Radiobiology studies in proton therapy: range verification with Zn nanoparticles and studies of cell surveillance after irradiation with different LETs

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Medical Applications of Ionizing Radiation Unit (CIEMAT)

IGFAE workshop on technologies and applied research at the future Galician proton-therapy facility

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2. Proton therapy
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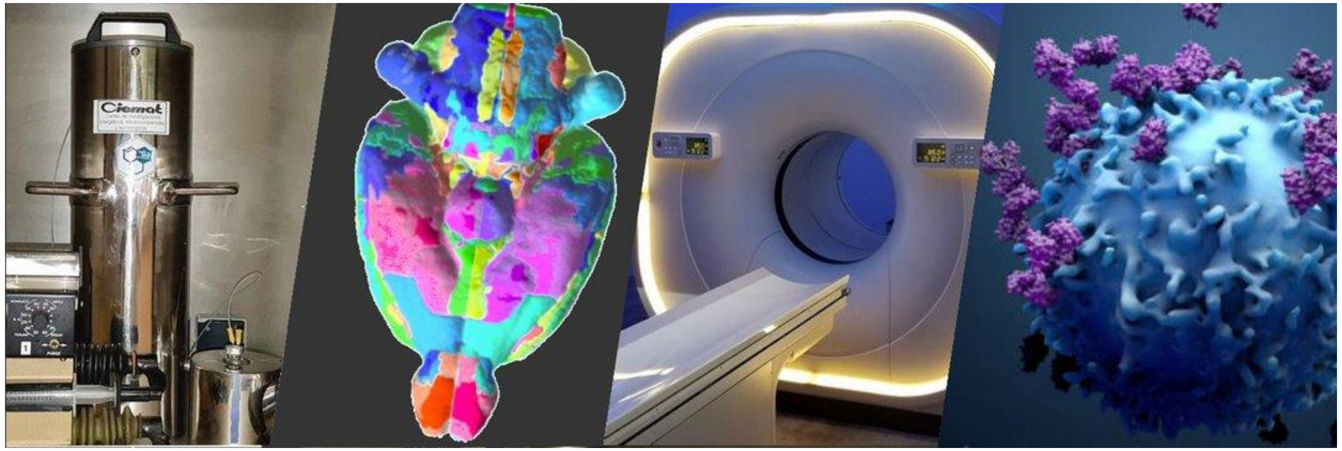
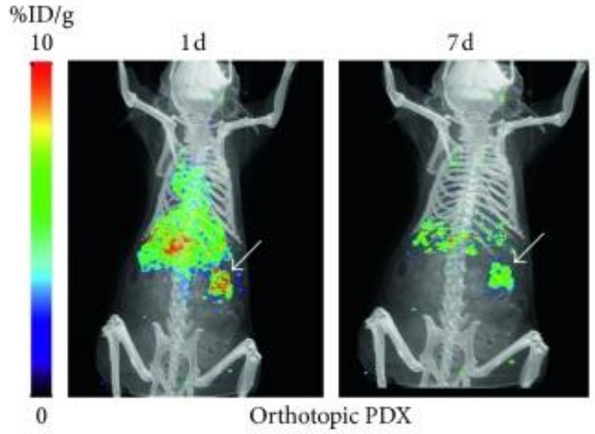
    B. Biological optimization for protons
        Development of a set-up for proton irradiation with different LETs in a clinical facility
        LQM adjustment for cell surveillance measurements in 96-well cell culture plates
3. Conclusions

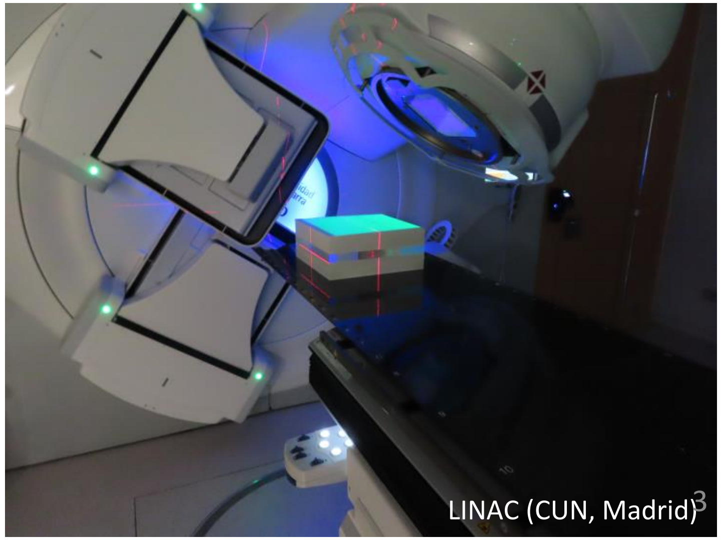
Medical Applications of Ionizing Radiation Unit





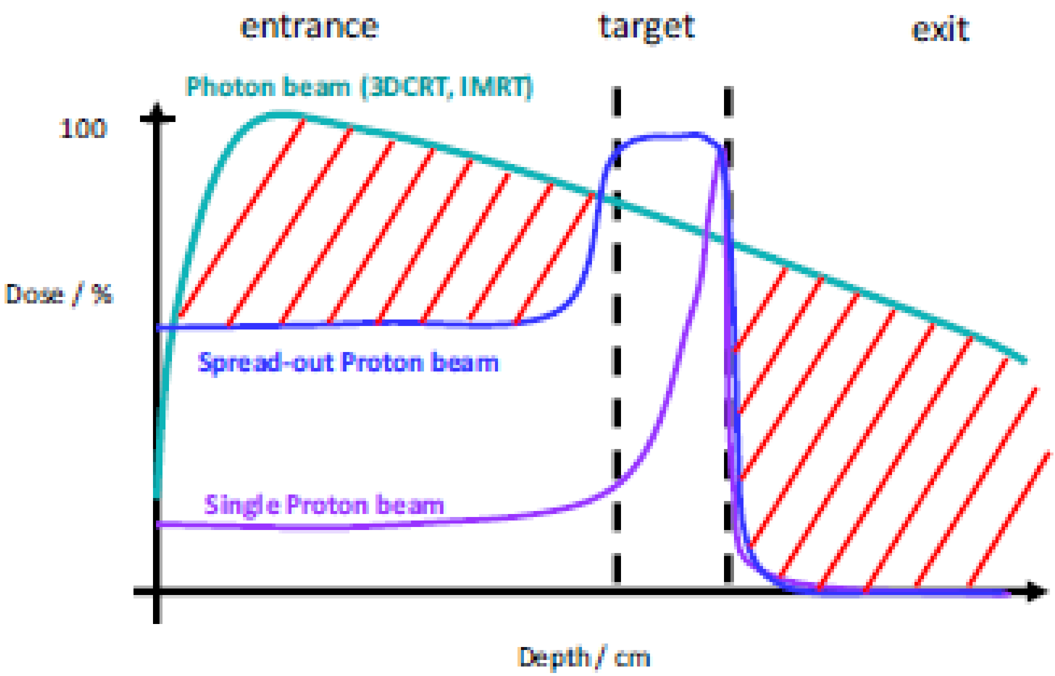
Morcillo, M. Á. *et al.* 2018

- Radioisotopes production and Radiopharmacy
- PET/CT Molecular Imaging
- Instrumentation and Simulation in Nuclear Medicine and Radiotherapy
- Radiobiology



LINAC (CUN, Madrid)

Protontherapy



Blanchard *et al*, 2017

Uncertainties:

- Delineation
- Positioning
- Interfractional variation
- Range calculation based on CT

Strategies:

- Monte Carlo planning
- Improvements in Image-Guided PT (IGPT)
- Range verification
- Radiobiological optimization for protons (LET/RBE)

A. Range verification

PRONTO-CM Project

CSIC
gfn UCM Grupo de Física Nuclear
Ciemat Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas
B2017/BMD-3888
Programas de I+D en Biomedicina 2017
UNIÓN EUROPEA
Fondos estructurales
Invertimos en su futuro
Comunidad de Madrid
Pronto
Protontherapy and nuclear techniques for oncology

Uncertainties

↓

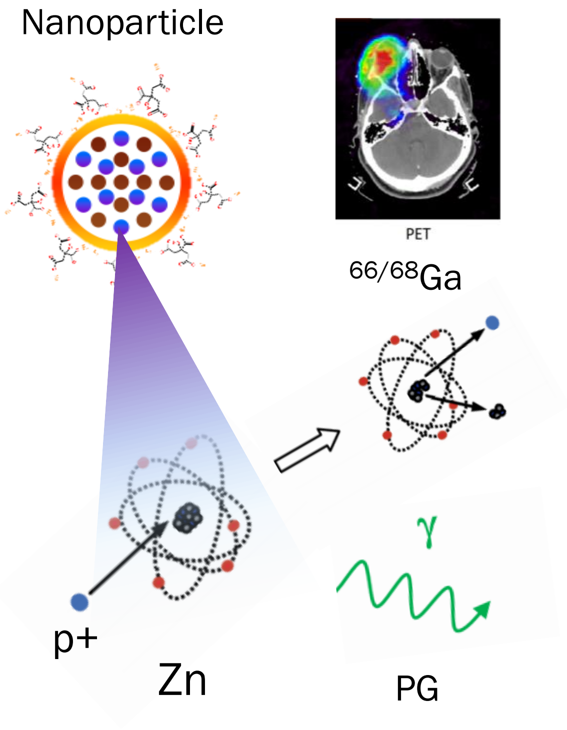
Range verification *in vivo*?

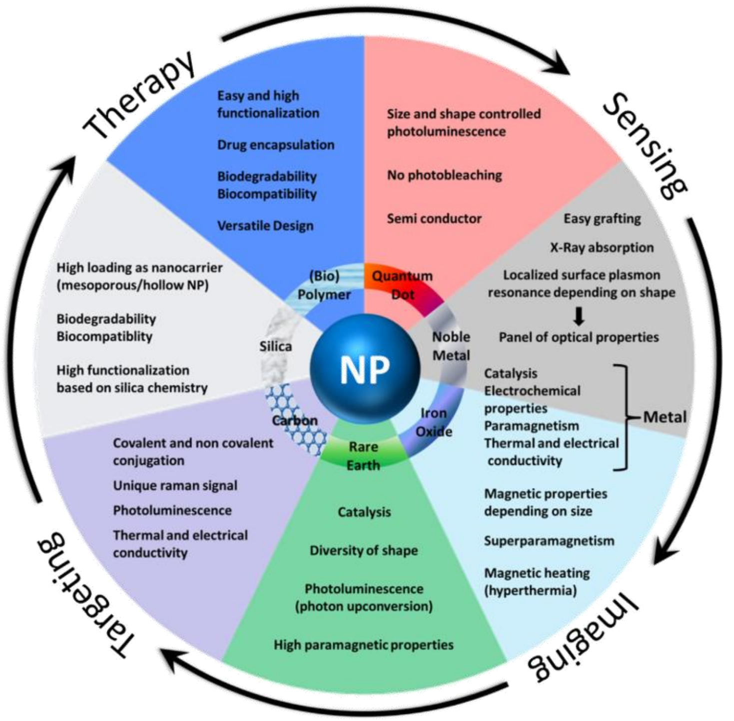
↓

PET or PG from patient activation with the beam

↓

Protoactivable NPs





Paper in process

Zinc-doped iron oxide nanoparticles as a proton-activable agent for dose range verification in PT

a. *Nanoparticle synthesis and characterization*

Microwave (CEM)

- ✓$FeCl_3$
- ✓$ZnCl_2$
- ✓$Na_3C_6H_5O_7$
- ✓N_2H_4
- ✓10 min
- ✓100ºC
- ✓240 W
- ✓ N_2 cooling

Amicon (30K) for medium exchange

IN VITRO STUDIES

Sephadex PD-10 for purification

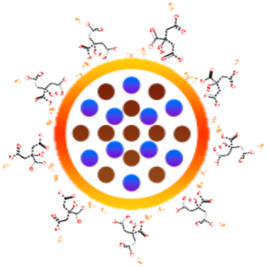
0.22 µm filter for sterilization

EX VIVO STUDIES

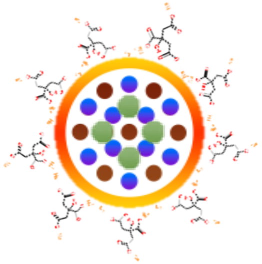
CURIUM™ LIFE FORWARD

^{67}Ga citrate to chloride

IONP@Zn-cit

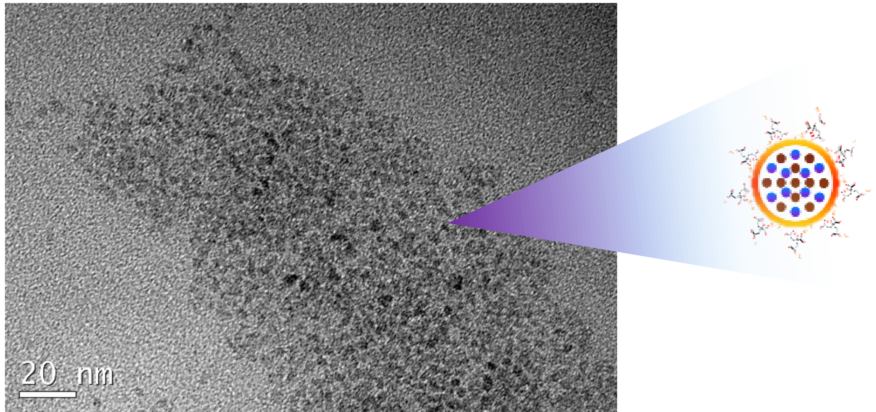


- Zn oxide (61.2 %)
- Fe oxide (38.8 %)
- Citrate



^{67}Ga-IONP@Zn-cit

TEM images



61 % Zn
39 % Fe

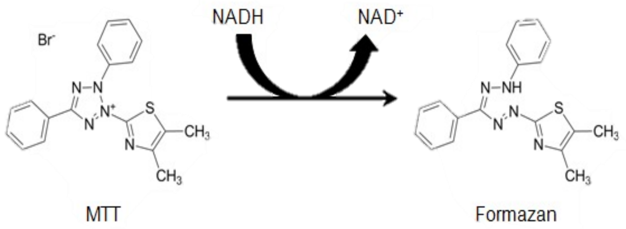
ICP-MS for quantification

Zinc-doped iron oxide nanoparticles as a proton-activable agent for dose range verification in PT

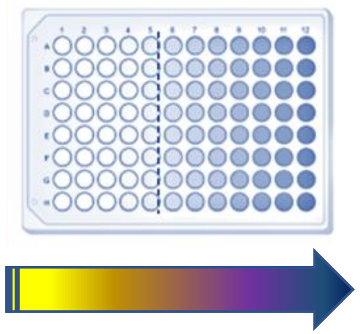
b. *IONP@Zn-cit Cytotoxicity*

IONP@Zn-cit

U251 cell line

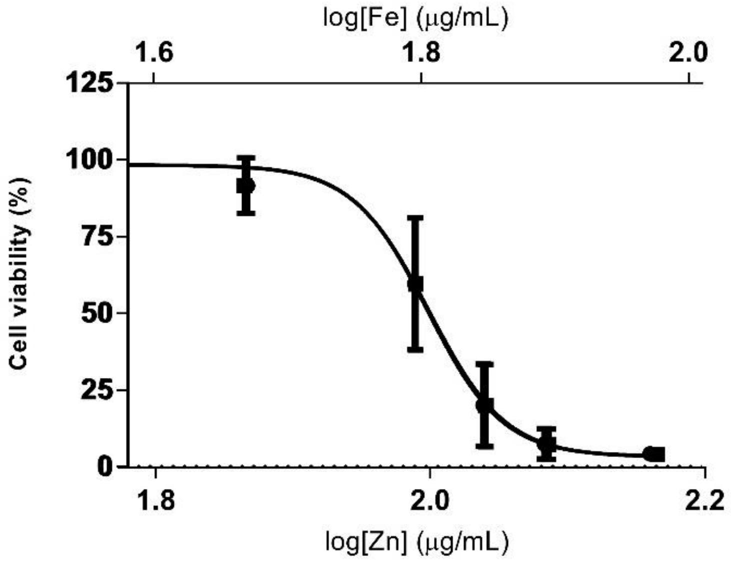


MTT Proliferation Kit I (Roche)



Absorbance measurement

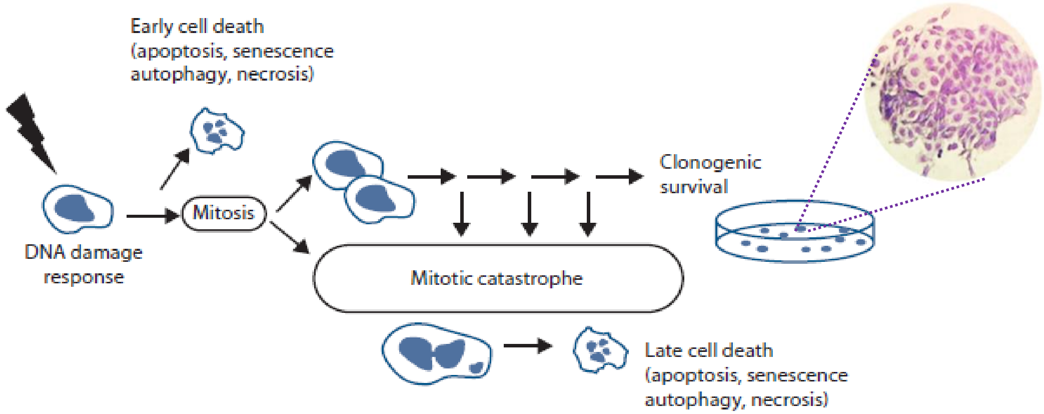
(570-690 nm)



Component Type	IC50 (µg/mL)	95% Confidence Intervals
Fe-IC50	64	61 to 67
Zn-IC50	100	96 to 104
Metals-IC50	164	157 to 171

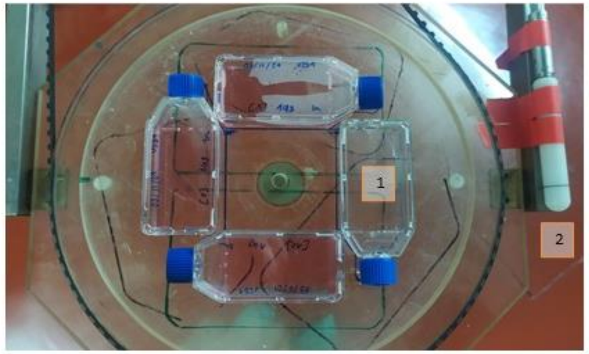
Zinc-doped iron oxide nanoparticles as a proton-activable agent for dose range verification in PT

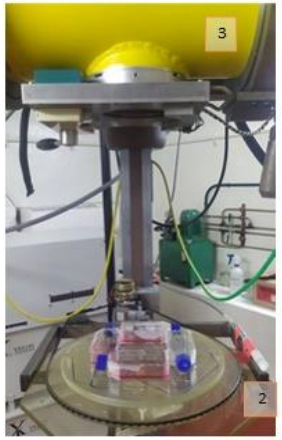
c. *Influence of IONP@Zn-cit on X-Ray radiation-induced clonogenic cell death*

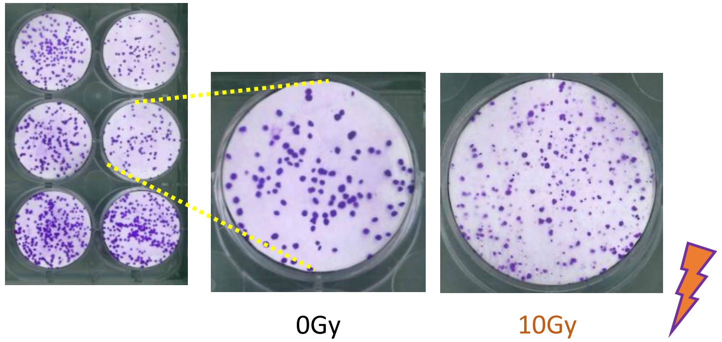


Formation of a 50 cell-colony (5-6 divisions)

Survival Quantification







0Gy
100 cells seeded

10Gy
1000 cells seeded

1. Cell culture flasks used to perform clonogenic assays with NPs.
2. Farmer Ionization chamber (model NE-2571) as monitoring chamber.
3. X-Ray irradiator (Philips MCN 321 X-Ray tube, CIEMAT).

Linear-Quadratic Model (LQM):

$$SF(D) = exp^{-\alpha D - \beta D^2}$$

Zinc-doped iron oxide nanoparticles as a proton-activable agent for dose range verification in PT

c. *Influence of IONP@Zn-cit on X-Ray radiation-induced clonogenic cell death*

- Results for U251 cell line and parameters to study NP effect:

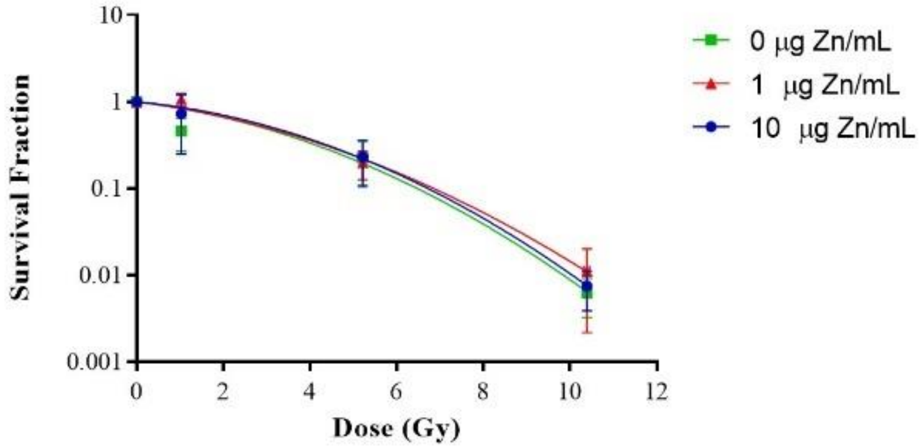
[IONP@Zn-cit] (μgZn/ml)	α (Gy^{-1})	β (Gy^{-2})	SF_{2Gy}	$D_{10\%}$ (Gy)	MID
0	0.747 ± 0.331 (44.3)	-0.025 ± 0.035 (143.2)	0.277 ± 0.164 (59.3)	3.60 ± 1.37 (38.2)	2.78 ± 0.60 (21.5)
1	0.085 ± 0.118* (139.2)	0.041 ± 0.019* (46.9)	0.729 ± 0.148* (20.2)	6.73 ± 1.07* (15.9)	4.25 ± 0.83 (19.5)
10	0.408 ± 0.129 (31.6)	0.007 ± 0.016 (222.2)	0.436 ± 0.082 (18.8)	5.15 ± 0.56 (10.8)	3.52 ± 1.13 (32.0)

*p-value<0.05

α: probability of lethal damages to DNA (linear part of the curve)

β: probability of sub-lethal damages (quadratic part of the curve)

$$MID = \int_0^{\infty} SF(D)dD$$



$$DEF_{2Gy} = \frac{Reference\ dose\ (2\ Gy)}{Dose\ needed\ with\ (cells + NPs) to\ achieve\ the\ same\ cell\ survival\ as\ cells\ alone\ at\ a\ dose\ of\ 2\ Gy}$$

DEF_{2Gy} = 5.04 (1.38;2.84) and 3.2 (1.03;1.56) for 1 and 10 μg Zn/ml NPs

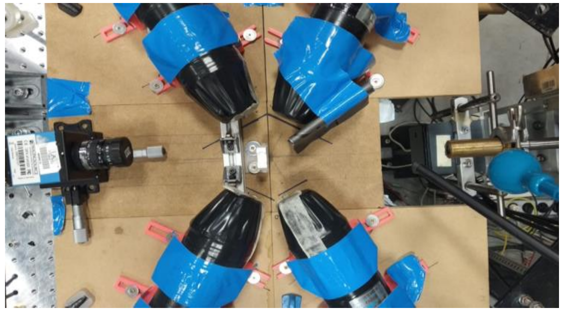
Radioprotective effects at low NP doses???

Matsui et al. 2019; Ahmad et al. 2020; Rajaee et al. 2019; Subiel et al. 2016

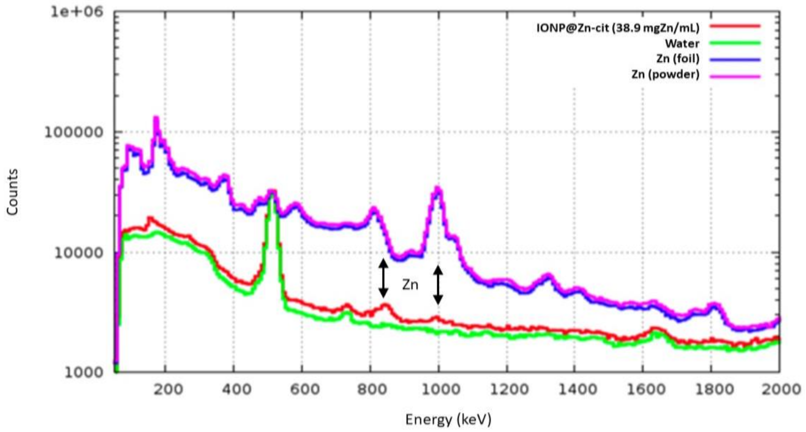
Zinc-doped iron oxide nanoparticles as a proton-activable agent for dose range verification in PT

c. *Proton irradiation of IONP@Zn-cit*

Center for Microanalysis of Materials
(CMAM, Madrid)



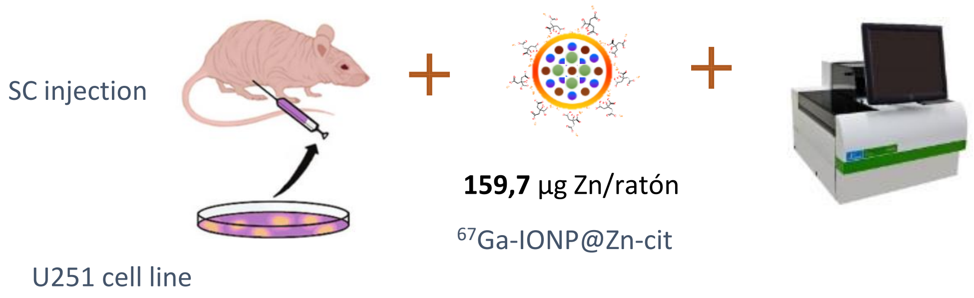
Set-up of CMAM irradiations with four LaBr detectors (in blue) for prompt gamma measurements.

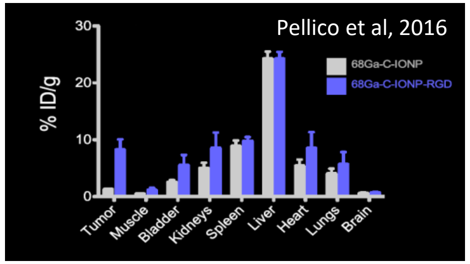


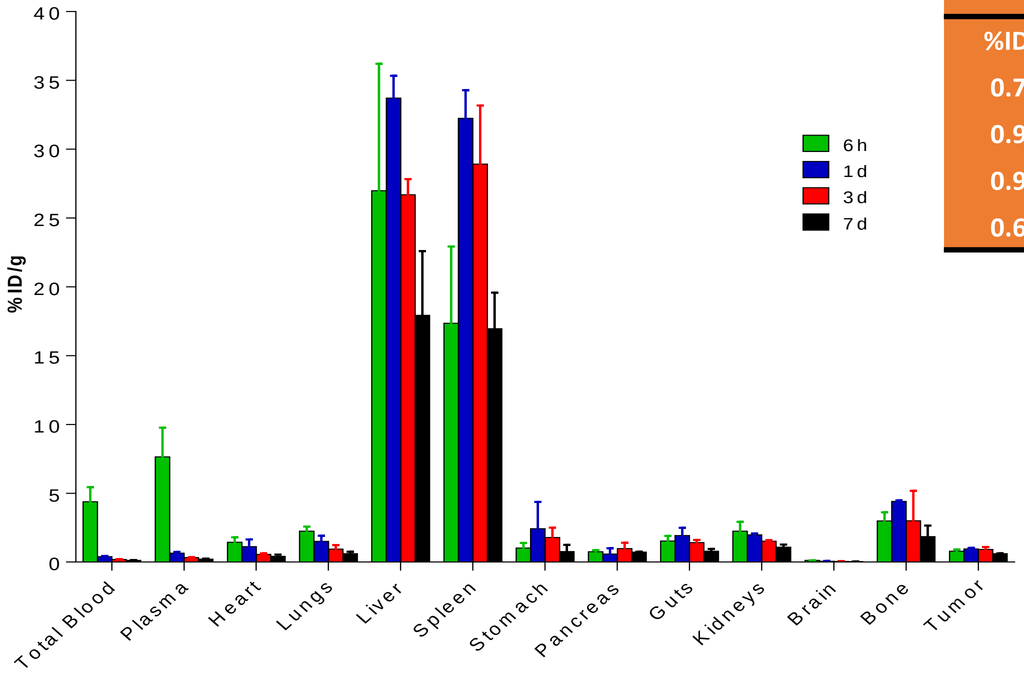
IONP@Zn-cit water solutions (red) were irradiated with 10-MeV proton beam and prompt gamma were measured. Zn foil sample (blue)/powder (pink) and water (green) were used as positive and negative controls, respectively.

10 mg/mL in this set-up

d. *Biodistribution and pharmacokinetic studies of ^{67}Ga-IONP@Zn-cit*







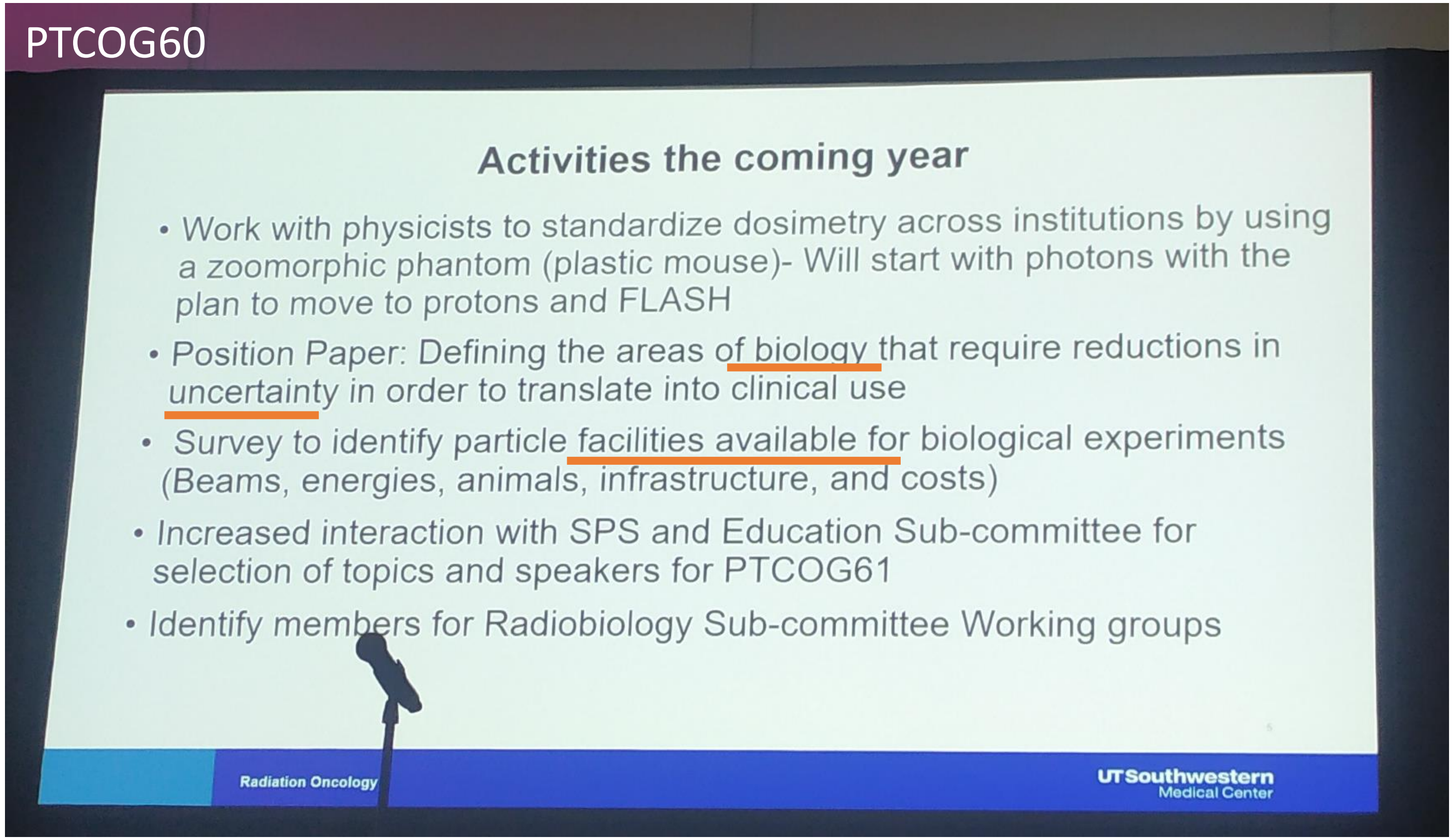
Tumor Radioactivity		^{67}Ga- IONP@Zn-cit Concentrarion	
%ID/g	SD	µg Zn/g	µg Fe/g
0.79	0.13	1.26	0.82
0.95	0.08	1.51	0.98
0.92	0.17	1.46	0.95
0.61	0.02	0.97	0.63

Possible improvements:

- Active targeting
- More detectors
- Add Oxygen-10

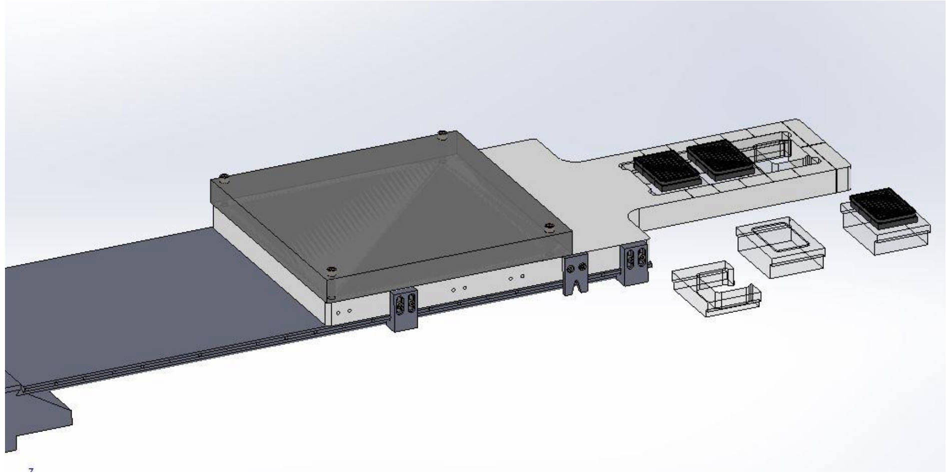
B. Radiobiological optimization for protons (LET/RBE)

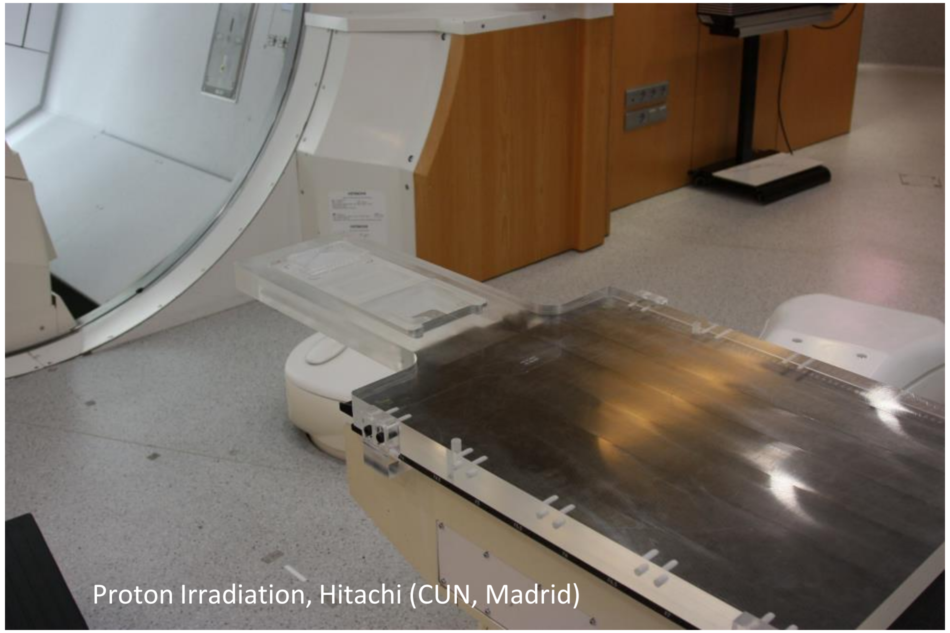
- Limitations -> Research facilities and reproducibility



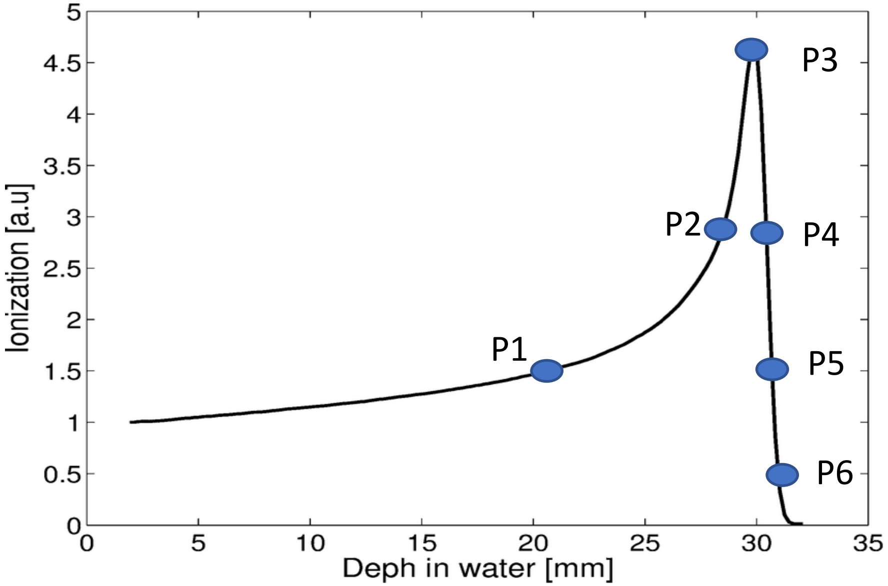
Radiobiological optimization for protons (LET/RBE)

a. Development of a set-up for proton irradiation with different LETs in a clinical facility



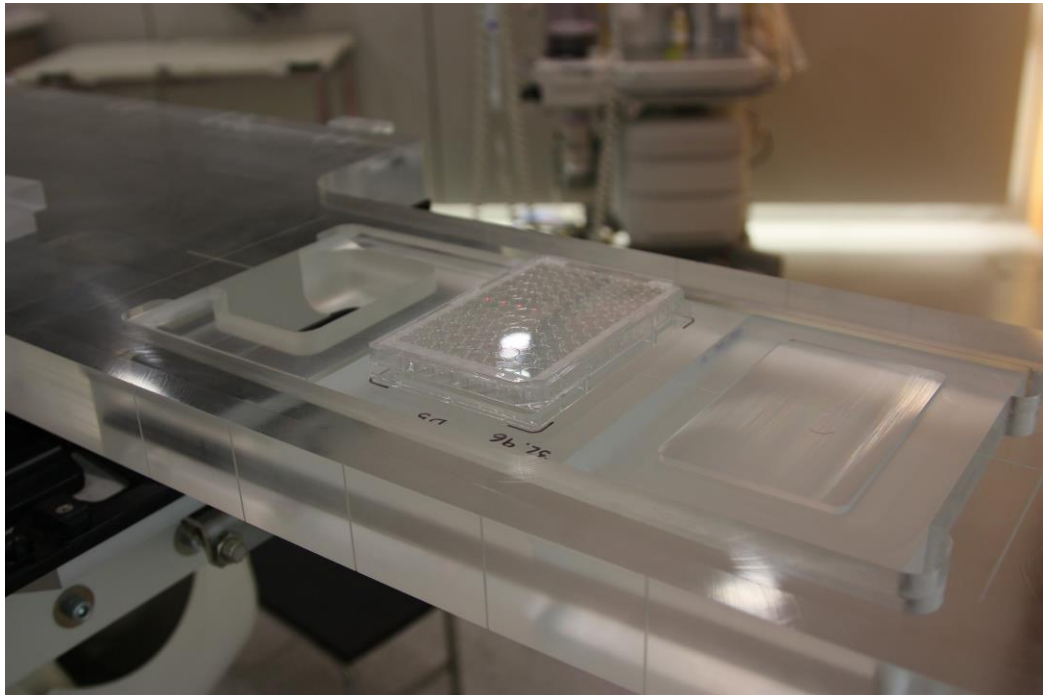


Proton Irradiation, Hitachi (CUN, Madrid)



LET	%PDD	PMMA Thickness (mm)
P1	30 %	23,46
P2	70 %	31,19
P3	100 %	32,11
P4	70 %	32,6
P5	30 %	32,96
P6	10 %	33,23

Radiobiological optimization for protons (LET/RBE)
a. Development of a set-up for proton irradiation with different LETs in a clinical facility

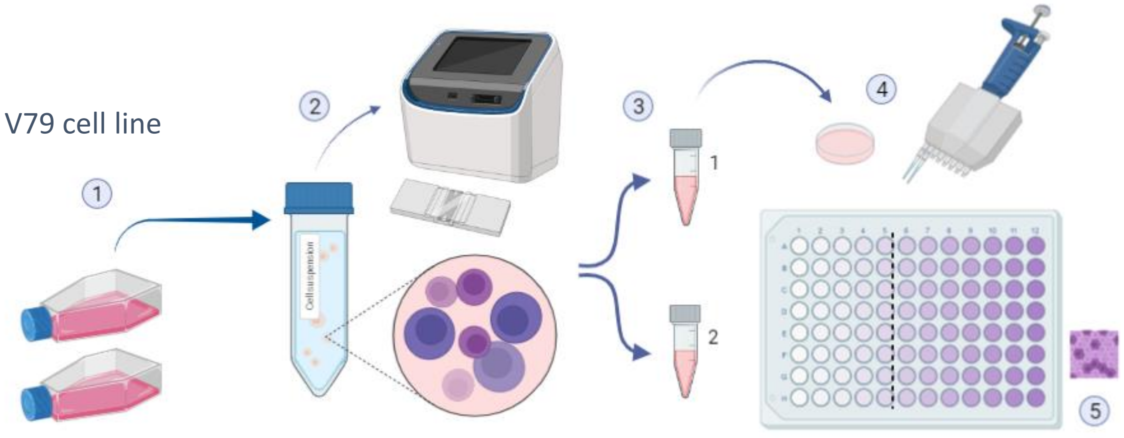


Proton Irradiation, Hitachi (CUN, Madrid)

Radiobiological optimization for protons (LET/RBE)

b. LQM adjustment for cell surveillance measurements in 96-well cell culture plates

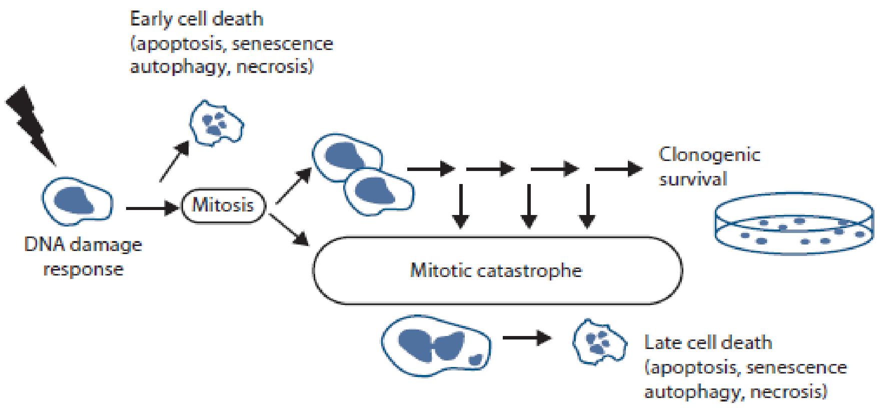
V79 cell line

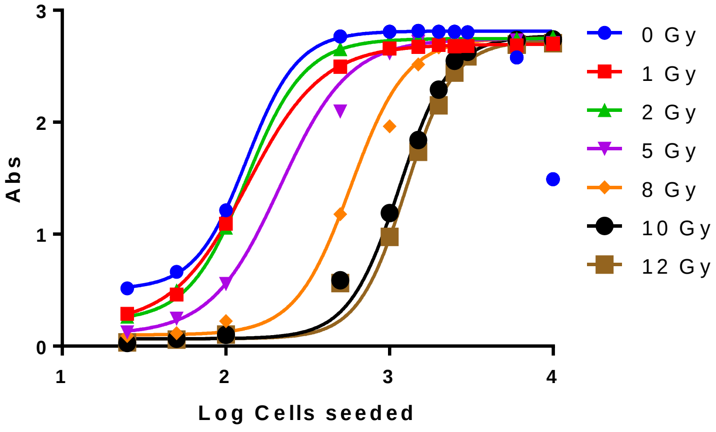


6. Dilution with Abs methanol

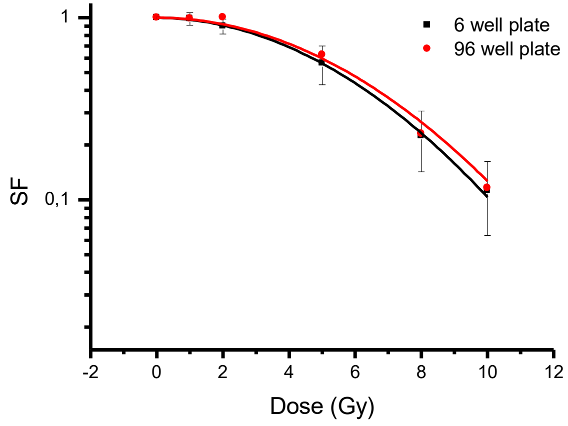
7. Absorbance measurement

(570-BKG nm)





$$SF_{XGy} = \frac{EC50\ control}{EC50_{XGy}}$$



Take home message

1. IONP@Zn-cit produce prompt-gamma at a certain concentration and the biological characterization was performed successfully, obtaining results about what could be our next steps in this topic.
2. The set-up for proton irradiation in a clinical facility built allows irradiations at different LETs with uncertainties measured below 9 %.
3. The new method of cell surveillance is giving results comparable with the gold standard used in radiobiology, the clonogenic assay in which colonies are counted.
4. Our Unit is interested in continuing with this research and further more…
    1. Endpoints (ferroptosis, ROS, study of DNA damage…)
    2. Radiotherapy in combination with immunotherapy
    3. FLASH radiotherapy

Acknowledgments…

Members of the Biomedical Applications on Ionizing Radiation Unit

Members of Nanomedicine and Molecular Imaging Group

Protontherapy and nuclear techniques for oncology (PRONTO-CM) - B2017/BMD3888

Clínica Universidad de Navarra (CUN)

Radiobiology of proton therapy. Rationale for combining proton radiation and immunotherapy (RADPROTIM) - PID2019-104558RB-I00

THANKS FOR YOUR ATTENTION!

@RadioBiomed_CIEMAT
@CIEMAT_OPI

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Timescale of the effects of radiation exposure