Radiobiology Facilities at LNS

Giorgio Russo

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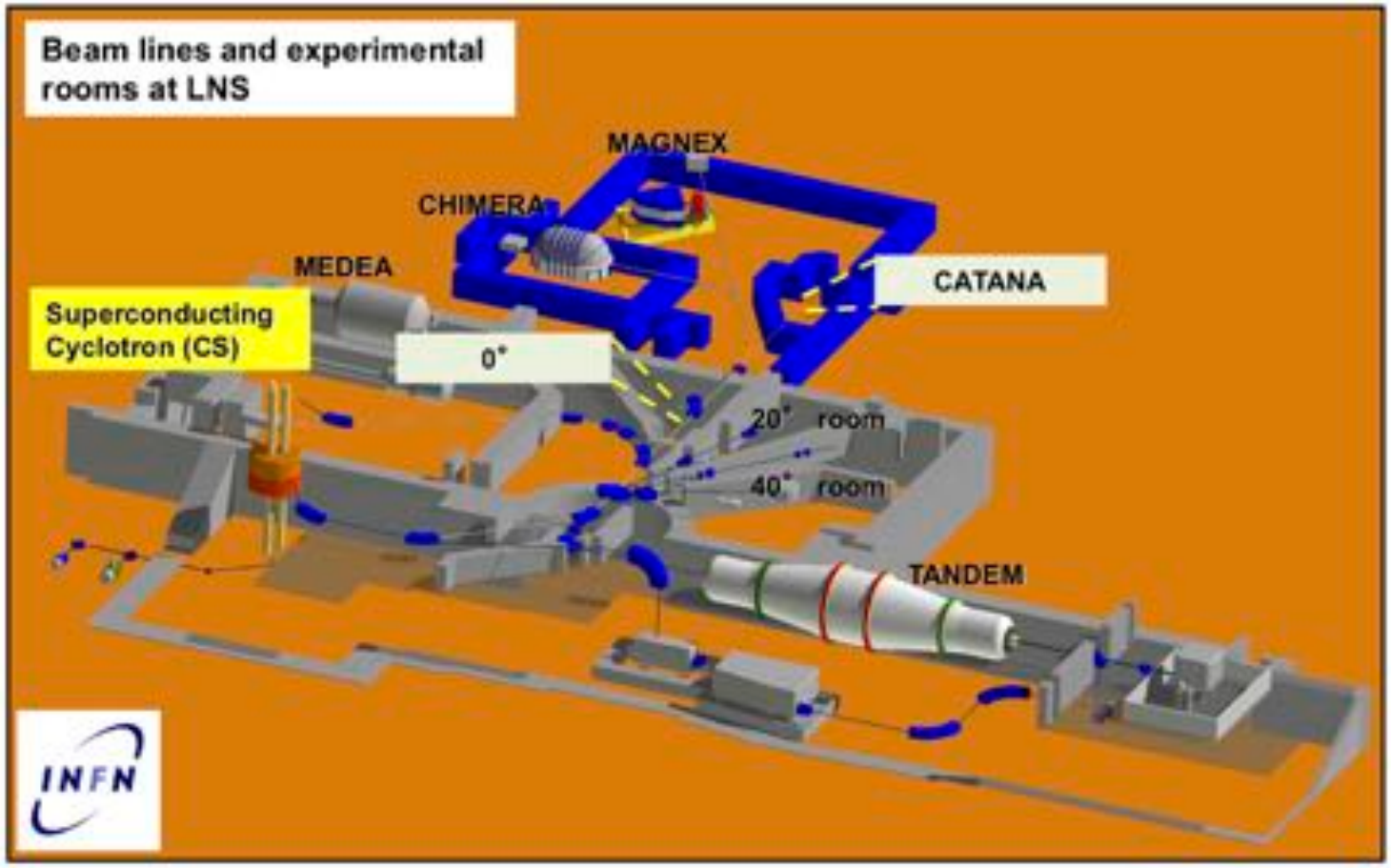
Istituto di Bioimmagini e Fisiologia Molecolare (IBFM) - CNR - SS Cefalù

Multidisciplinary beam lines at INFN-LNS

Two rooms are available at LNS for multidisciplinary activities irradiations:

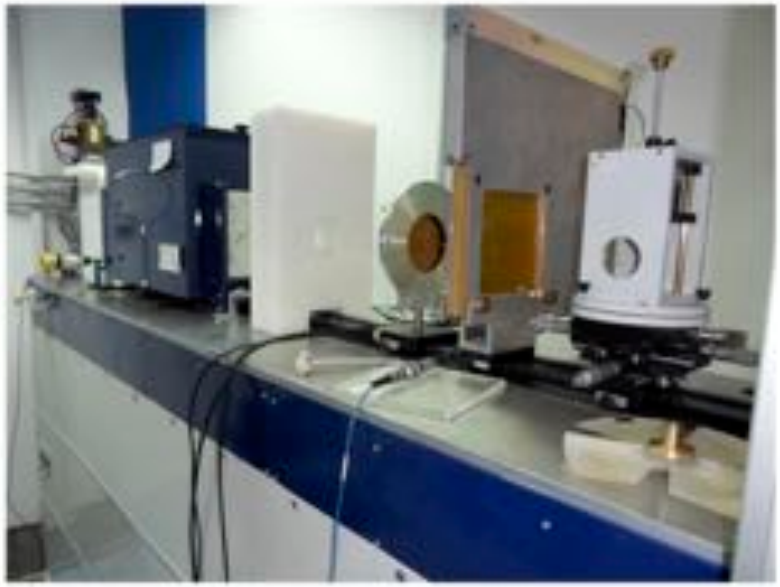
- **CATANA beam line** (clinical proton beams at 62 MeV)
- **0° beam line** (protons and light ions up to 80 AMeV)

both equipped with detectors for beam diagnostics and dose monitoring.



CATANA beam line

- Mainly dedicated to proton irradiation (eye melanome treatments)
- Dosimetry and radiobiology experiments
- *In-air* only
- Energy passively degraded
- Fast and easy positioning systems but …



- Double scattering system for lateral spread → homogeneity ≈ 3%
- Collimated beams 1 mm / 35 mm
- Fixed elements limiting some applications:
  - Fluence not maximized
  - High level of homogeneity but no point-like spot size
  - Radiation protection issues during the patient treatments may limit beam current

Ask to Pablo Cirrone and Giada Petringa about the dosimetry

0° beam line

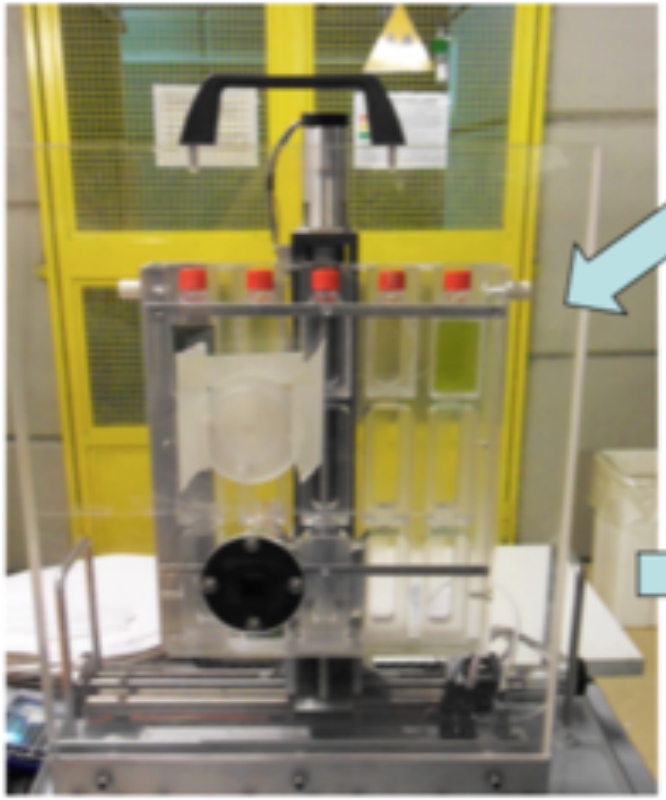
- Transported beams:
  p, He, C, O, Ne, Ar, Kr, Xe, …
- Relative and absolute dosimetry
- Certified beam line for ESA experiment by the MAPRAD Group.
- Mainly dosimetry and radiobiology *in-air* irradiation but also possibility to use vacuum chambers

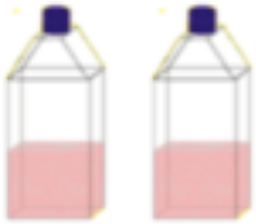
- Fast and easy positioning systems
- No particular constrain from fixed elements but ....
  - Homogeneity ≈ 15% (non focalized beams)
  - Final collimator can be removed but alignment procedure must be repeated (4 h)

Ask to Pablo Cirrone and Giada Petringa about the dosimetry

Radiobiology: irradiation device

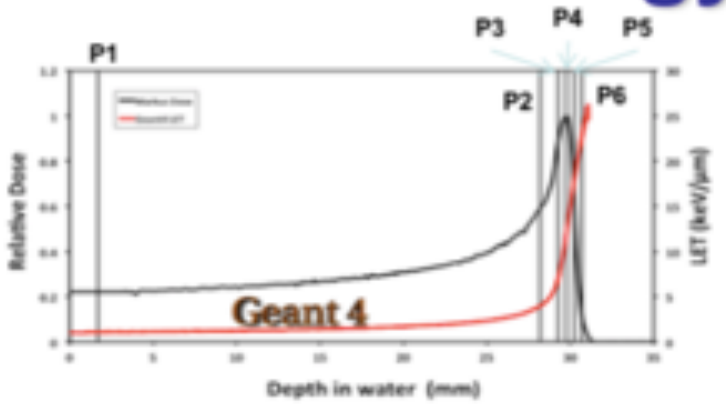
The software for remote cell positioning has been updated



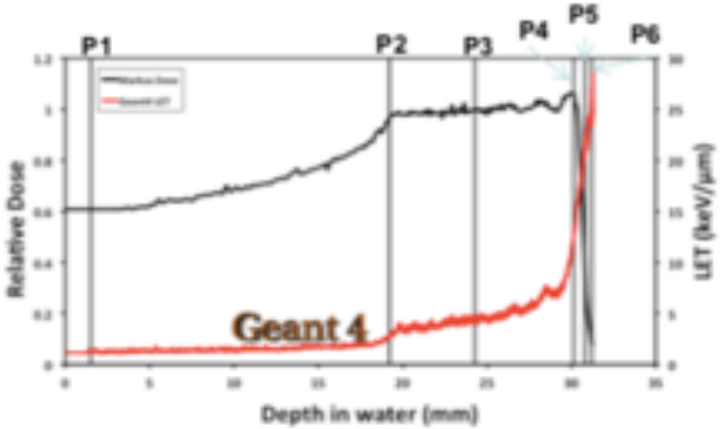


- Motorized system with 100 um precision
- Remotely controlled
- Interfaced with beam control system
- Real time dose-rate monitoring

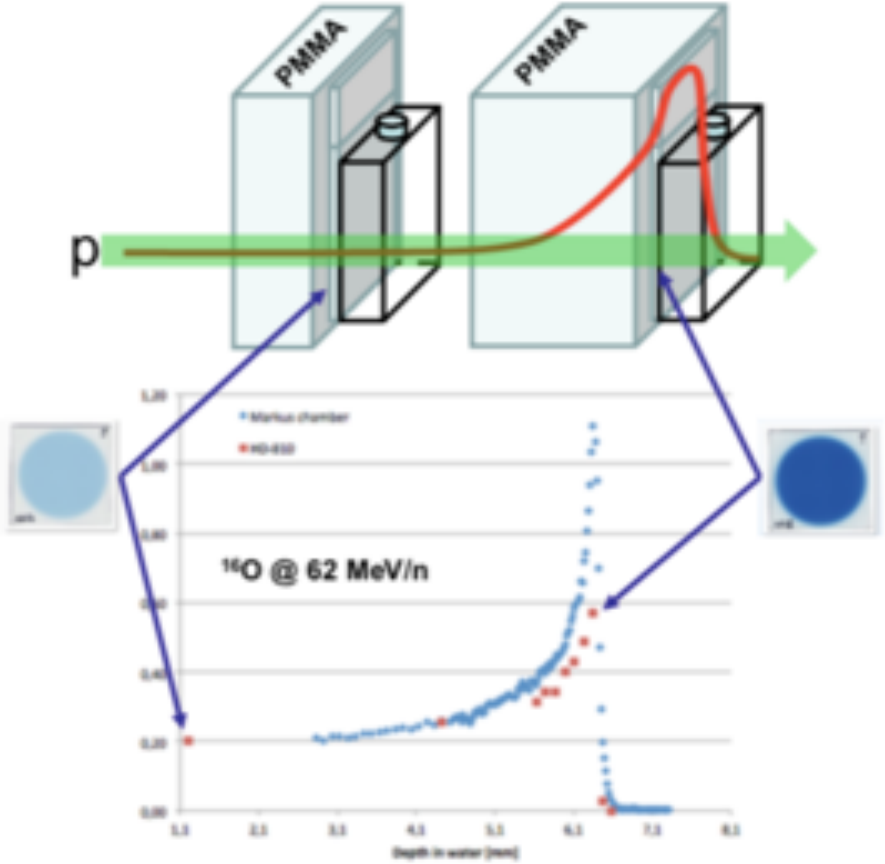
Radiobiology: cell positioning



	P1	P2	P3	P4	P5	P6
Depth water [mm]	1.38	20.23	24.59	27.69	29.48	30.08
LET [keV/µm]	1.2	2.6	4.5	13.4	21.7	25.9



	P1	P2	P3	P4	P5	P6
Depth water [mm]	1.38	27.42	29.21	29.8	30.7	31.29
LET [keV/µm]	1.11	4.0	7.0	11.9	18.0	22.6



50 µm positioning accuracy achieved combining Gafchromic films with Markus Chamber

Working together with LNS – GEANT4 group

INFN-LNS Radiobiological Laboratory

The "*Radiobiological Laboratory*" is a cell biology laboratory dedicated to biological studies, from cell growth to irradiation procedures and following analysis. It is equipped with all devices necessary to perform experiments.

A. Laboratory Hood
B. Inverted microscopy
C. Centrifuge
D. Incubator
E. -80°C for storage of biological samples
F. Dewar for long term storage of different cellular batch

INFN-LNS Radiobiological Laboratory

Fluorescence Microscopy

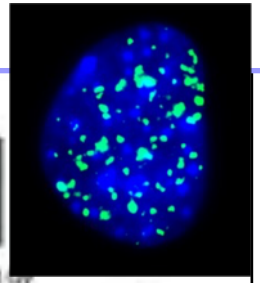
16.25 megapixel CMOS image sensors for microscopy

High sensitivity
Excellent linearity
High – frame rate
Low Noise

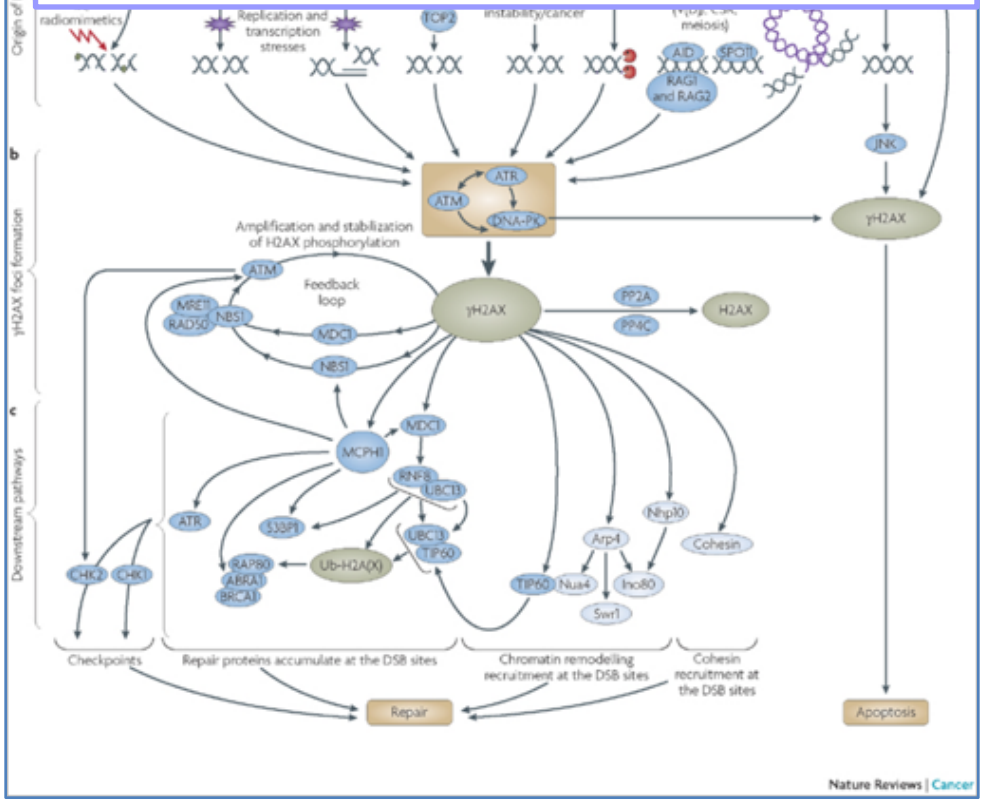
Integration with imaging SF

Fluorescence Microscopy and Radiobiology Why?

To Analyse DNA DAMAGE (DSBs)
When DNA damage, forms double stranded breaks (DSBs) , it is always followed by the phosphorylation of the histone, H2AX



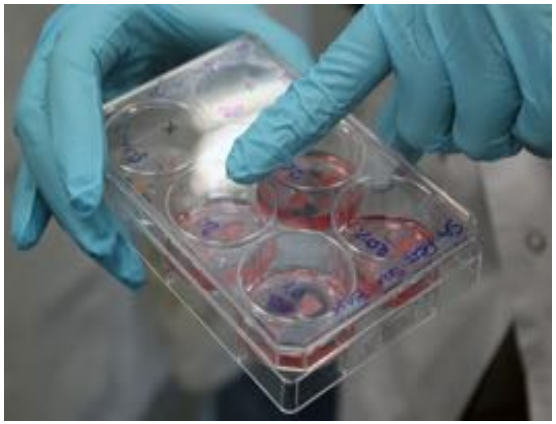
To Analyse Molecular actors of DAMAGE
H2AX is the central component in response to DSBs but there are other interesting molecules involved in the pathways that need to be investigated using the fluorescence microscopy



NEXT STEP:

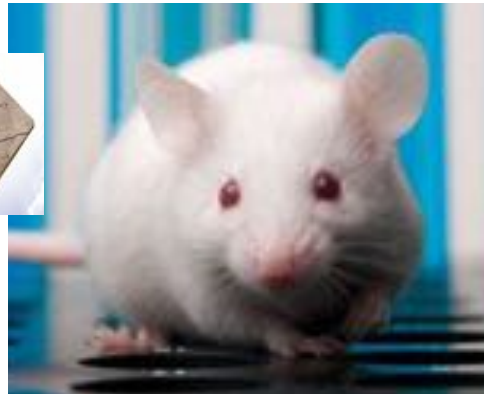
In vitro studies allowed us to acquire knowledge in order to be able to plan *in vivo* studies.

In vitro studies



In vivo studies

Mice model



Studies on:

Tumour region

Health tissue

Preclinical models are a crucial component of research in radiation therapy and in nuclear medicine. Small-animal irradiation systems must mimic the clinical application of radiation therapy as closely as possible.

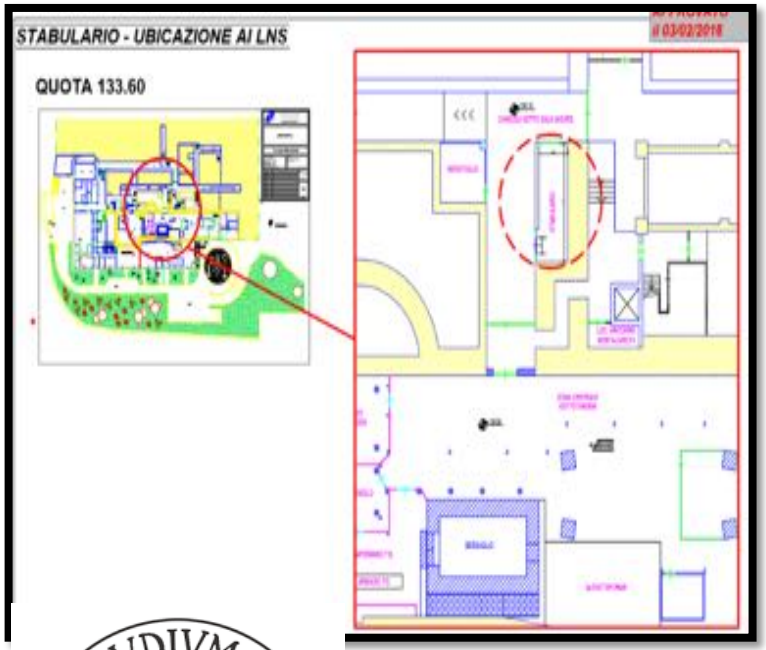
INFN-LNS Facility for Small Animal

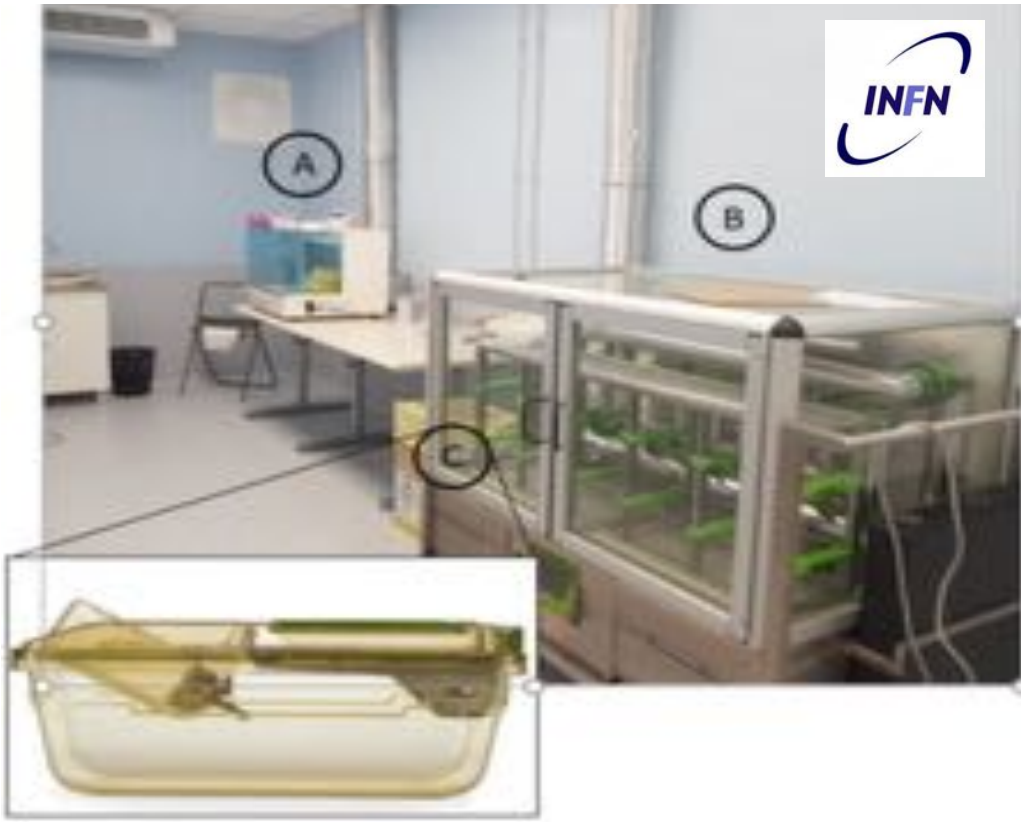
Small Animal facility

A - Chemical Hood

B –Transport Unit

C – Individual Ventilated Cage - IVC





The first animal experiments was approved on 2016

POSITIONING SYSTEM

An important part of a hadrontherapy treatment is the positioning …

Human positioning system

LNS-INFN

Small animal positioning system

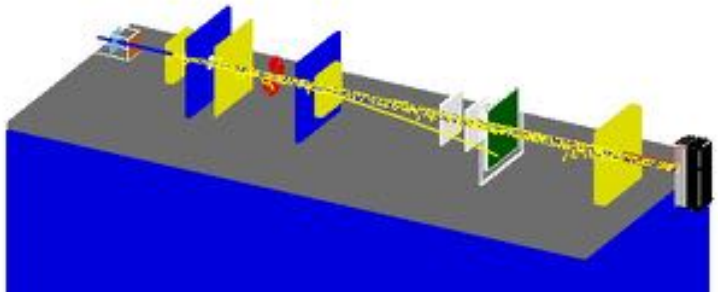
Nuclear Instruments and Methods in Physics Research A

ELSEVIER

Preliminary study for small animal preclinical hadrontherapy facility

G. Russo[a], P. Pisciotta[a,b,*], G.A.P. Cirrone[b], F. Romano[b], F. Cammarata[a], V. Marchese[a], G.I. Forte[a], D. Lamia[a], L. Minafra[a], V. Bravatà[a], R. Acquaviva[c], M.C. Gilardi[a], G. Cuttone[b]

Radiation treatment

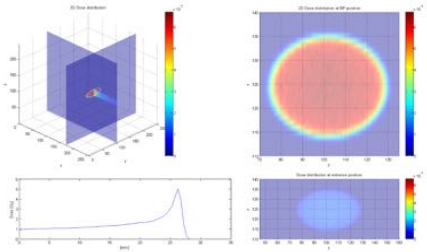


Geant 4

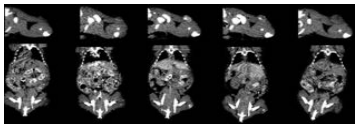
Geant4

Our custom application

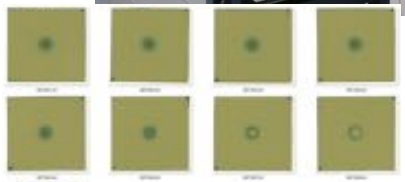
Simulation results



Validation phase needed



Experimental results

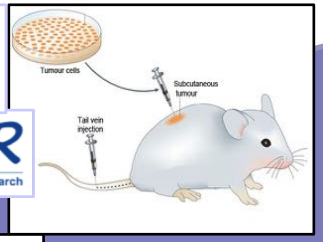


THE UNIQUE NATIONAL NETWORK PROVIDING RESEARCH AND SERVICE ACTIVITY IN THE PRECLINICAL IMAGING AND ADROTHERAPY

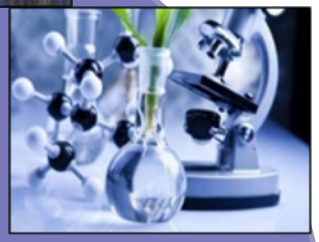
Since July 2017

UNIVERSITÀ degli STUDI di CATANIA

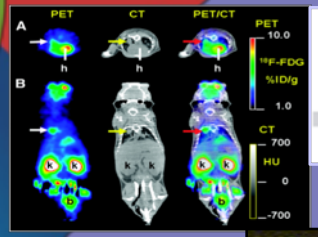
CAPiR Center for Advanced Preclinical *in vivo* Research



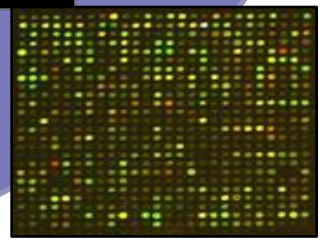
OSPEDALE CANNIZZARO CATANIA
AZIENDA OSPEDALIERA PER L'EMERGENZA



INFN Istituto Nazionale di Fisica Nucleare
Laboratori Nazionali del Sud



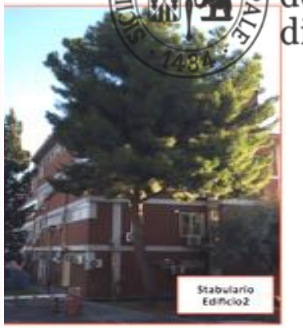
IBFM



Equipment & skills UNICT

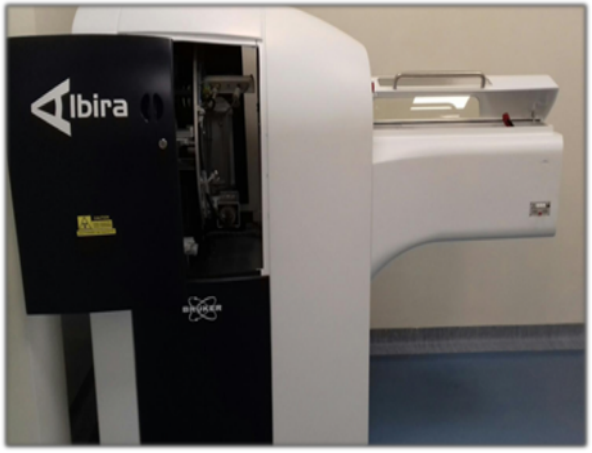
UNIVERSITÀ degli STUDI di CATANIA

Two enclosures of 700 square meter are available with spaces, equipments and skills to relay mice, rats, guinea pigs and rabbits, as well as a room equipped with zebrafish tanks. The two enclosures also have got experimental surgery and microsurgery rooms, washing and sterilization locals, chemical and biological laboratories.



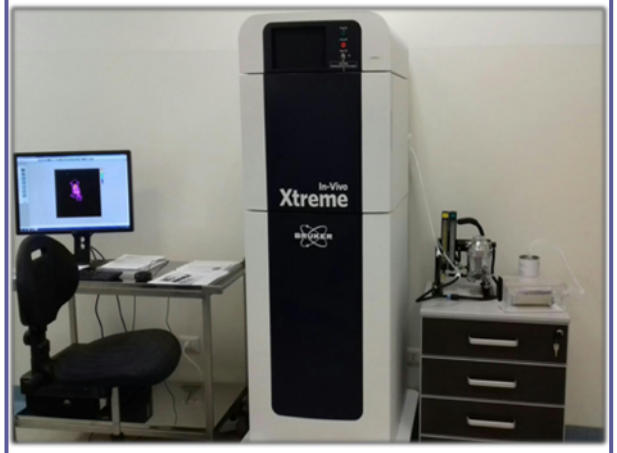
PET facility

equipped with an integrated multimodal PET/CT Bruker IBIRA for small animal, allowing the study of the progression of pathologies, the evaluation of the therapeutic efficacy of new possible molecules and substantially facilitating research activities for the development of new drugs in the diagnostic and therapeutic area.



Optical Imaging facility

equipped with an *in vivo* radiographic system "Bruker Xtreme" allowing the acquisition of traditional images in white light, fluorescence, luminescence and X-ray. It also allows screening of multiple animals at the same time, measuring the position and the biodistribution of the administered tracer/radiolabelling agent.



Ultrasound Imaging facility

equipped with an ultrasound system "Vevo2100 Visualsonics", allowing to acquire functional and morphometric information of animal models compared to the evolution of the pathology studied.

Equipment & skills H-Cannizzaro

Since 2005, it has been carried out Nuclear Medical Imaging activities, by the Nuclear Medicine and Pet Center Operational Unit, that is capable of a Cyclotron for the radiopharmaceuticals production, a Radiochemistry Laboratory and staff with qualitative analysis of nuclear medical imaging and radiopharmaceuticals expertise of Nuclear Medicine area.

H-CANNIZZARO produces radiopharmaceuticals for clinical use: [18F] FDG - [11C] Colin - [11C] Methionine - [68Ga] DOTATOC.

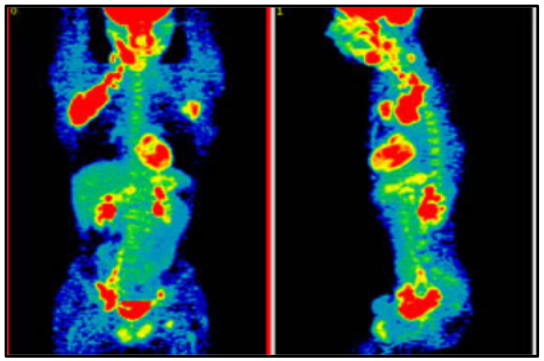
It distributes [18F] FDG to approved facilities.

New Radiotracers are going to be prepared: [18F] FLT - [18F] MISO - [18F] Fluoride - [18F] Colin.

Multidisciplinary skills for *in vivo* tracers synthesis and biodistribution (nuclear physician, radio-pharmacist, physical physician, chemist).

Nuclear Medical Staff for the qualitative image analysis supported by nuclear medical opinion.

Physical Physician Staff for physical and dosimetric quantitative evaluations and support for image processing.



Equipment & skills
IBFM-CNR

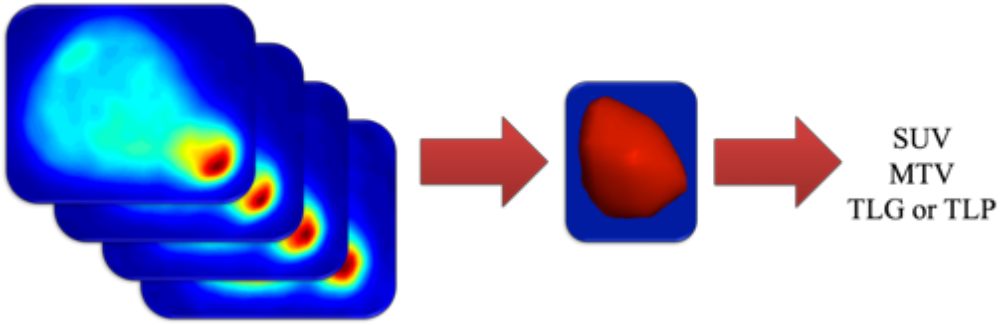
It develops *in vitro* and preclinical *in vivo* research activities in its secondary head office in Cefalù, having staff with animal experimentation, in bioimaging and in radioprotection expertise.

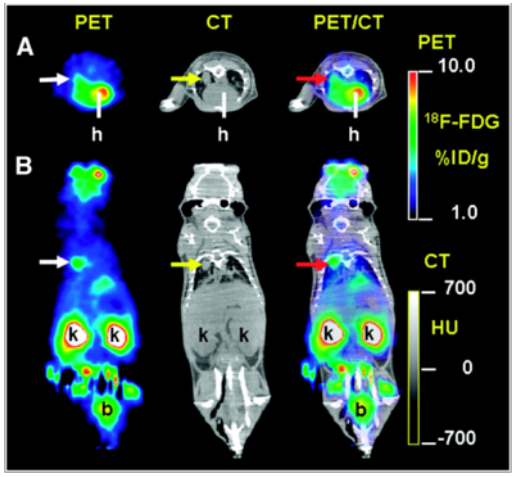
It owns staff with competences in:

- ✓ Animal Experimentation with FELASA Cat-C
- ✓ Assistance for experimental design and support for animal management at LNS.
- ✓ Processing of bioimaging for signal quantification.
- ✓ Molecular Analysis of biological samples

felasa

Federation of European Laboratory Animal Science Associations





To Radiomics studies

Consiglio Nazionale delle Ricerche

Istituto di Bioimmagini e Fisiologia Molecolare (IBFM)

CNR – IBFM Cefalù Unit

It was founded in (Delibera n. of the CNR n. 215/2007)

He currently works at the G. Giglio Institute Foundation in Cefalù

Institutional aims
"Research activities in the fields of Cancer Diagnosis and Treatment"

People

At the SS of Cefalù of the IBFM, a young group with multidisciplinary expertise of Researchers, Research Technicians, Research Fellows and Administrative personnel is working as follows:

- *6 staffed researchers* *(2 Engineers , 1 Medical Physics, 3 Biologists)*
- *1 Research Technician Collaborator*
- *1 Administrative collaborator*
- *4 researcher fellows in training* *(1 Medical Physics, 3 Biologists)*

Radiobiology facility @ INFN-LNS

CNR researchers working at LNS together with LNS researchers

Giorgio Russo - Medical Physics
Head of CNR – IBFM Cefalù Unit
Radiation Protection Expert at LNS and University of Catania

Francesco Cammarata –Phd in Biochemistry
Researcher - CNR - IBFM Cefalù Unit
Chief of activity in Radiobiology Laboratory and Preclinical Radiobiology Laboratory

Close cooperation

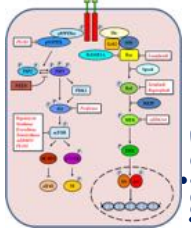
Cefalù CNR Unit

giorgio.russo@ibfm.cnr.it

francesco.cammarata@ibfm.cnr.it

Biological Skills

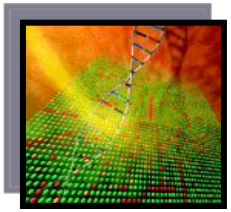
Western Blot



Proteomics

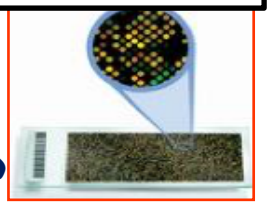
Proteogenomics

Molecular Biology



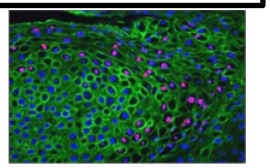
Genomics

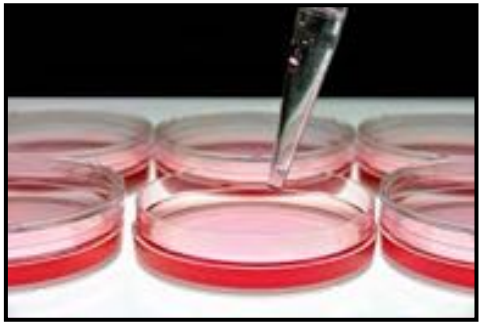
Genomic analysis



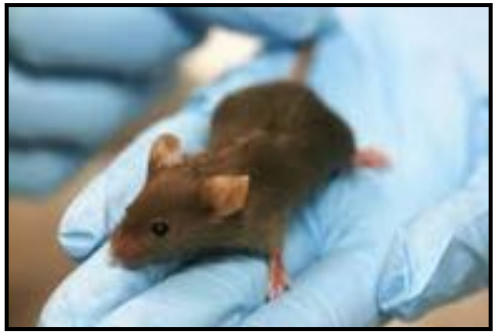
Inflammation

Immunoistochemistry





In vitro



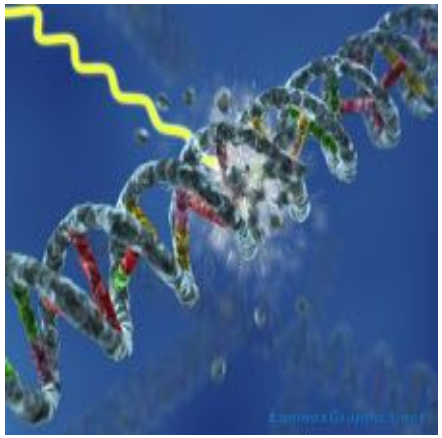
In vivo

ETHICS

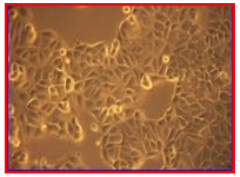
Pre-clinical experimental and theoretical studies to improve treatment and protection by charged particles

Understanding the underlying action mechanisms on normal cells by charged particles used in medicine to reduce the risks for human health

PI: Lorenzo Manti



Hadrontherapy (INFN Ethics project)



***In vitro* irradiations** were performed on different **breast cell lines**:

Non tumorigenic MCF10 and breast cancer cell lines to compare the response

In vivo Projects

First preclinical experiment at LNS - INFN

P.Pisciotta[1-2], FP.Cammarata[1-2], GI. Forte[1-2], V. Bravatà[1-2], L. Minafra[1-2], V. Marchese[1], R. Tringali[3], F. Torrisi[2], M. Bulgari[2], M. Abate[4], V. Zimmitti[4], L.Manti[5], G. Petringa[1], GAP.Cirrone[1], R. Acquaviva[3], G.Russo[1-2].

(ETHICS Collaboration)

1) INFN - Laboratori Nazionali del Sud,Via S. Sofia 62, 95125 Catania, Italy

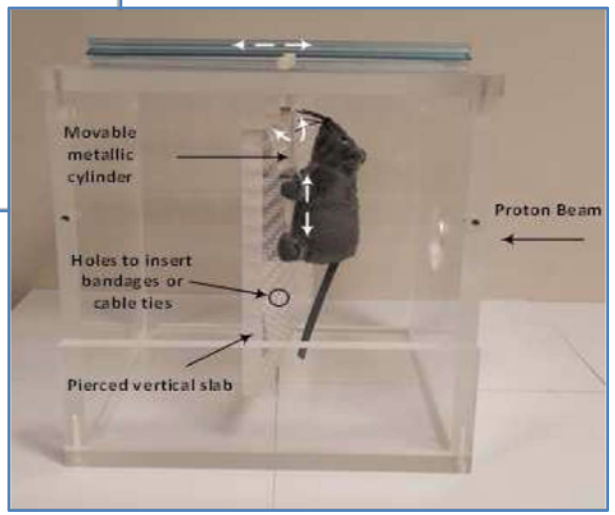
2) CNR-IBFM, UOS Cefalù (PA), Italy

3) University of Catania - Department of Drug Science, Catania, Italy.

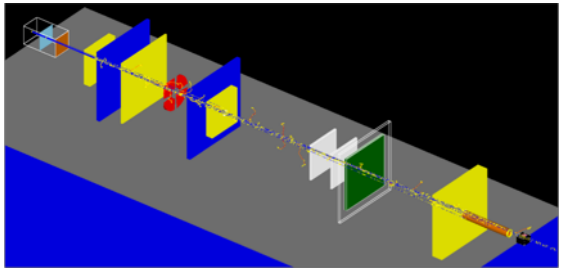
4) University of Catania – CAPiR, Catania, Italy

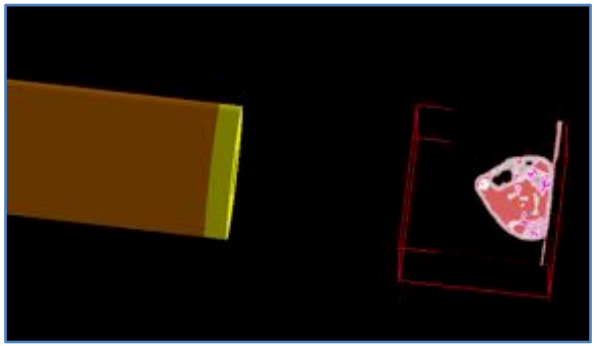
5) University of Napoli Federico II – Physics Department- Napoli, Italy

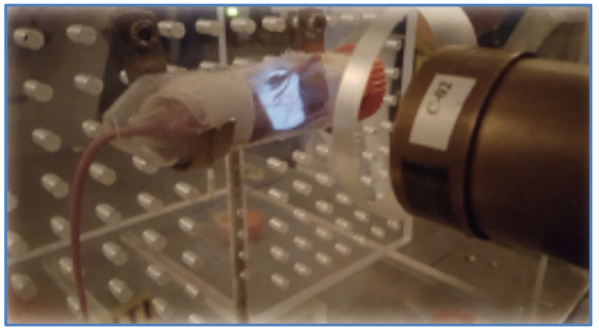
Published on Annuals of LNS 2017



In the framework of the research project ETHICS “Pre-clinical experimental and theoretical studies to improve treatment and protection by charged particles” we studied the preliminary steps to perform a particle treatment of cancer cells in small animals and to realize a preclinical hadrontherapy facility.







IBFM References of INFN CSN5 Projects

Ethics Project

- **Russo G, Pisciotta P, Cirrone GAP, Romano F, Cammarata F, Marchese V, Forte GI, Lamia D, Minafra L L, Bravatá V, Acquaviva R, Gilardi MC, Cuttone G. Preliminary study for small animal preclinical hadrontherapy facility. Nuclear Instruments and Methods in Physics Research A, 2017. 846:126-134.**
- **Forte GI, Minafra L, Bravatà V, Cammarata FP, Lamia D, Pisciotta P, Cirrone GAP, Cuttone G, Gilardi MC, Russo G. Radiogenomics: the utility in patient selection. Transl Cancer Res 2017;6 (Suppl 5):S852-S874.**
- **Bravatà V, Minafra L, Cammarata FP, Pisciotta P, Lamia D, Marchese V, Manti L, Cirrone GAP, Petringa G., Gilardi MC, Cuttone G, Forte GI, Russo G. Gene expression profiles induced by proton and electron irradiations in breast cancer cells. The British Journal of Radiology", 2018 Jun 11:20170934.**
- **Bravatà V, Cammarata FP, Minafra L, Pisciotta P, Scazzone C, Manti L, Cirrone GAP, Petringa G, Cuttone G, Forte GI, Russo G. Molecular insights of breast cells proton treated. *Submitted to International Journal of Radiation Biology, 2018***
- **Cammarata FP, Bravatà V, Minafra L, Pisciotta P, Scazzone C, Manti L, Cirrone GAP, Petringa G, Cuttone G, Gilardi MC, Forte GI, Russo G. Breast Cancer cell treated with proton beam: immunological features and gene signature. XVIII Convegno Nazionale della Società Italiana per le Ricerche sulle Radiazioni (SIRR).**
- **Pisciotta P, Cammarata FP, Minafra L, Bravatà V, Forte GI, Marchese V, Acquaviva G, Tringali R, Cirrone GAP, Petringa G, Cuttone G, Manti L, Russo G. Cell and molecular response to proton radiation treatments in breast cancer: in vitro models and in vivo applications. 44th Annual Meeting of the European Radiation Research Society (ERR 2018)**

In vivo Projects

Analysis of the Hormone Stress Response in Charged Particles Radiation Therapy (ANSIA)

M. Cestelli Guidi[1], R. Amendola[1,2], F.P. Cammarata[3], G. Russo[4], P. Pisciotta[4], G.A.P. Cirrone[4], R. Acquaviva[5], R. Tringali[5].

1) INFN, Laboratori Nazionali di Frascati, Frascati, Italy
2) ENEA, SSPT, TECS, Rome, Italy
3) Institute of Molecular Bioimaging and Physiology, IBFM CNR-UOS, Cefalú, Italy.
4) INFN, Laboratori Nazionali del Sud, Catania, Italy
5) University of Catania, Catania, Italy

Published on Annuals of LNS 2017

Experiments done
Paper on writing

Figure 2: Schematic view of the skin extraction areas. The irradiation point is indicated as S1. Points S2, S3 and S4 are at increasing distance from the irradiation point.

The project intends to characterize cognitive dysfunctions and metabolic stress due to charged particle (CP) therapeutic treatment that adversely affect patient response by limiting the effectiveness of therapy. ANSIA will determine a panel of metabolites in blood from irradiated mice.
Among metabolic alterations, the project is focused on lipid metabolism and the negative energy balance, both of them regulated by the proto-hormone Leptin (LEP).

INFN LNS

TIFPA Trento Institute for Fundamental Physics and Applications

IBFM

MoVe IT

Modeling and Verification for Ion beam Treatment planning
INFN Call 2017

WP3 Objectives

WP3					
Title	c			**Start month**	1
Leader	Walter Tinganelli/Giorgio Russo (TIFPA/LNS)			**End month**	36
Unit	INFN-TIFPA	INFN-LNS	INFN-NA	I	Total

Objectives

- ***Design new biological verification devices for therapeutic proton beam***
- ***Design new biological verification devices for heterogeneous (hypoxic) tumors***
- ***Validation of TCP and NTCP models by preclinical studies***
- ***Obtain an preclinical assessment of RBE impact for proton***
- ***Get molecular insight in effectiveness of hypoxia irradiation***

WP3 –Biological Dosimetry in vitro molecular characterization

Specific Tool for Experimental Verification of Irradiation on Hypoxia Condition

3 Glioblastoma Cell Lines

grown under

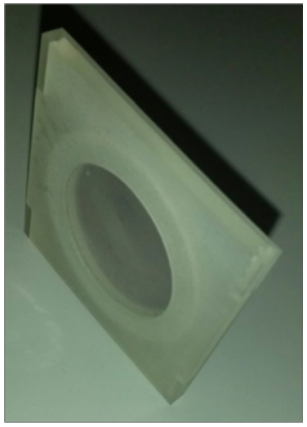
3 O_2 Concentrations

- 21% O_2 (Normal Condition)
- 0,5% O_2 Hypoxia [1]
- 0% O2 Hypoxia [2]

treated with

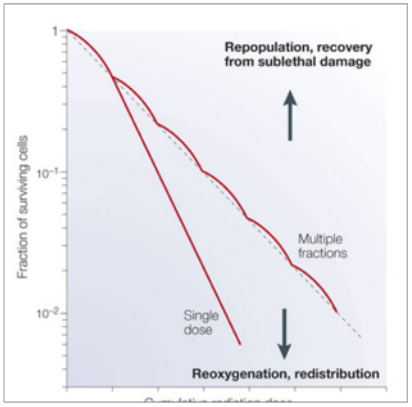
3 Proton-therapy doses

- 2 Gy
- 10 Gy
- 21Gy



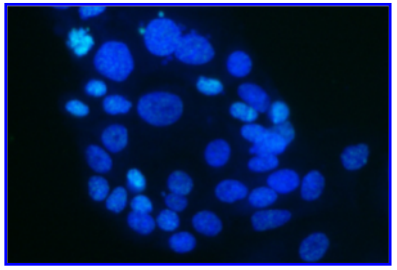
Preliminary Hypoxia Chamber by TIFPA

SURVIVING FRACTIONS BY Survival curves



DNA DAMAGE BY GAMMA-H2AX

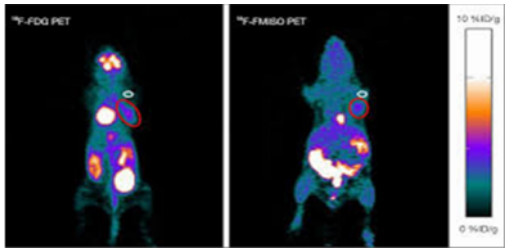
Proton (62 MeV), @ CATANA beam line



WP3 –Biological Dosimetry
2)in vivo molecular characterization

Animal models (Mouse) will be used for verification of the radiobiological modeling for treatment planning developed by TIFPA researchers.

Two different types of animal tests will be performed



The animal plans will be based on PET-CT images.

1. To assess the impact of variable RBE myelopathy (paresis grade II)

done *2018*

2. To assess Hypoxia Biomarkers

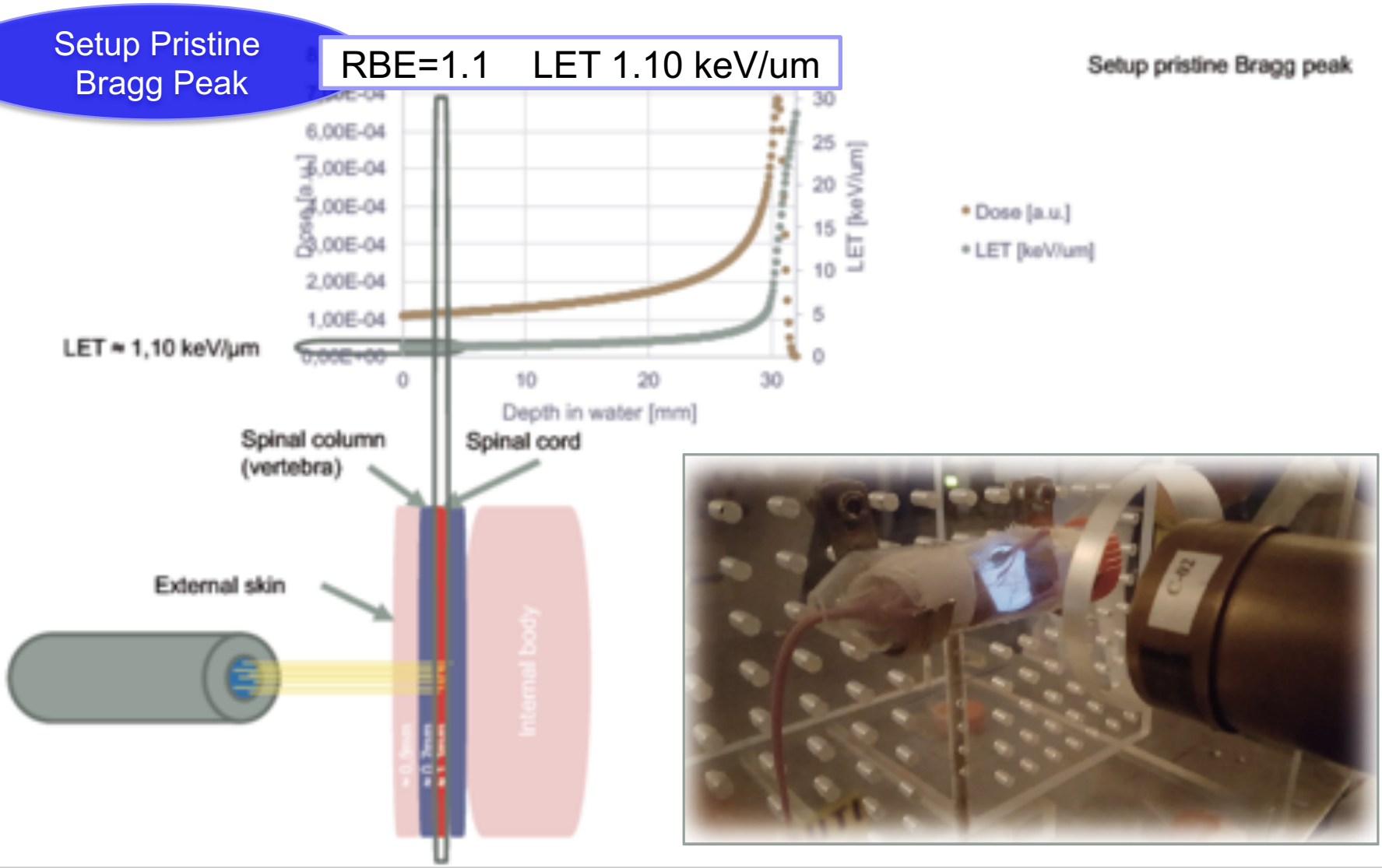
2019

WP3 – Objectives

- *1) Obtain an preclinical assessment of RBE impact for proton*

Setup Pristine Bragg Peak

RBE=1.1 LET 1.10 keV/um



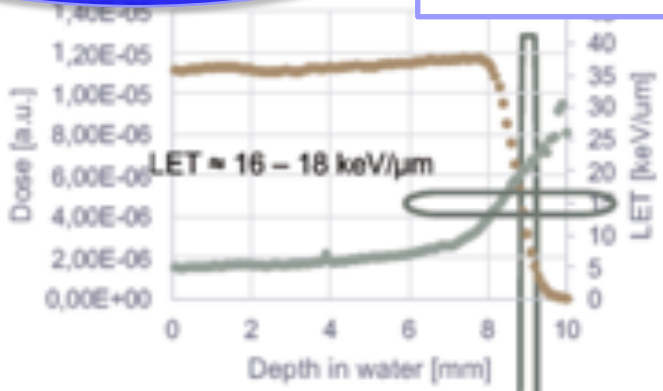
[2] Enhanced radiobiological effects at the distal end of a clinical proton beam: in vitro study. Journal of Radiation Research, 2014, 55, 816–822 (2014)

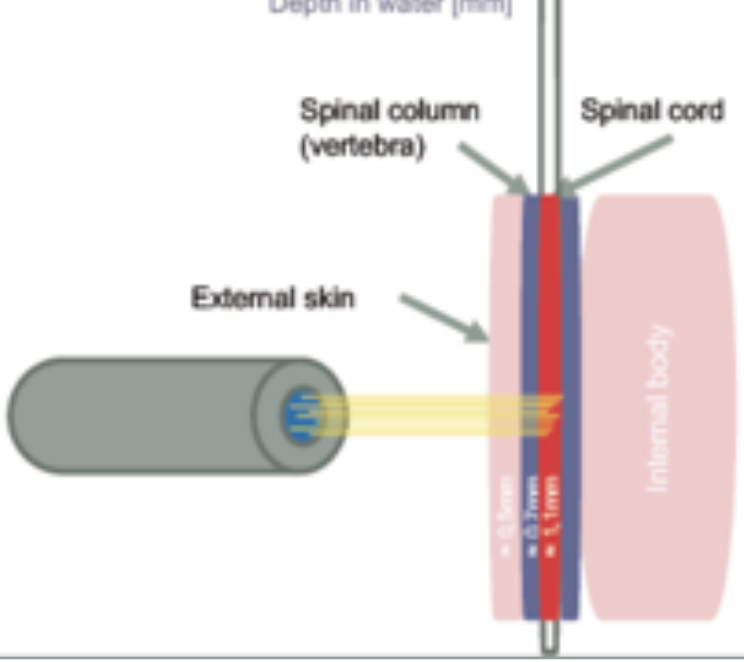
WP3 – Objectives

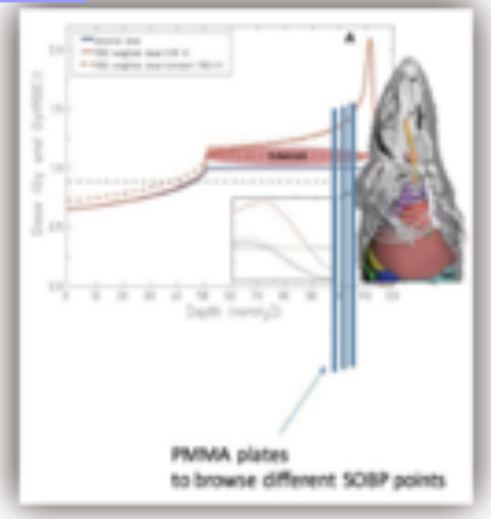
- *1) Obtain an preclinical assessment of RBE impact for proton*

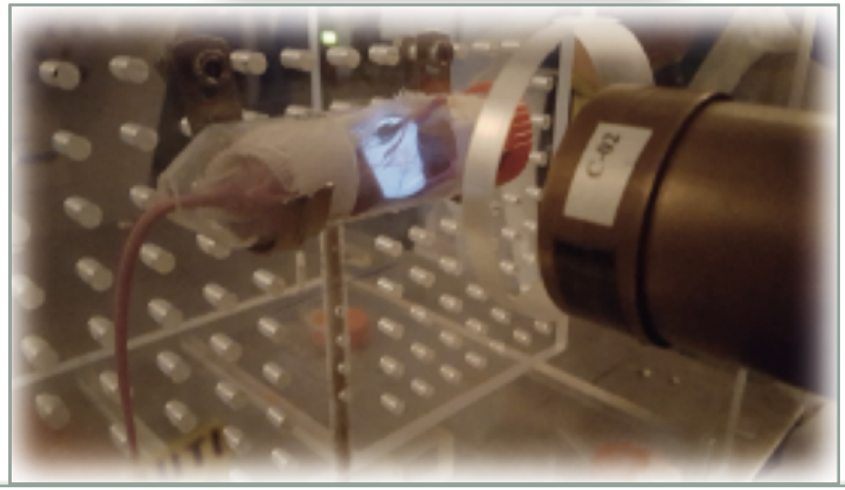
Setup SOPB Distal

RBE=1.4 LET 16/18 keV/um









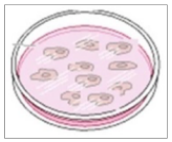
[2] Enhanced radiobiological effects at the distal end of a clinical proton beam: in vitro study. Journal of Radiation Research, 2014, 55, 816–822 (2014)

WP3-Biological Dosimetry
2) in vivo molecular characterization

2. To assess Hypoxia Biomarkers on in vivo model

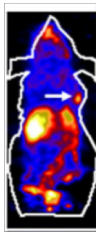
Proton (62 MeV), @ CATANA beam line

Cell line



Inoculated Mice

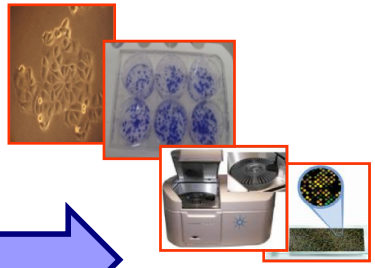
PET Imaging with F-FDG F[MISO]



Proton Therapy

Microarray analysis

Preliminary

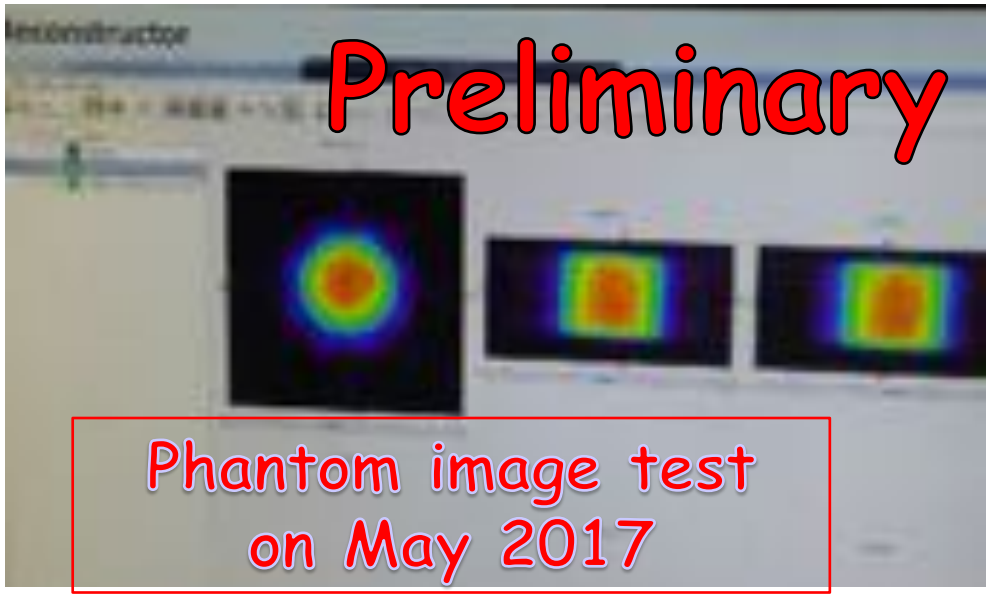


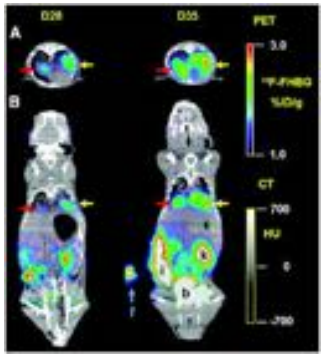
Cell line in vitro characterization in response to Proton Therapy by cell and molecular approach

2019

MicroPet-Ct for small animal

Collaboration with CAPIR
Catania University





Pet-CT allow trought the radiopharmaceutical use to visualize different kind of lesions

MoVe IT Project

- Forte GI, Minafra L, Bravatà V, Cammarata FP, Lamia D, Pisciotta P, Cirrone GAP, Cuttone G, Gilardi MC, Russo G. **Radiogenomics: the utility in patient selection**. Transl Cancer Res 2017. Transl Cancer Res 2017;6 (Suppl 5):S852-S874.
- P. Pisciotta, F.P. Cammarata, A. Stefano, F. Romano, V. Marchese, F. Torrisi, G.I. Forte, L. Cella, G.A.P. Cirrone, G. Petringa, M.C. Gilardi, G. Cuttone, G. Russo, **Monte Carlo GEANT4-based application for in vivo RBE study using small animals at LNS-INFN preclinical hadrontherapy facility**, Phys Med. 2018, Oct; 54:173:178
- Calascibetta C, Turturici G, Minafra L, Bravatà V, Russo G, Cammarata FP, Forte GI, Cavalieri V. **HIF-1a-dependent gene expression analysis of U87 glioblastoma cells under chemical-induced hypoxia.** 5° Meeting BIO-Tecnologie 2018

Supporting activity in Radiobiological Laboratory and CATANA Dosimetry

VINČA
INSTITUTE OF NUCLEAR SCIENCES
University of Belgrade
NATIONAL INSTITUTE OF THE REPUBLIC OF SERBIA

VINČA INSTITUTE OF NUCLEAR SCIENCES
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email: office@vin.bg.ac.rs

Aleksandra Ristic –Fira

HADMAC Project

Aleksandra Ristić-Fira

The project won the “Grande Rilevanza” Call of Ministry of Economic development

Ministero dello Sviluppo Economico
DIREZIONE GENERALE PER GLI INCENTIVI ALLE IMPRESE

Research Laboratory and open facility for radiation biology with Accelerated Ions - LARIA

LARIA

Team from LARIA (research laboratory and open facility for radiation biology with accelerated ions) studies healthy tissues and tumor responses to unconventional radiation therapy (hadron biology). By engineering tridimensional models, LARIA develop relevant models for healthy tissues fate or tumor control analysis in physiologic conditions.

Located in Caen (Normandie, France) on GANIL (Grand Accélérateur National d'Ions Lourds) campus, LARIA is also an open facility for french or foreign research team involved in radiation biology. LARIA operate the CIRIL radiation biology experimental plateform jointly with CIMAP (UMR 6252, CEA DSM – CNRS – ENSICAEN – Université de Caen Basse Normandie)

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Yannich Saintigny

CARSIP Project

Yannick Saintigny

Starting new projects - Radiobiological Laboratory and Dosimetry

MIRTO Project

MicrodosImetric study and RBE measuremenT with 62 clinical prOton beam

The goal of this experiment will be the experimental study of the RBE (Relative Biological Effect) of a clinical eye protontherapy beam by using microdosimetric measures and irradiation of biological samples

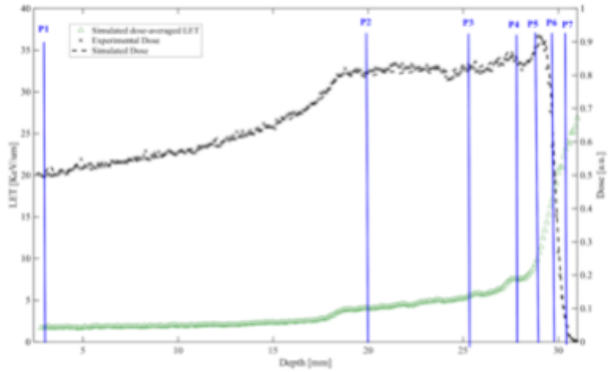


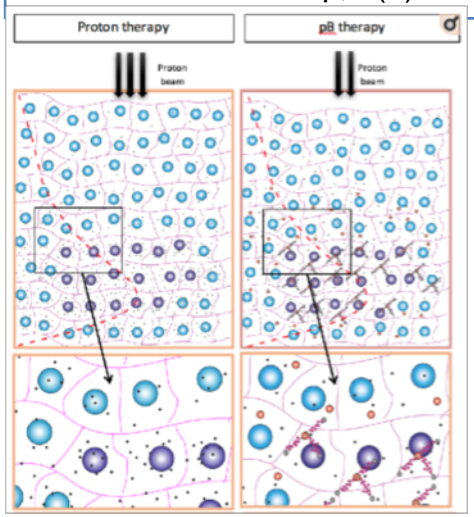
Figure 1. Spread Out Bragg Peak and LET of the studied clinical case

NEPTUNE Project

Nuclear process-driven Enhancement of Proton Therapy UNraVeled

The main aim to study and understand the recently observed increase of protontherapy effectiveness for irradiations occurring in the presence of 11B atoms

Cirrone GAP et al., First experimental proof of Proton Boron Capture Therapy (PBCT) to enhance protontherapy effectiveness. Sci Rep, 8(1):1141 (2018)



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-Gaetano Savoca

-Alessandro Stefano

INFN – LNS Catania

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