ISOLPHARM_APEX

Laboratori Nazionali di Legnaro – INFN

A new CSN5 experiment proposal

Responsabili Nazionali : A. Andrighetto, A. Arzenton

June 2025

Outline

- ISOLPHARM method and collaboration
- ISOLPHARM experiments: past and present
- ISOLPHARM_APEX organization
- Conclusions

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The ISOLPHARM project: 4 pillars

1. National collaboration

Broad, focused on radio-pharmaceutical development: synthesis, characterization and preclinical tests (*vitro/vivo*)

2. Innovative method

Innovative production @SPES of several (medical) Carrier Free + High Purity radionuclides (^{111}Ag, ^{89}Sr, ^{136}Cs..)

4. Activities, INFN experiments

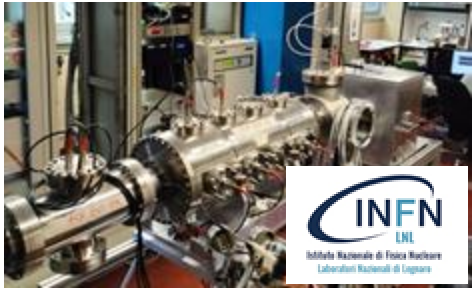
Financed by CSN5 (physics application): ISOLPHARM_Ag + ISOLPHARM_EIRA + ADMIRAL

3. Facility at SPES

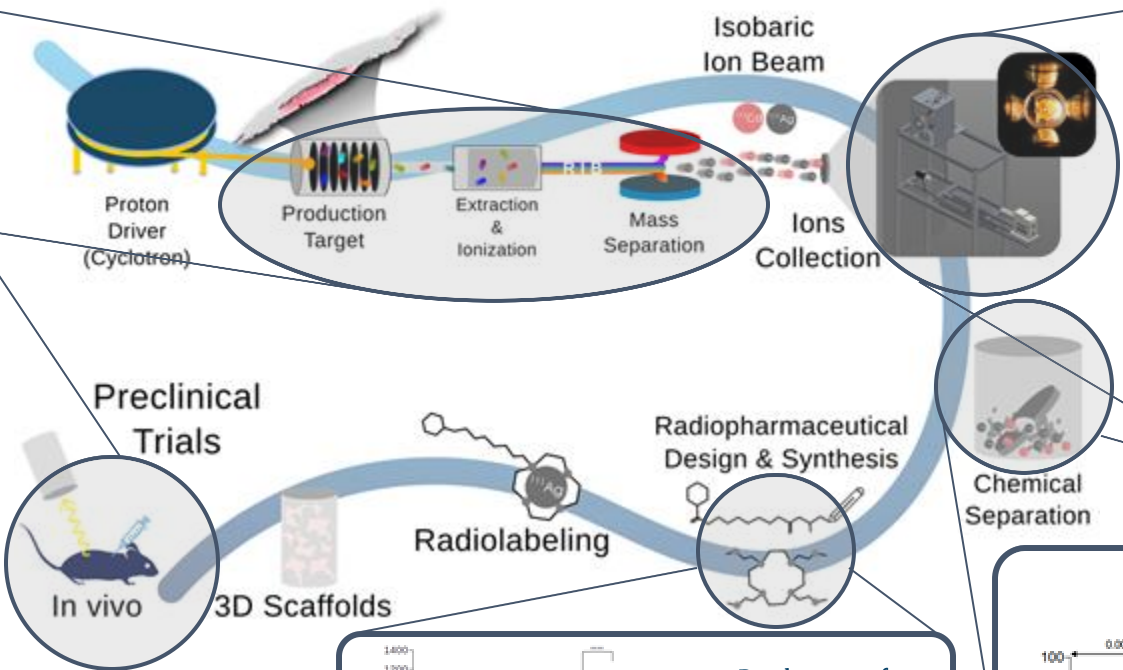
An irradiation station, using the SPES Low Energy Beams, is planned in the SPES building

Also… Scientific and training hub

Collaboration infrastructures

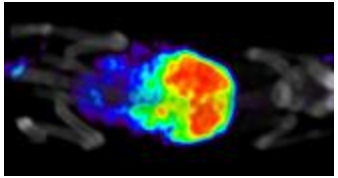


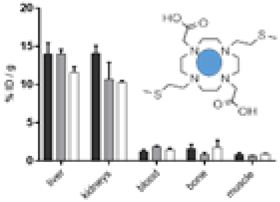
ISOLPHARM method



Design & construction of the IRIS system for radioisotope deposition and spectroscopic characterization

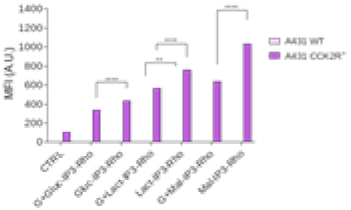
PET/CT fused image 24 h after the radiolabeled compound injection





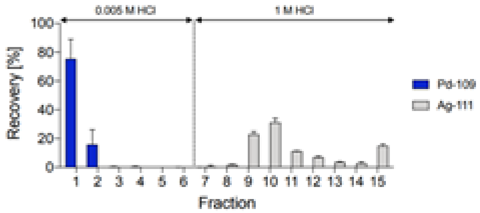
DO2A2S-^{64}Cu ex-vivo biodistributions

Results from ISOLPHARM CSN5 experiments



Development of 2nd gen. ligands: increased uptake and total competition with more hydrophilic linkers

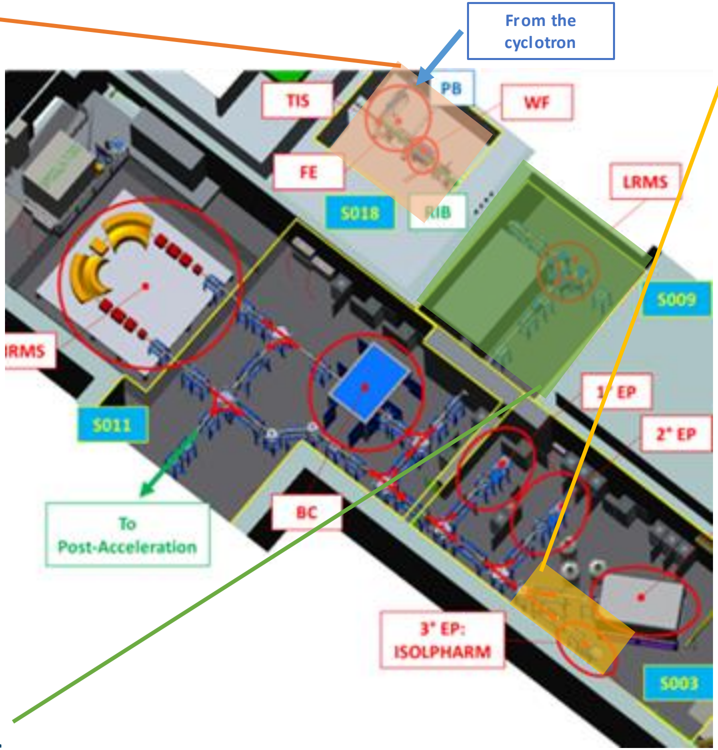
Pd/Ag chemical separation



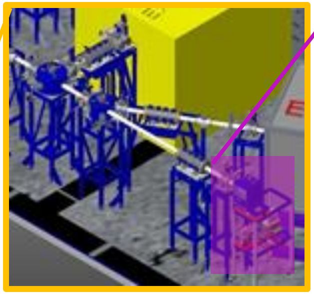
The ISOLPHARM facility

SPES ISOL bunker
(already installed!)

SPES Low Resolution Mass Spectrometer
(WIP installation)

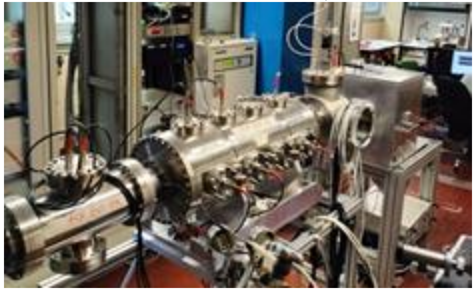


ISOLPHARM beamline and IRIS (ISOLPHARM Radionuclide Implantation Station) system

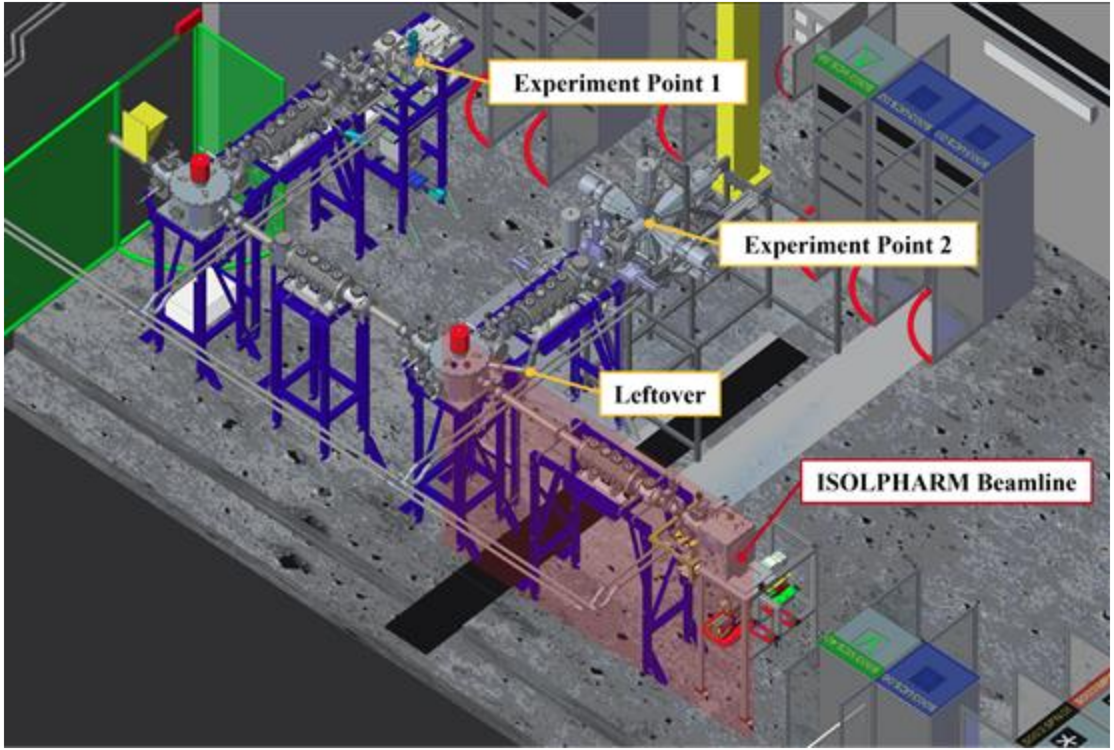


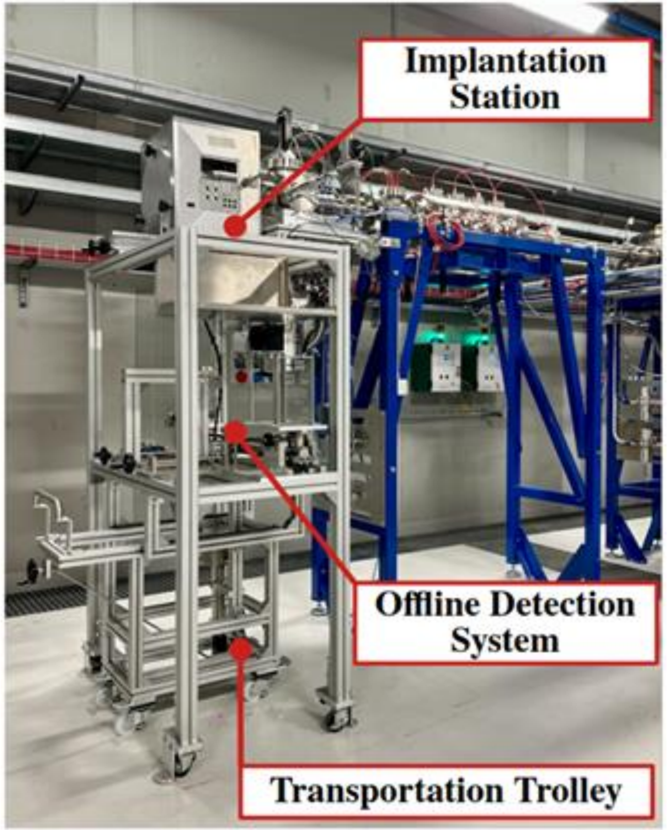
Beamline design almost complete!

IRIS system built and tested at the SPES offline lab!



IRIS: ISOLPHARM Radionuclide Implantation Station





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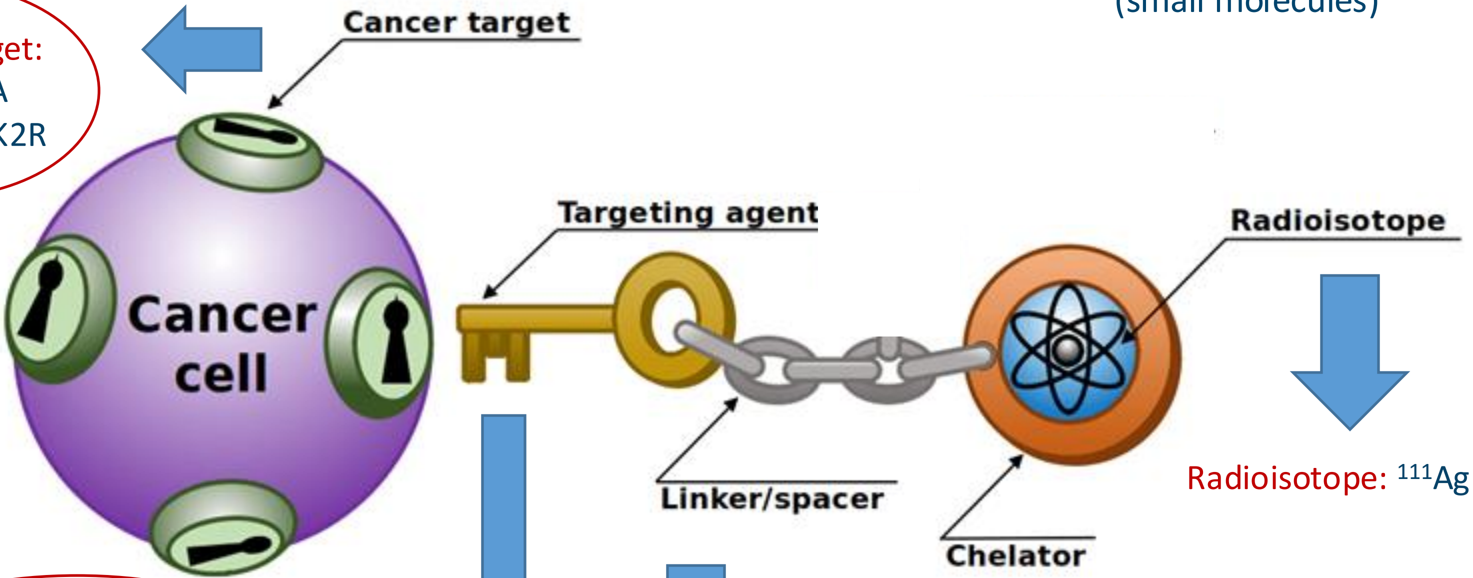
Radiopharmaceutical frame

(small molecules)

Cancer target:
Now: PSMA
Before: CCK2R



Radioisotope: ^{111}Ag

Targeting agent:
Now: PSMA-617
Before: Z360 (for CCK2R)

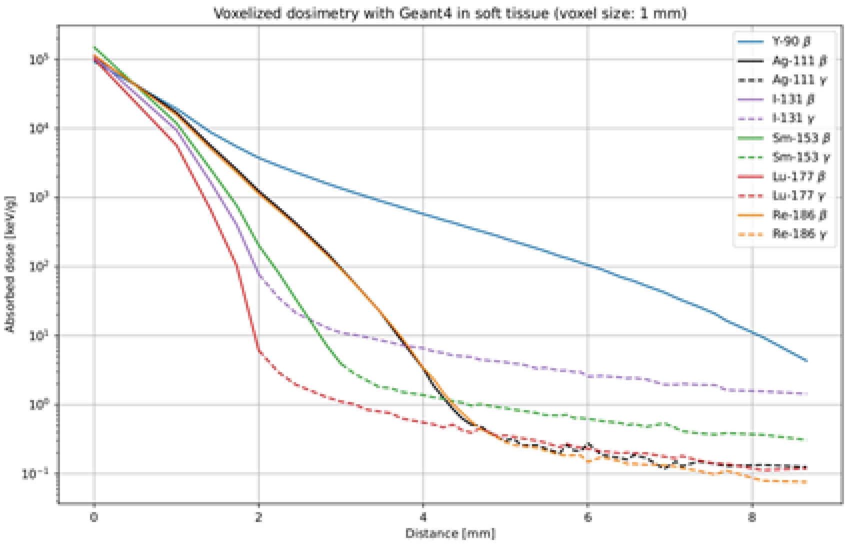
Linker: Bifunctional

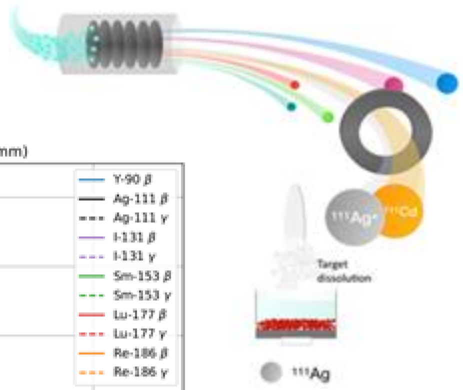
Chelator: DO4S

Experiments: why ^{111}Ag?

^{111}Ag properties

- **β^- emitter** (average energy **360 keV**)
- Good half-life (**7.45 days**)
- Average tissue penetration (1.8 mm)
- Medium energy **γ rays** -> SPECT?





→ In the market no radiopharmaceuticals radiolabeled with silver-111

→ Silver-111 can be produced @ SPES with high purity & high production rate

→ No isobaric radioactive contamination in the implantation foil (also with LASER off!)

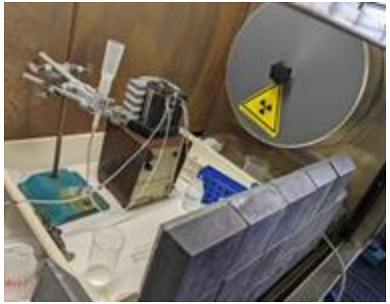
→ ^{111}Ag exhibits theranostic properties similar to ^{177}Lu which was recently approved by FDA

→ ^{111}Ag behaves similarly to ^{186}Re, recently studied in phase I/II clinical trials

111 Isobaric chain	Half-Life $T_{½}$	Decay	Target Yield
Cadmium-111	Stable		Low yield production
Silver-111	7.45 days	β−	Good yield production
Palladium-111	23.4 min	β−	Bad release, short $T_{1/2}$
Rhodium-111	11 sec.	β−	No release, very short $T_{1/2}$

ISOLPHARM: over 10 years of activity

2014 – 2017 — Interdisciplinary study group on production of medical radioisotopes at SPES



INFN experiments

2018 – 2019 — ISOLPHARM Ag — Simulations and feasibility evaluation of Ag as radiopharmaceutical precursor

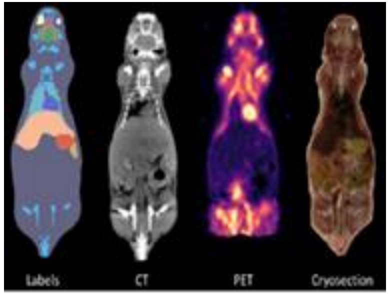
2020 – 2022 — ISOLPHARM_EIRA — First production of ^{111}Ag in reactor and beginning of *in-vitro* and *in-vivo* testing

2023 – 2025 — ADMIRAL — Characterizing the 2D/3D *in-vitro* therapeutic effect of ^{111}Ag and its imaging capabilities

2025 – 2027 — SPES MED — First nuclear measurements of radionuclide production at SPES

PRIN

2024 – 2025 — ISOLPHARM CORE — Technological aspects of radionuclide production (target, ion source, implantation...)



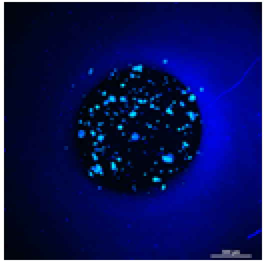
ADMIRAL achievements

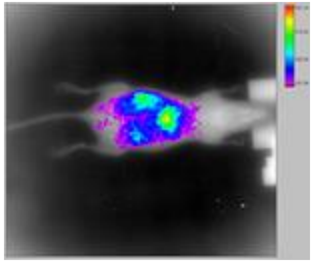
WP1 - Radiopharmaceutical production
- Optimization of chelators for Ag
- Optimization of Ag production and separation
- Development of 3D scaffolds for prostatic cancer cells

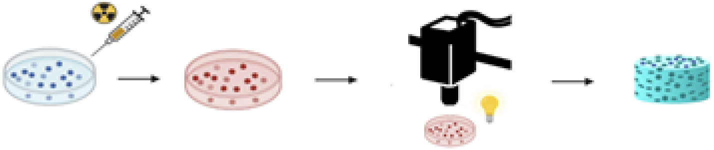
WP2 - β imaging
- 2D β imager "DUMBO" construction
- Characterization tests with ^{111}Ag

WP3 - γ imaging
- ^{111}Ag γ camera prototype development
- Characterization tests with ^{111}Ag

WP4 - Targeted radiobiology
- 2D survival of different cell lines treated with free ^{111}Ag
- Protocol for experiments in 3D scaffolds
- Cell dosimetry and DNA damage/repair models







Open questions after ADMIRAL

1) Which are the radiobiological advantages of ^{111}Ag with respect to other β^- emitters?

2) Can ^{111}Ag be used in radiopharmaceutical therapy against prostatic tumors with PSMA-617 (already used with ^{177}Lu) as a targeting agent?

3) Can its γ imaging be used in a SPECT?

4) Can its β imaging be used for cell uptake measurements?

5) Can the first SPES nuclides provide interesting radiobiological data?

ISOLPHARM_APEX (2026-2028)

^{111}Ag-PSMA-617 EXperiments

Main route

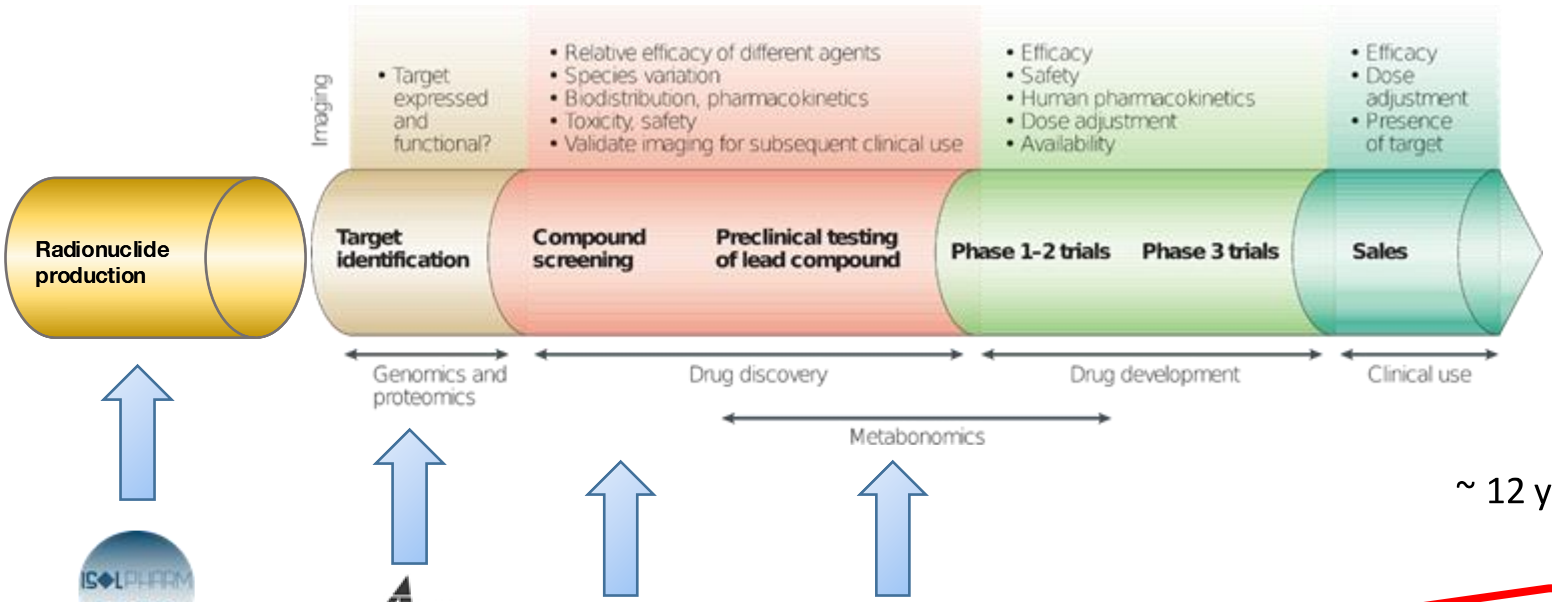
Secondary route

Radionuclide production at LENA and SPES

Main goals

1) Understanding the dosimetry of ^{111}Ag-PSMA-617 in living systems such as cells, small animals and human patients.
2) Studying this radiopharmaceutical in cancer cell cultures in 2D monolayers and in 3D tissue-mimicking scaffolds.
3) Observing the biodistribution and the effects of theranostic ^{111}Ag-PSMA-617 on small animals.
4) Investigating the radiobiological response to low-dose-rate radiation using the first nuclides obtainable at SPES.

The long road to (radio)pharmaceuticals



~ 12 years

ISOLPHARM_APEX is aimed at the completion of the *preclinical testing* block

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ISOLPHARM
SPES exotic beams for medicine

Project organization

INFN
Istituto Nazionale di Fisica Nucleare

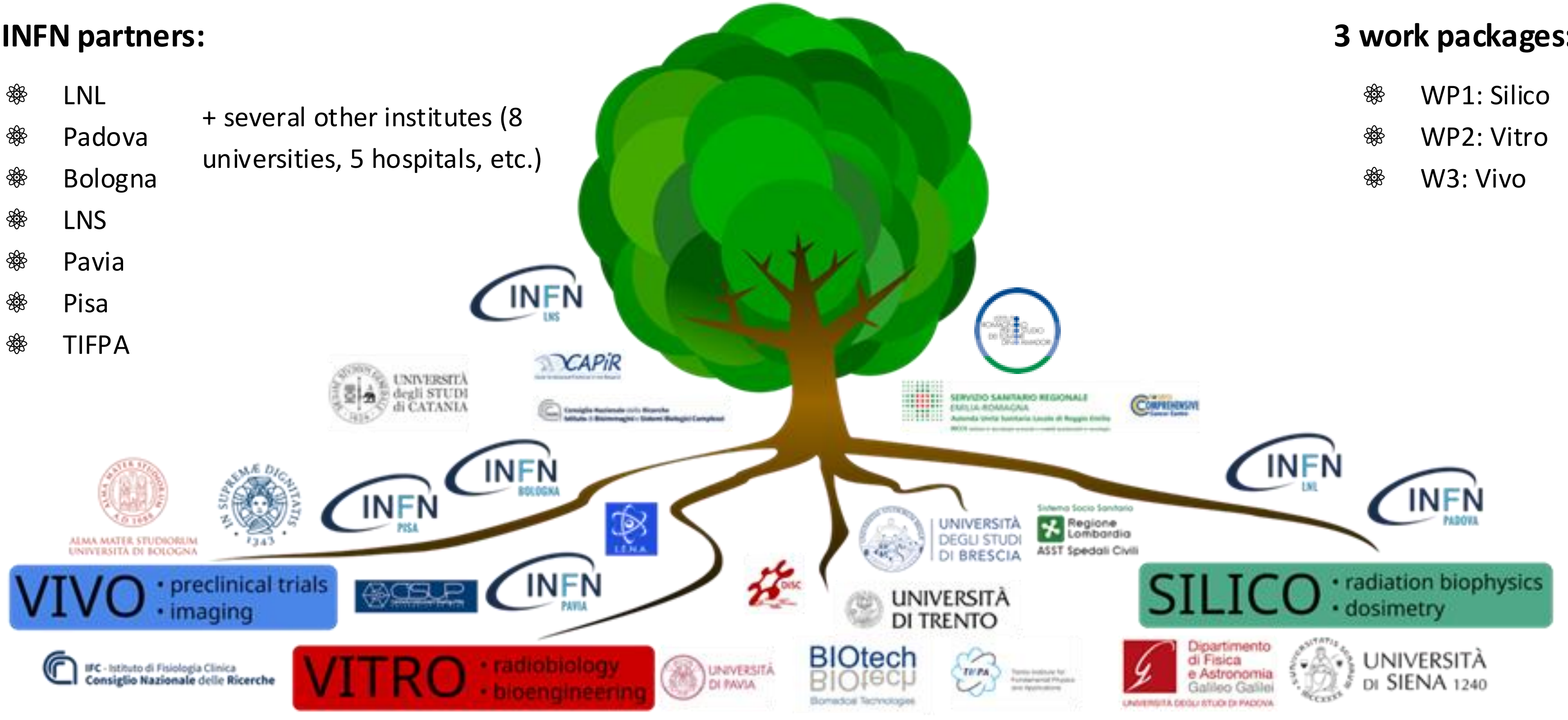
7 INFN partners:

- LNL
- Padova
- Bologna
- LNS
- Pavia
- Pisa
- TIFPA

\+ several other institutes (8 universities, 5 hospitals, etc.)

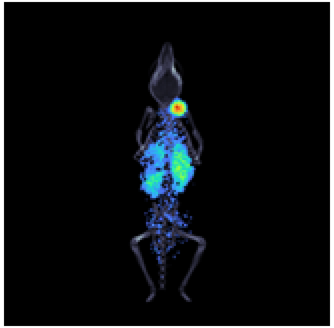
3 work packages:

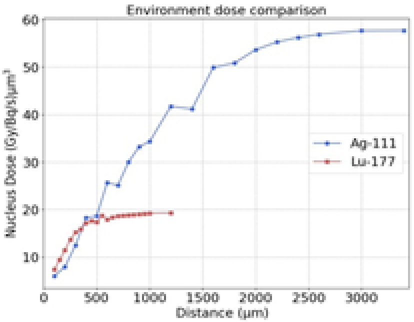
- WP1: Silico
- WP2: Vitro
- W3: Vivo

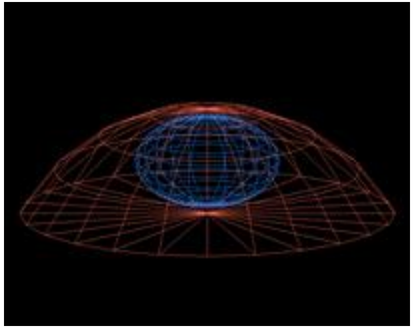


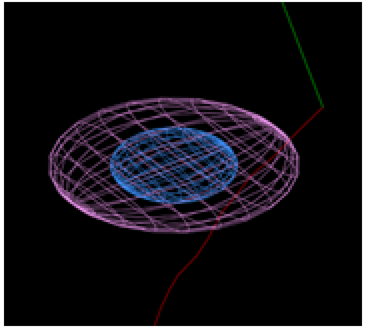
WP1 – SILICO

- Computational dosimetry at cell and organ level
- Survival models and DNA damage study
- Monte Carlo simulations for imaging prototypes



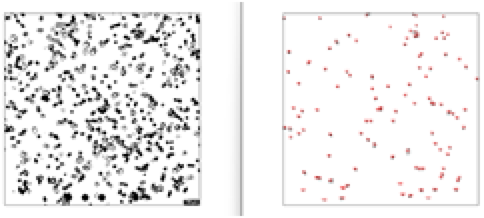




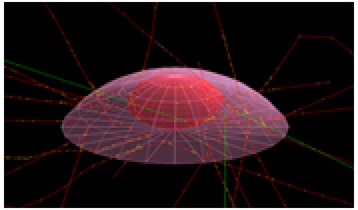


WP1 methodology

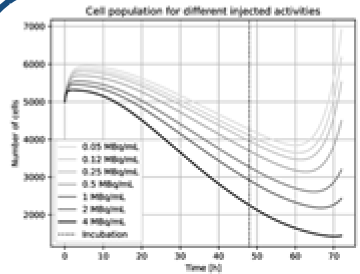
Silico for vitro



Cell shape and dimensions are retrieved from microscopy via image-processing software.



Monte Carlo simulations compute the absorbed dose per activity unit (***S-value***) and the consequent **DNA damage**.

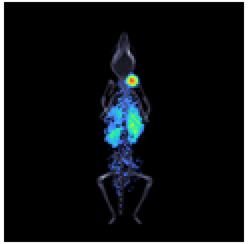


Biophysical models help understanding the **dose-rate curve** and the **DNA repair dynamics** from data.

Silico for vivo

MOBY phantom

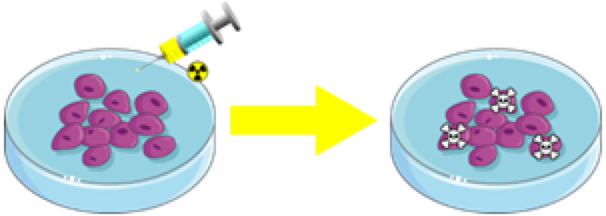
Monte Carlo simulations are performed using commercial **virtual phantoms** or **CT** images; the **input biodistribution** can be measured **ex vivo** or taken from **PET/SPECT** imaging.



Simulations can provide **organ S-values** for treatment plans as well as **imaging previews** to tune the devices.

WP2 – VITRO

- Clonogenic survival assays
- Uptake measurements with β imaging
- *Foci* assays



2D and 3D radiobiology

Cold studies

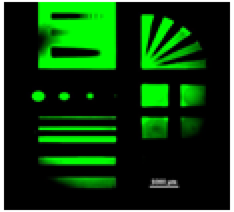
^{111}Ag-PSMA-617

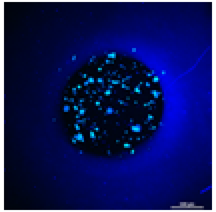
Radiotracers

Other nuclides

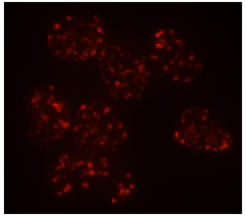
WP2 methodology

Biological effects: DNA damage and cell survival

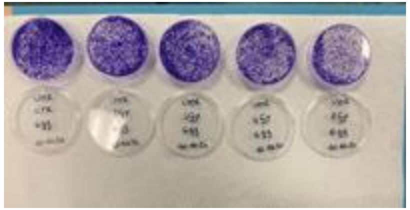




3D tissue-mimicking scaffolds are optimized for the cell lines under study.



2D/3D ***foci* assays** visualize the real-time DNA damage imparted by the ^{111}Ag-PSMA-617 treatment.



Cell death and vitality are evaluated by means of **clonogenic survival assays**.

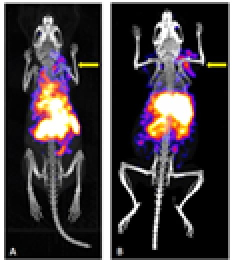
Uptake measurements

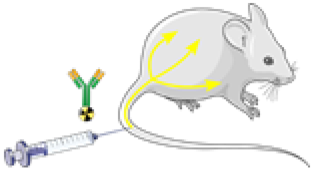
The **uptake** of radiopharmaceutical in Petri is generally evaluated with **γ counters** analyzing pellets of cells. This is useful for dosimetry.

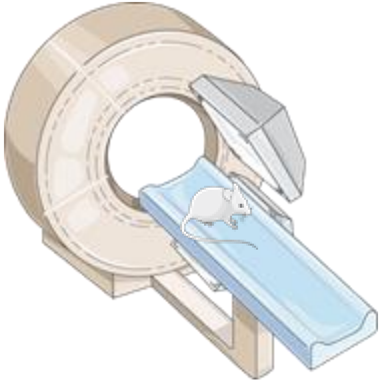
In APEX, high-resolution **β imagers** allow to discriminate intracellular absorption and can be used on 3D scaffolds.

WP3 – VIVO

- Biodistribution *ex vivo* of ^{111}Ag-PSMA-617
- Preclinical experiments *in vivo* with ^{111}Ag-PSMA-617 and ^{177}Lu-PSMA-617 for comparison
- γ imaging and radiomics



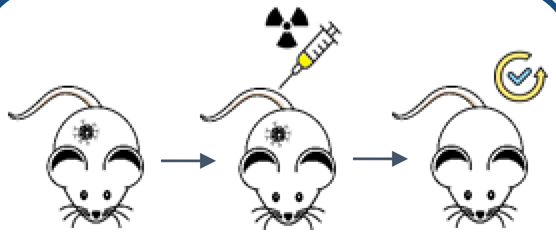




WP3 methodology

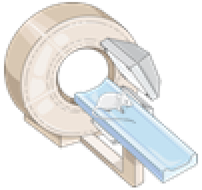
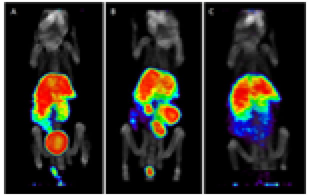
Preclinical experiments: biodistribution and animal trials


The **ex-vivo biodistribution** is preliminarily evaluated with free ^{111}Ag, chelated ^{111}Ag and ^{111}Ag-PSMA-617 in healthy mice and in mice **transfected with tumors** expressing or not expressing the target receptor.

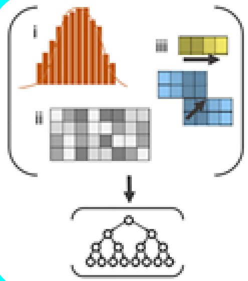


If the targeting is good, the **therapeutic effect** is finally assessed evaluating the **recovery** of treated mice.

Imaging and radiomics


The real-time biodistribution can be visualized via 2D **autoradiography** and 3D **PET/CT** or **SPECT/CT** imaging; a ^{111}Ag-tailored **γ camera** is also available from ADMIRAL.



PET/CT or SPECT/CT imaging can also be used for **pattern recognition** (extraction of **radiomic features**).

Gantt diagram

PADOVA INVOLVED IN WP1 AND WP2

		Year 1				Year 2				Year 3				Notes		
		M3	M6	M9	M12	M15	M18	M21	M24	M27	M30	M33	M36	Required for	Publications	Divisions
	WP1 – SILICO (leader: S. Bortolussi)															
T1.1	Image-based Monte Carlo dosimetry for prostate cancer cells	→	●											T2.1-T2.6	A, B	LNL, PD, PV
T1.2	Internal Monte Carlo dosimetry of biodistribution study with healthy mice	→			●									T3.5	C	LNL, PD, PV
T1.3	Internal Monte Carlo dosimetry of biodistribution study with tumor-bearing mice					→			●					T1.5, T3.6	D	LNL, PD, PV
T1.4	Biophysical model validation with radiobiological data			→					●						A	LNL, PD
T1.5	Dosimetric planning for preclinical studies with tumor-bearing mice							→			●			T3.6	E	LNL, PD, PV
T1.6	Validation of DNA damage models using radiobiological data and Geant4-DNA							→					●		F	LNL, PD
T1.7	Preliminary dosimetric predictions for human patients										→		●		E	LNL, PD, PV
	WP2 – VITRO (leader: D. Maniglio)															
T2.1	2D radiobiology of Ag-111-PSMA-617 on prostate cancer cells	→			●									T1.4	A	PV
T2.2	2D radiobiology of Lu-177-PSMA-617 on prostate cancer cells	→			●									T1.4	A	LNS
T2.3	β imaging of 2D cell culture uptake	→			●									T2.1	A	PD, PI
T2.4	3D radiobiology of Ag-111-PSMA-617 on prostate cancer cells					→			●						B	PV, TIFPA
T2.5	Microscopy and β imaging of 3D cell culture uptake					→			●					T2.4, T2.6	B	TIFPA, PD, PI
T2.6	3D radiobiology of Ag-111-PSMA-617 on prostate cancer cells in bioreactor									→			●		B	PV, TIFPA
T2.7	2D radiobiology of SPES radionuclides							→					●	T1.6	F	LNL
	WP3 – VIVO (leader: F. P. Cammarata)															
T3.1	Authorization request to work with Ag-111 in mice at CAPiR and CISUP	→	●											T3.4-T3.6	-	PI, LNS
T3.2	γ camera and SPECT imaging of Ag-111 in phantoms													T3.7	G	BO, PI
T3.3	ARG imaging improvement using the γ camera collimator													T3.4-T3.6	G	BO, LNS
T3.4	γ imaging and *ex vivo* biodistribution study of Ag-111-PSMA-617 in healthy mice													T1.2	C	LNS, PI, LNL
T3.5	γ imaging and *ex vivo* biodistribution study of Ag-111-PSMA-617 in tumor-bearing mice													T1.3	D	LNS, PI, LNL
T3.6	Preclinical experiments and γ imaging using Ag-111-PSMA-617 on tumor-bearing mice												●	T1.7, T3.7	E	LNS, PI, BO
T3.7	Radiomic features evaluation from SPECT/CT of preclinical studies												●		H	LNS
→	Activity started															
●	Deliverable/milestone reached															

Publication/report schedule

Index	Year	Theme
A	2027	2D radiobiology of Ag-111-PSMA-617
B	2029	3D radiobiology of Ag-111-PSMA-617
C	2027	Biodistribution in healthy mice
D	2028	Biodistribution in tumor-bearing mice
E	2029	Preclinical trials of Ag-111-PSMA-617
F	2029	Validation of DNA damage models
G	2029	Scintigraphy with Ag-111 γ camera
H	2029	Radiomics

Endorsement of hospitals

Protocollo d'Intesa di Collaborazione Scientifica e Tecnologica

[Loading…]

Santa Maria Nuova (Reggio Emilia)

Cannizzaro (Catania)

IRST D. Amadori (Meldola, FC)

Spedali Civili (Brescia)

Project FTE

LNL			
Name	WP	FTE	Status
Alberto Andrighetto (National resp.)	2	0.5	Experimental physicist, technology executive
Stefano Corradetti (LNL local resp.)	2	0.1	Materials engineer, technologist
Valerio Di Marco	2, 3	1	Chemist, associate professor at UNIPD
Laura Orian	2, 3	1	Chemist, associate professor at UNIPD
Omorjit Singh Khwairakpam	2	0.1	Experimental physicist, post-doc
Total LNL FTE		2.7	T=0.6; R=2.1

Padova			
Name	WP	FTE	Status
Alberto Arzenton (National resp.)	1, 2	0.6	Theoretical physicist, research scholar at UNIPD
Marcello Lunardon (PD local resp.)	2, 3	0.5	Experimental physicist, associate professor at UNIPD
Sandra Moretto	2, 3	0.1	Experimental physicist, associate professor at UNIPD
Lisa Zangrando	1	0.1	Computer scientist, technologist
Total PD FTE		1.3	T=0.1; R=1.2

Bologna			
Name	WP	FTE	Status
Matteo Negrini (BO local resp.)	3	0.2	Experimental physicist, researcher
Anselmo Margotti	3	0.2	Experimental physicist, technology executive
Carla Sbarra	3	0.2	Experimental physicist, researcher
Giuseppe Baldazzi	3	0.3	Experimental physicist, associate professor at UNIBO
Edoardo Borciani	3	0.5	Physics PhD student at UNIBO
Total BO FTE		1.4	T=0.2; R=1.2

LNS			
Name	WP	FTE	Status
Giorgio Russo (LNS local resp.)	3	0.3	Experimental physicist, CNR researcher
Francesco Paolo Cammarata	3	0.5	Biologist, CNR researcher
Cristiana Alberghina	3	1	Biologist, post-doc at UNICT
Alessandro Stefano	3	0.3	Computer engineer, CNR researcher
Total LNS FTE		2.1	T=0; R=2.1

Pavia			
Name	WP	FTE	Status
Antonietta Donzella (PV local resp.)	1, 3	0.6	Computational physicist, technologist at UNIBS
Giorgio Biasiotto	2	0.2	Biochemist, associate professor at UNIBS
Roberto Bresciani	2	0.2	Biochemist, associate professor at UNIBS
Carlo Rodella	1, 2	0.4	Medical physicist at Spedali Civili di Brescia
Federica Saiani	1, 2	0.4	Medical physicist at Spedali Civili di Brescia
Andrea Salvini	2, 3	0.3	Radiochemist, technologist at UNIPV-LENA
Andrea Gandini	2, 3	0.3	Radiochemist, technologist at UNIPV-LENA
Fabio Zelaschi	2, 3	0.2	Radiochemist, technologist at UNIPV-LENA
Silva Bortolussi	1, 2	0.2	Experimental physicist, associate professor at UNIPV
Marco Di Luzio	2	0.5	Metrologist, INRiM researcher
Giancarlo D'Agostino	2	0.5	Metrologist, INRiM researcher
Laura Cansolino	2	0.3	Biologist, technologist at UNIPV
Cinzia Ferrari	2	0.4	Biologist, technologist at UNIPV
Total PV FTE		4.5	T=2.1; R=2.4

Pisa			
Name	WP	FTE	Status
Nicola Belcari (PI local resp.)	2, 3	0.4	Experimental physicist, associate professor at UNIPI
Emilio Mariotti	3	0.1	Experimental physicist, associate professor at UNISI
Giancarlo Sportelli	2, 3	0.3	Experimental physicist, associate professor at UNIPI
Luca Menichetti	3	0.2	Radiochemist, CNR researcher
Daniele Panetta	3	0.2	Medical physicist, CNR researcher
Total PI FTE		1.2	T=0; R=1.2

TIFPA			
Name	WP	FTE	Status
Devid Maniglio (TIFPA local resp.)	2	0.5	Experimental physicist, associate professor at UNITN
Alessio Bucciarelli	2	0.7	Material scientist, researcher at UNITN
Annalisa Tirella	2	0.7	Biomedical engineer, associate professor at UNITN
Antonella Motta	2	1	Biomedical engineer, full professor at UNITN
Total TIFPA FTE		2.9	T=0; R=2.9

Total project FTE	16.1	T=3.0; R=13.1

1st year budget

LNL		
Type	**ID**	**Item**
Equipment	0	Equipment for radiobiology lab
Consumables	1	Mouse phantoms
	2	Radiobiology laboratory material
	3	Laboratory material for molecule synthesis
Travels	4	Travels for experimental activity
Total LNL		

Padov		
Type	**ID**	**Item**
Consumables	5	New components for β detector
Shipping	6	Shipping of detectors, etc.
Travels	7	Travels for research activities
Total PD		

Bologn		
Type	**ID**	**Item**
Consumables	8	Scintillator
	9	Tungsten foil for collimator (or other material)
	10	Electronic components
	11	Mechanic components
Travels	12	Travels for experimental activity
Total BO		

LNS		
Type	**ID**	**Item**
Consumables	13	Laboratory material
	14	Mice for in-vivo experiments
Shipping	15	Shipping of detector, drugs, samples
Travels	16	Travels for experimental activity
Total LNS		

Pavia		
Type	**ID**	**Item**
Consumables	23	Chambers and electronics for "hot" bioreactor
	24	Radiobiology laboratory material
	25	Enriched Pd-110 targets
	26	Laboratory material for LENA
	27	Material for activation measurements (INRiM)
Services	28	Target irradiation and chemical separation at L
Shipping	29	Shipping of Ag-111, etc.
Travels	30	Travels for research activities
Total PV		

Pisa		
Type	**ID**	**Item**
Consumables	17	Laboratory material
	18	New components for β detector
	19	Mice for in-vivo experiments
Services	20	Access to the Eurobioimaging facility
Shipping	21	Shipping of Ag-111, etc.
Travels	22	Travels for experimental activity
Total PI		

TIFPA		
Type	**ID**	**Item**
Consumables	31	Chambers and electronics for "cold" bioreacto
	32	Biology laboratory material
Shipping	33	Shipping of cell lines, etc.
Travels	34	Travels for experimental activity
Total TIFPA		

Total project

Padova prospect

Padova			
Name	WP	FTE	Status
Alberto Arzenton (National resp.)	1, 2	0.6	Theoretical physicist, research scholar at UNIPD
Marcello Lunardon (PD local resp.)	2, 3	0.5	Experimental physicist, associate professor at UNIPD
Sandra Moretto	2, 3	0.1	Experimental physicist, associate professor at UNIPD
Lisa Zangrando	1	0.1	Computer scientist, technologist
Total PD FTE		1.3	T=0.1; R=1.2

Padova							
Type	ID	Item	WP	Year 1 [k€]	Year 2 [k€]	Year 3 [k€]	Total
Consumables	5	New components for β detector	2	1	1	1	3
Shipping	6	Shipping of detectors, etc.	3	1	1	1	3
Travels	7	Travels for research activities	1, 2, 3	3	3	3	9
Total PD				5	5	5	15

Outline

- ISOLPHARM method and collaboration
- ISOLPHARM experiments: past and present
- ISOLPHARM_APEX organization
- Conclusions

Resume

WP1 – SILICO

Monte Carlo dosimetry in murine and cellular geometries; development of models for DNA damage repair and cell survival.
Divisions involved: PD, LNL, PV.

WP2 – VITRO

2D and 3D radiobiology using ^{111}Ag-PSMA-617, other nuclides for comparison and radiotracers, including also cold studies for the 3D part. β imaging for cell uptake measurements.
Divisions involved: PV, TIFPA, LNS, LNL, PD, PI.

WP3 – VIVO

In vivo experiments with ^{111}Ag-PSMA-617, radiomics and imaging using γ camera prototype, SPECT/CT and ARG. Imaging calibration using phantoms.
Divisions involved: LNS, PI, BO.

Possible international partners: **BIOEMTECH** (SPECT imaging), **CNEA** (currently studying ^{177}Lu-DTPA complexes)

Fallout

- With successful preclinical trials using ^{111}Ag-PSMA-617 against prostatic tumors, **clinical trials** could begin

- Possible **commercialization** γ and β imaging prototypes, if their goals in terms of resolution and efficiency will be satisfied

- Production of low-dose-rate **radiobiological data** as benchmark for DNA damage and repair models with SPES_MED nuclides

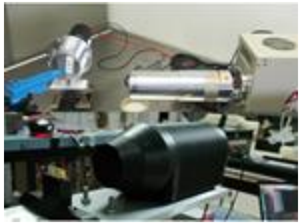
Thank you!

Backup slides

ISOLPHARM_EIRA achievements

Task 1 - Physics

- Production of ^{111}Ag via radiative neutron capture using ^{110}Pd or ^{nat}Pd targets
- Spectroscopic system for radiation measurements at the reactor and ex vivo
- Study of laser photo-ionization schemes of Ag for SPES laser ion source

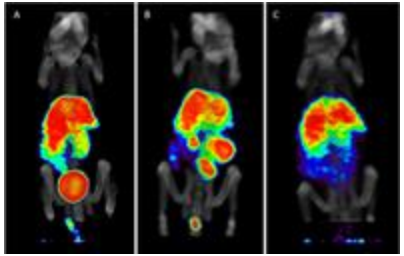


Task 2 - Chemistry

- Ag/Pd separation protocol after irradiation at LENA
- Development of stable chelators for Ag

Task 3 - Biology

- Synthesis of linkers and targeting agents for CCK2R
- "Cold" affinity tests in vitro
- Biodistribution and stability tests in vivo using PET/SPECT radionuclides



The SPES_MED experiment (2025-2027)

First nuclear measurements at SPES financed by INFN-CSN3!

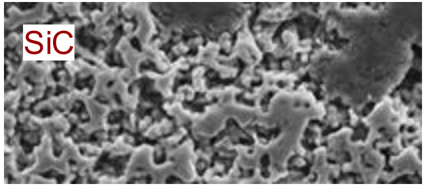
Work packages

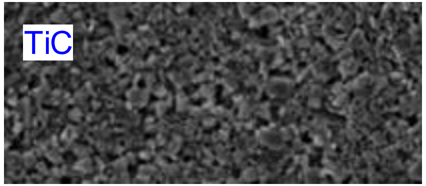
1) Cross-section measurements (LARAMED project)
2) ISOL production yield measurements (ISOLPHARM project)
3) Theory and simulations

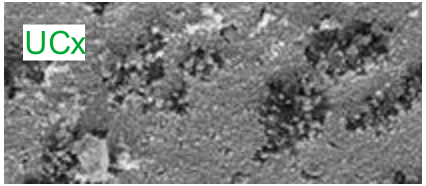
The SPES_MED scenario

WP2 aim: measuring the **ISOL production yields of radiotracers** with applications in biology, medicine, environment, industry.

2025: ^{24}**Na** and ^{7}**Be** from SiC target (currently out of SPES_MED)
2026: ^{28}Mg from SiC target (low activity expected)
2027: ^{51}Cr (low activity expected), ^{43}**K** from TiC target + ^{111}Ag from UC_x



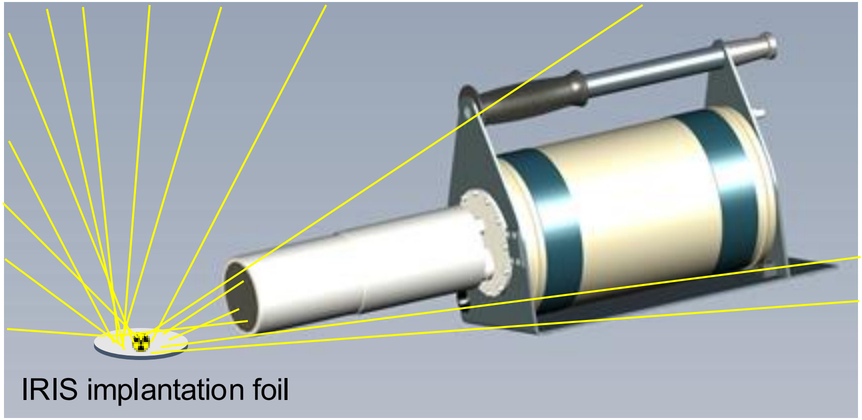




High-production nuclides could be used in a **CSN5** experiment! → **Radiobiology lab at LNL?**

SPES_MED vs ISOLPHARM_APEX

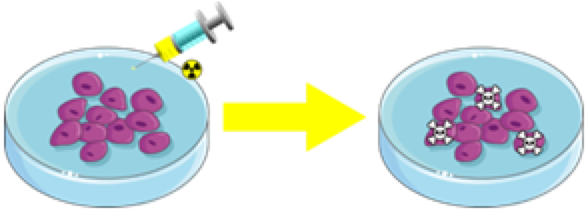
SPES_MED: nuclear measurements (approved by CSN3)

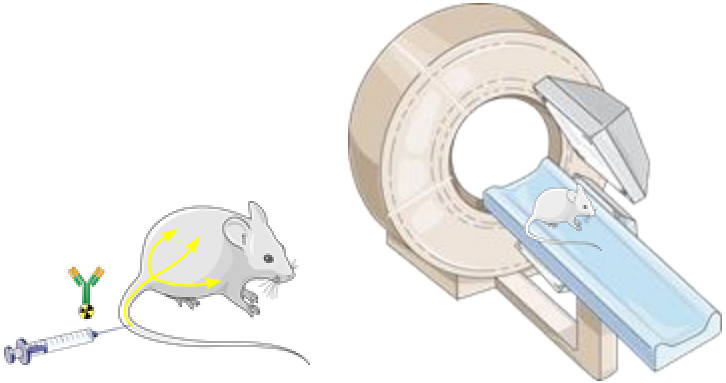


After measurements, either:

- dispose of the radionuclide
- reuse it for applications

ISOLPHARM_APEX: application to radio-biology and medicine (to be submitted to CSN5)





1^{st} year summary

IN-SILICO DOSIMETRY IN CELLULAR AND MURINE GEOMETRIES WP1 will begin the computational activities in geometries reproducing the microscopic cellular environment and the macroscopic living systems formed by the small animals for preclinical tests. Different prostate cancer cell lines will be analyzed via microscopy in terms of shape and volume. In this way, it will be possible to calculate the dosimetry of a treatment and associate the absorbed dose per cell to the administered activity, which is experimentally measured. Moreover, as soon as the first biodistribution data will be obtained after injection of ^{111}Ag or ^{111}Ag-labelled molecules in mice, the internal dosimetry of the small animals' organs will be calculated by Monte Carlo simulations. This is a fundamental step to plan the activity concentrations to be administered in the subsequent in-vivo experiments.

2D RADIOBIOLOGY EXPERIMENTS AND UPTAKE MEASUREMENTS Regarding WP2, the activities of 2026 will deal with radiobiology studies in traditional 2D Petri dishes. The purpose is to study the cell survival (clonogenic assay) and the repair dynamics of radiation-induced DNA damage (*foci* assay) in cultures treated with ^{111}Ag-PSMA-617 and, for comparison, with ^{177}Lu-PSMA-617. The targeting efficiency of the novel ^{111}Ag-labelled compound will be evaluated by repeating the experiments using several prostate cancer cell lines with different expressions levels of the PSMA target receptor. Such experiments will be performed by the Pavia and LNS personnel, taking advantage of the infrastructures of the Universities of Pavia and Catania and of the Cannizzaro hospital. In addition, the uptake of radiopharmaceutical will be measured by the β imagers developed within the ISOLPHARM collaboration by the Pisa and Padova groups and by γ counters.

FIRST PRECLINICAL EXPERIMENTS WITH ^{111}Ag-PSMA-617 WP3 in-vivo experiments will start as soon as the devoted laboratories, CAPiR in Catania and CISUP in Pisa. The main aim will be the measurement ex vivo of the biodistribution of i) free ^{111}Ag, ii) chelated ^{111}Ag (^{111}Ag-DO4S) and iii) ^{111}Ag-PSMA-617 in mice without tumor. This will be useful to assess the stability of the molecules and the targeting efficiency in vivo, when the same tests will be repeated in mice with transfected tumors during the 2^{nd} year of the project. The experiments aforementioned will be supported by γ imaging techniques, including SPECT in Pisa, autoradiography in Catania and the γ camera prototype developed by the Bologna group. Such devices will be first calibrated with murine phantoms, to quantify the resolution achievable and the exposure time required. An additional activity will regard the possible improvement of the autoradiography resolution using the ^{111}Ag-specific collimator of the γ camera prototype.

2nd year summary

THERAPY PLANNING FOR PRECLINICAL STUDIES AND BIOPHYSICAL MODELS FOR RADIOBIOLOGY Taking advantage of the first ^{111}Ag-PSMA-617 biodistributions measured in mice with prostatic tumors, WP1 will optimize and complete the internal dosimetry activities on murine geometries. This essential work will provide an exhaustive picture of the dose imparted by the novel drug to the various organs (as well as the tumor) during a treatment. The 3rd year preclinical studies will be planned according to the dose maps obtained. On the other side, the WP1 personnel will use the experimental data acquired by WP2 to tune the biophysical models developed. The goal is to provide a robust method for the analysis of clonogenic-assay outcomes obtained using radiopharmaceuticals, also making them comparable to the benchmark of external-beam radiotherapy.

UPGRADE TO 3D RADIOBIOLOGY EXPERIMENTS IN TISSUE-MIMICKING SCAFFOLDS In 2027, WP2 will substantially repeat the 2D tests of 2026 in 3D tissue-mimicking scaffolds (developed by the TIFPA group). Uptake measurements will strongly rely on β imagers, since a γ-counter analysis of the samples may not be possible due to physical features of the scaffolds. Also, a microscopic analysis of the stable equivalent of the drug could be helpful in this context.

^{111}Ag-PSMA-617 BIODISTRIBUTION EXPERIMENTS IN TUMOR-BEARING MICE WP3 will complete the activities begun during 2027. The characterization and optimization of the γ imaging devices will be concluded by the end of the year. ^{111}Ag-PSMA-617 biodistribution tests will also continue with the experiments on tumor-bearing mice. The information resulting from these will be a fundamental input for WP1 internal dosimetry simulations which will lead to the definition of the treatment plans to be adopted in the preclinical experiments of the last year of the project.

3rd year summary

INTERNAL DOSIMETRY ON MICE AND HUMANS AND DNA REPAIR MODELLING In the last year of the project, WP1 will have a fundamental role in the planning of preclinical experiments (WP3), as also in the second half of 2027. Moreover, in the last part of the year, the dosimetric parameters obtained for mice will be converted using appropriate methods to the human case, in order to have an idea of the possible amounts of activity required by patients in hospitals. On the cellular side, the theoretical modelling of the DNA repair pathways will be studied and compared to the data from *foci* assays.

3D RADIOBIOLOGY IN BIOREACTORS AND FIRST STUDIES ON SPES RADIONUCLIDES 2028 will take WP2 to the final stage of in-vitro experimentation: the use of 3D scaffolds in bioreactors to reproduce more faithfully the in-vivo conditions in cancer spheroids. Before the experiments, which will take place in Pavia, a campaign of cold tests will be performed in the facilities of the University of Trento associated to TIFPA. Together with such an enticing challenge, a goal of ISOLPHARM_APEX is to re-open the radiobiology laboratory of the LNL to study the effects on cells of radiotracers produced at the SPES ISOL facility using light targets.

PRECLINICAL EXPERIMENTS AND FINAL EVALUATION OF ^{111}Ag-PSMA-617 In the final months of ISOLPHARM_APEX, WP3 will have the task of testing the therapeutic effect of the novel radiopharmaceutical candidate ^{111}Ag-PSMA-617 in mice. The outcomes of these experiments, also compared to ^{177}Lu-PSMA-617 results, will determine the goodness of the new drug as, in case of success, it would be considered ready for the clinical phase. Finally, the set of SPECT/CT images collected during the three years of the project will enable the opportunity to perform radiomics studies, taking advantage of the machine-learning expertise of the LNS personnel.