

Exploration of immune effects of radionuclide therapy and rationale for combination with immunotherapy in prostate cancer

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Transfer of status

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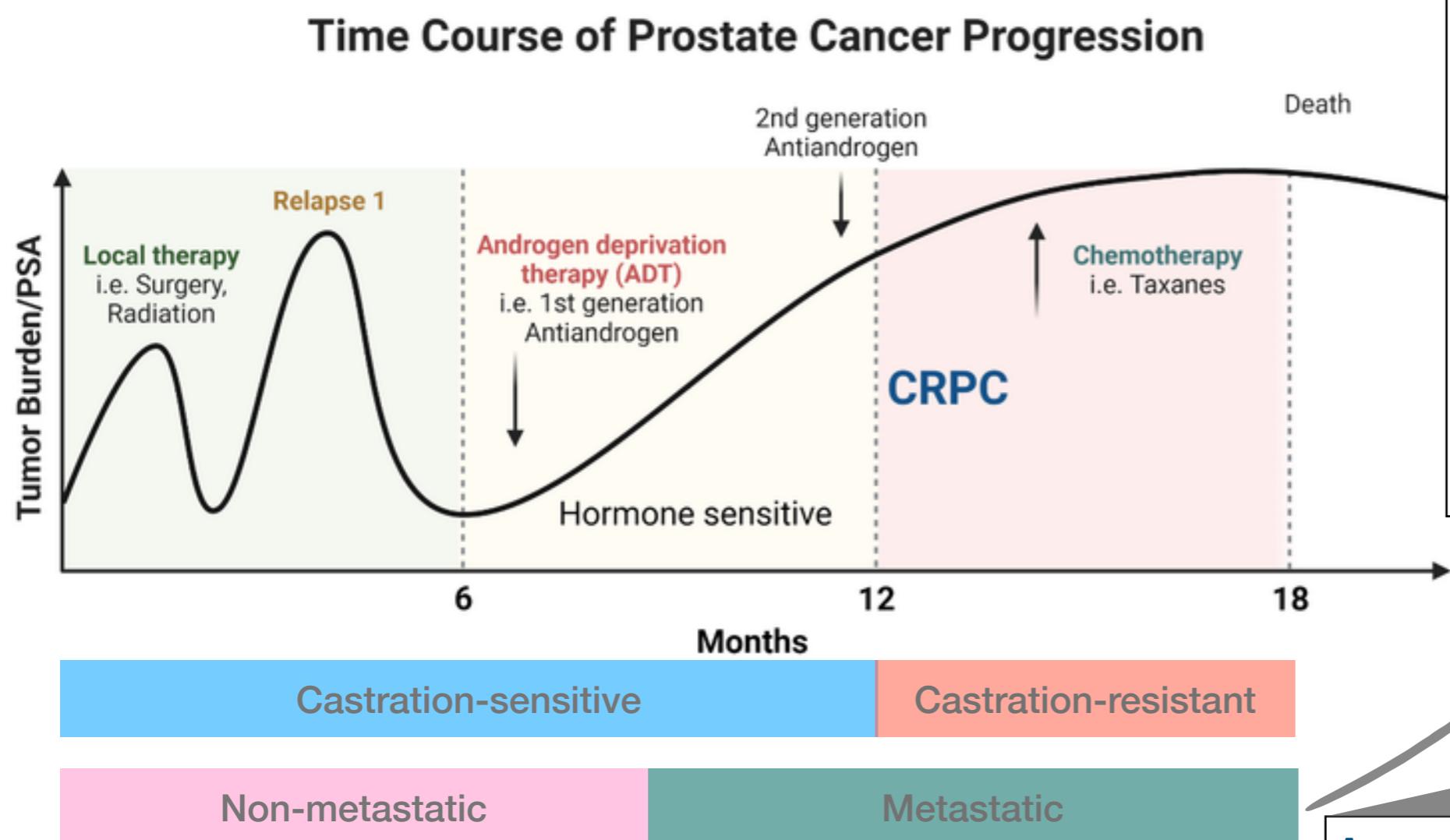
Outline

- **Introduction**
- **Early results**
 - Overview of materials and methods
 - [¹⁷⁷Lu]Lu-PSMA-I&T induces the release of DAMPs
 - Summary-1
 - [¹⁷⁷Lu]Lu-PSMA-I&T induces significant immune response in a syngeneic mouse model
 - Summary-2
- **Future plans and timeline**

Introduction

Treatment for metastatic prostate cancer (PCa) is challenging

[¹⁷⁷Lu]Lu-PSMA therapy has been approved for use in metastatic castration-resistant prostate cancer (mCRPC)



1st generation antigen-deprivation therapy

+

Abiraterone
Enzalutamide
Darolutamide
Docetaxel
EBRT
Olaparib
Sipuleucel-T
Radium-223

[¹⁷⁷Lu]Lu-PSMA therapy

VISION trial

Approved by FDA in 2022

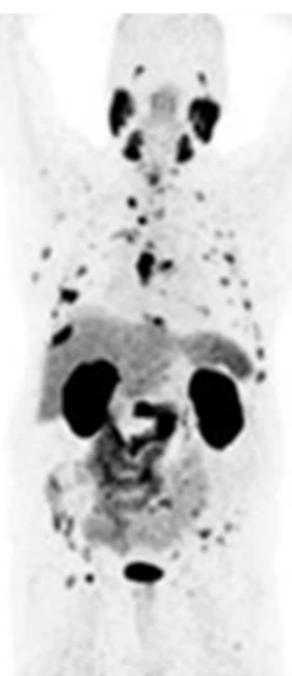
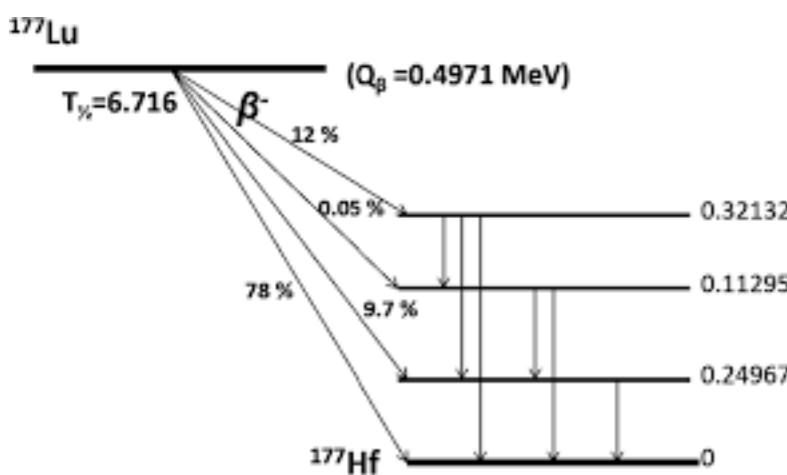
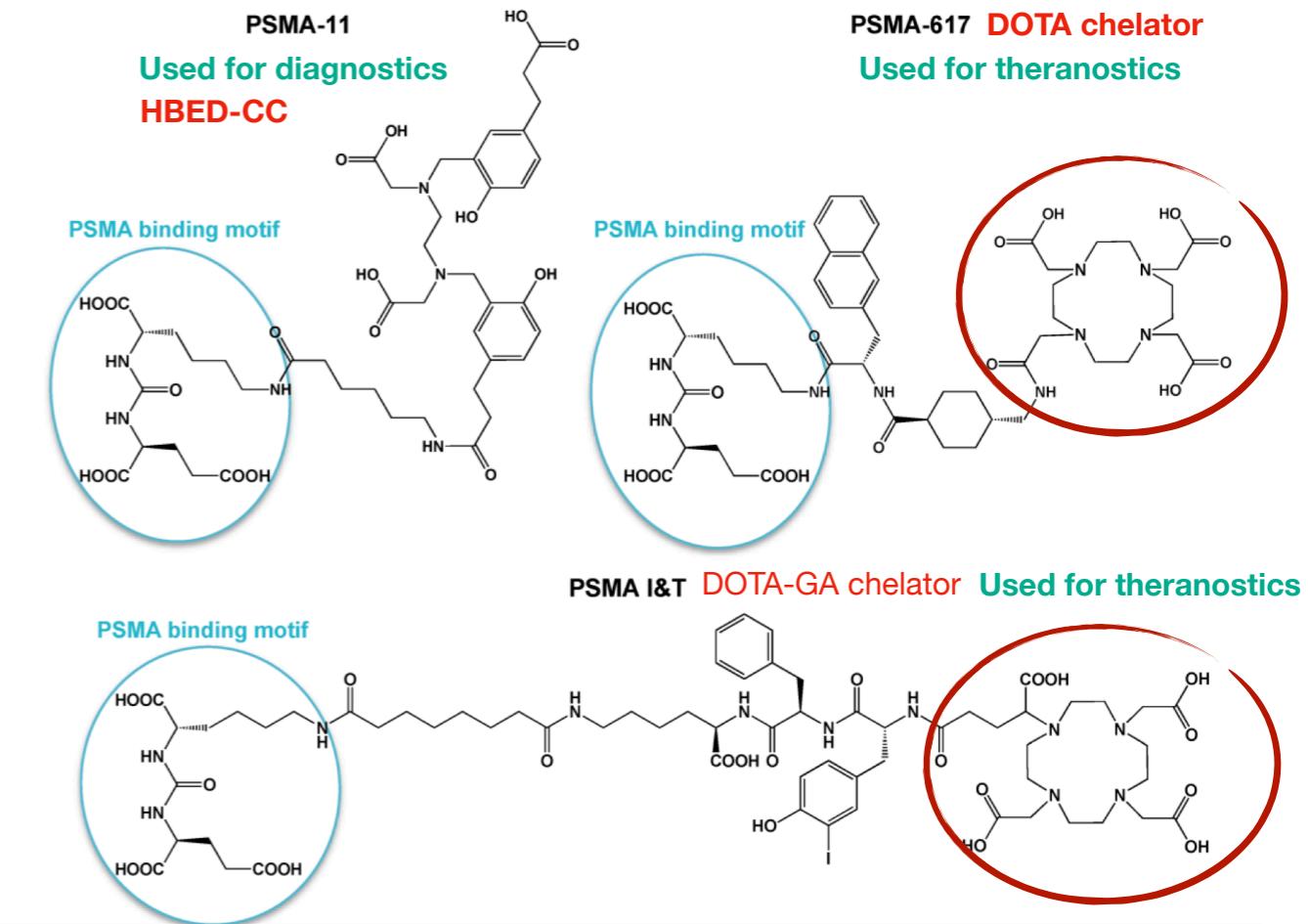
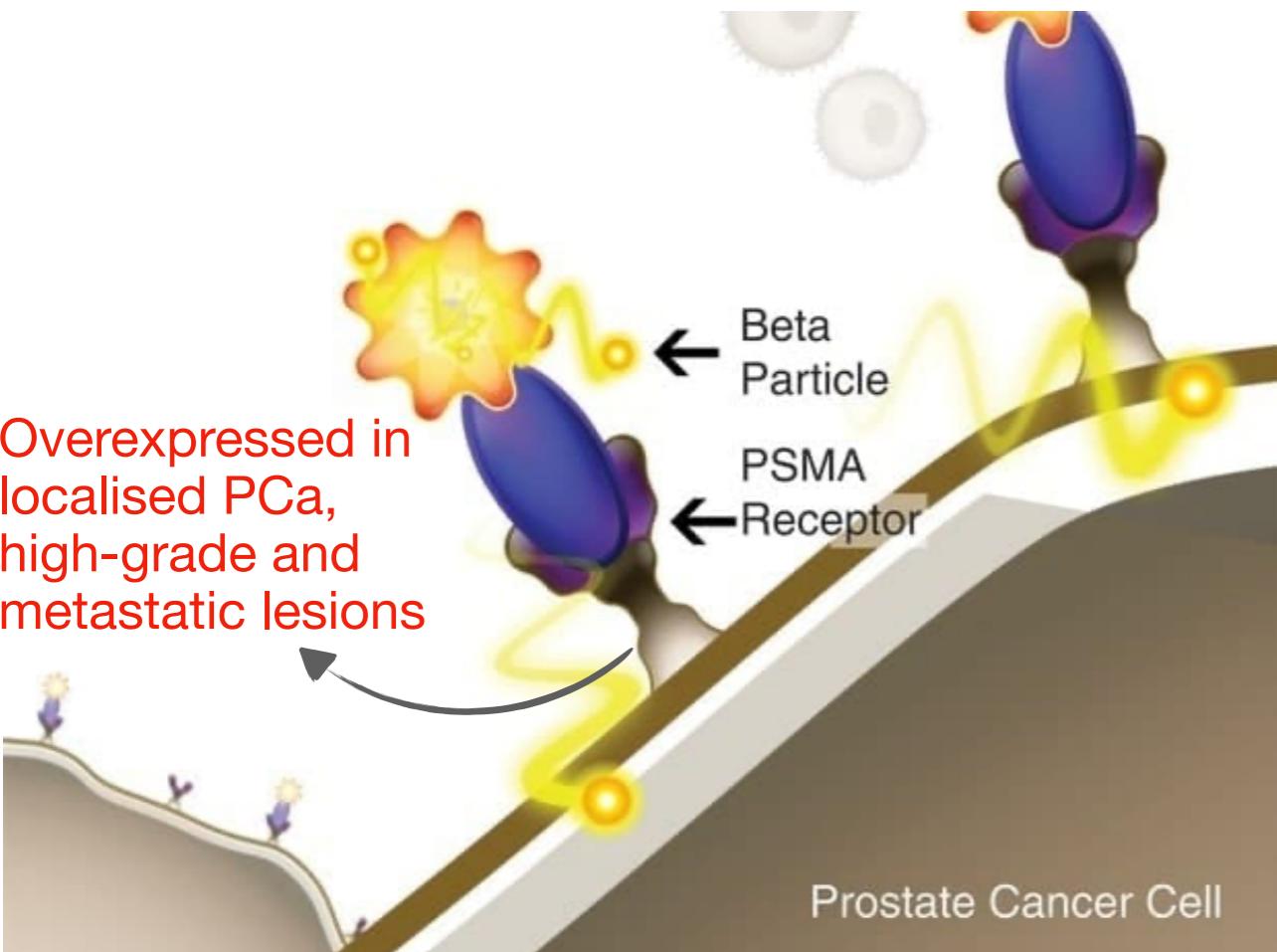
Significantly improves (as compared with standard treatment):

Progression-free survival

Overall survival

Quality of life not affected

What is [¹⁷⁷Lu]Lu-PSMA (prostate-specific membrane antigen) therapy?



[⁶⁸Ga]Ga-PSMA-11 after [¹⁷⁷Lu]Lu-PSMA-617



[¹⁷⁷Lu]Lu-PSMA therapy has become a promising alternative

available at www.sciencedirect.com
journal homepage: www.europeanurology.com



Review – Prostate Cancer

A Meta-Analysis and Meta-Regression of the Efficacy, Toxicity, and Quality of Life Outcomes Following Prostate-Specific Membrane Antigen Radioligand Therapy Utilising Lutetium-177 and Actinium-225 in Metastatic Prostate Cancer

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Published in Sep. 2024

87/100 studies for [¹⁷⁷Lu]Lu-PSMA:

49% of patients have ≥50% PSA response and 70% of patients have any PSA response across all cycles of treatment

Improved survival if PSA response can be achieved

Manageable toxicities

Improved quality of life

How to improve the therapeutic efficacy of [¹⁷⁷Lu]Lu-PSMA therapy?

Ongoing trials for [¹⁷⁷Lu]Lu-PSMA +/- ICIs

EVOLUTION	Australian centres	NCT05150236 Recruiting	¹⁷⁷Lu-PSMA versus ¹⁷⁷Lu-PSMA + Ipilimumab and Nivolumab (mCRPC)
UCSF Phase Ib	UCSF	NCT03805594 Active, not recruiting ASCO 2021	Phase 1b study of ¹⁷⁷ Lu-PSMA-617 + Pembrolizumab
PRINCE	Australian centres	NCT03658447 Completed	Phase Ib/II study of ¹⁷⁷ Lu-PSMA-617 + Pembrolizumab for mCRPC
NEPI trial	Germany	NCT06388369 Not yet recruiting	¹⁷⁷ Lu-PSMA versus ¹⁷⁷ Lu-PSMA + Ipilimumab for very high-risk PCa (candidates for prostatectomy)

[¹⁷⁷Lu]Lu-PSMA therapy combined with ICI is feasible



Single-dose ¹⁷⁷Lu-PSMA-617 followed by maintenance pembrolizumab in patients with metastatic castration-resistant prostate cancer: an open-label, dose-expansion, phase 1 trial *Lancet Oncol* 2023; 24: 1266–76



Rahul Aggarwal, Stephanie Starzinski, Ivan de Kouchkovsky, Vadim Koshkin, Rohit Bose, Jonathan Chou, Arpita Desai, Daniel Kwon, Samuel Kaushal, Lauren Trihy, Medini Rastogi, Robin Ippisch, Maya Aslam, Terence Friedlander, Felix Feng, David Oh, Alexander Cheung, Eric Small, Michael Evans, Lawrence Fong*, Thomas A Hope*



Single-dose [¹⁷⁷Lu]Lu-PSMA



56% objective response rate
(median follow-up: 16.5 months)

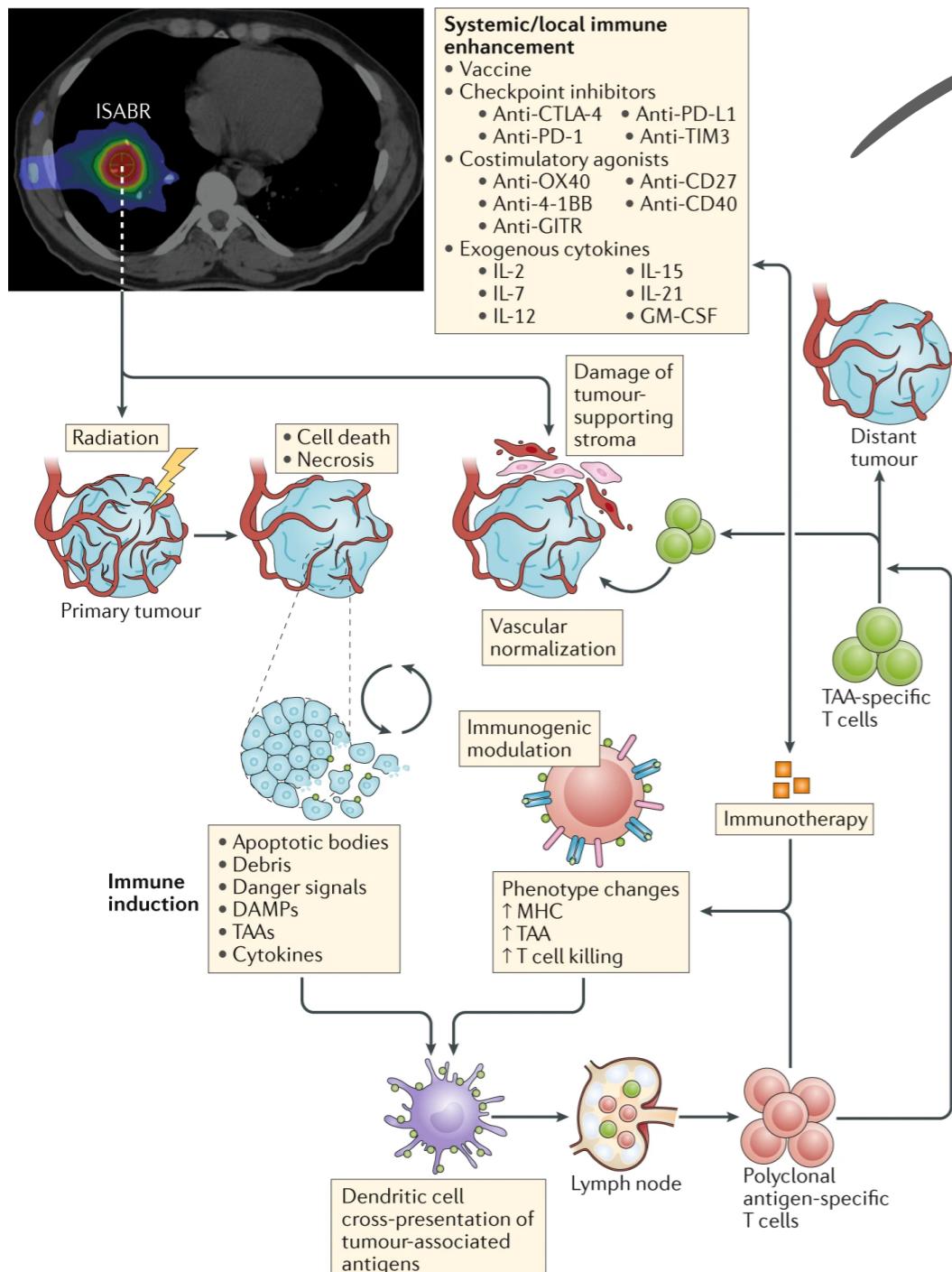
Circulating immune cells in
responders:

Higher cell cluster frequencies of
CD8+ effector cells, CD8+ effector
memory cells, $\gamma\delta$ T cells, and
natural killer T cells.

Contributed by [¹⁷⁷Lu]Lu-PSMA or ICI?

Rationale of combining [¹⁷⁷Lu]Lu-PSMA therapy with immunotherapy

Thinking about conventional radiotherapy first



Radiation reprograms the tumour microenvironment and induces immunogenic cell death (ICD)

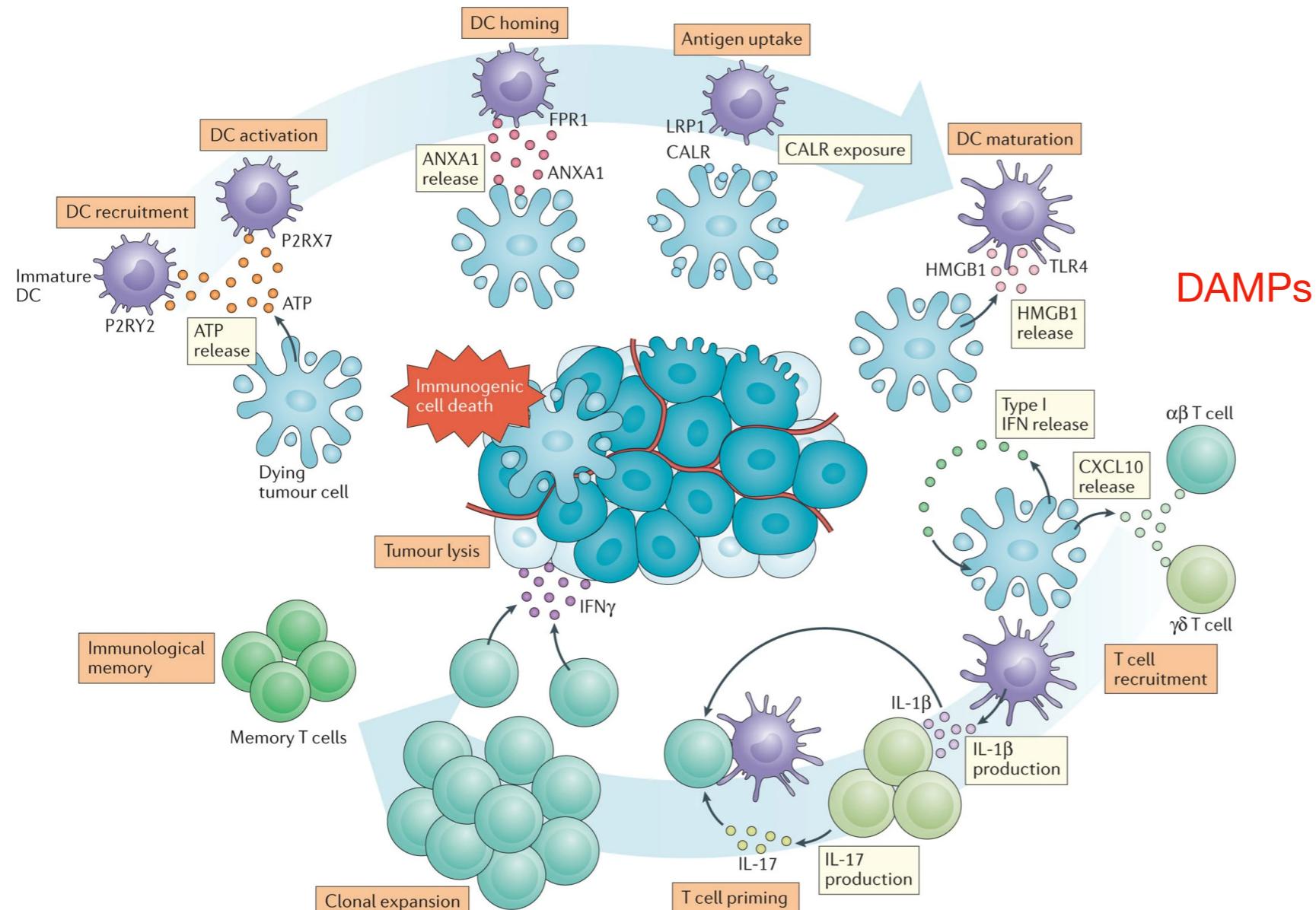
- Increased expression of **MHC I** —> recognition of tumour-associated antigens by cytotoxic T cells
- Accumulation of **DNA in cytosol**—> activates **cGAS-STING**
- Release of **DAMPs and inflammatory cytokines**—> activates antigen-presenting cells

Prime polyclonal cytotoxic T cells

Enhanced by adding immune checkpoint inhibitors (ICIs)

ICD is crucial in enhancing the effectiveness of immunotherapy

Common ICD inducers: anthacycline, oxaliplatin, **radiation**, oncolytic virus



Common ICD markers expressed by tumour cells:

ATP release
Exposure of calreticulin (CRT)
HMGB1 release

Others:

ANXA1 release
Type I IFN release
CXCL10 release
IL-1 β production
IL-17 production

Nature Reviews | Immunology

Main objectives of this project

- Characterise ICD induced by [¹⁷⁷Lu]Lu-PSMA therapy
- Explore the immune response elicited in a syngeneic mouse model by [¹⁷⁷Lu]Lu-PSMA therapy
- Identification of therapeutic targets that may contribute to the success of combined therapy with ICIs
- In vitro and in vivo validations.

Early results

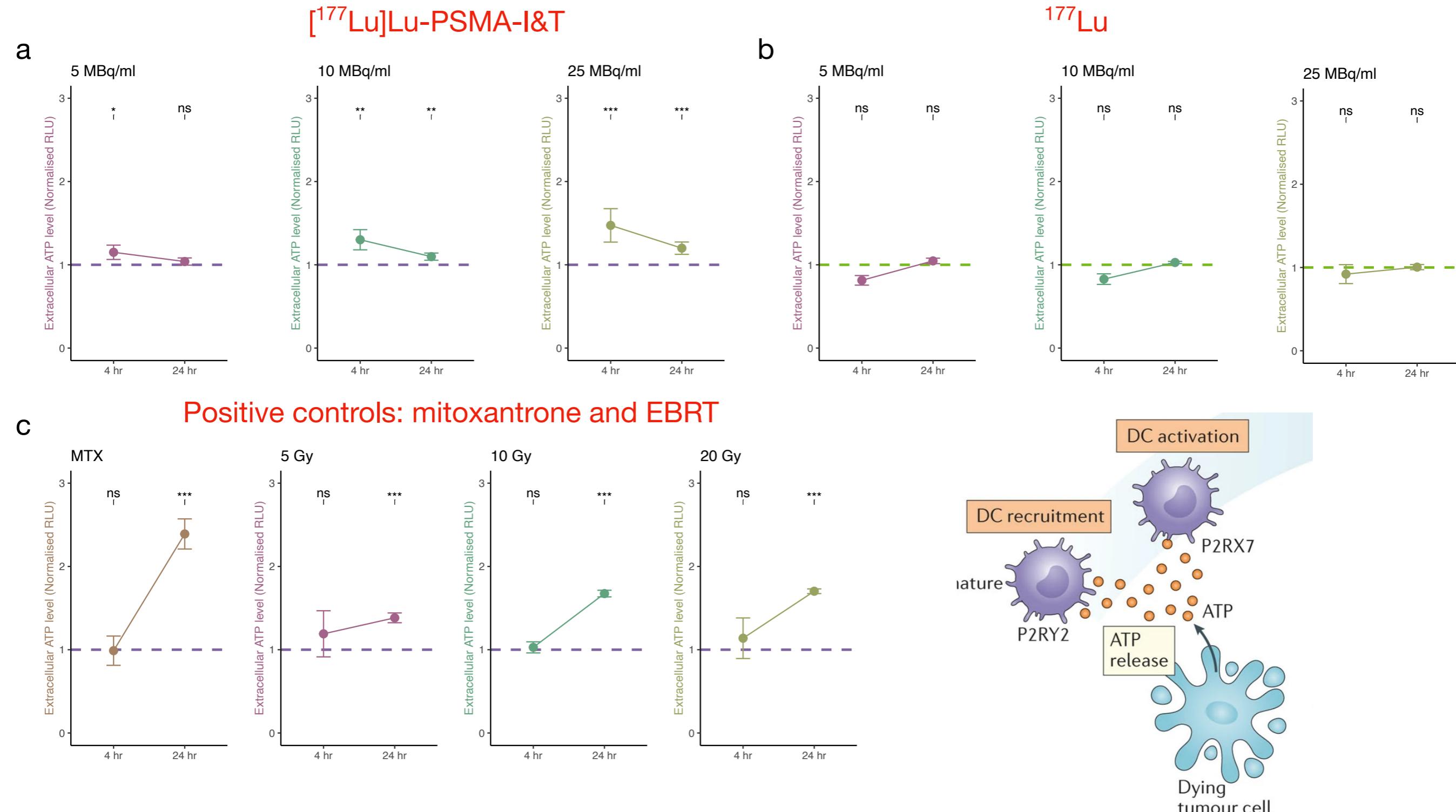
Overview of materials and methods

- **Cell line:** RM1-PGLS murine PCa cell line (with the phenotype of **CRPC**, **PSMA-expressing**)
- **Radiolabelling:** 80 MBq $^{177}\text{LuCl}_3$ with 1 nmol of PSMA-I&T (yield checked with iTLC)
- **Mouse:** male C57BL/6, 4-6 weeks old
- **Release/expression of DAMPs:** bioluminescent assays and immunofluorescence imaging (LSM 880)
- **Release/expression of Cxcl10:** ELISA and immunofluorescence imaging (LSM 880)
- **Positive control:** radiotherapy (5-20 Gy), mitoxantrone (3 μM , optional)
- **Biodistribution study:** MILabs, PMOD, LIFEx
- **Statistic/Bioinformatics analyses:** R software (packages: NanoTube, GSVA, pheatmap), Python

[¹⁷⁷Lu]Lu-PSMA-I&T induces the release of DAMPs

[¹⁷⁷Lu]Lu-PSMA-I&T induces the release of ATP

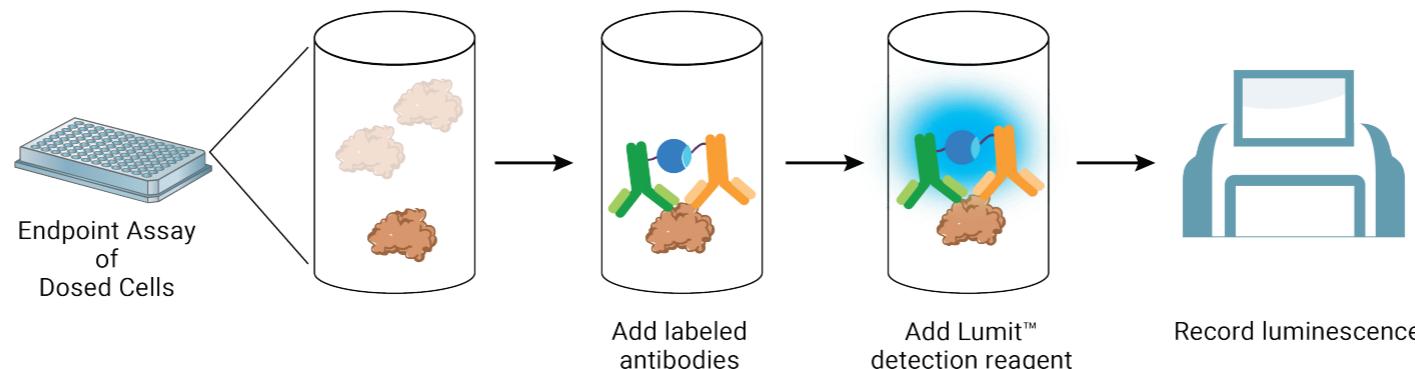
ATP is released in an activity dependent manner at 4h after [¹⁷⁷Lu]Lu-PSMA-I&T



*p<0.05, **p<0.01, ***p<0.001

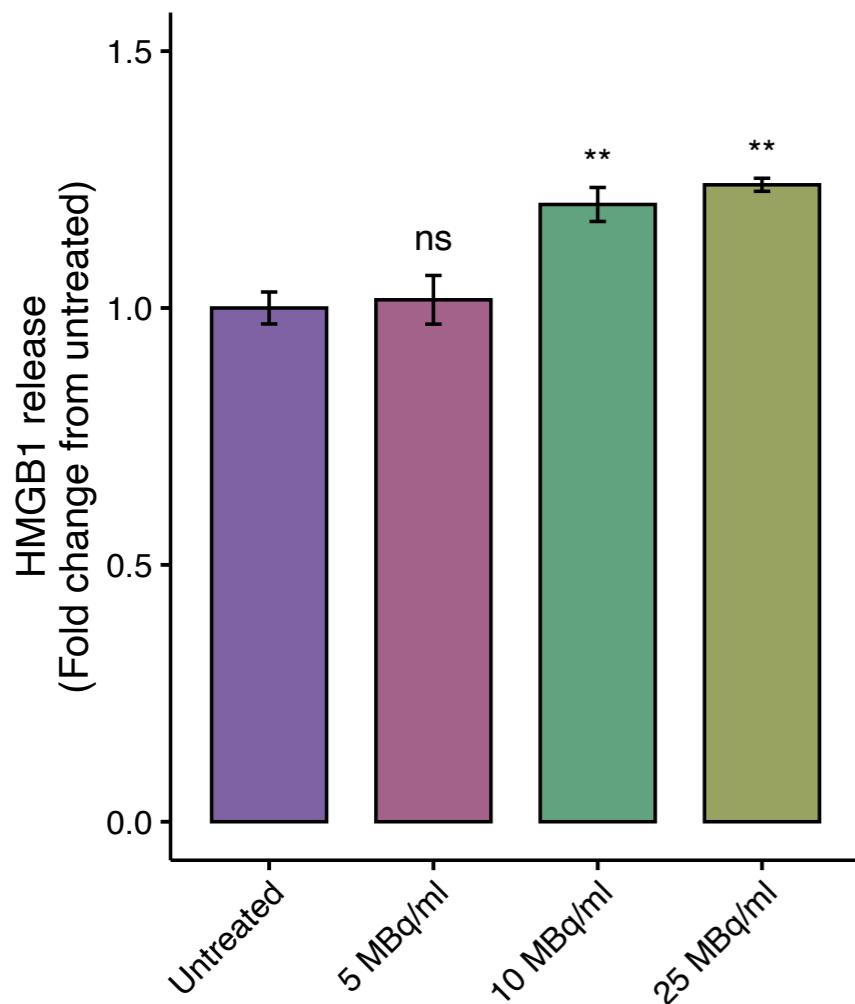
[¹⁷⁷Lu]Lu-PSMA-I&T induces the release of HMGB1

Time point evaluated: 24 hr



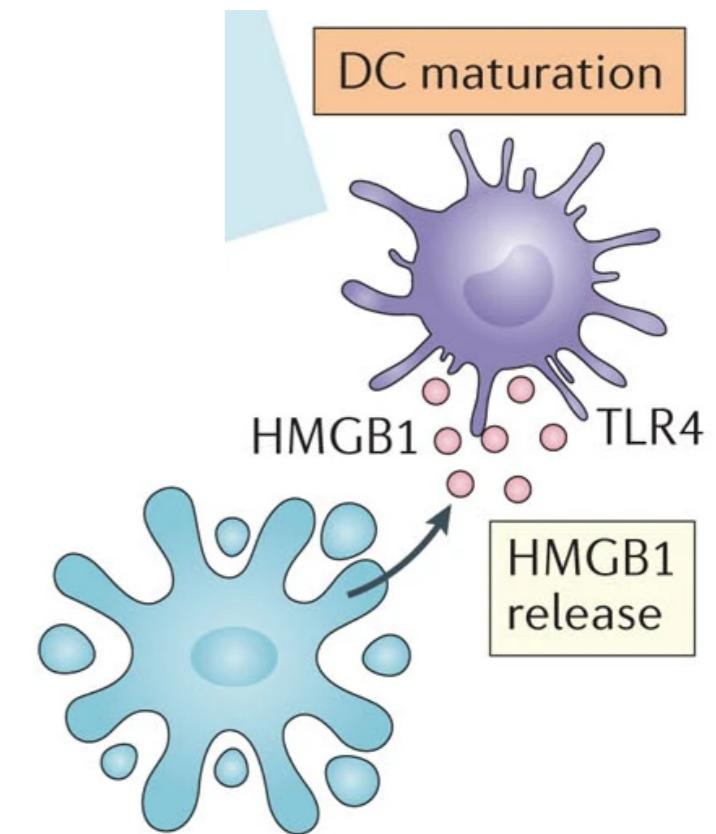
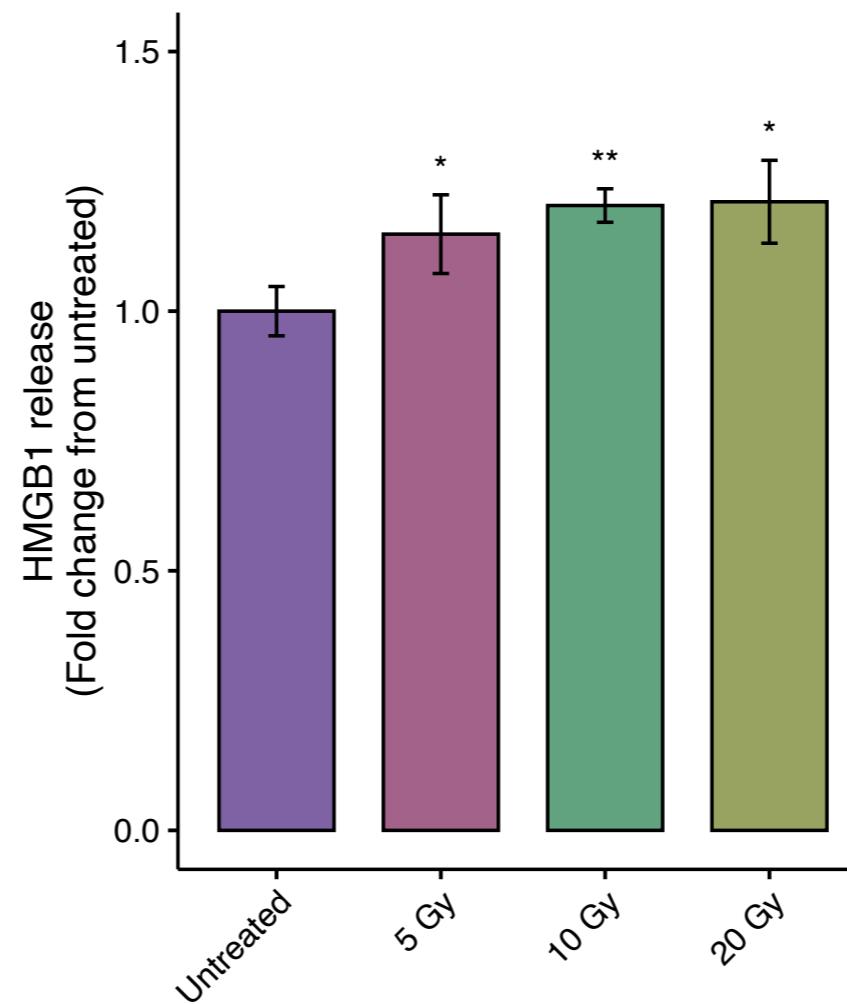
[¹⁷⁷Lu]Lu-PSMA-I&T

■ Untreated ■ 5 MBq/ml ■ 10 MBq/ml ■ 25 MBq/ml



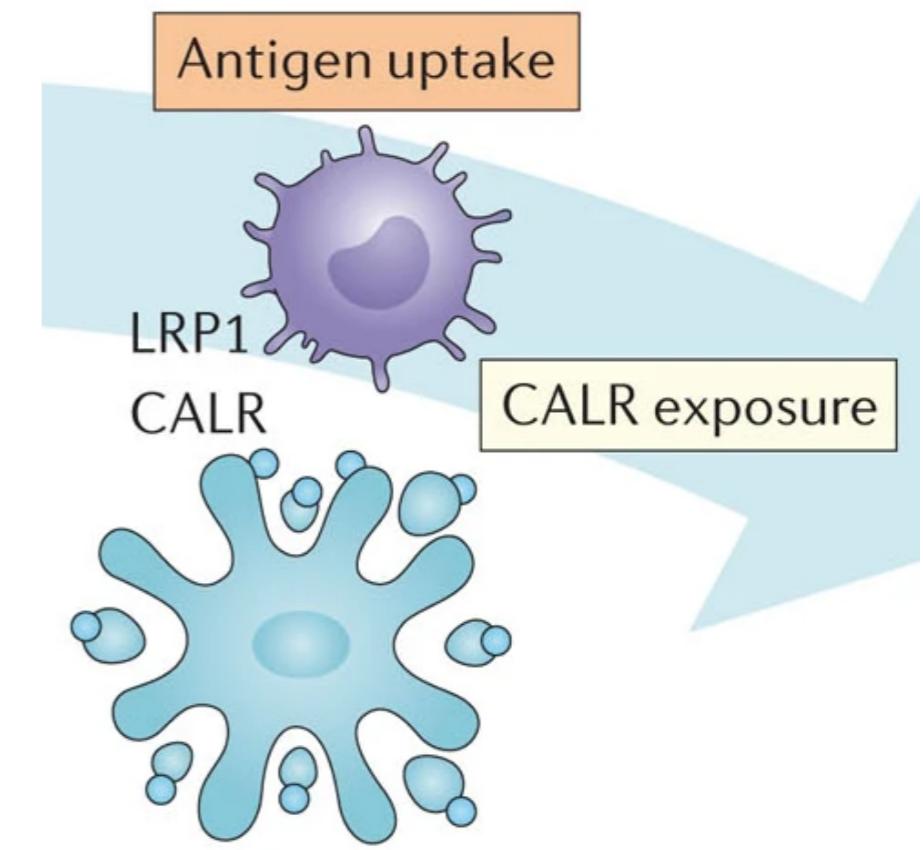
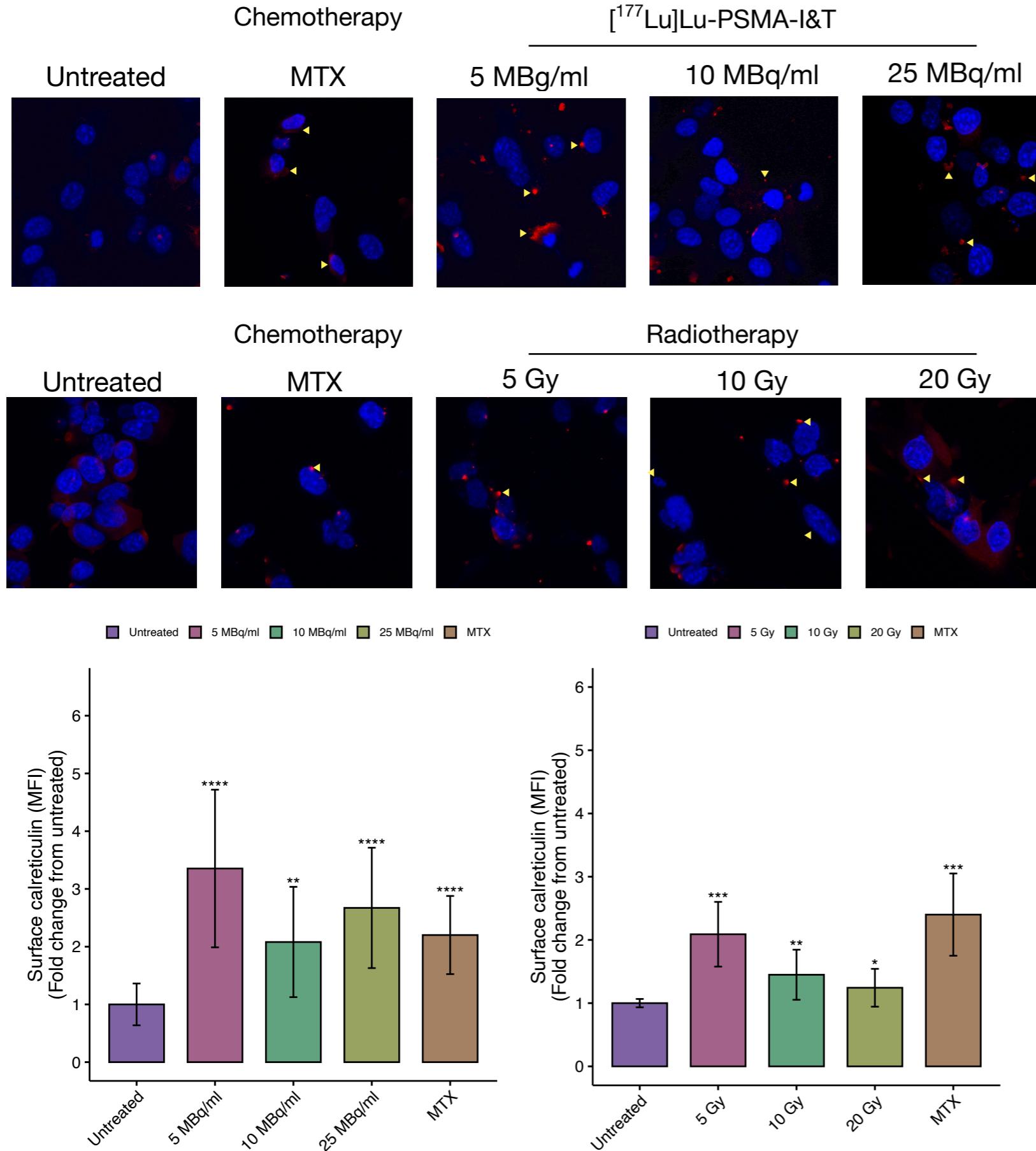
EBRT

■ Untreated ■ 5 Gy ■ 10 Gy ■ 20 Gy



*p<0.05, **p<0.01, ***p<0.001

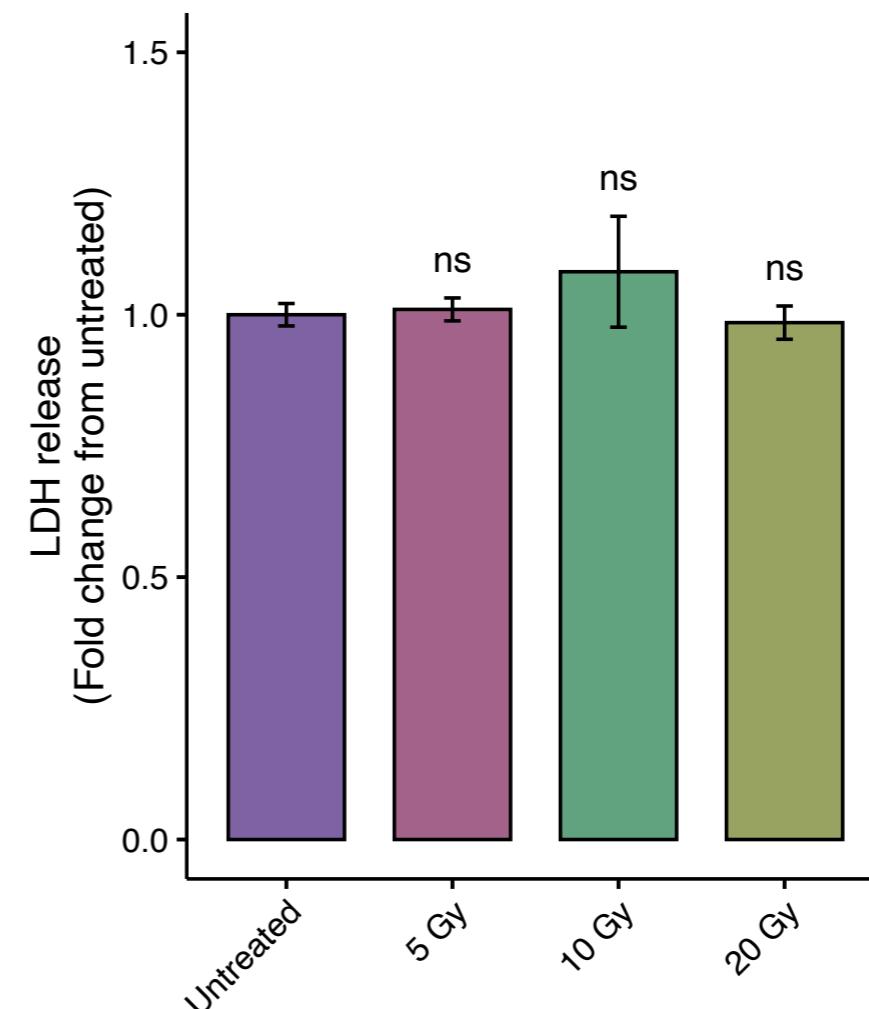
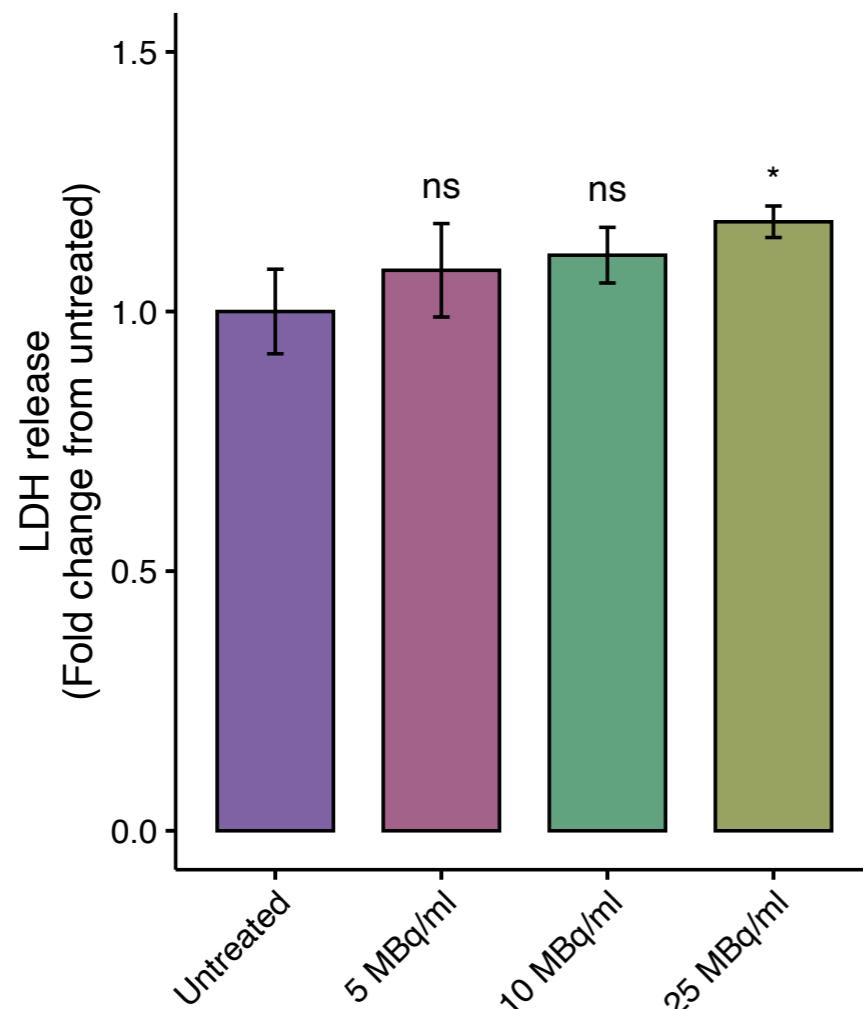
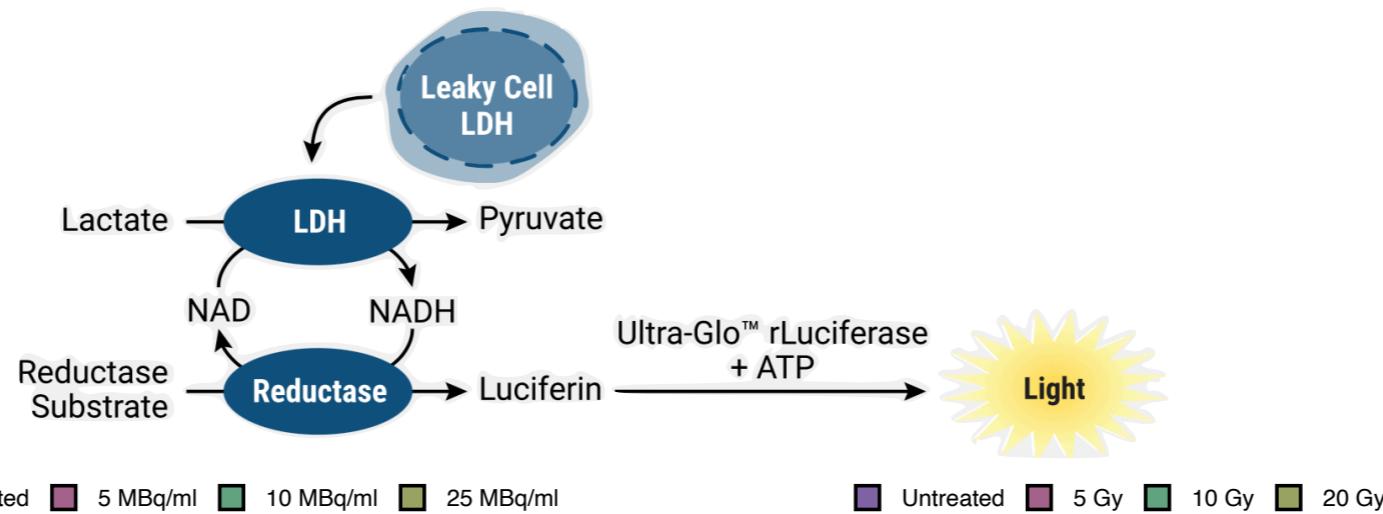
[¹⁷⁷Lu]Lu-PSMA-I&T induces exposure of CRT



*p<0.05, **p<0.01, ***p<0.001

[¹⁷⁷Lu]Lu-PSMA-I&T induces cytotoxicity

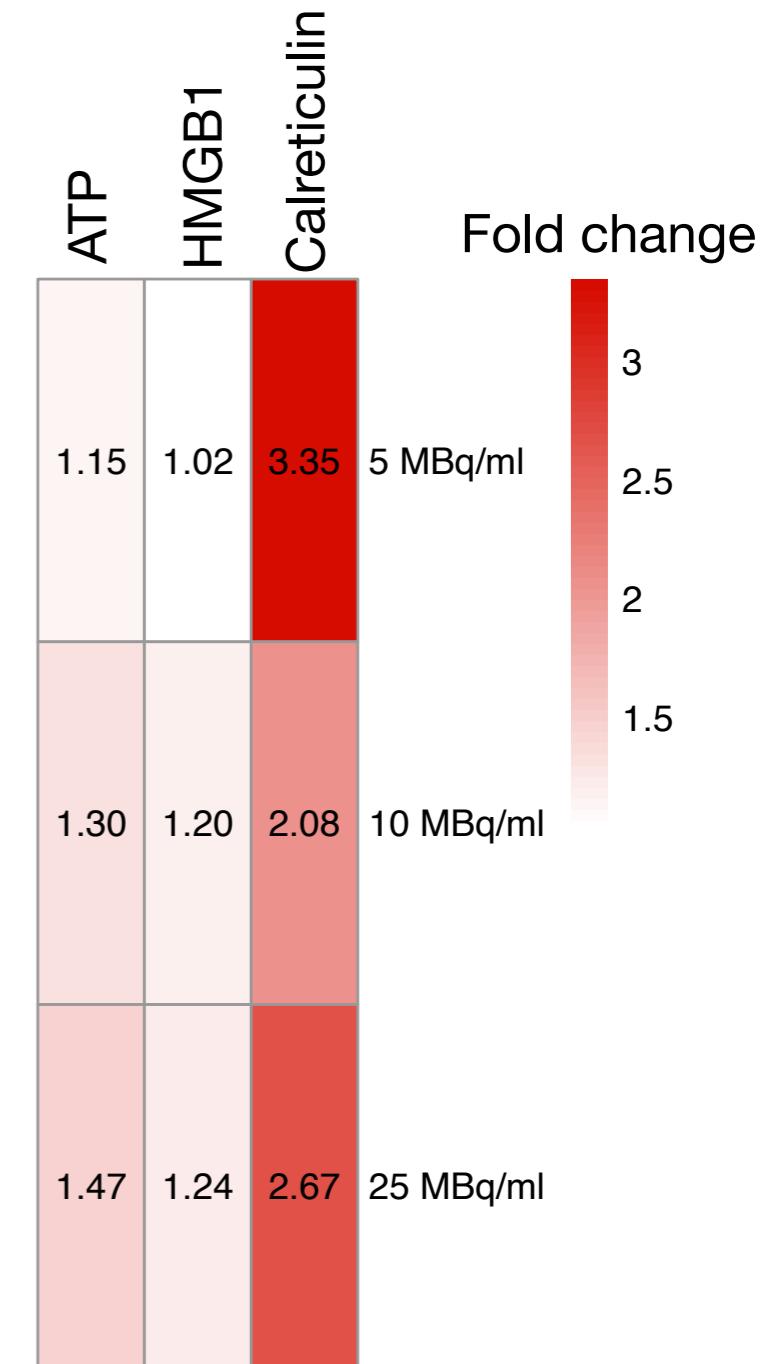
Measurement of cell death; time point evaluated: 24 hr



*p<0.05, **p<0.01, ***p<0.001

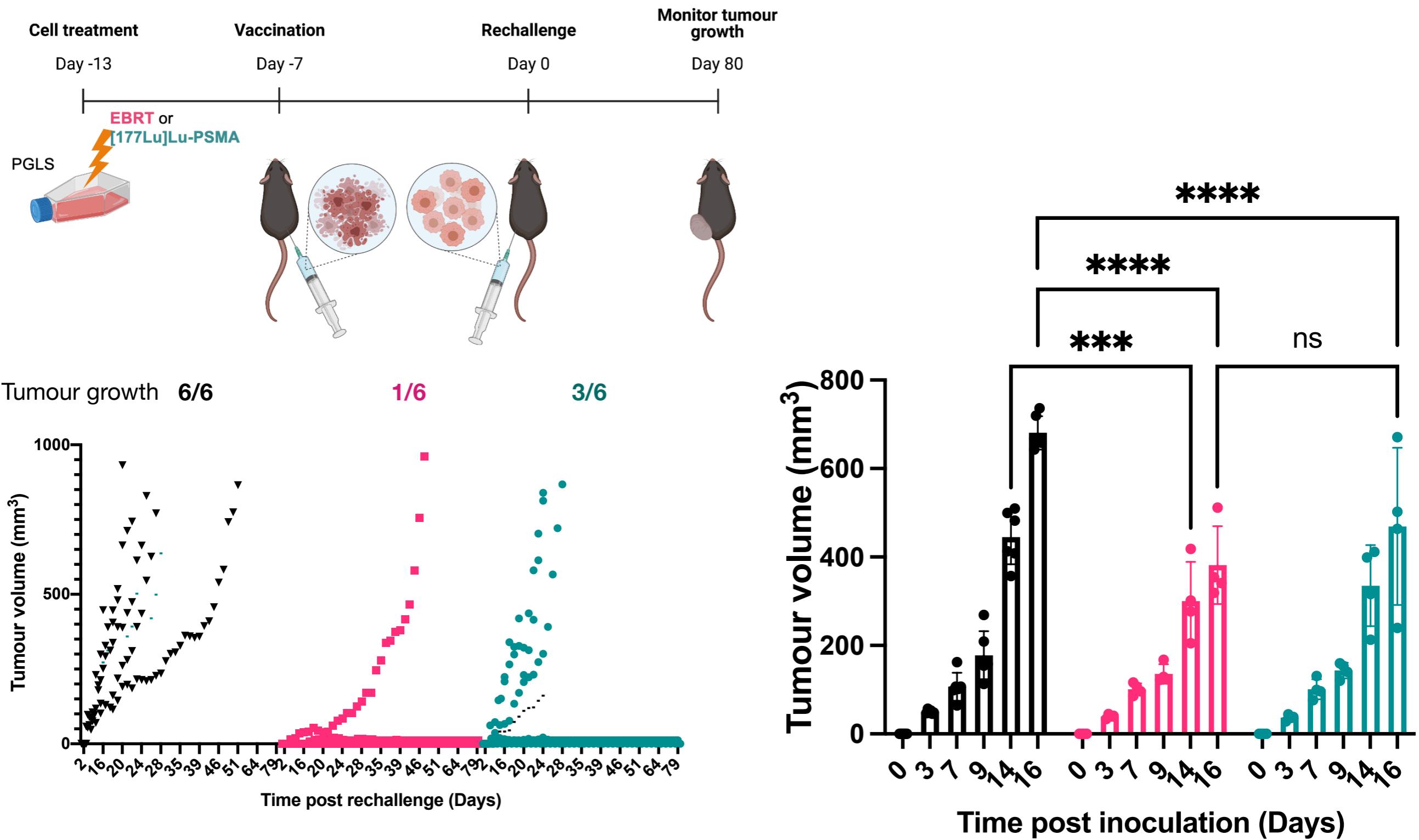
Summary-1

- $[^{177}\text{Lu}]\text{Lu-PSMA-I\&T}$ effectively induces the **release of DAMPs** and promote **cell death** when administered at an activity of 25 MBq/ml for 24 hours.
- These results suggest $[^{177}\text{Lu}]\text{Lu-PSMA-I\&T}$ is a potential **ICD inducer in vitro**.



In vivo vaccination study (Gold standard for ICD)

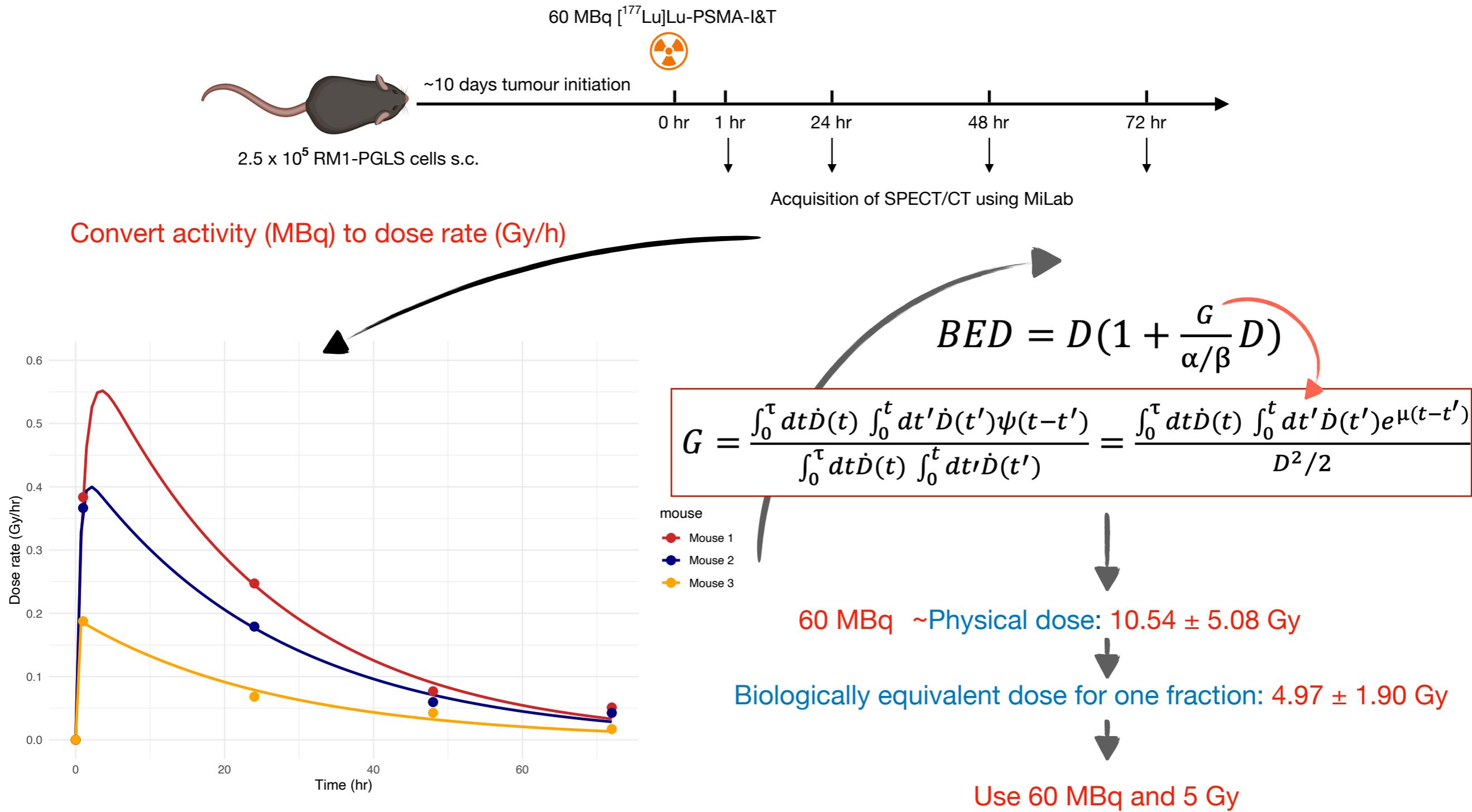
Performed by Gemma Dias (previous post Doc)



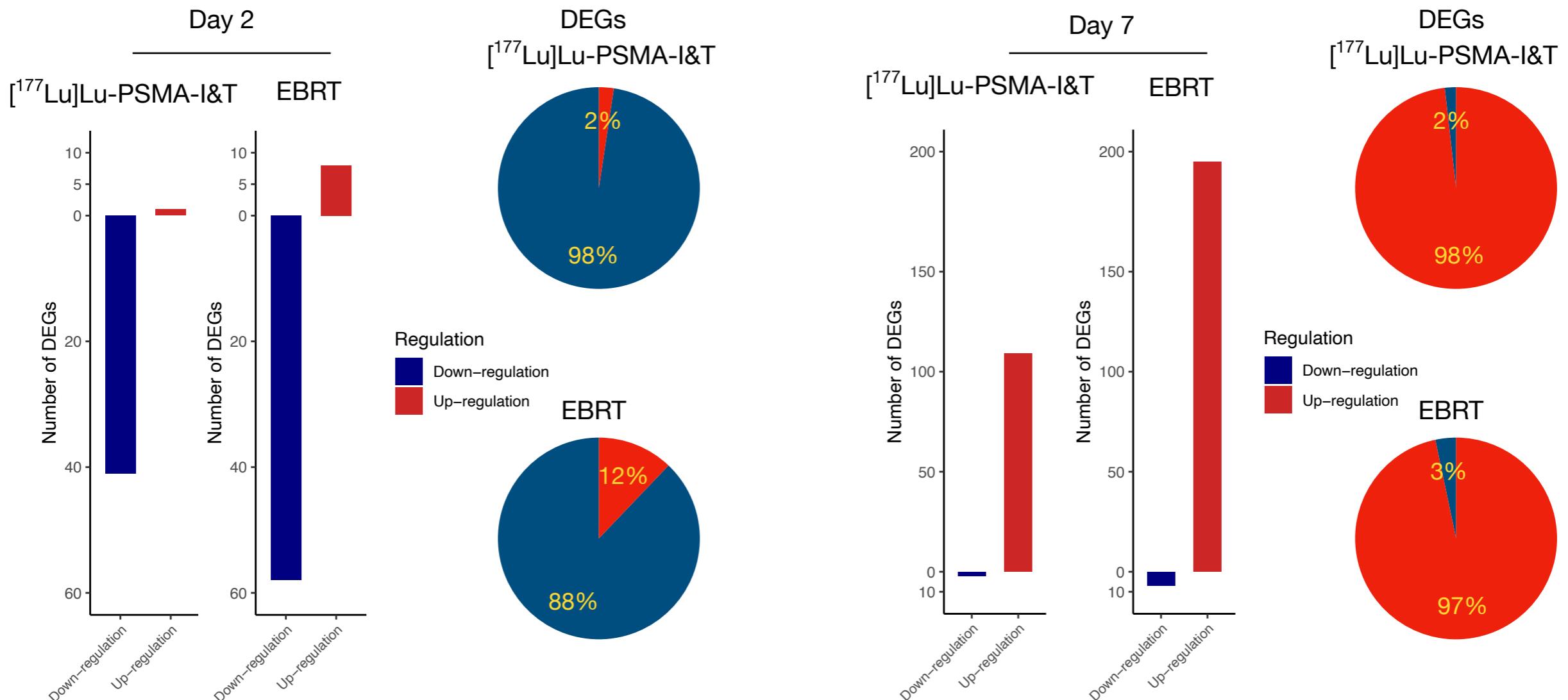
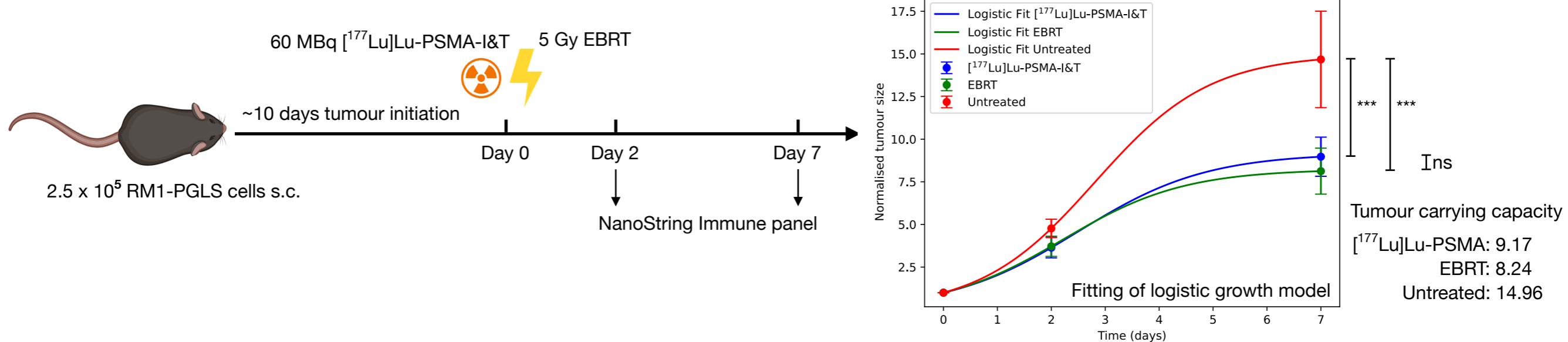
**[¹⁷⁷Lu]Lu-PSMA-I&T induces
significant immune response in a
syngeneic mouse model**

Identification of biologically equivalent dose of EBRT

For comparison with 60 MBq [^{177}Lu]Lu-PSMA-I&T; equation proposed by Mark Macsuka

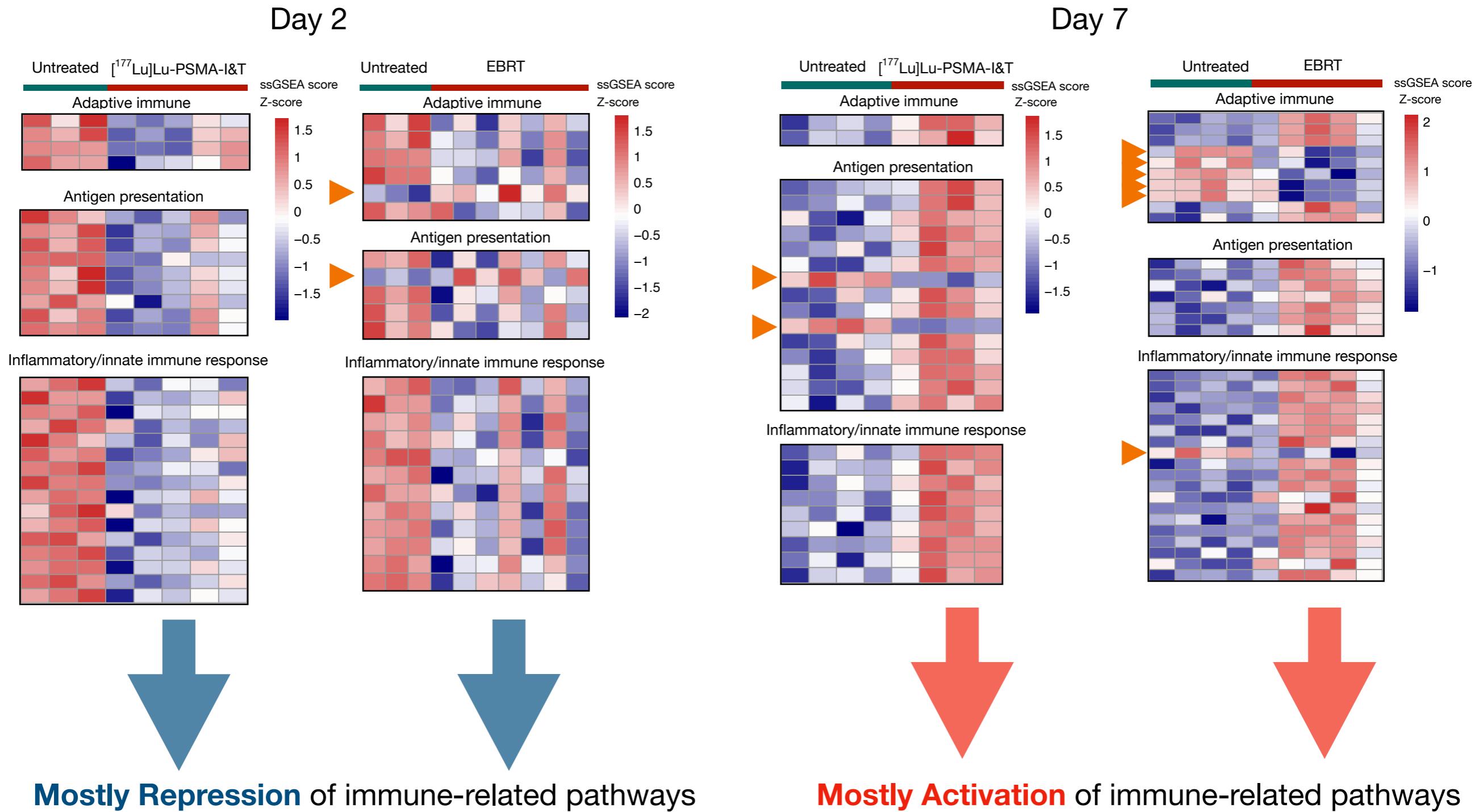


Up-regulation of immune-related genes on day 7



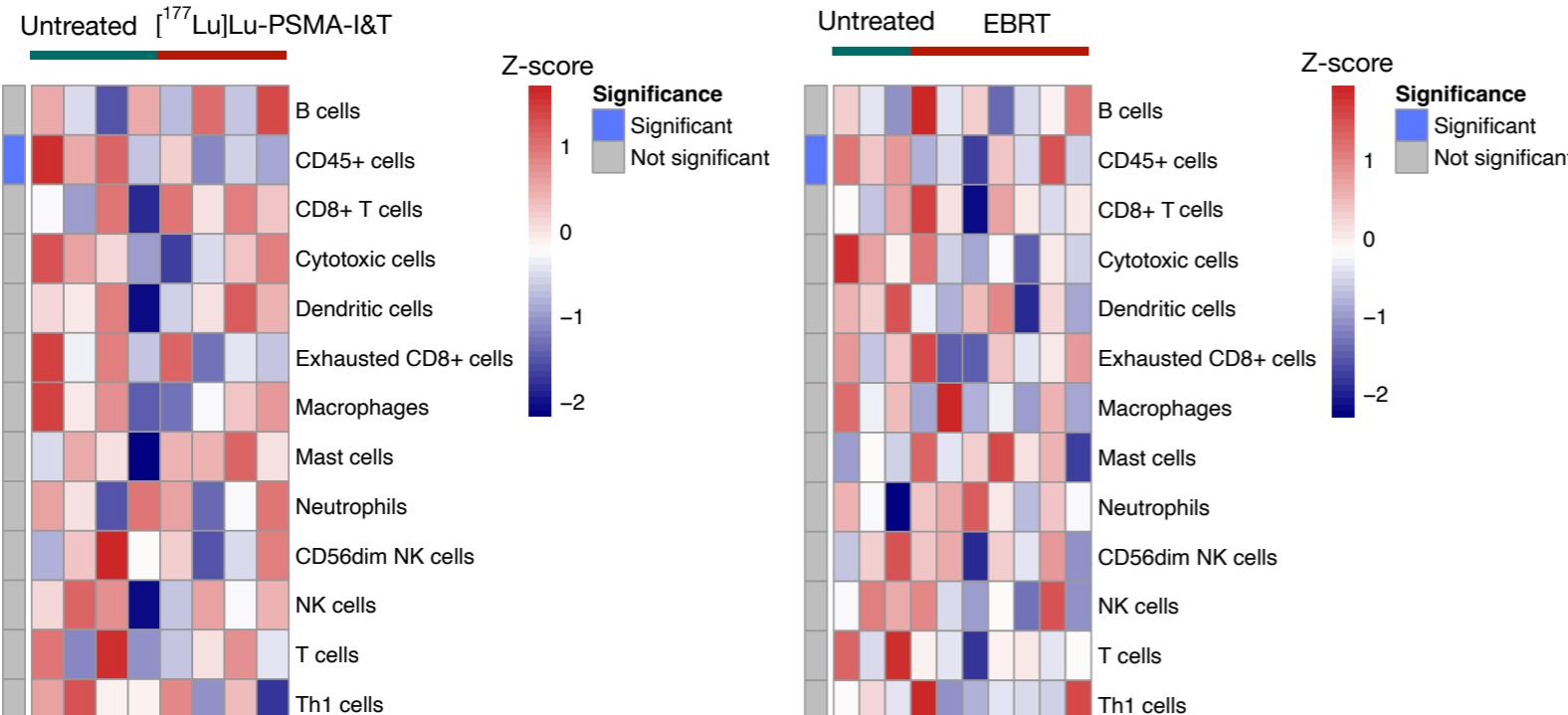
Activation of immune pathways on day 7

[¹⁷⁷Lu]Lu-PSMA-I&T treatment leads to different immune response profile from EBRT: more antigen presentation-related pathways

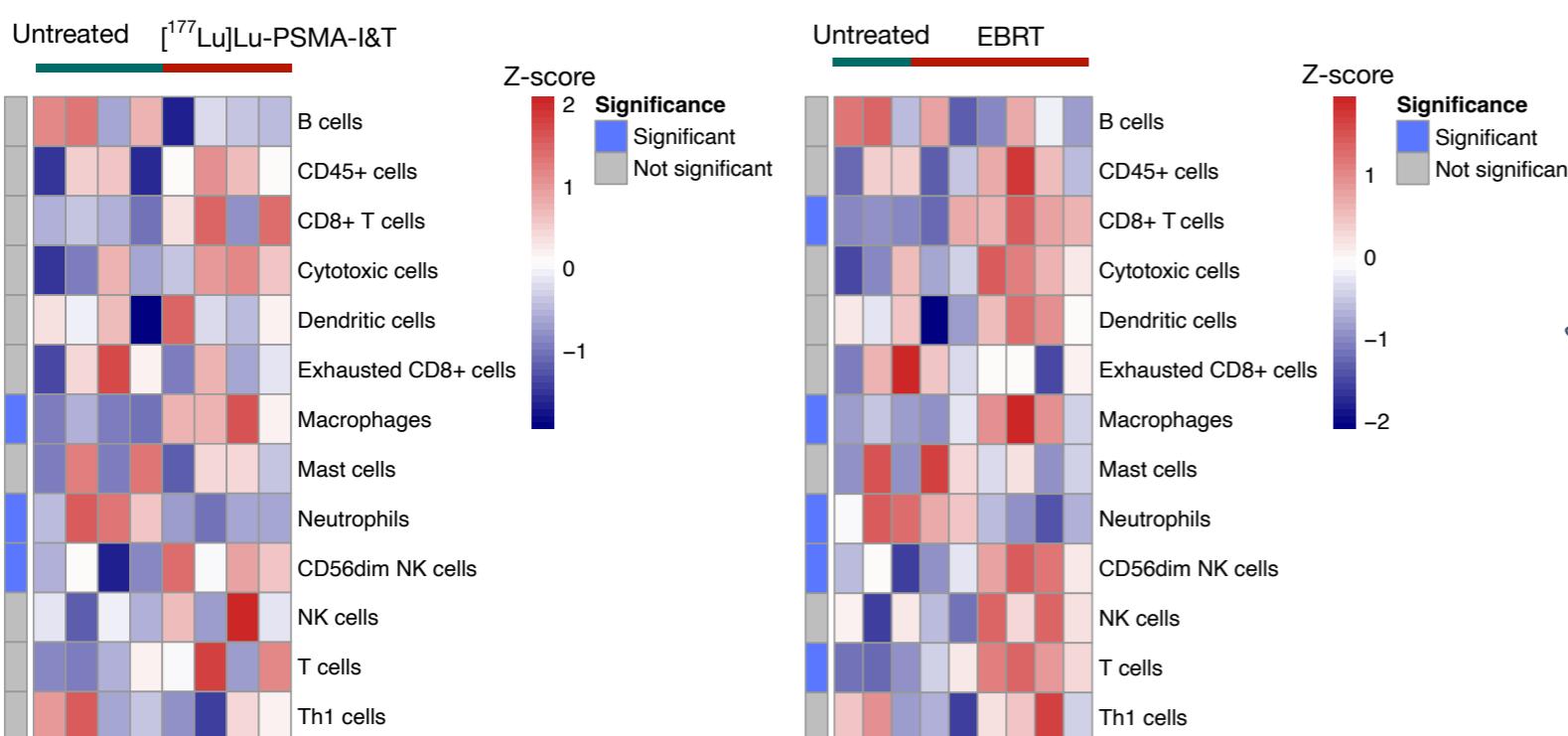
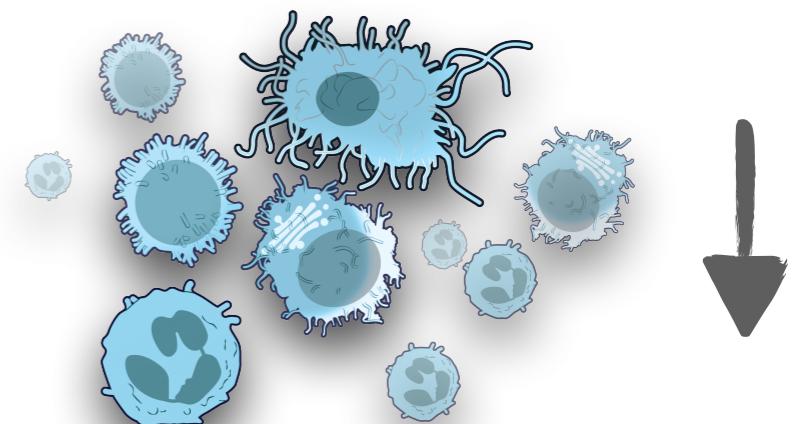


Increased immune cell infiltrations on day 7

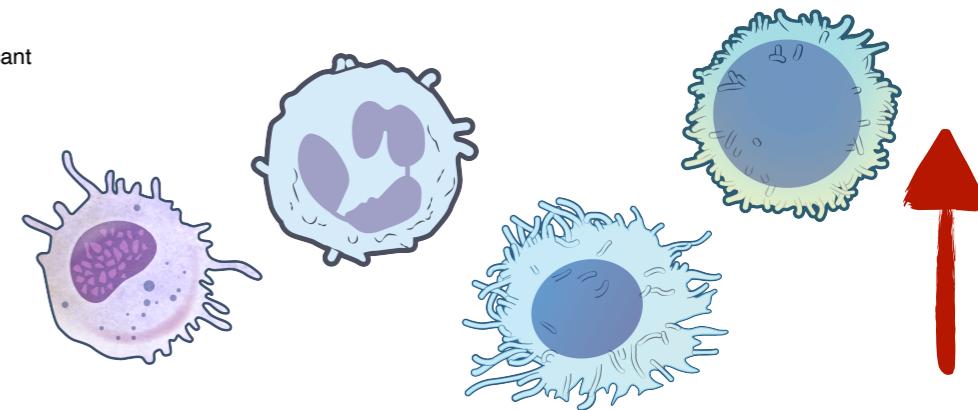
Immune gene signature proposed by Danaher et al.



Decreased TILs

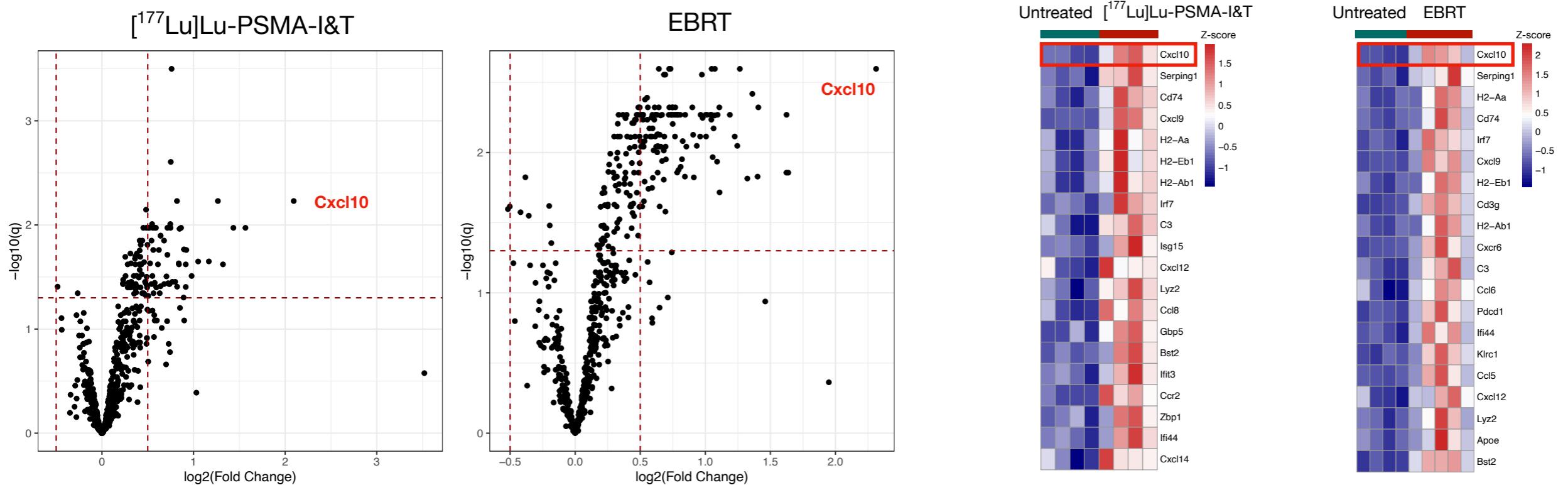


Increased immune cell infiltrations:
macrophages, neutrophils and NK
cells (mixed innate and adaptive
immune cells)

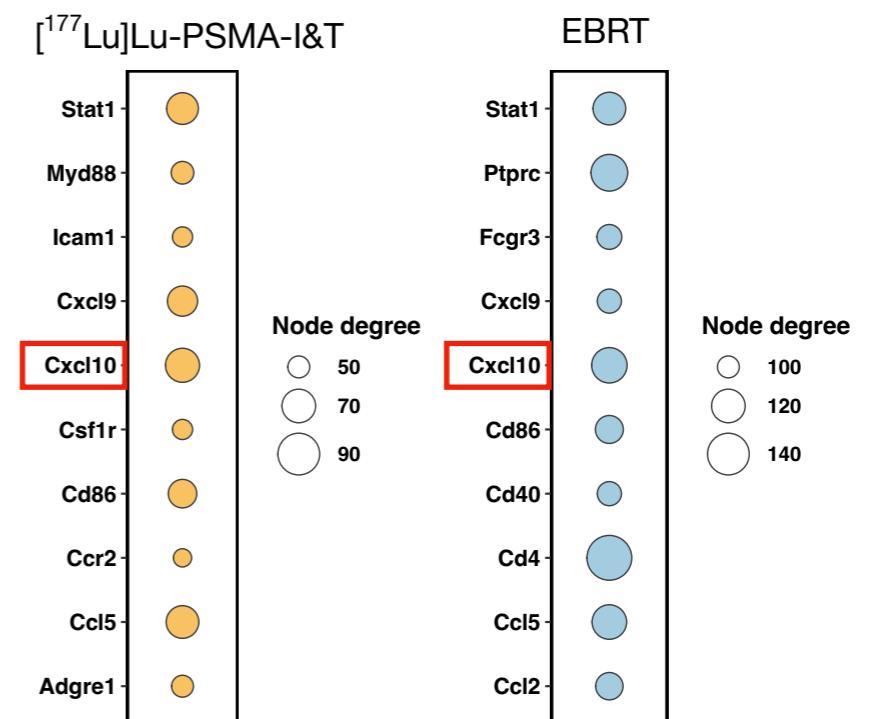
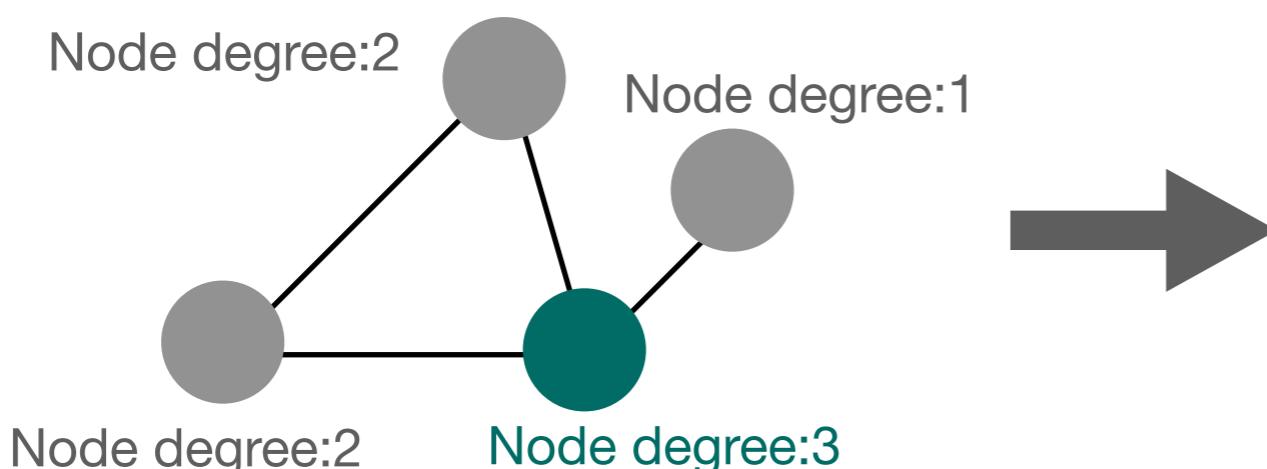


Cxcl10 emerges as the top regulated gene after treatment

Cxcl10 is the hub gene after [¹⁷⁷Lu]Lu-PSMA-I&T and can be expressed in PCa prior treatment

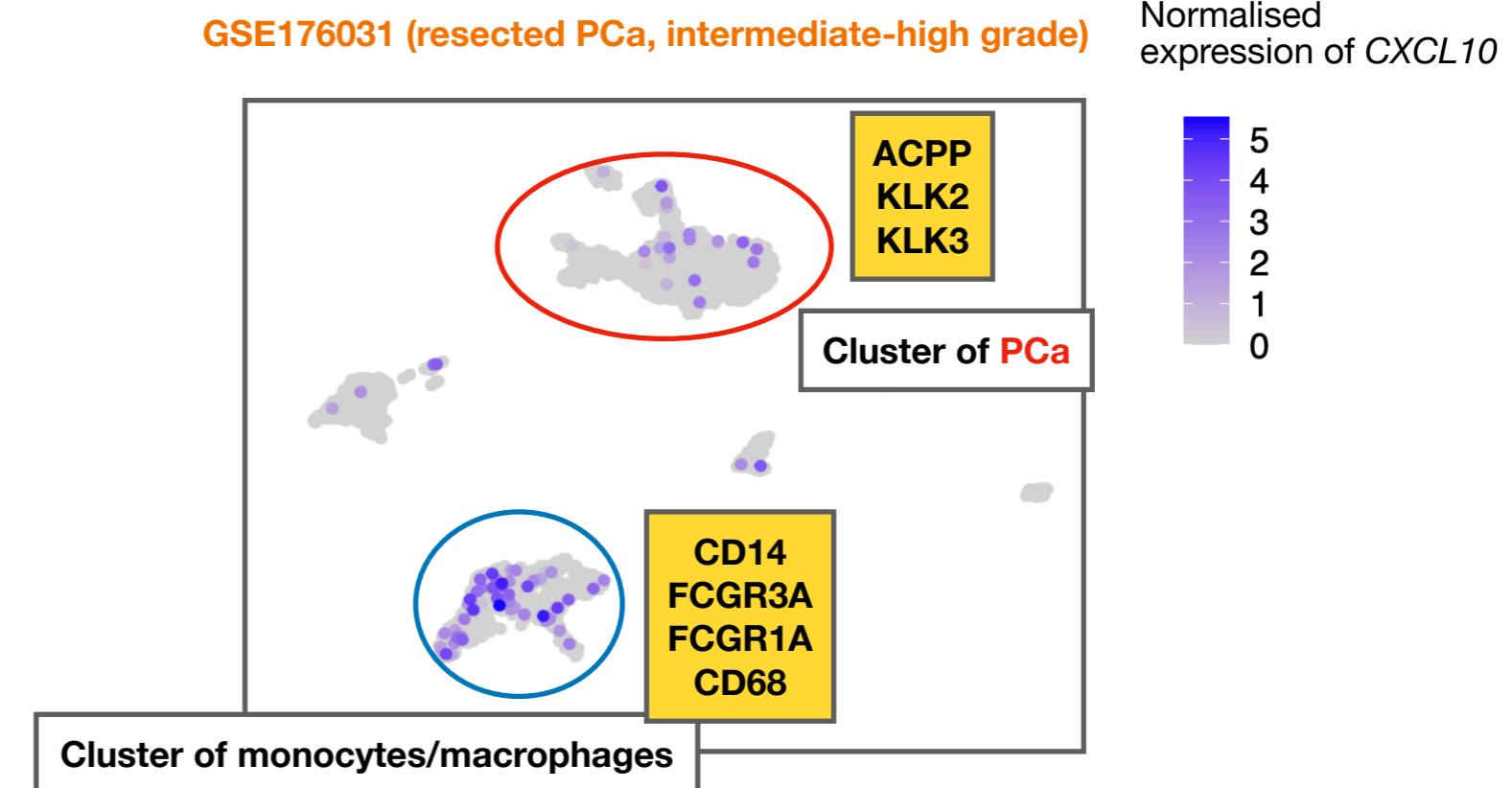
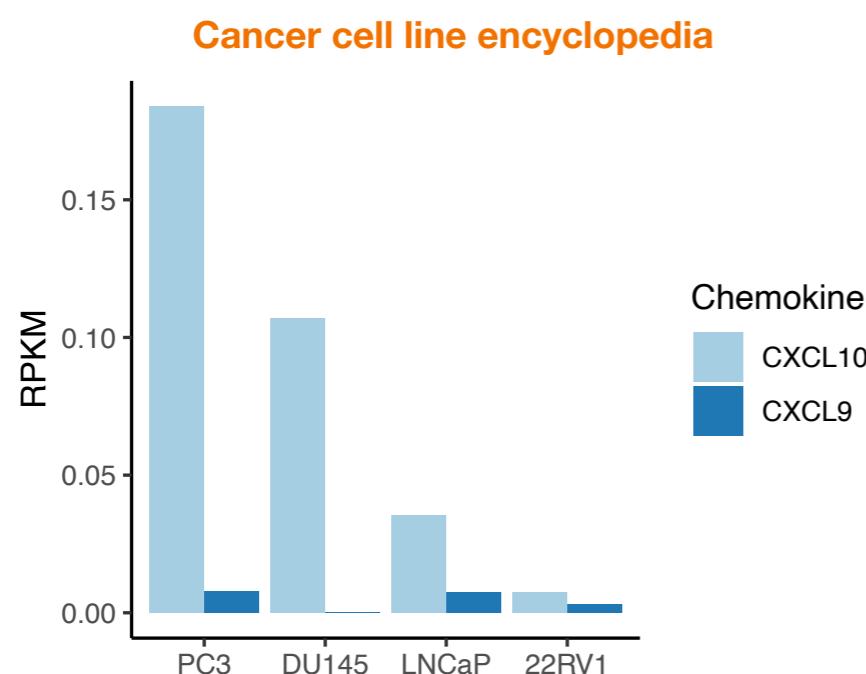
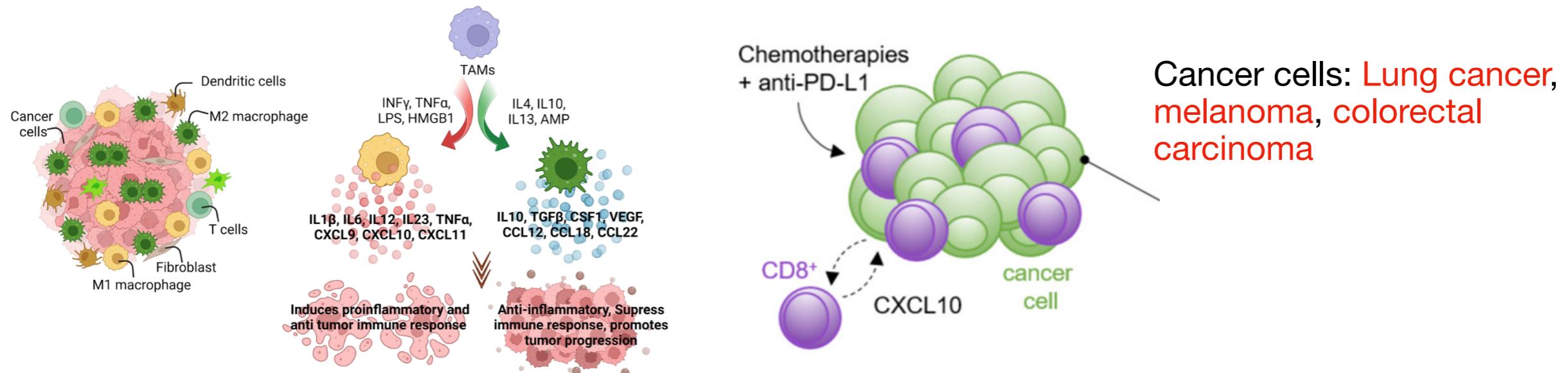


Calculation of network node degree



Cxcl10 recruits CD8+ T cells to tumour

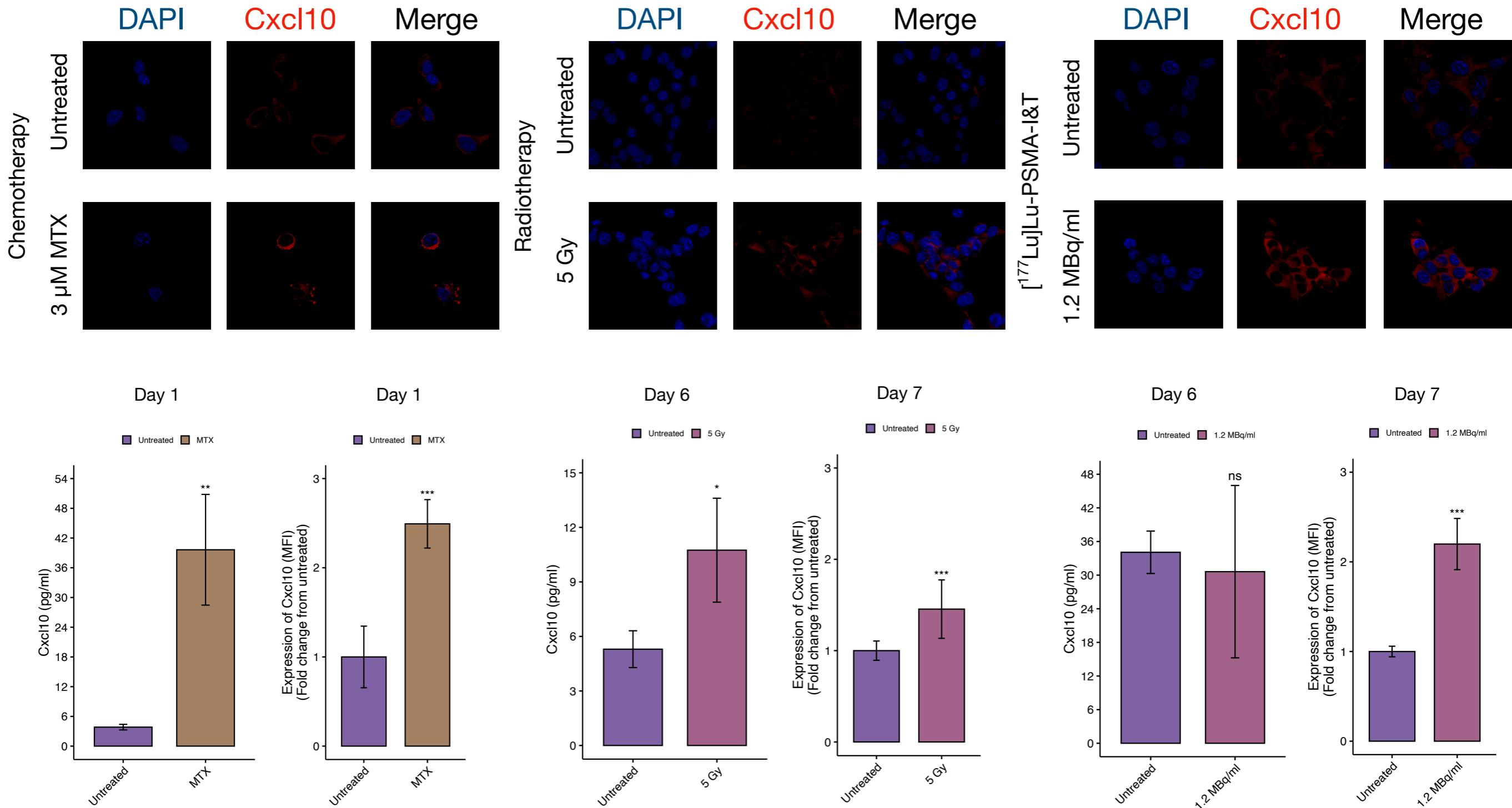
Tumour associated macrophages are the primary source of Cxcl10
Cancer cell can also express and release Cxcl10



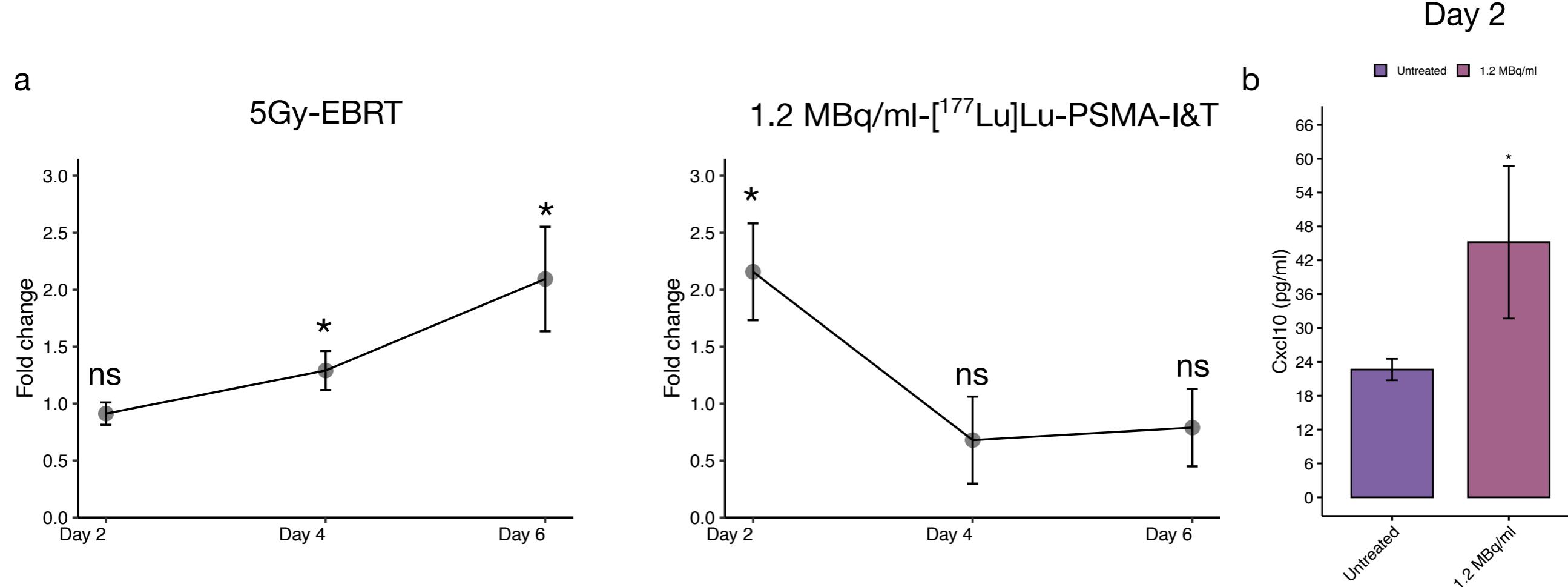
Semin Immunopathol 2023 Mar;45(2):187-201.
Cancer Cell 2022 Feb 14;40(2):136-152.e12.
J Immunother Cancer 2021 Sep;9(9):e003521.

Expression of *Cxcl10* is up-regulated in vitro

Release of *Cxcl10* is increased at specific time points



Release of Cxcl10 diminishes after [¹⁷⁷Lu]Lu-PSMA-I&T post day 2



Summary-2

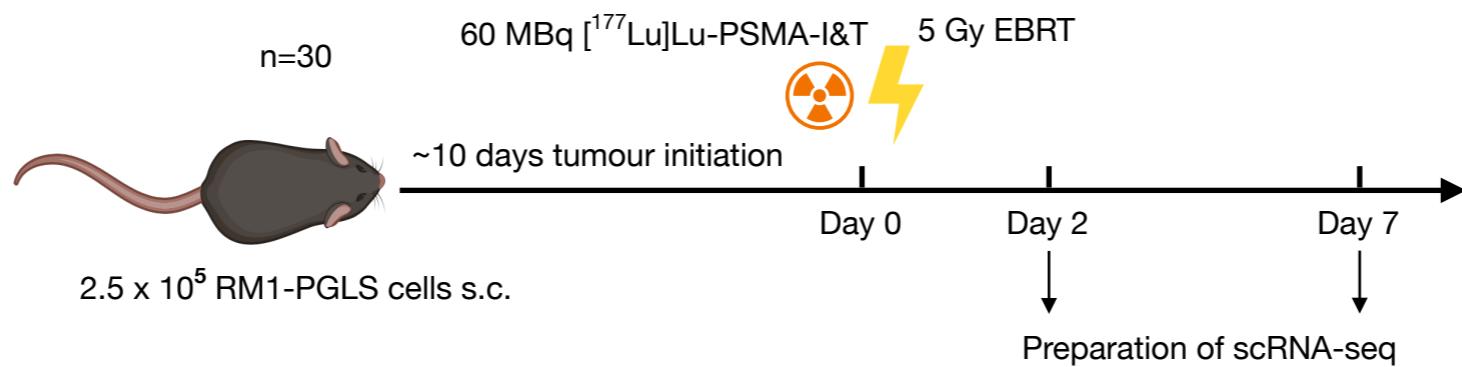
- In a syngeneic mouse model of PCa, [¹⁷⁷Lu]Lu-PSMA-I&T triggers a robust immune response, including antigen-presentation and adaptive immunity, seven days post-treatment.
- [¹⁷⁷Lu]Lu-PSMA-I&T up-regulates and promotes the release of Cxcl10, a critical immune regulator and a potential therapeutic target.
- Expression and release of Cxcl10 can be activated in the PGLS cells by [¹⁷⁷Lu]Lu-PSMA-I&T, though the activity used needs to be optimised.

Future plans and timeline

- Explore the immune landscape after [¹⁷⁷Lu]Lu-PSMA in vivo and identify the source of Cxcl10, using **scRNA-seq**
- Identify therapeutic targets and design **combined treatments with ICIs**.

	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
Year1	Preparations (inductions, cell culture techniques, radiation training, animal work training)				Literature review -> meta-analysis->manuscript drafting and submission				Biodistribution study	Student symposium	ARR conference	Study design for scRNA-seq
	Obtain LuCaP PDX from University of Washington				Characterisation of ICD for [¹⁷⁷ Lu]Lu-PSMA-I&T and EBRT (objective 1&2)							
Year2	Animal study: scRNA-seq and comprehensive data analyses (objective 3-5)				Data-driven initiation of experimental validation (exploration of target cytokine/chemokine and immune subsets) (objective 3-5)				Student symposium			
Year3	Animal study: combination with immunotherapy ((objective 6-9))				Animal study: combination with immunotherapy (objective 6-9)				Student symposium	SNMMI		
Year4	Confirmation				Animal study: establish mouse model for PDX (objective 10)				Animal study: explore immune response and therapeutic effect on PDX model (objective 10)			
	Consider exploring other radionuclide therapy such as Actinium-225				Thesis writing and viva preparation				DPhil viva	Manuscript drafting for submission to journal if necessary		
										Correction		

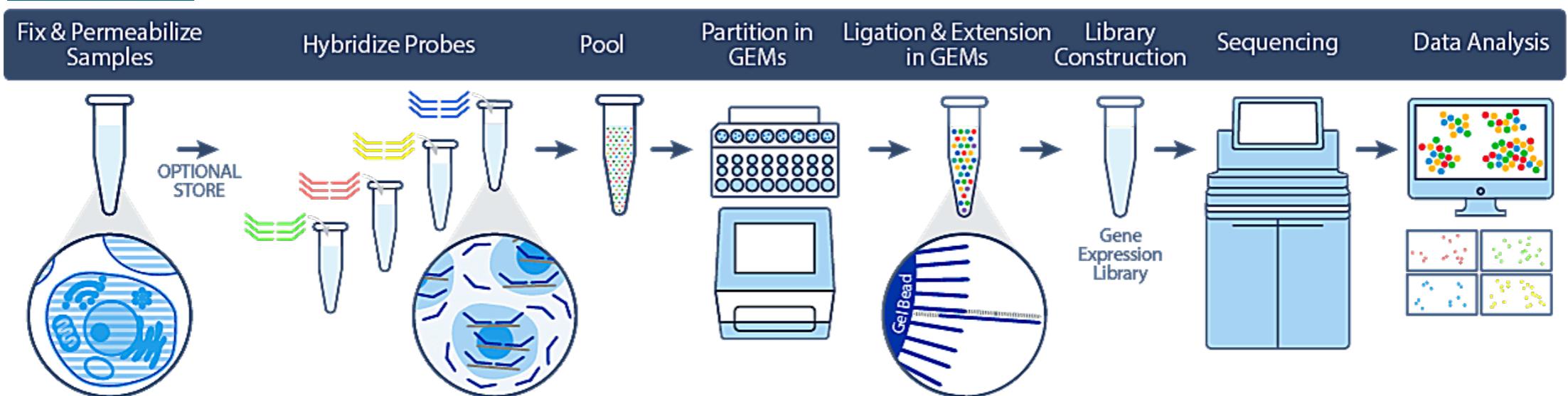
- scRNA-seq and data driven study
- Combined with ICIs; consider using PDX (University of Washington)
- Thesis drafting



Tumours have been harvested on [24/10/24](#) and [31/10/24](#)



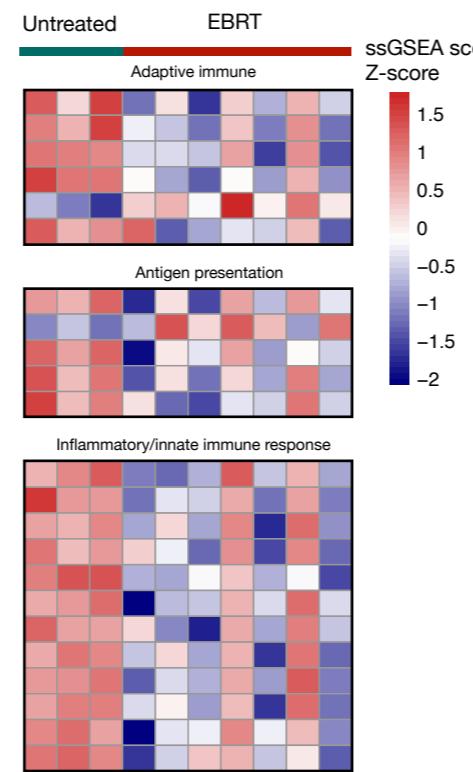
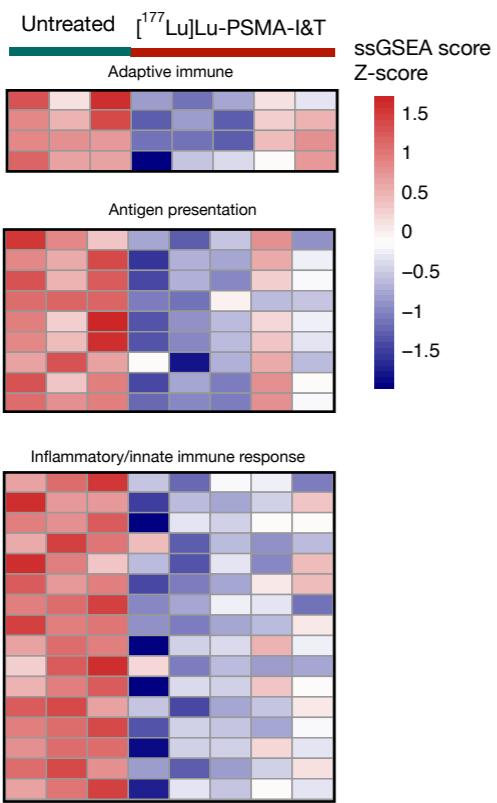
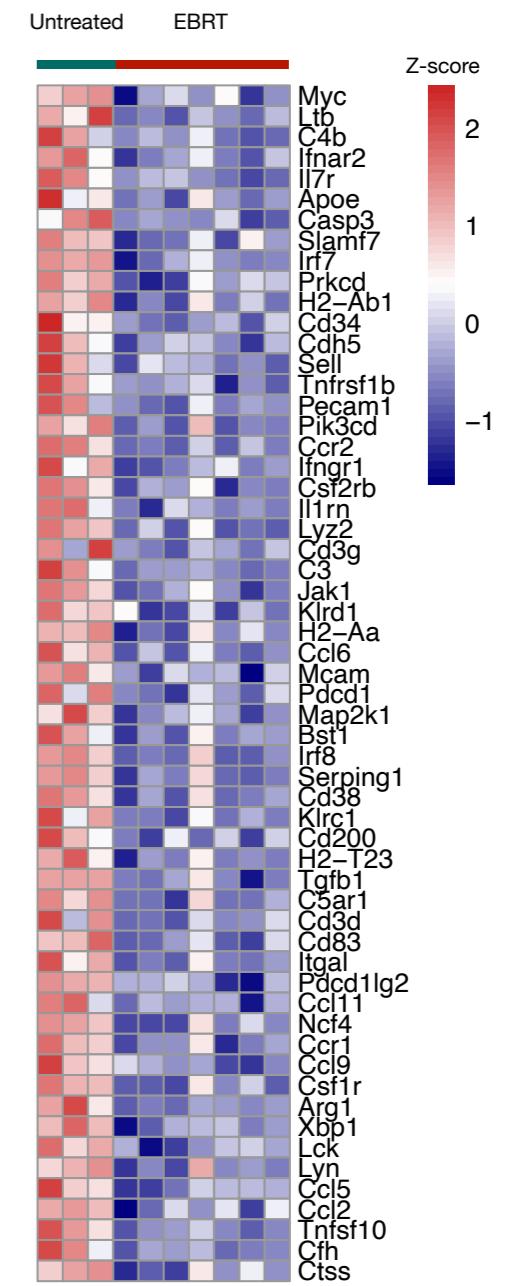
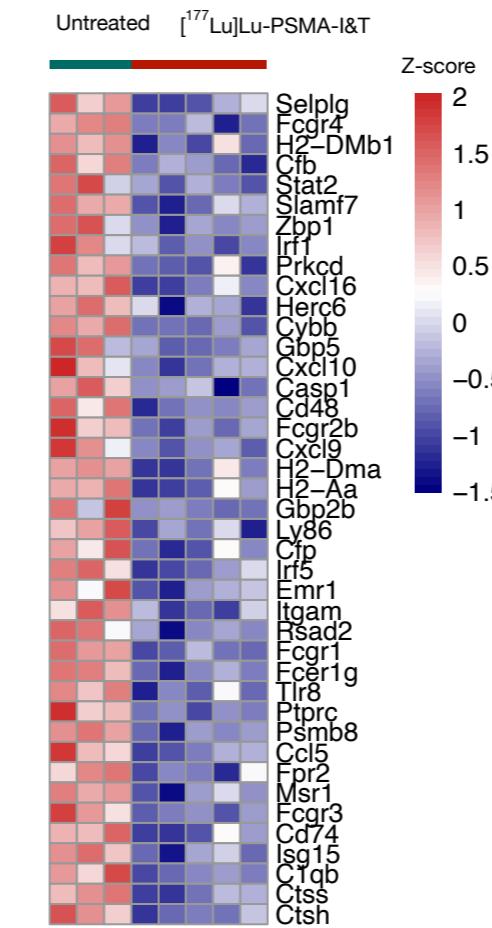
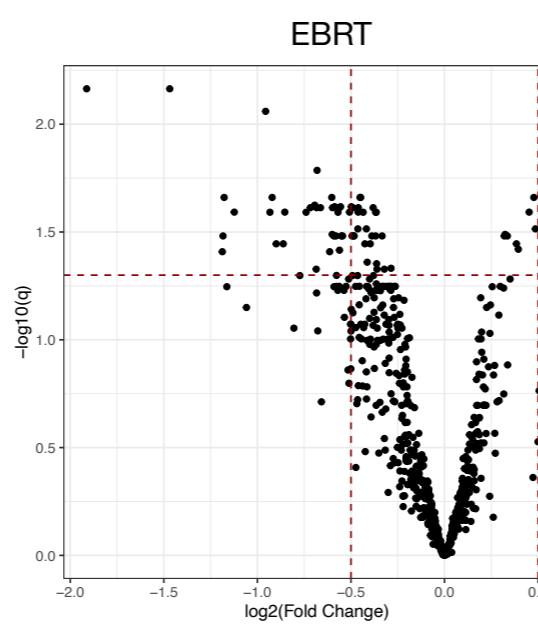
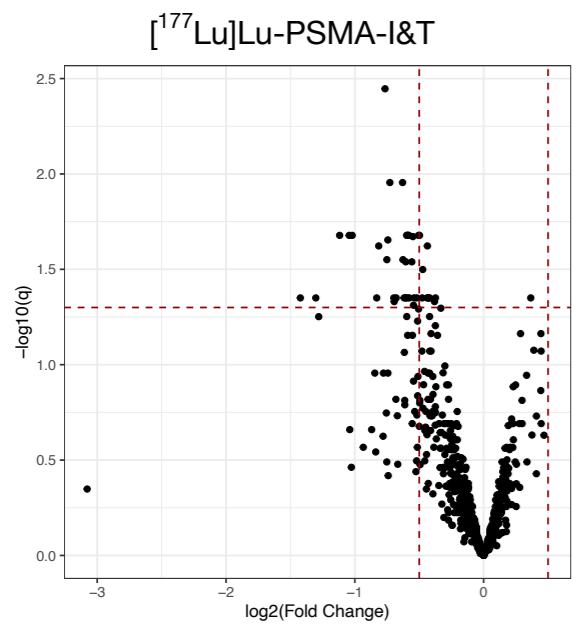
Preparation of scRNA-seq: pending, in collaboration with WIMM



[Expect to have results before March-April, 2025](#)

Additional slides

Supplementary figure



Supplementary figure

