



PRECLINICAL STUDIES OF SUB-CELLULAR TARGETED ^{161}Tb -COMPLEXES FOR CANCER RADIOTHERANOSTICS

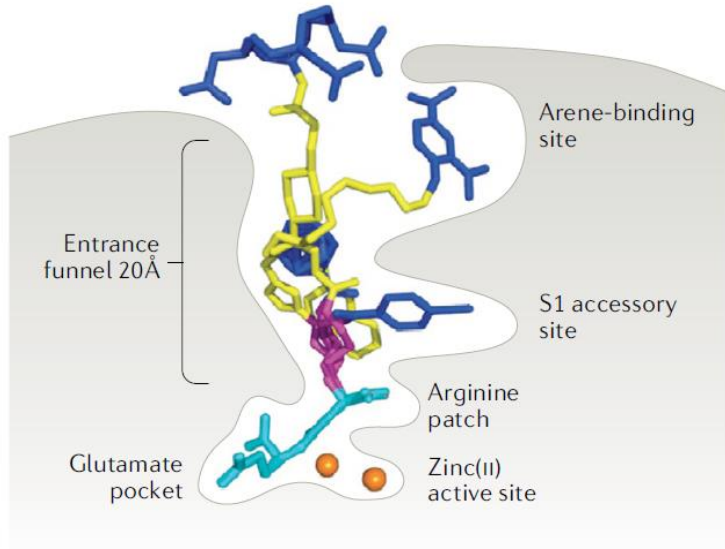
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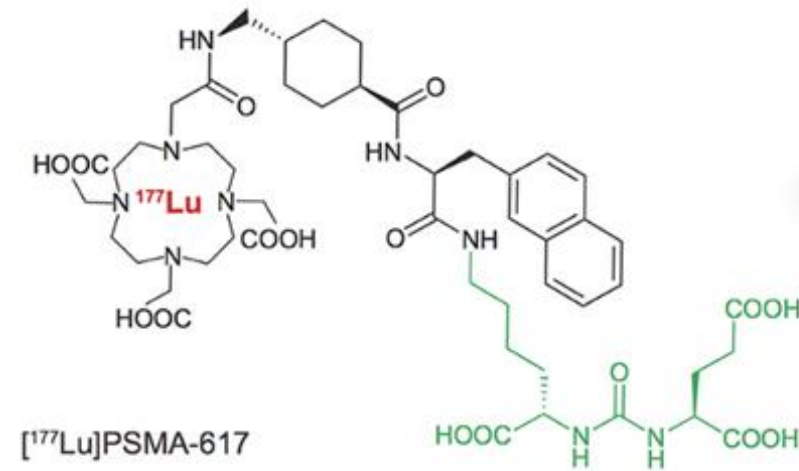
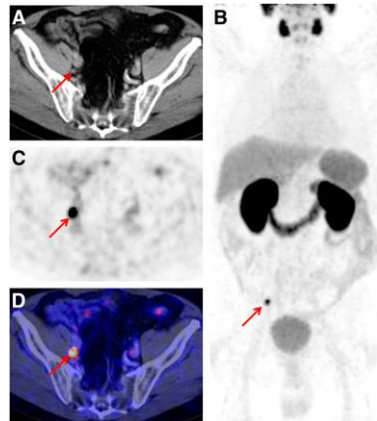
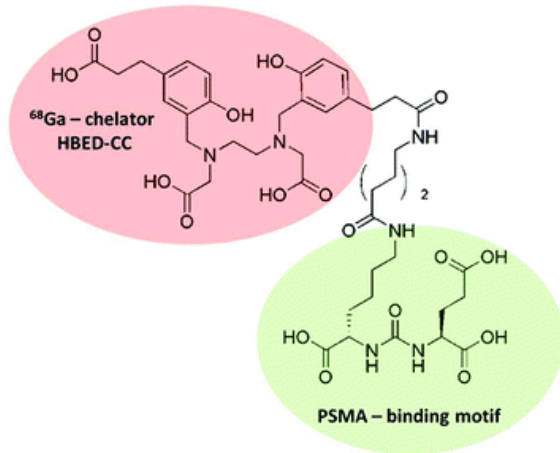
C²TN Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico
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PRISMAP RADIOLANTHANIDES WORKSHOP, PSI, Zürich, 3rd September 2024

Theranostic of Prostate Cancer: PSMA Inhibitors



- ❑ Several PSMA inhibitors containing the Glu-urea-Lys unit (KuE) have been labeled with numerous radionuclides for imaging or therapy, namely with ^{68}Ga and ^{177}Lu .
- ❑ Intense research in this area enabled the development of ^{68}Ga -PSMA-11 or ^{177}Lu -PSMA-617.
- ❑ ^{177}Lu -PSMA-617 (Pluvicto™) has been recently approved by the EMA and FDA.

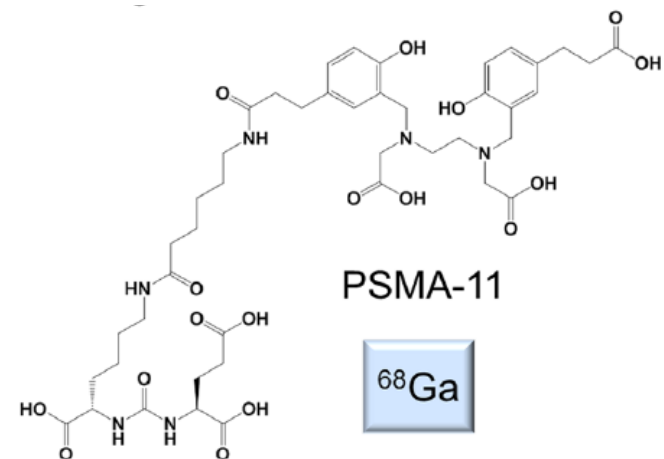
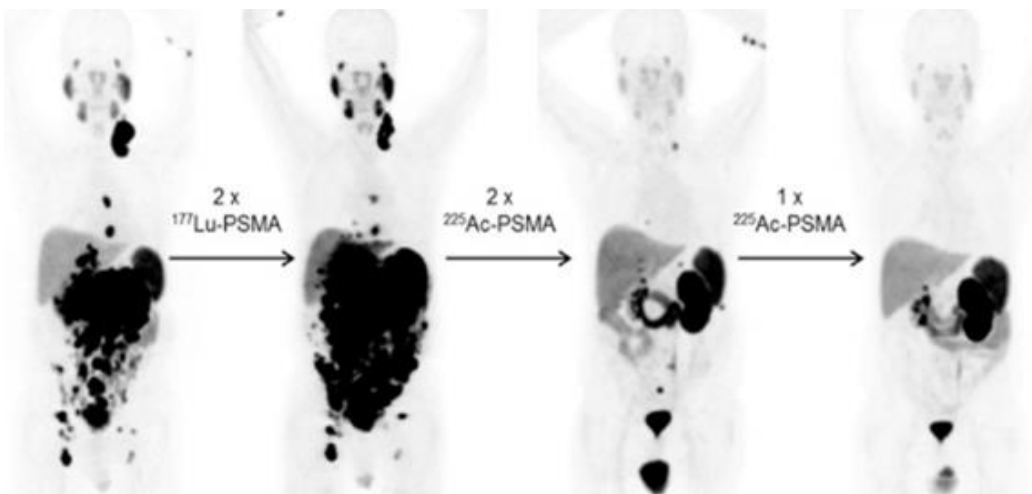


M. Eiber et al., *J Nucl Med* (2015) 56:668–674

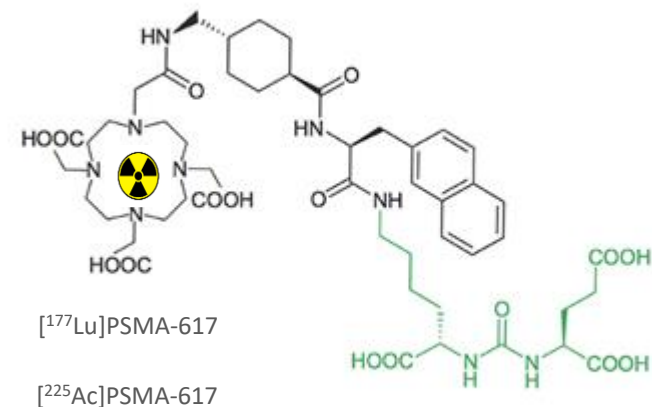
M. Einsenhut, *Bioconjugate Chem.* (2012) 23 (4), pp 688–697

Attard, G. et al., *Lancet* (2016) 387, 70–82

PSMA Inhibitors: beta vs alpha Therapy



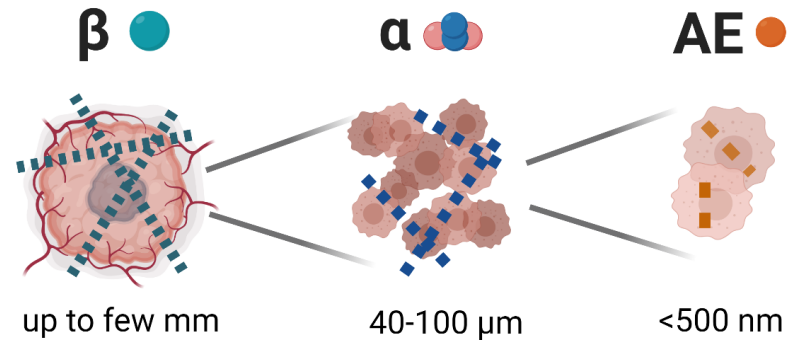
- ❑ Despite the good results obtained with ^{177}Lu -PSMA-617, the use of β - emitters in targeted therapy with radionuclides has some limitations: **nephrotoxicity, resistance to β -radiation** and **low efficacy to eradicate small lesions** .
- ❑ Therapy with α -emitters may be an alternative: promising clinical and preclinical results for ^{225}Ac -PSMA-617.
- ❑ **Alpha Emitters Limitations:** Challenging production, demanding radiochemistry, *in vivo stability* issues.



Auger Electron Therapy: Pros and Cons

- Short range (less than the cell diameter) and high LET of Auger electrons (AE) are favourable features for targeted radionuclide therapy (TRT), namely to eradicate **small tumors, metastases** and even non-imageable **small clusters of tumor cells**.

Bolcaen et al., *J Nucl Med.* 2023;64(9):1344-1351.

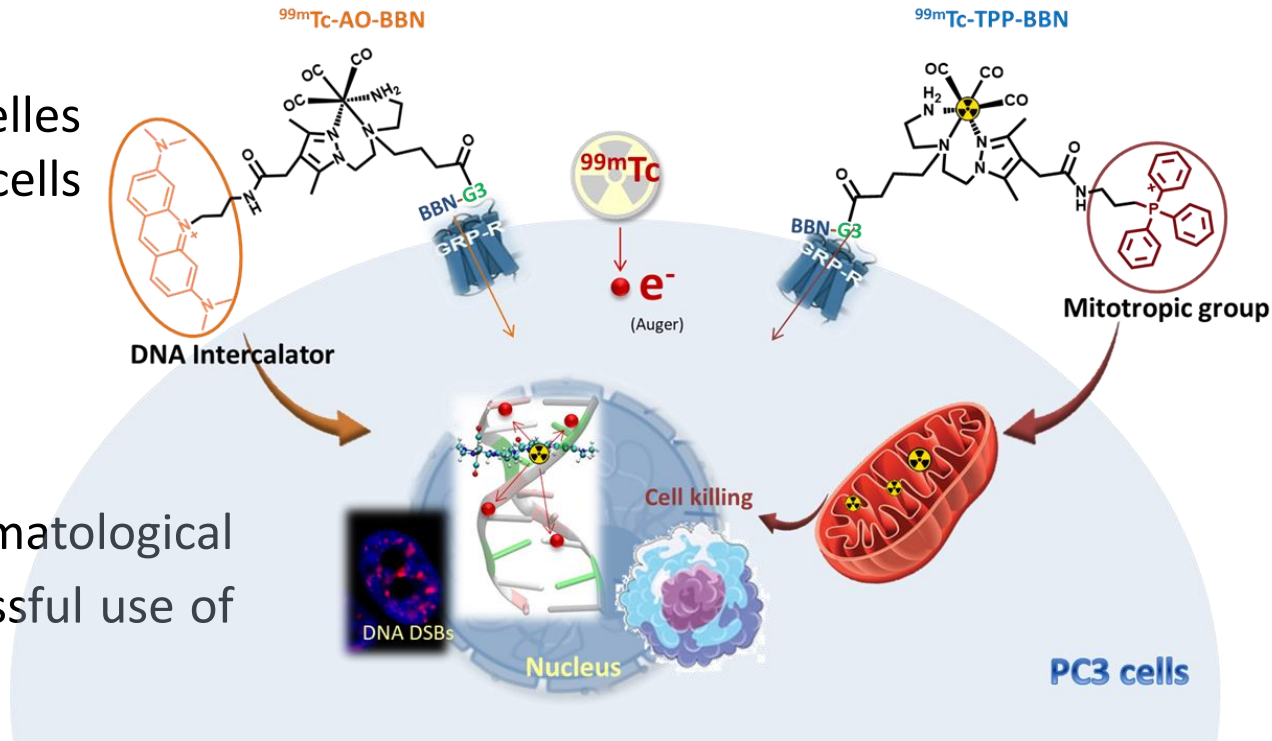


- Biological Effects (e.g., induction of cell death) are strongly dependent on the subcellular localization of AE emitters and distance to radiosensitive targets (e.g., **cell membrane, nucleus or mitochondria**).
- Many Auger emitters correspond to commercially available radionuclides in use for diagnostic (e.g. ^{123}I , ^{67}Ga , $^{99\text{m}}\text{Tc}$ or ^{111}In): **Theranostic potential**.
- New and more suitable Auger emitters start to be available (e.g., ^{161}Tb , $^{195\text{m}}\text{Pt}$, ^{197}Hg , $^{103\text{m}}\text{Rh}$, ^{165}Er).
- A great challenge is to avoid undue irradiation of non-target tissues by γ -photons often emitted by AE emitting radionuclides.

Organelle-Targeted AE-Emitting Radioconjugates

□ Specific delivery of AE emitters to radiosensitive organelles (**cell membrane**, **nucleus** or **mitochondria**) of cancer cells might enhance radiotherapeutic effects at lower doses.

□ Minimization of undesired side effects (e.g. hematological toxicity, kidney damage or cardiotoxicity), for a successful use of AE emitters in TRT.



Fernandes et al., *Int J Mol Sci.* 2022;23(13):7238

□ **Challenge:** impact of organelle-specific moieties in the PKs and biodistribution of the radioconjugates.

^{161}Tb vs ^{177}Lu

Radionuclide	^{177}Lu	^{161}Tb
Half-life (day)	6.647	6.906
Type of decay (%)	β^- (100%)	β^- (100%)
β particles mean energy (keV)	133.3	154.3
Daughter	^{177}Hf (stable)	^{161}Dy (stable)
CE (keV per decay)	13.52	39.28
CE energy range in keV (weighted average energy) ^a	6.2 – 206.3 (87)	3.3 – 98.3 (28)
AE (keV per decay)	1.13	8.94
AE energy range in keV (weighted average energy) ^a	0.01 – 61.7 (1)	0.018 – 50.9 (0.8)

Hindié et al., *EJNMMI Physics* **2020**, 7:33

- ^{161}Tb associates the traditional advantages of a medium-energy β^- emission spectrum with the additional benefit of a high localised dose provided by **conversion and Auger electrons (AE)**.
- Very encouraging results for ^{161}Tb -**PSMA-617** (PSMA inhibitor) and ^{161}Tb -**DOTA-LM3** (SST2-antagonist) undergoing clinical trials.

Van Laere et al., *Theranostics*. 2024;14(4):1720-1743

"Dual-Targeted" ^{161}Tb complexes

P6
Dual-Targeting Strategy for the Nuclear Delivery of Trivalent Radiometals to Prostate Cancer Cells

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PROSTATE CANCER AND PSMA

- Recent approval of ^{177}Lu -PSMA-617, Pluvicto, targeting the Prostate-Specific Membrane Antigen (PSMA) overexpressed in the majority of prostate cancer (Pca) and its metastases [1]
- Limitations of targeted radionuclide therapy with β^- emitters like ^{177}Lu , nephrotoxicity and resistance in a non-negligible number of patients [2,3]
- Attractive alternative: AUGER THERAPY
- DNA is the canonical target for biological damage induced by ionizing radiation (IR)
- Auger electrons (AE) have a short path length and AE emitters become more effective when placed near the nuclear DNA [2]
- Enhancement of radiotherapeutic effects at lower doses

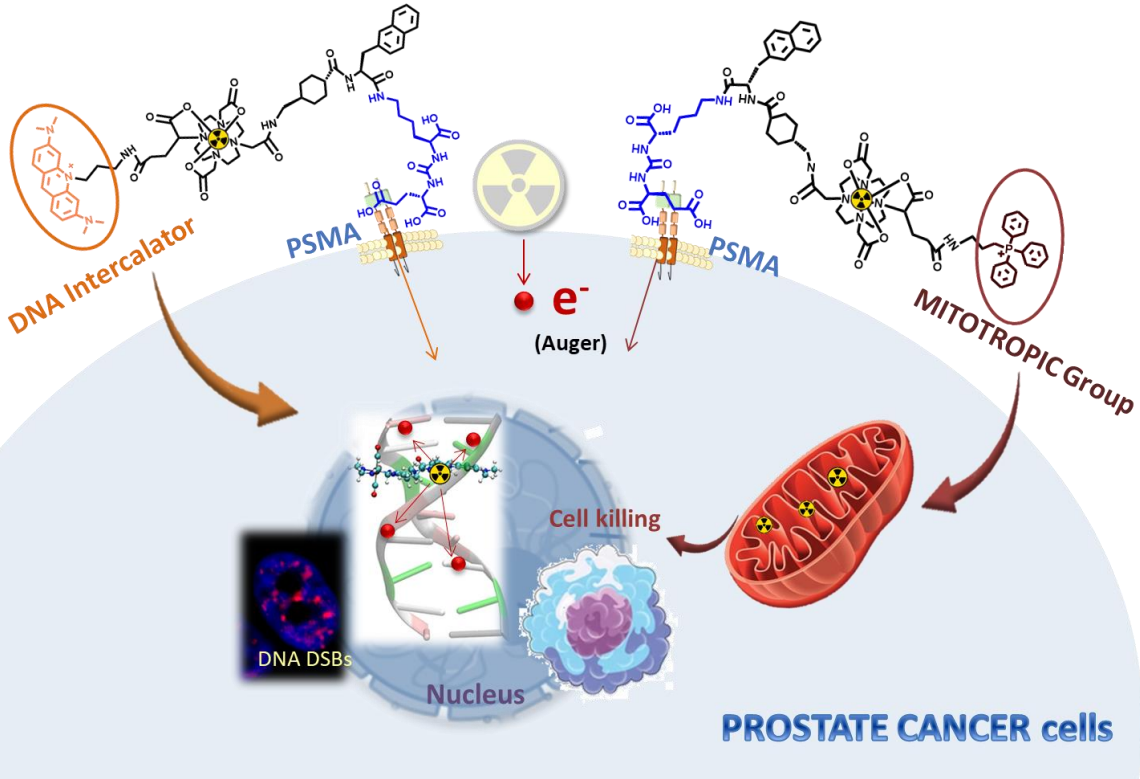
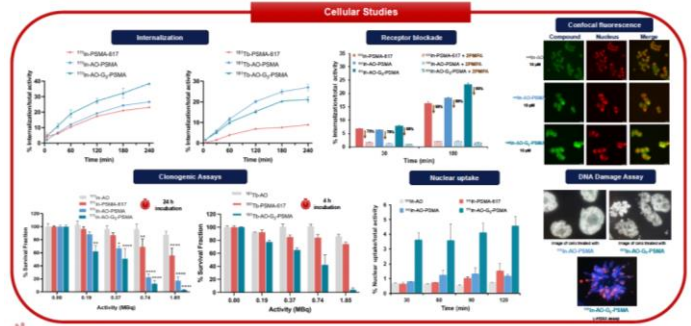
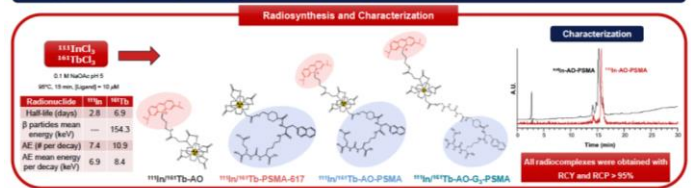
Assess dual targeted radiocomplexes carrying a PSMA inhibitor for selective uptake by PCa cells and an Acridine Orange (AO) group for accumulation in the nucleus aiming to obtain enhanced radiobiological effects by the AE emitter ^{161}Tb or the mixed β^- -AE emitter ^{169}Tb .

results with $^{99\text{m}}\text{Tc}$ -BBN radioconjugates, we have developed ^{161}Tb AO pharmacophores to promote the uptake of Prostate cancer cells.

P6 Day 2

P5 Day 2

advantage of AEs emitted by ^{161}Tb to obtain selective compared with single-targeted congeners to improve therapeutic



P5
Comparison of the biological performance of In-III and Tb-161 radiocomplexes as prostate cancer radiotherapeutics

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Introduction

- ^{177}Lu -PSMA₆₁₇ (Pluvicto), approved by FDA/EMA for targeted radionuclide therapy of Prostate Specific Membrane Antigen (PSMA) positive prostate cancer (PCa) and its metastases [1, 2]
- ^{161}Tb is a beta-minus emitter that also emits conversion electrons and Auger electrons (AE), potentially enhancing therapeutic effects at lower administered doses [3]. ^{161}Tb emits AE but does not emit beta minus particles.
- AEs have high linear energy transfer over a nanometric range, being most effective when they are emitted in close proximity to highly radiosensitive organelles, such as the mitochondria [4]

Radiosynthesis and Characterization

All of the radiocomplexes were obtained with RCY and RCP > 95%

Radioisotope	^{161}Tb	^{169}Tb
Half-life (days)	2.8	6.9
β^- particles mean energy (keV)	—	154.3
AE (per decay)	7.4	10.9
AE mean energy (per decay) (keV)	6.9	8.4

Biological Evaluation

Internalization

Mitochondrial Uptake

DNA Damage

Clonogenic Assay

uSPECT/CT Imaging

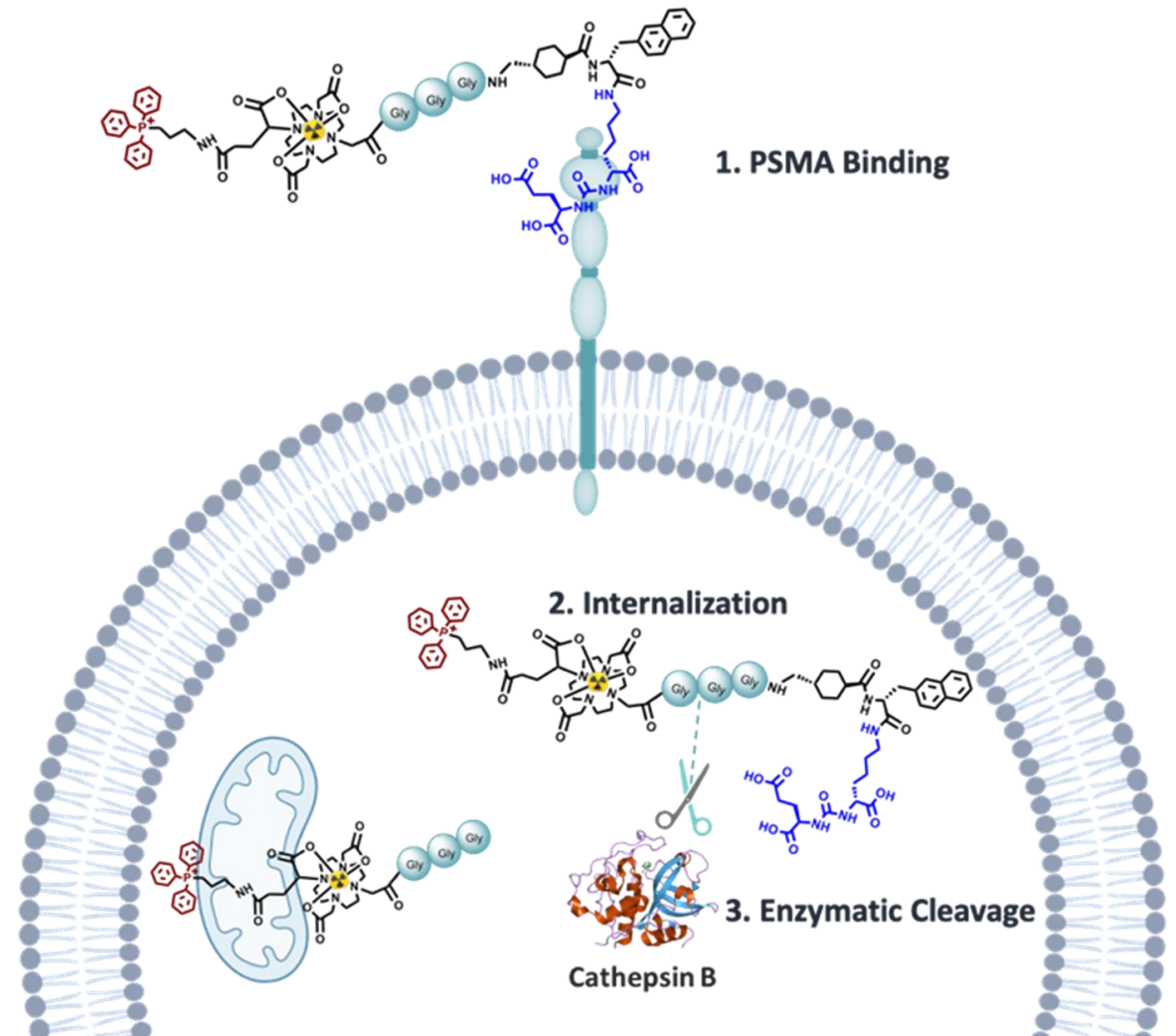
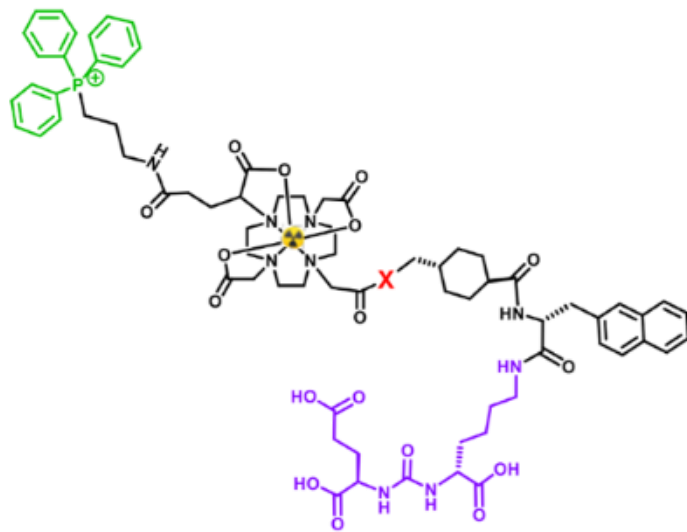
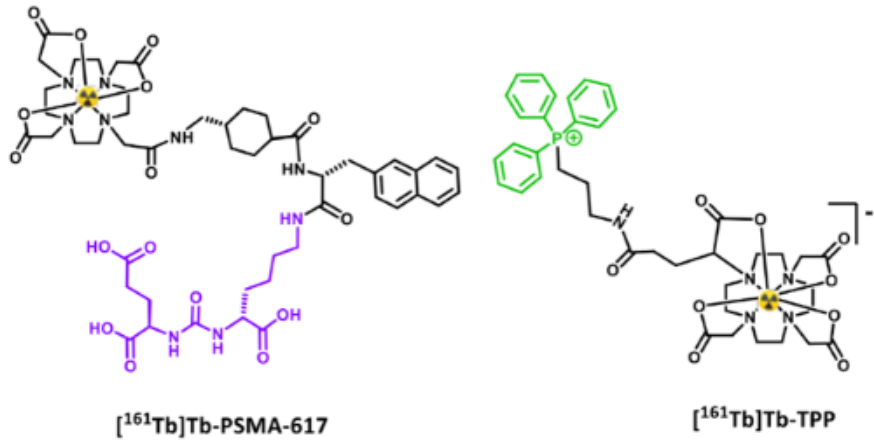
Autoradiography

Conclusions

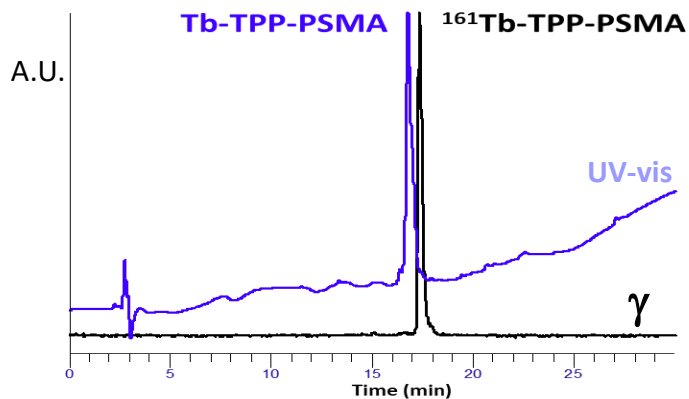
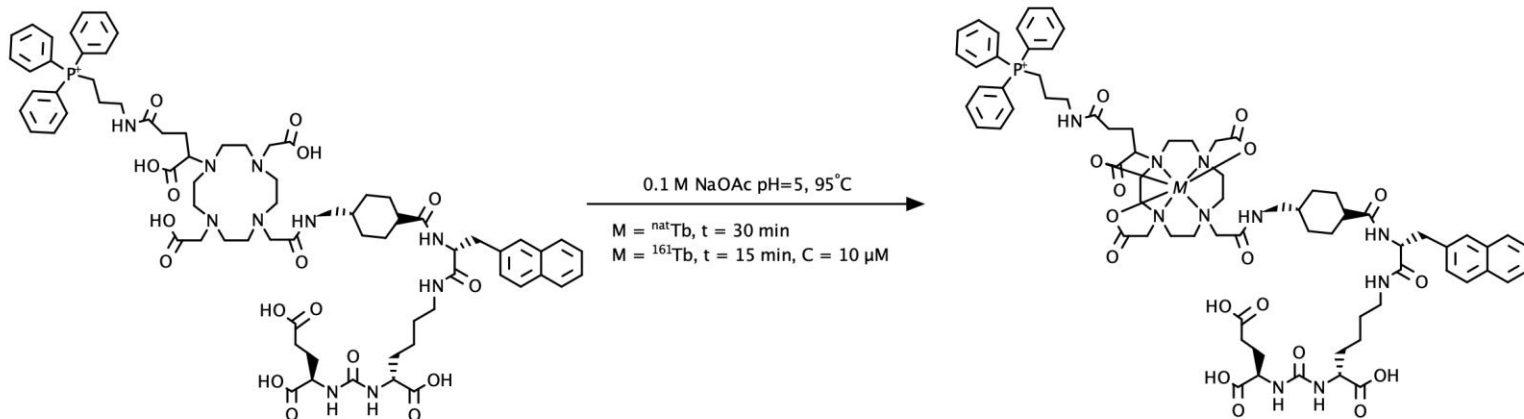
- New families of PSMA-targeted radiocomplexes were obtained with high yield and radiochemical purity and excellent *in vitro* stability.
- The PSMA-targeted ^{161}Tb -complexes showed high specific cellular uptake and internalization in prostate cancer cells.
- The radiocomplexes carrying the TPP showed increased mitochondrial uptake, in accordance with the enhanced radiobiological effects observed.
- In vivo* uSPECT data show that the radiocomplexes present high tumor uptake, as confirmed by *in vivo* autoradiography, with more efficient clearance from non-target tissues and kidneys when labeled with ^{161}Tb .
- Overall, these results are indicative of the potential of these radiocomplexes for Auger therapy of cancer.

^{161}Tb complexes Carrying Mitotropic (TPP) Units

☐ Synthesized and Evaluated Complexes

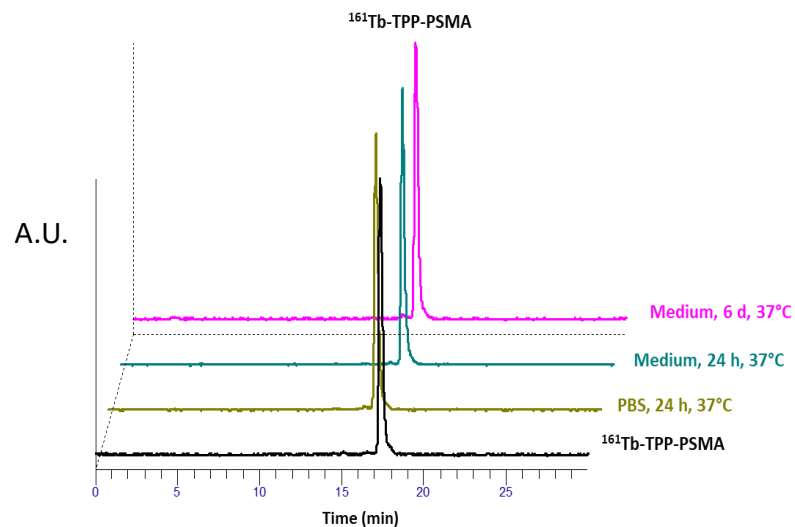


Radiolabeling/ In vitro Stability Studies



- ✓ Non-radioactive congeners characterized by ESI-MS analysis.
- ✓ HPLC co-injection runs of ^{nat}Tb and ¹⁶¹Tb complexes confirmed the chemical identity of the radioactive ones.

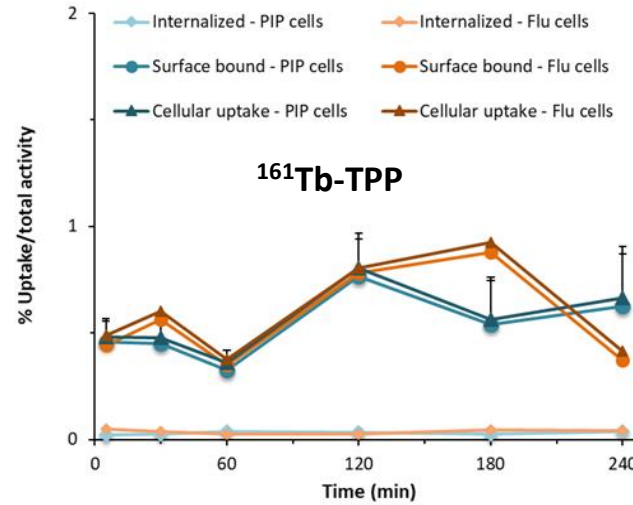
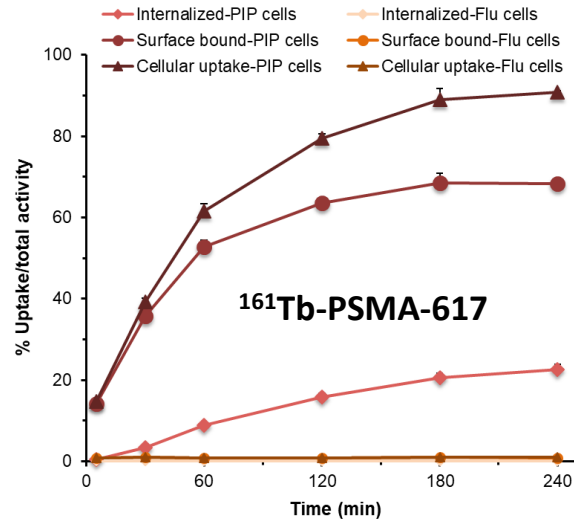
In vitro stability in PBS and cell culture medium



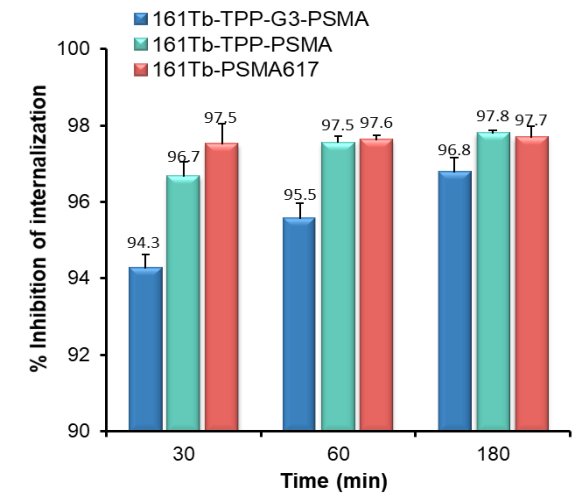
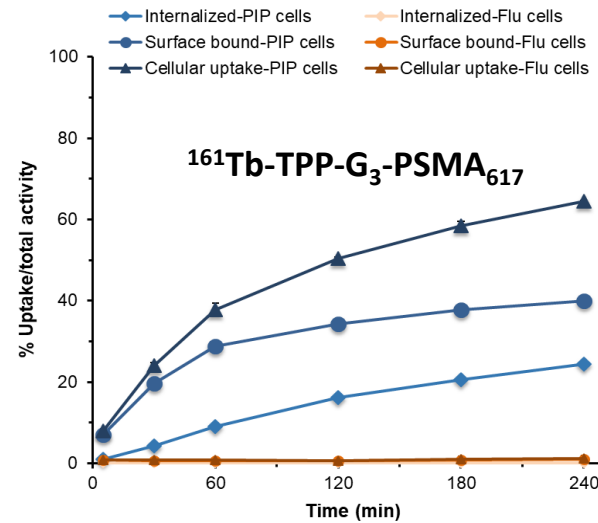
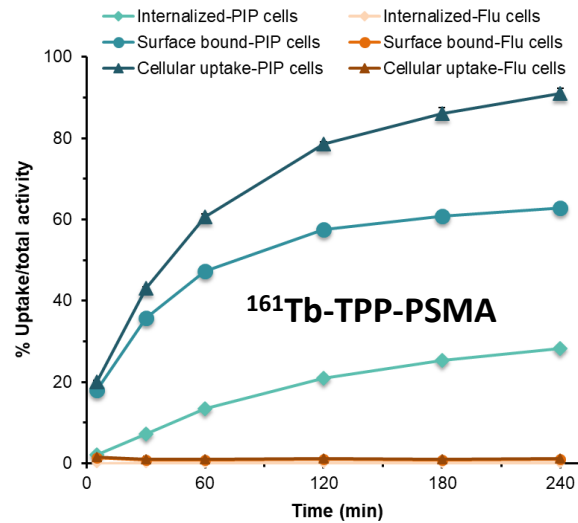
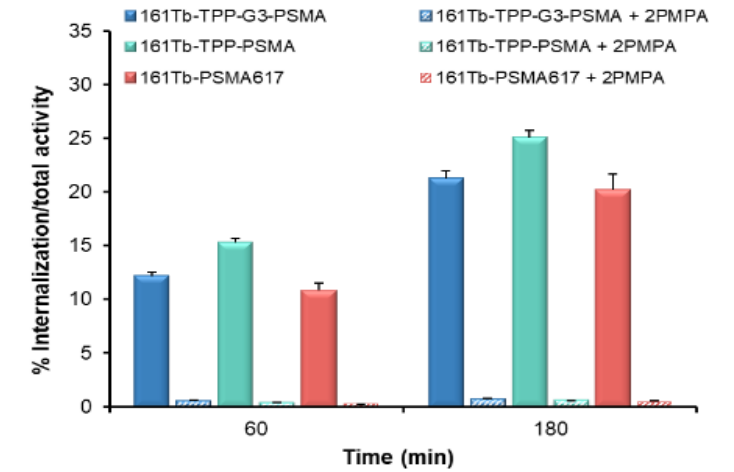
- ✓ High RCY/RCP
- ✓ High Specific Activity
- ✓ High In Vitro Stability

Cellular Studies: Uptake, Internalization and Blockade Assays

Uptake and internalization in PC3 PIP cells (PSMA+) and PC3 flu cells (PSMA-)



PSMA-blocking study in PC3 PIP cells



Cellular Studies: Mitochondrial uptake

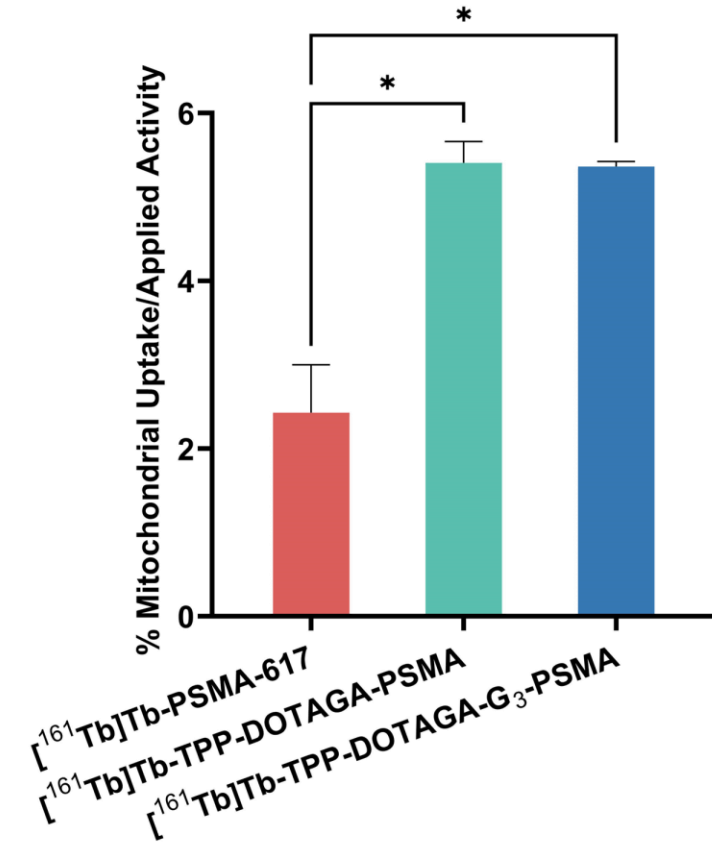
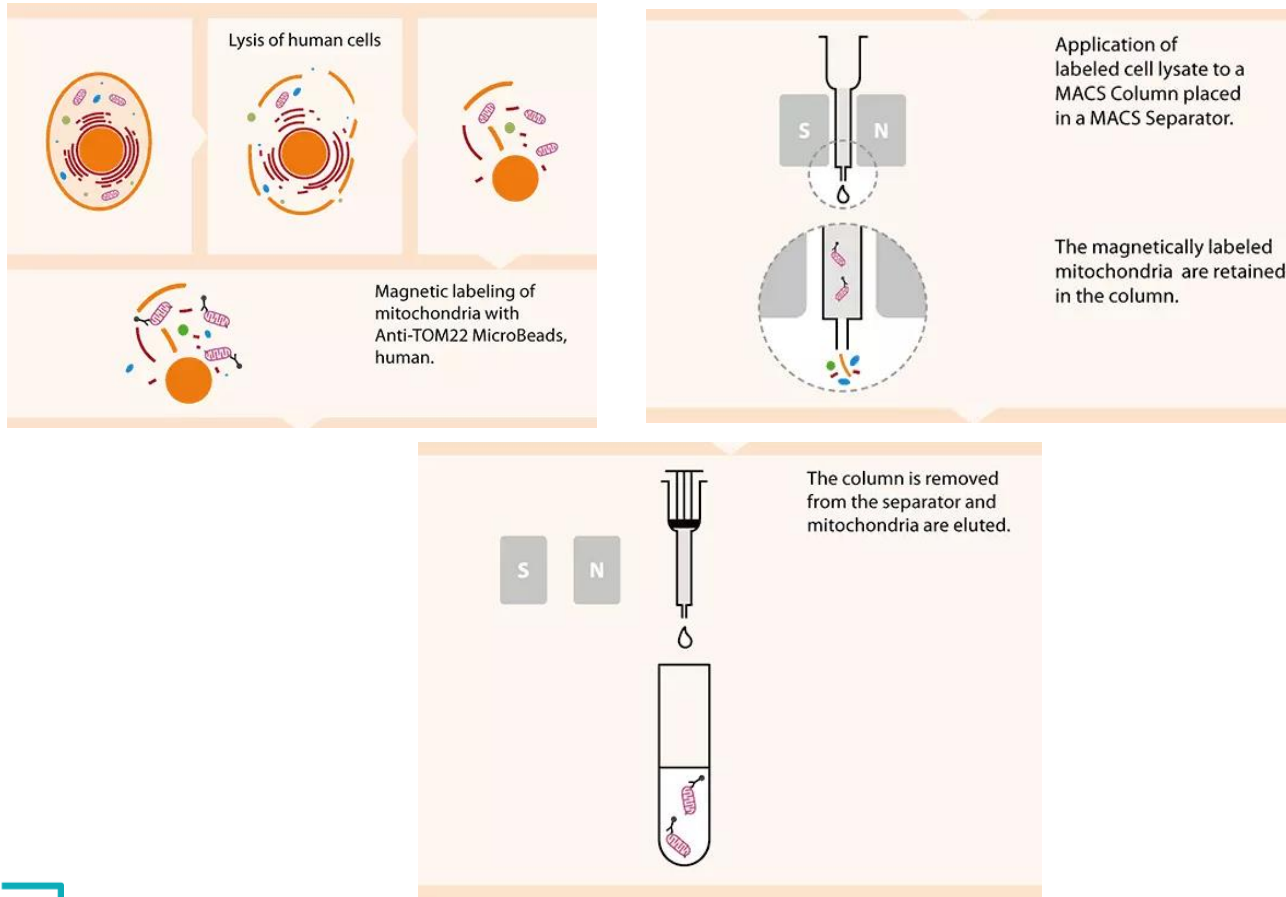
■ Mitochondrial Uptake



1 h

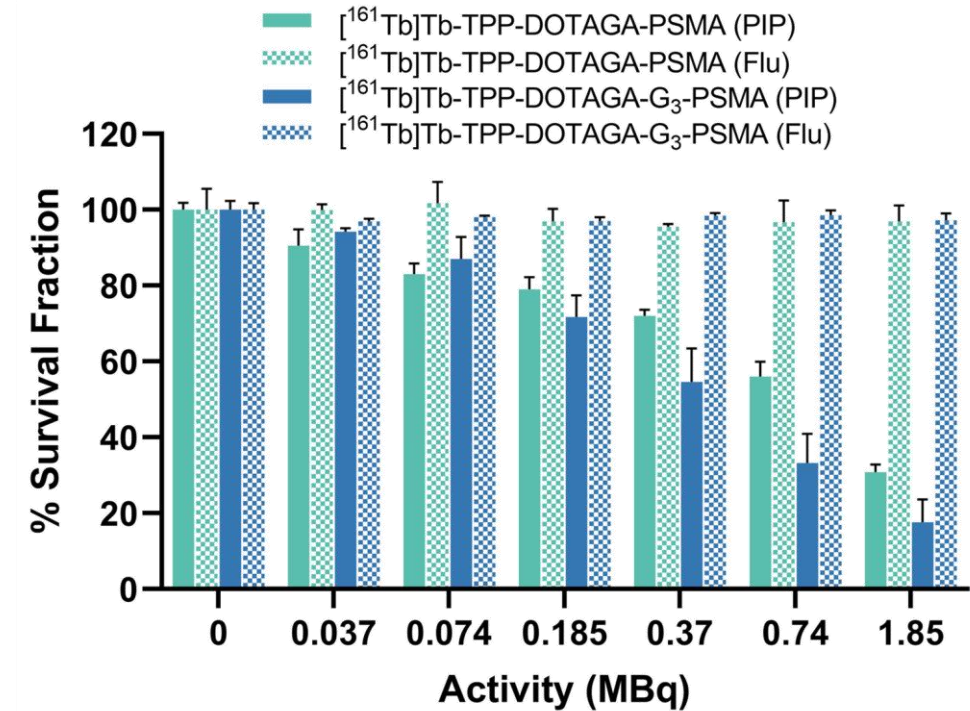
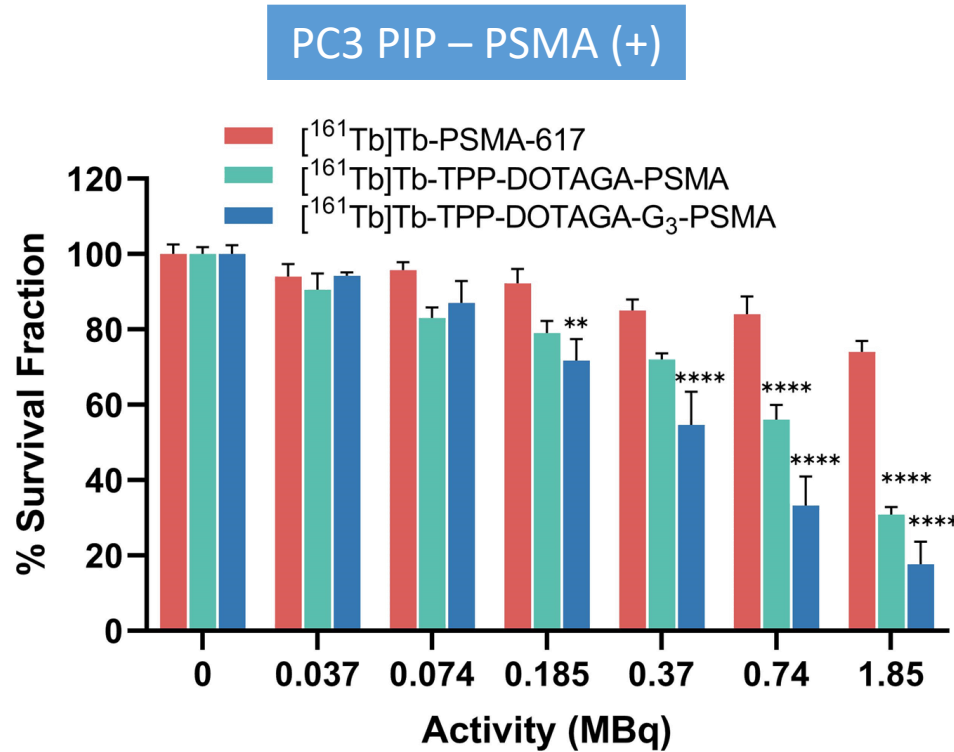
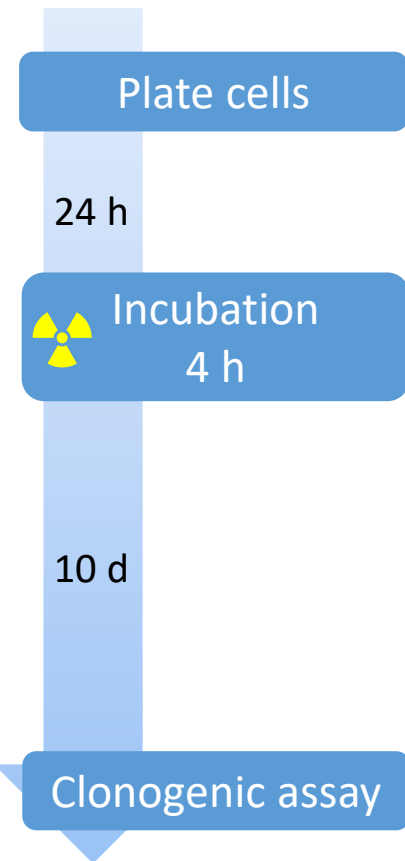
PC3-PIP cells

Mitochondria Isolation Kit (using magnetic beads)
(Miltenyi Biotec)



Cellular Studies: Radiobiological Effects

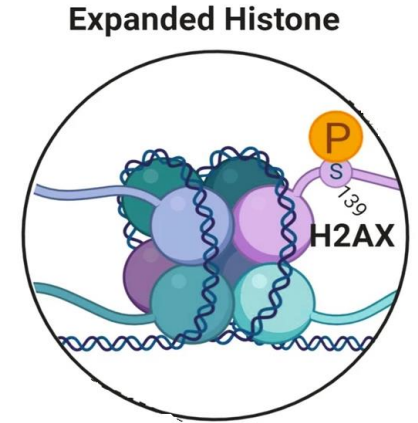
- ❑ **Clonogenic assay:** assess the ability of cells to divide and form colonies upon exposure to the radioconjugates.



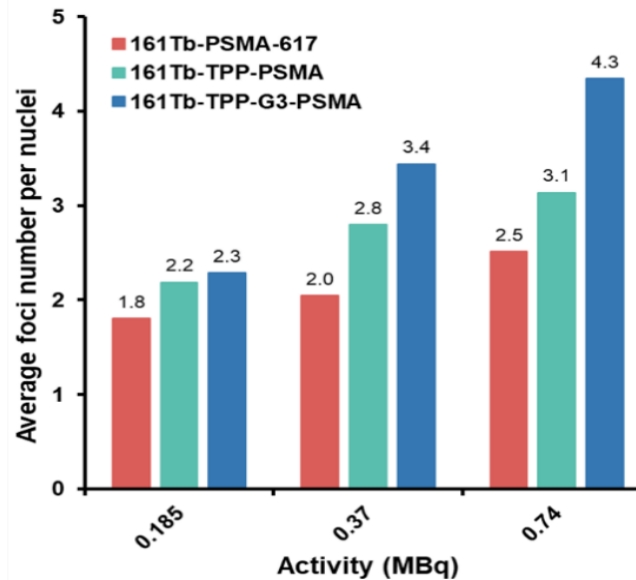
- ❑ The dual complexes ¹⁶¹Tb-TPP-PSMA and ¹⁶¹Tb-TPP-G₃-PSMA induces stronger radiobiological effects than ¹⁶¹Tb-PSMA-617, inhibiting more efficiently the proliferation of PC3-PIP cells for the highest tested activities without affecting the PC3-FLU cells.

Cellular Studies: Radiobiological Effects

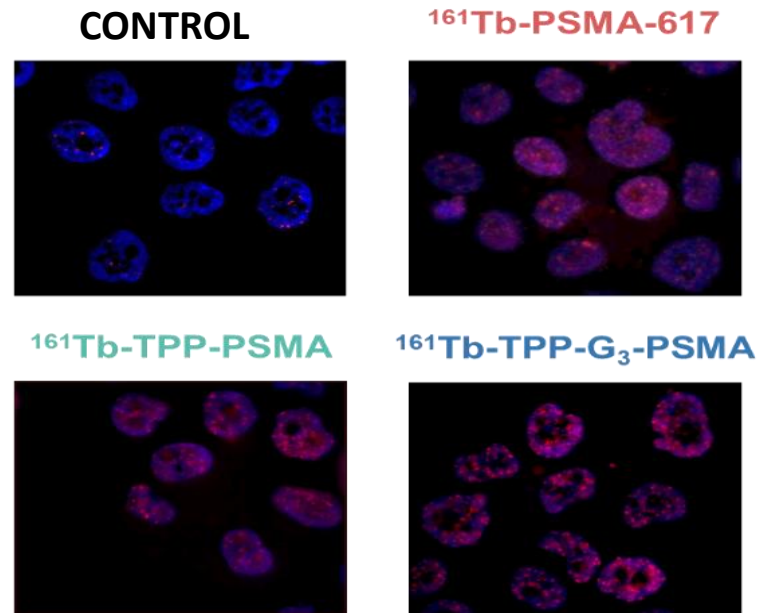
- ❑ **γ -H2AX Assay:** Quantification of individual γ -H2AX foci by fluorescence microscopy
- ❑ Phosphorylation of the Ser-139 residue of the histone variant H2AX leads to the formation of γ -H2AX foci, which is an early cellular response to the induction of DNA double-strand breaks.
- ❑ γ -H2AX foci are correlated with the induction of DNA double-strand breaks



**Activity-dependent (0.185-0.74 MBq)
DNA damage after 4 h incubation at 37 °C**



**Fluorescence images of cells exposed
to 0.74 MBq for 4h**



(Cells were immunostained for γ -H2AX;

DAPI was used to visualize the nuclei)

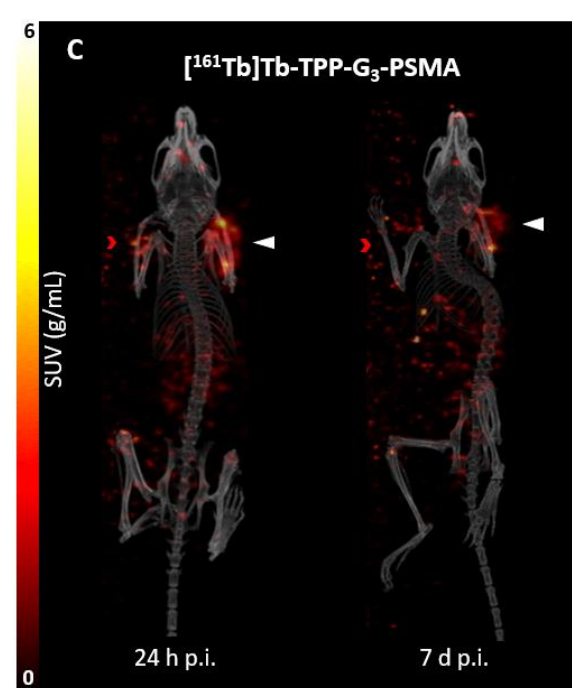
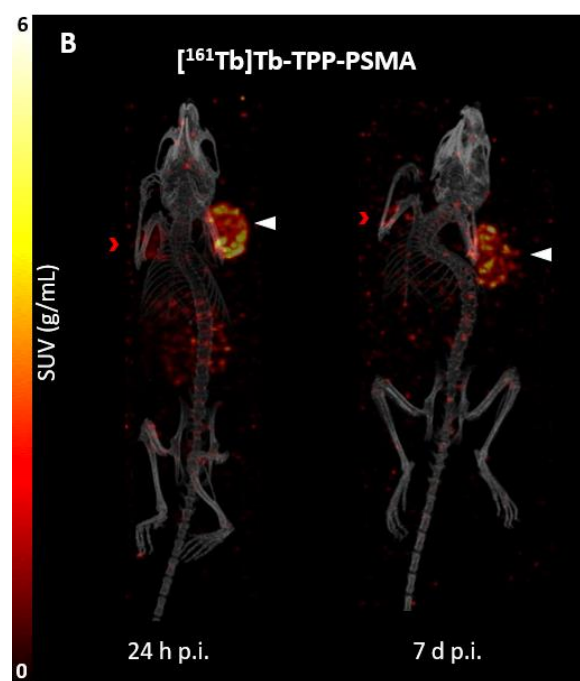
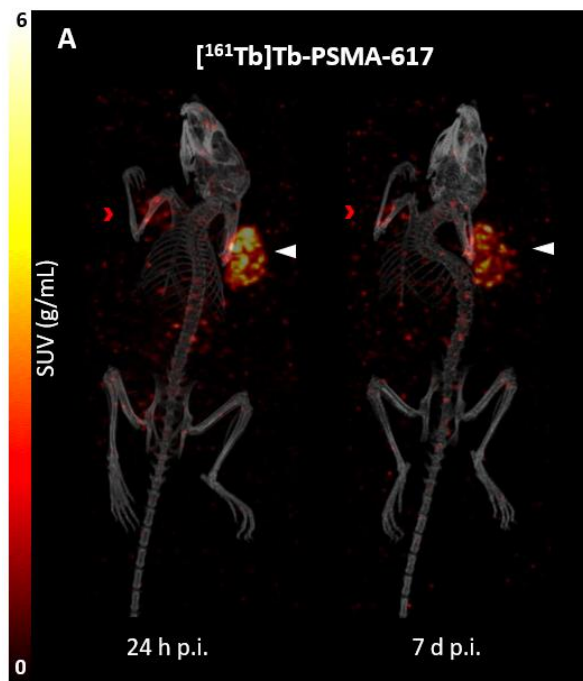
MicroSPECT Imaging Studies

- μ SPECT 24 h and 7 d p.i.

Left tumor
PSMA-
(PC3 Flu)



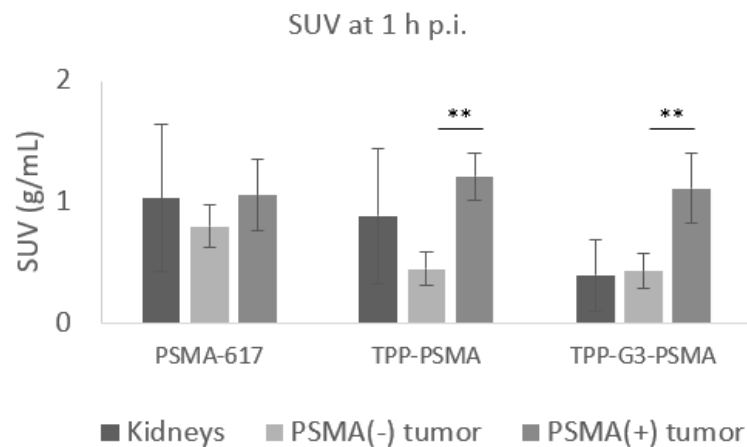
Right tumor
PSMA+
(PC3 PIP)



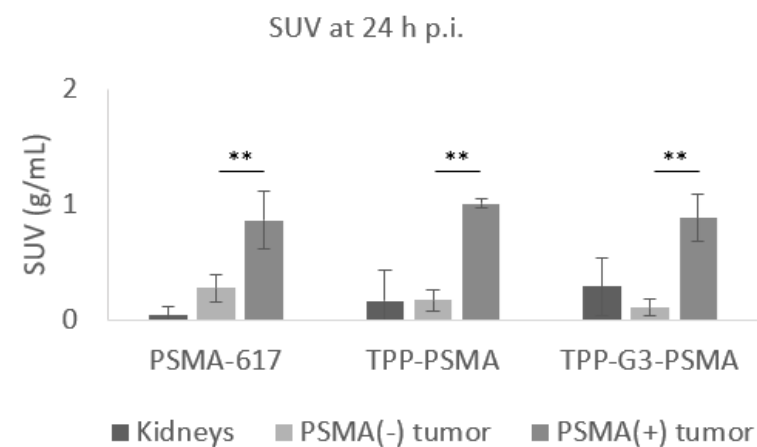
MicroSPECT Imaging Studies

- SUV values (kidneys and tumors) (1 h, 24 h, 96 h and 7 d p.i.)

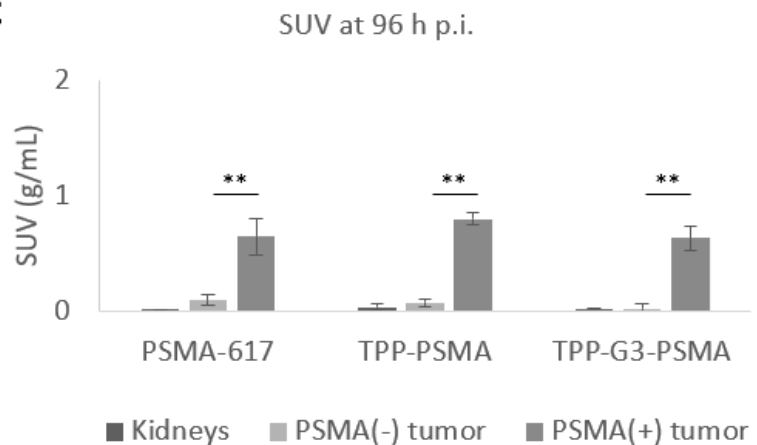
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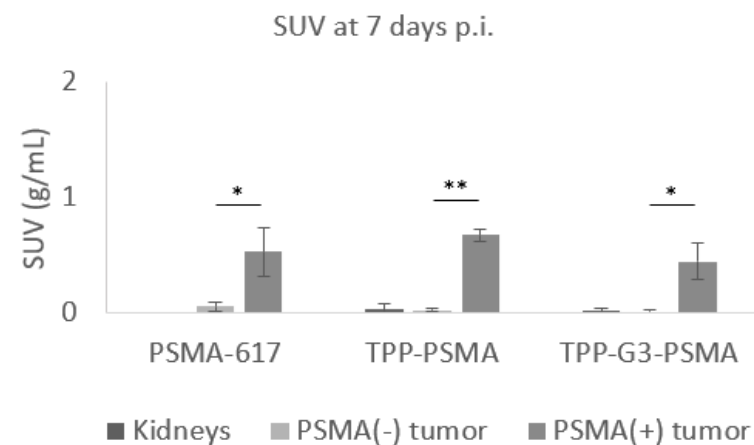
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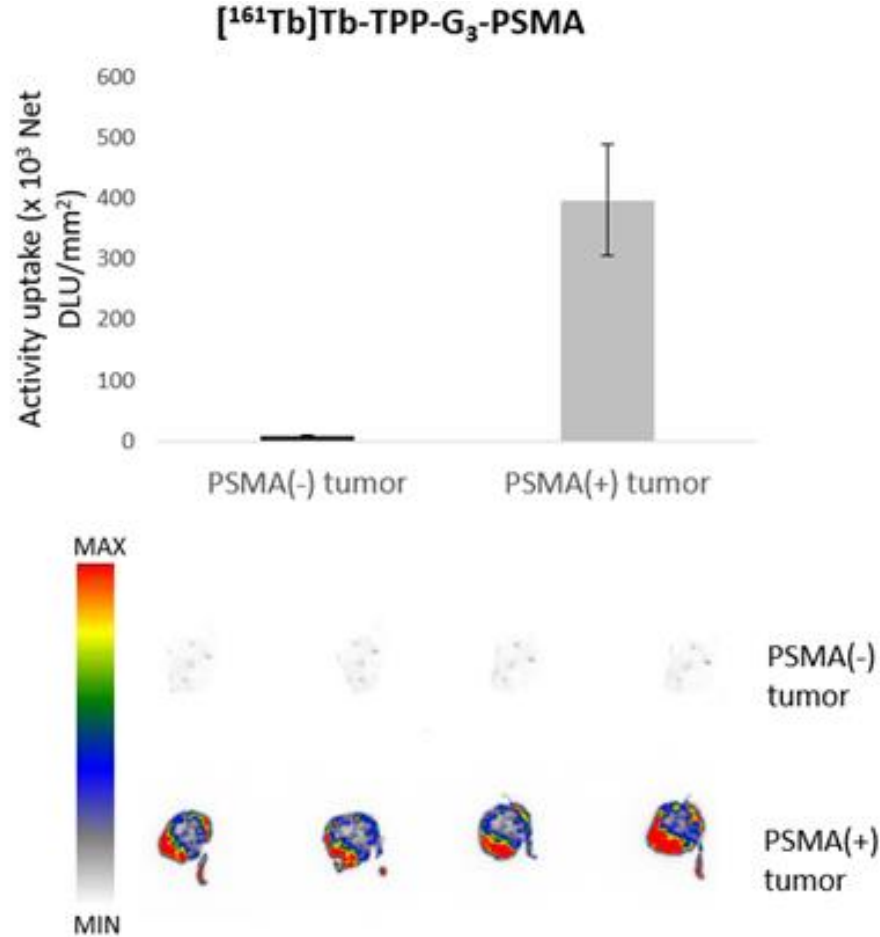


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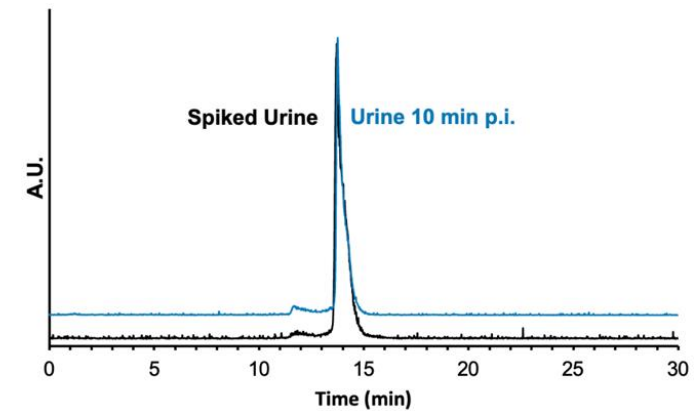
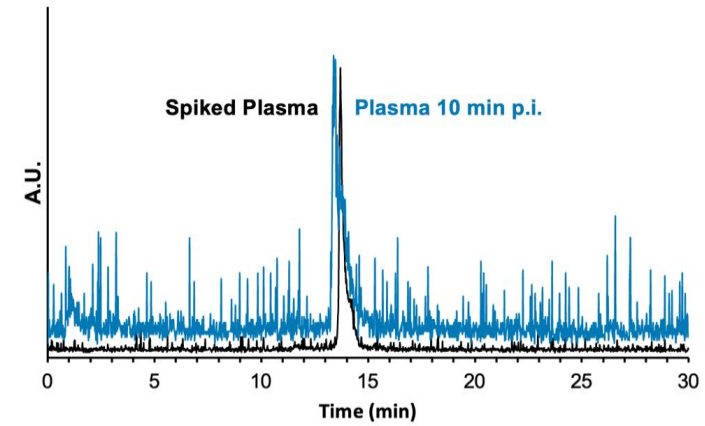


Autoradiography and Radiometabolite Studies

- Ex vivo autoradiography data at 7 days post-injection



- HPLC radiochromatograms of **plasma** and **urine** samples from mice injected with [¹⁶¹Tb]Tb-TPP-G₃-PSMA (10 min. p.i. and comparison with samples spiked with the radiocomplex)



Conclusion and Perspectives

- TPP-containing ^{161}Tb radiocomplexes, combining PSMA and mitochondria targeting ability, significantly enhance radiocytotoxicity of ^{161}Tb in PSMA-positive prostate cancer cells, leading to increased DNA damage.
- Their excellent *in vivo* stability, successful *in vivo* tumor targeting and favorable pharmacokinetics make these TPP-containing radiocomplexes good candidates to improve the efficacy of PCa treatment based on Auger electron therapy.
- Therapeutic assays in tumor bearing mice are foreseen for the best performing $^{161}\text{Tb-TPP-G}_3\text{-PSMA}$, together with further studies to enlighten the possible role of mitochondrial damage on the observed radiobiological effects.

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Ana Belchior



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Christopher Cawthorne

Camille Van Laere
Irwin Cassells



Maarten Ooms
Michiel Van de Voorde



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THANK YOU FOR YOUR ATTENTION!



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