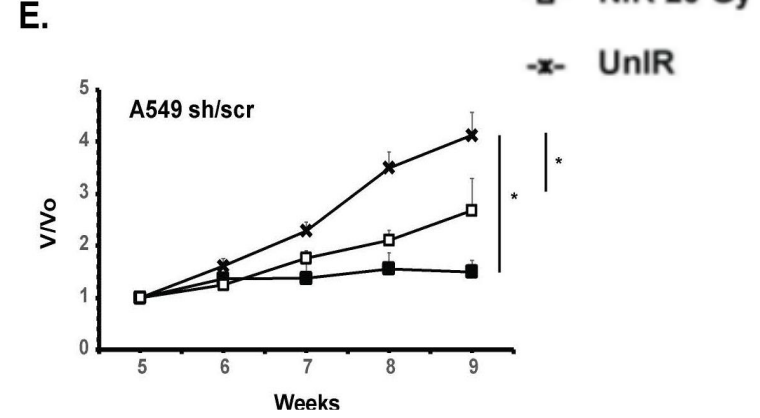
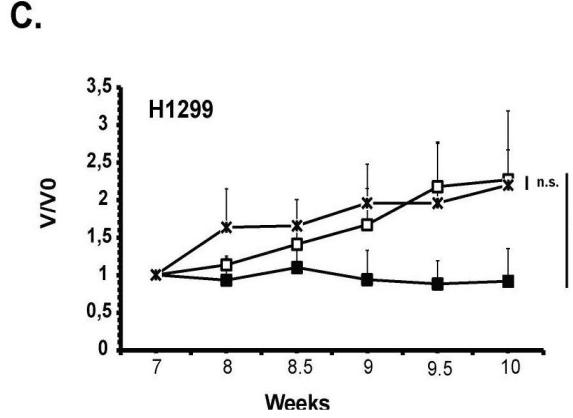
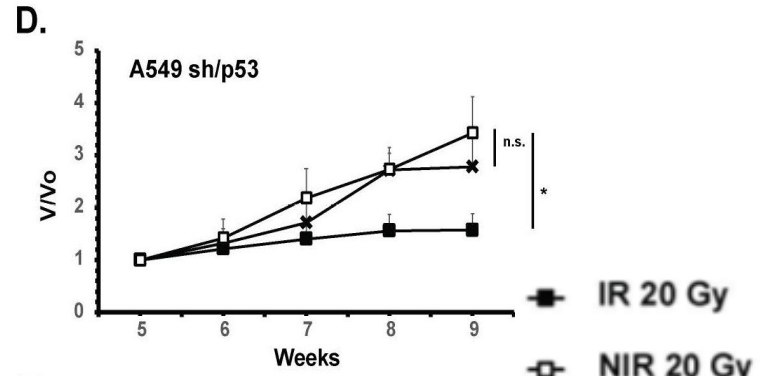
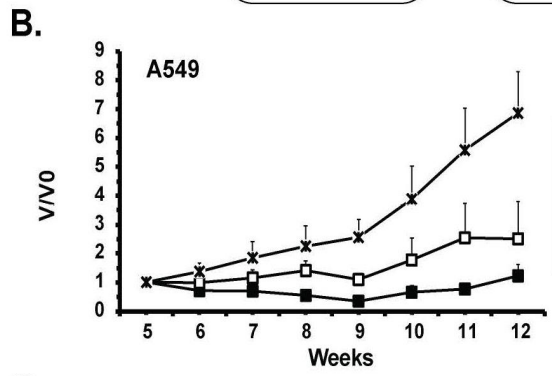
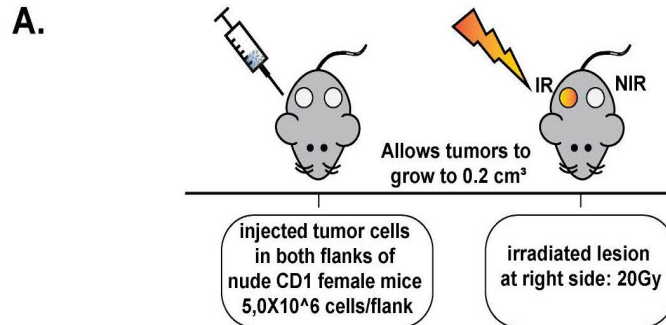


Camphausen K, et al. *Cancer Res.* 2003 Apr 15;63(8):1990-3.

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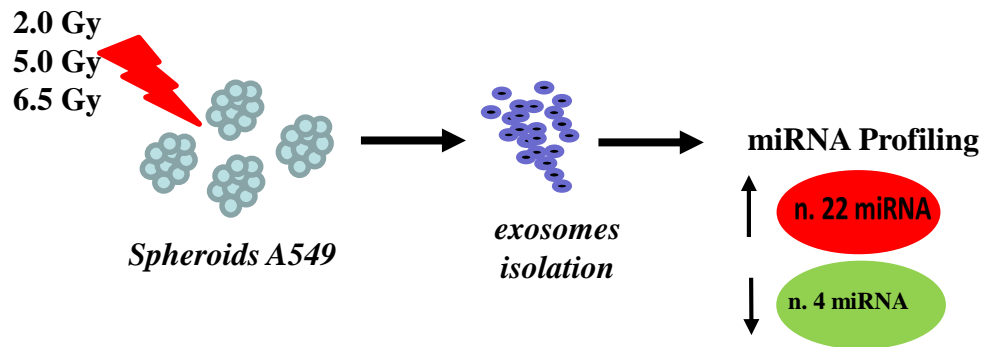


TP53 and high dose radiation are required to trigger abscopal effect in NSCLC tumour models

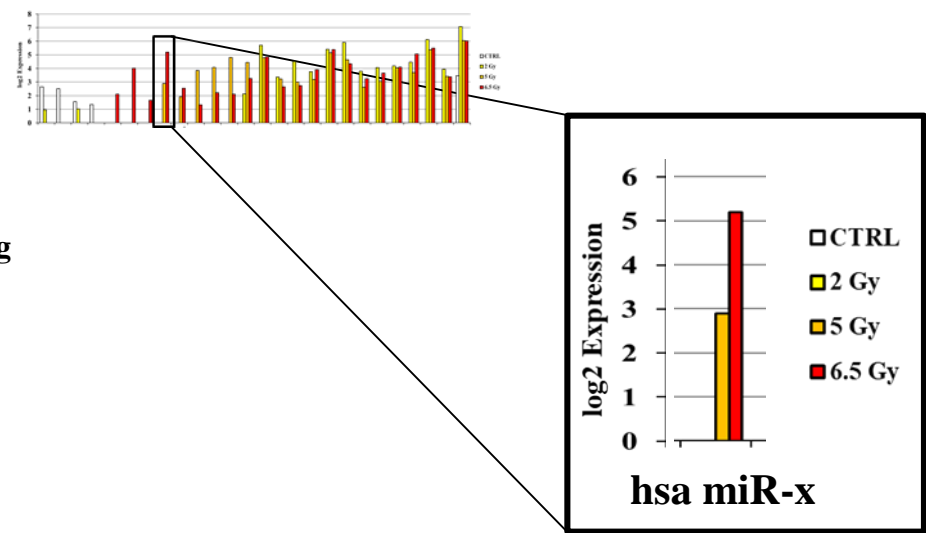


miRNAs might deliver abscopal signals through exosomes: miR-x?

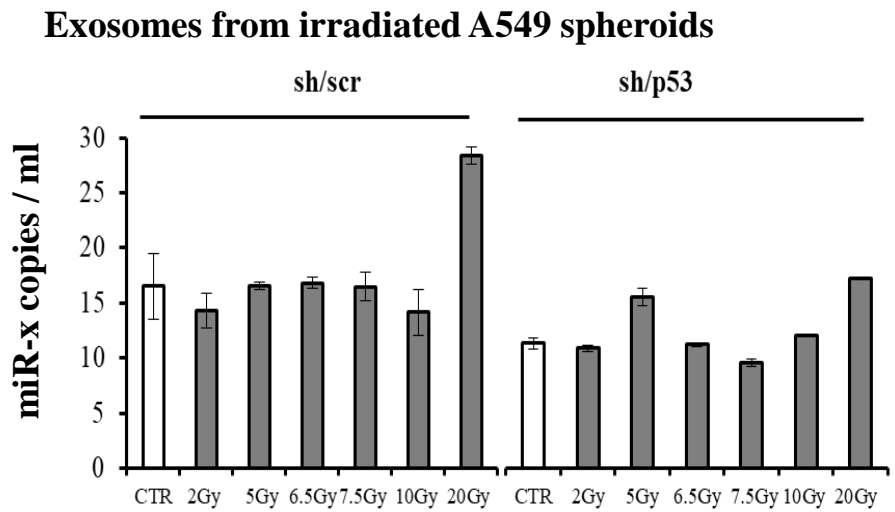
A.



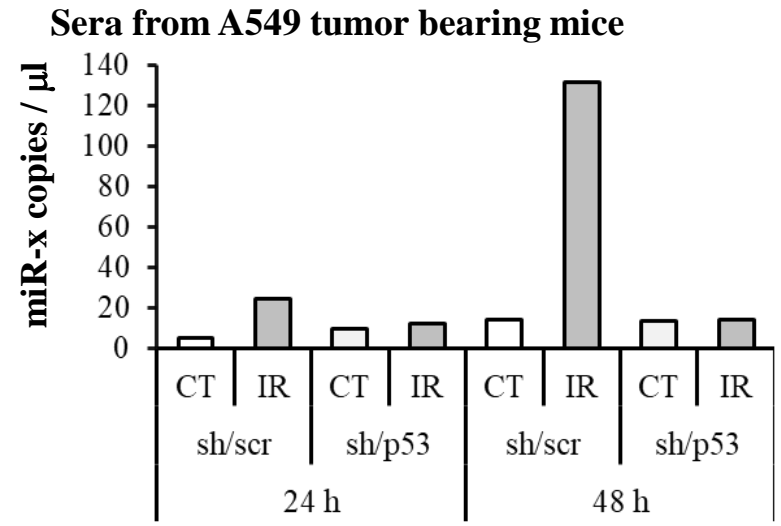
B.



C.



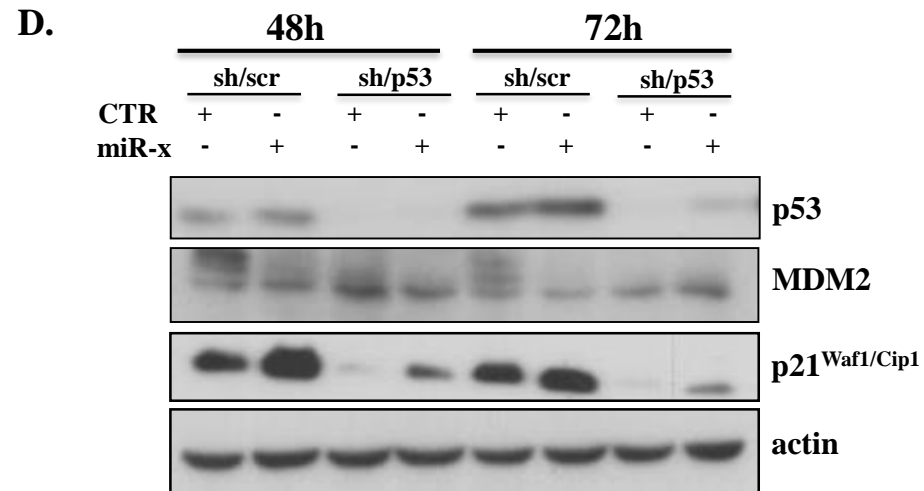
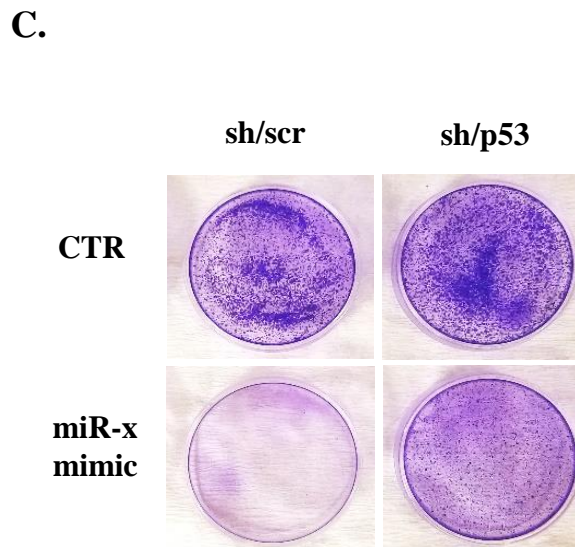
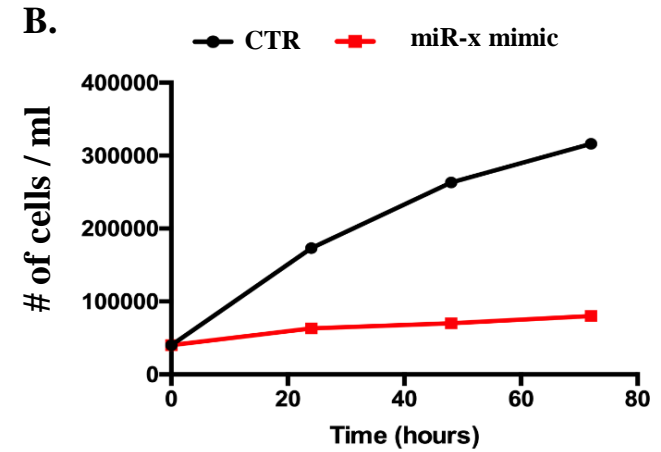
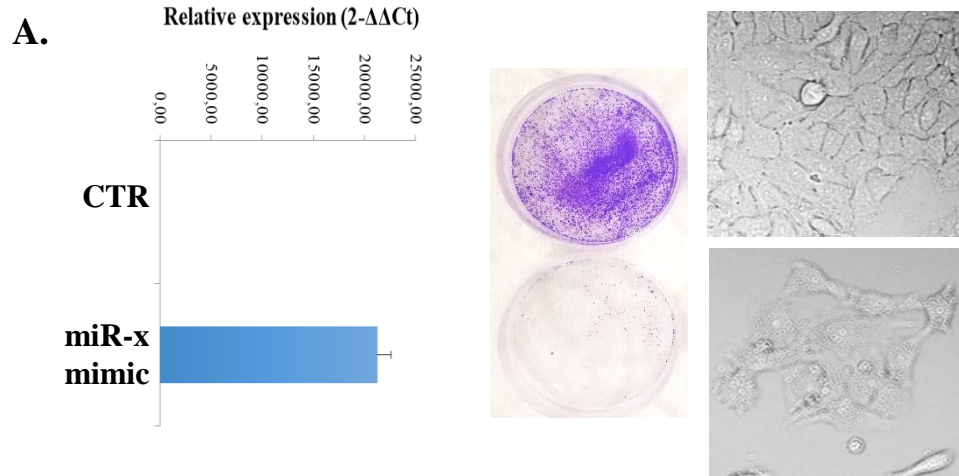
D.



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miR-x induces senescence-like phenotype in A549 cells in vitro



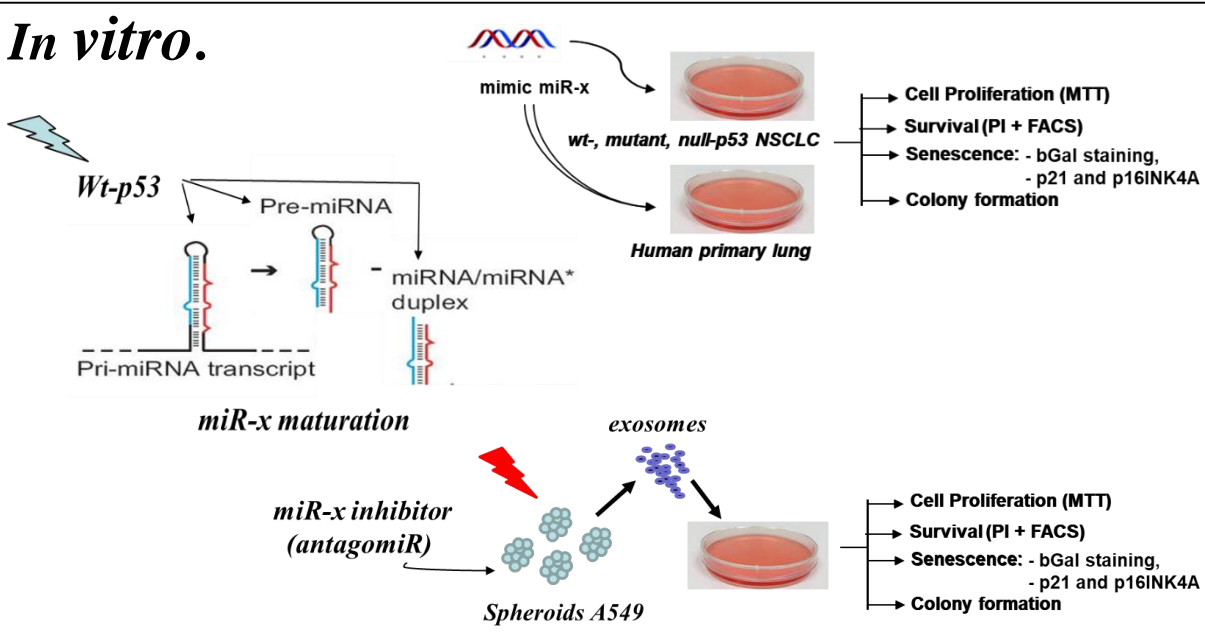
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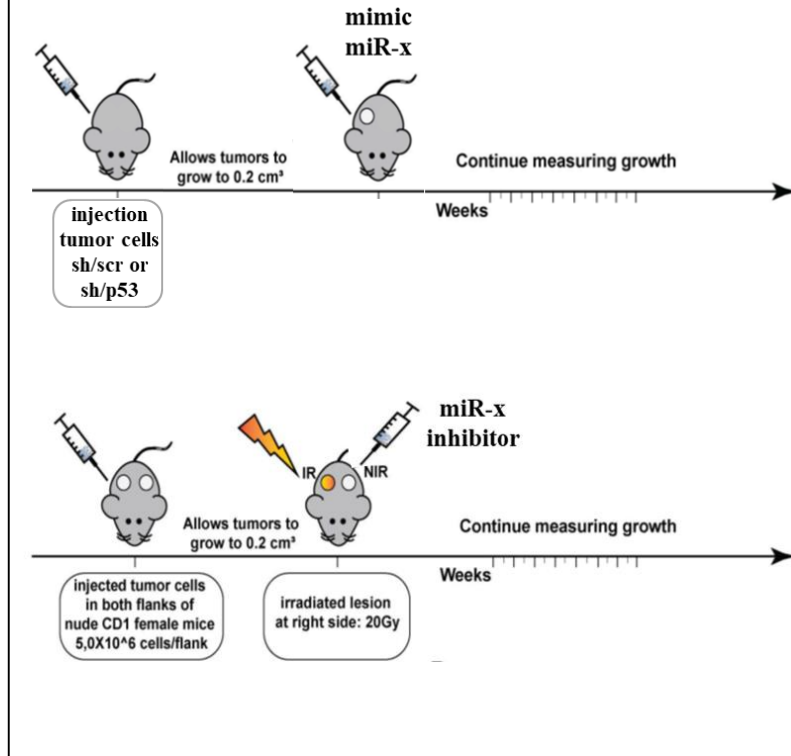
AIM 1. Dissecting miR-x roles in radiation-induced abscopal effect.

Name	Histology	TP53 status	zygosity	AA mutation
PCS-130-010	Normal tissue, lung smooth muscle	WT	-	-
PCS-300-015	Normal tissue, lobar epithelial	WT	-	-
A549	NSCLC	WT	-	-
H1299	NSCLC	NULL	homozygous	-
H1770	NSCLC	MUT	homozygous	p.R248W
H1975	adenocarcinoma	MUT	homozygous	p.R273H

In vitro.



In vivo.

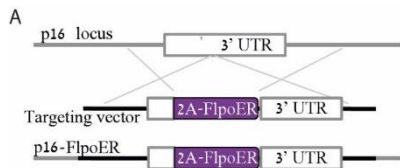


Milestones:

- 1) validate **miR-x** as molecular player in delivering the **abscopal signals** to un-irradiated metastatic lesion triggering AE;
- 2) define whether the **p53 status** determination in **primary lesions** might constitute a **predictor** of successful **RT-induced miR-x** expression in cancer patients.

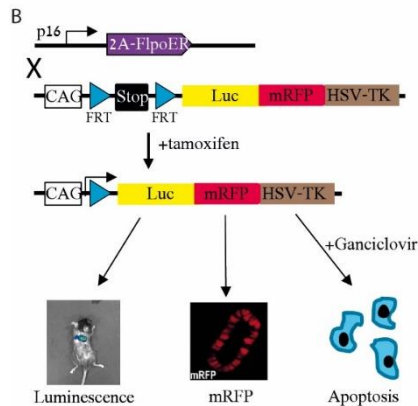
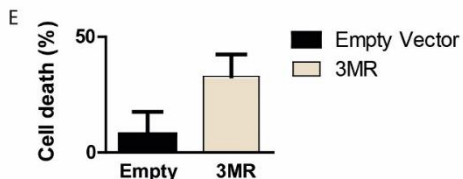
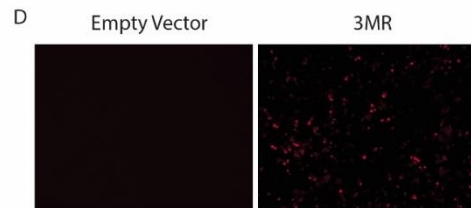
AIM2. Understanding the involvement of radiation induce-senescence in AE with immunocompetent mice.

- Development of mRFP / LSL-krasg12d TG mice, to:
- fluorescence tagging of senescent cells by tamoxifen;
 - kill of senescent cells by ganciclovir;

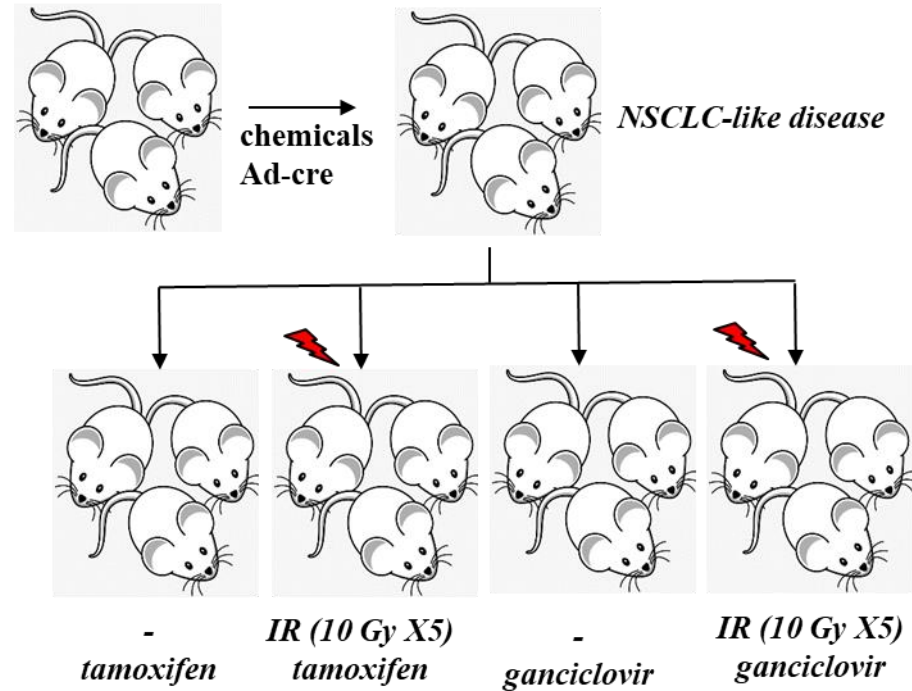


C

	RLU
Renilla	+
3MR	+++



mRFP / LSL-krasg12d TG mice



continuous weight monitoring,
IHC proliferation (K67), apoptosis
(TUNEL), angiogenesis (CD138),
markers of invasion.

Milestones:

establish whether radiation-induced senescence constitute the baseline mechanism required to trigger AE in distant no-irradiated metastatic lesions

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- *Department of Radiation Oncology*
Maria Grazia Petrongari / Giuseppe Sanguineti;
- *Oncogenomic and Epigenetic Unit: TBD;*

- *Department of Molecular Cell Biology: Lior Roitman;*
- *Metabolic Profiling Facility*

AIM3. *Assessing miR-x and senescence metabolic biomarkers as predictors of AE in cancer patients undergoing RT.*



Blood samples collection

15 patients / year

metastatic patients
undergoing hypo-
fractionated RT
(10 Gy x3), including
NSCLC patients.

Before RT
(CTR)

1° RT
(10 Gy x1)

2° RT
(10 Gy x2)

3° RT
(10 Gy x3)

plasma
serum

IRE

Exosomes isolation
miR-x quantification q-PCR

WIS

metabolic biomarkers of senescent by
Mass Spectrometry

Milestones:

- establish **miR-x** and metabolic **senescence** as **molecular predictors** of **RT-induced AE** in metastatic patients, thus helping in the identification of patients who could benefit from RT treatments, thus allowing **tailored therapeutic treatments**.
- establish a **biobank** of plasma and serum samples.

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