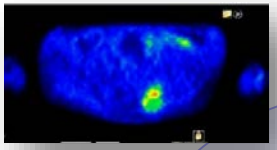
Consiglio Nazionale delle Ricerche

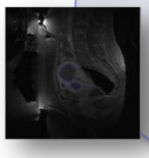
ISTITUTO DI BIOIMMAGINI E FISIOLOGIA MOLECOLARE (IBFM)

In-vitro and *in-vivo* radiobiological studies for the comprehension of molecular radio-resistance mechanisms by interdisciplinary approach

Giusi Forte

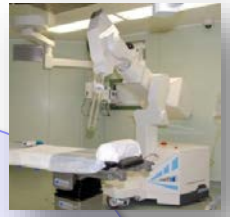
THE IBFM TRANSLATIONAL RESEARCH MODEL





Diagnosis

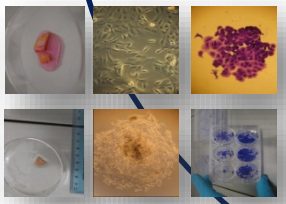
Molecular imaging by MRI, PET/CT



Therapies

IORT, Hadrotherapy

Disease Unit



Prognosis/Prediction

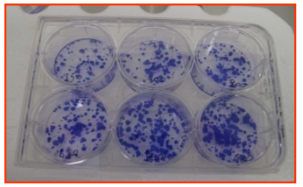
Cell-based

monitoring responce to therapies

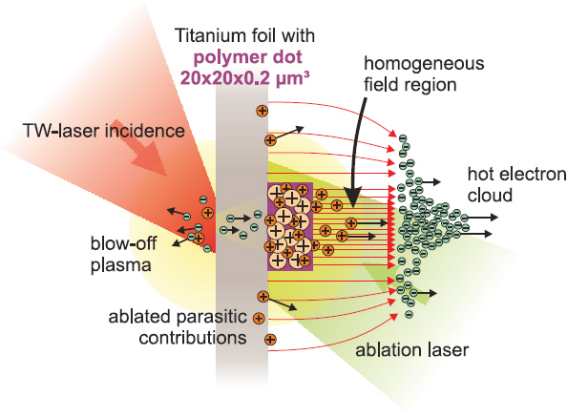
Radiobiology Knowhow

Biological effects induced by ionizing radiations are studied by *in-vitro*, *ex-vivo* and *in-vivo* approaches and by the integration of **multimultidisciplinary skills** of physicists, engineers, biologists and physicians.

The *in-vitro* activities have been conducted on **Breast cancer cells** with different beams:



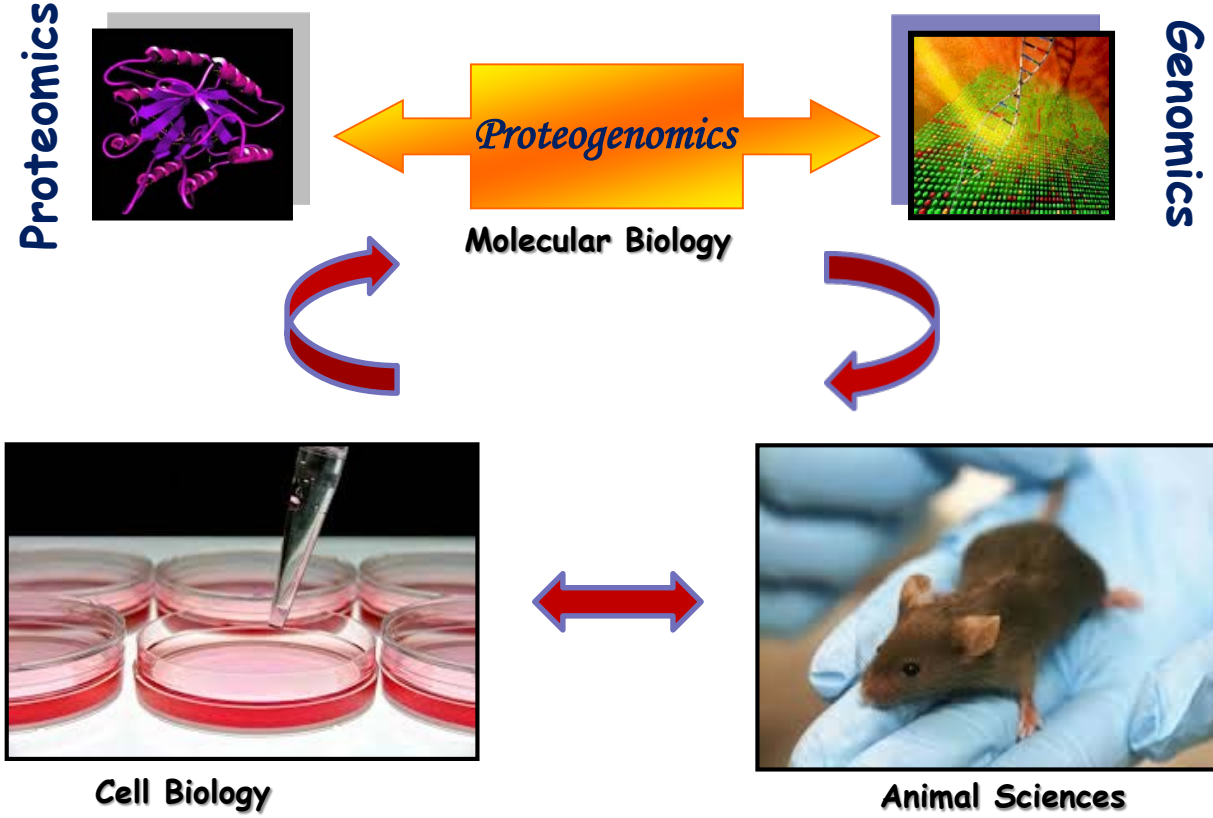
- **electrons,**
- **protons and**
- **electrons produced by laser target interaction**



LNS-INFN

Istituto di Bioimmagini e Fisiologia Molecolare (IBFM) - CNR

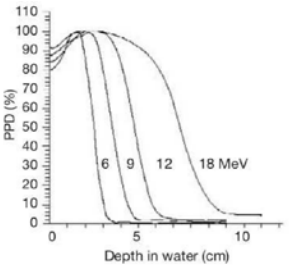
Biological Skills

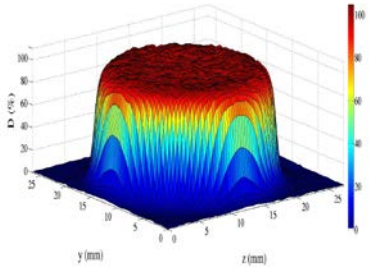


Physics Skills

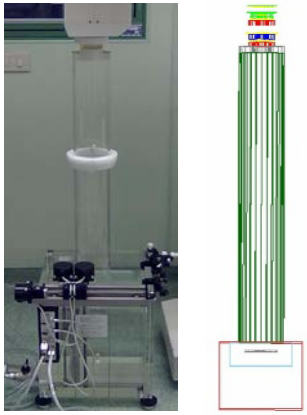
Monte Carlo simulations

Dosimetry

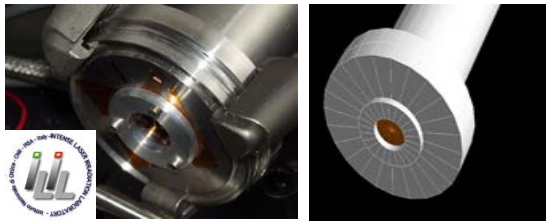




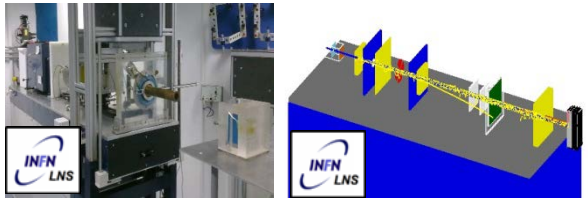
IORT simulation



Laser-driven simulation



Catana simulation



Experimental Radiobiology

1. In vitro/ ex vivo cell-based models

Using different Breast Cancer cell lines:

- ***Immortalized:***
  1. Tumorigenic: MCF7; MDA-MB-231
  2. Non tumorigenic: MCF10A
- ***Normal and tumour primary cells from patients biopsies***

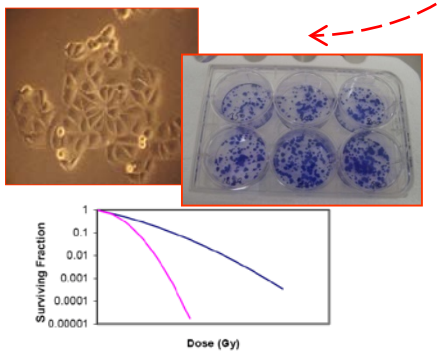
2. In vivo approach with mouse models

Work in progress:

- ***Ministry authorization***
- ***Animal facility***
- ***Dosimetry and simulation studies***

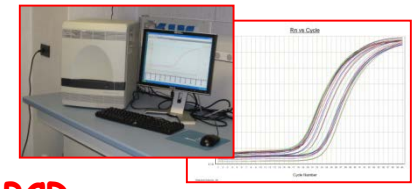
1. *In vitro/ ex vivo cell-based models*

Main goal is to highlight molecular mechanisms involved in the response to Radiation Treatment with different types of beam, in order to identify radio-resistance biomarkers and personalize treatments.



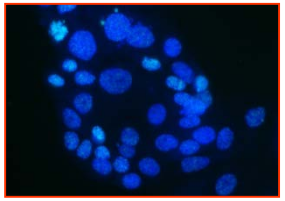
Morfology and vitality assays

Gene expression profiling by Microarray



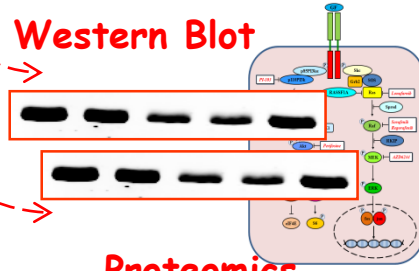
qRT-PCR

Immunofluorescence

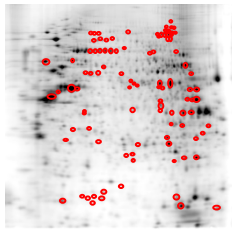


Luminex

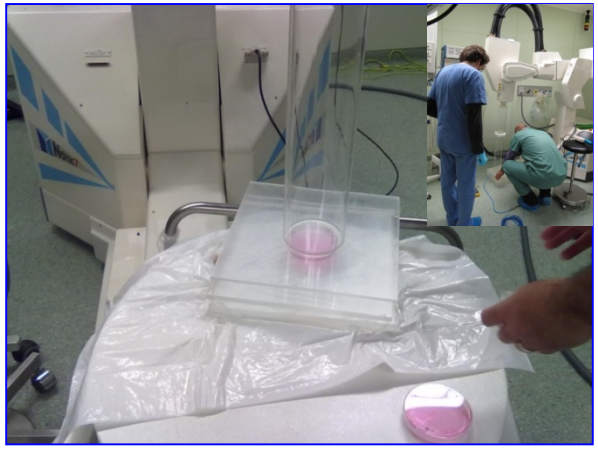
Western Blot

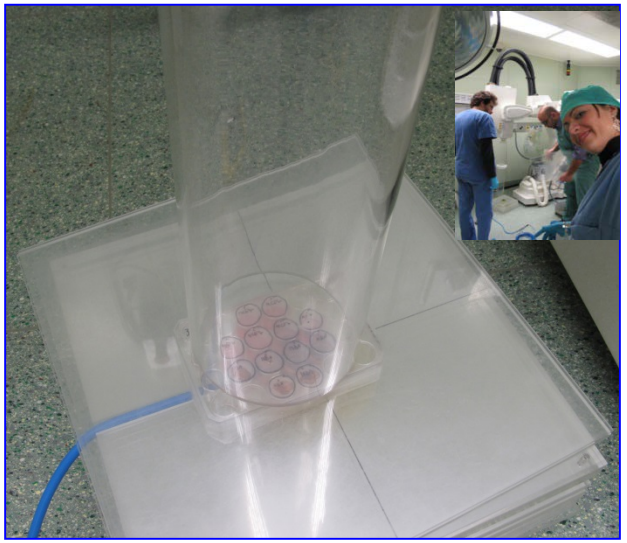


Proteomics



IOERT Treatment with Linear Accelerator NRT Novac 7





BREAST CELL LINES

Immortalised cells:

- MCF7 (Lum.B, Er+/Pr+)
- MDA-MB-231 (Bas.B, Er-/Pr-)
- MCF10A (Bas.B, Er-/Pr-)

Normal and tumour primary cells

IOERT

2 Radiotherapy Treatments:

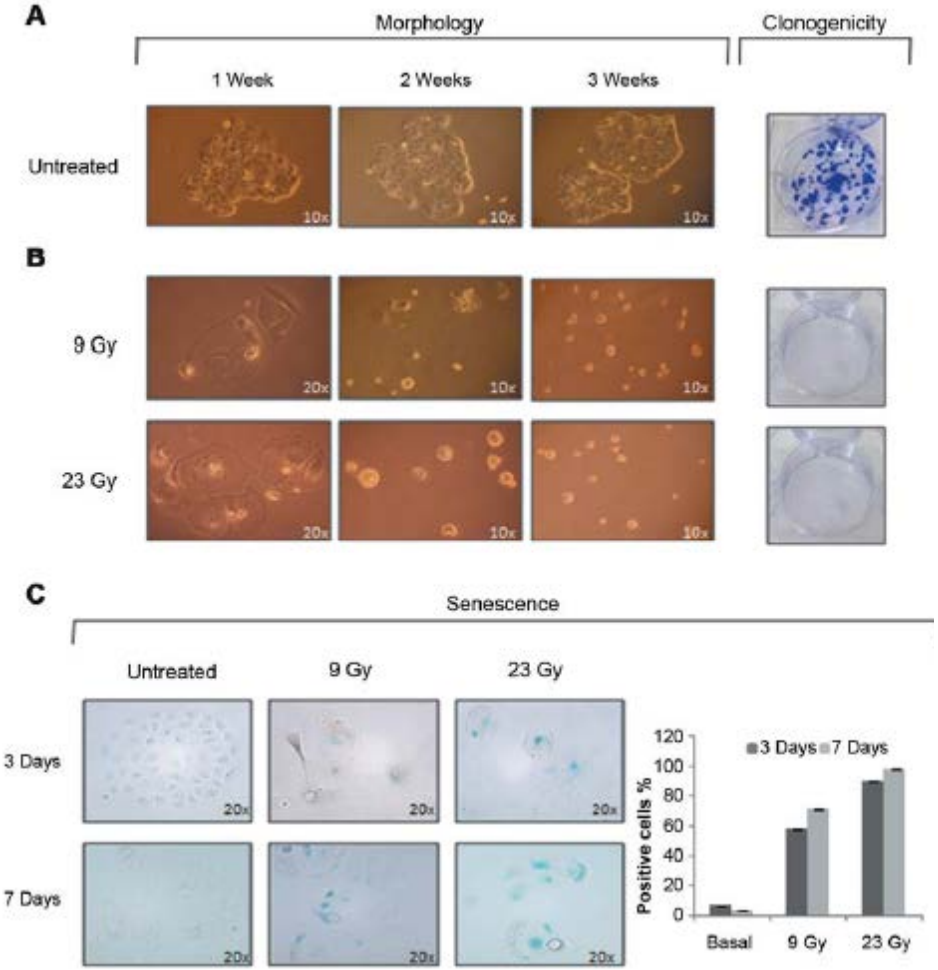
9 Gy (*boost*);

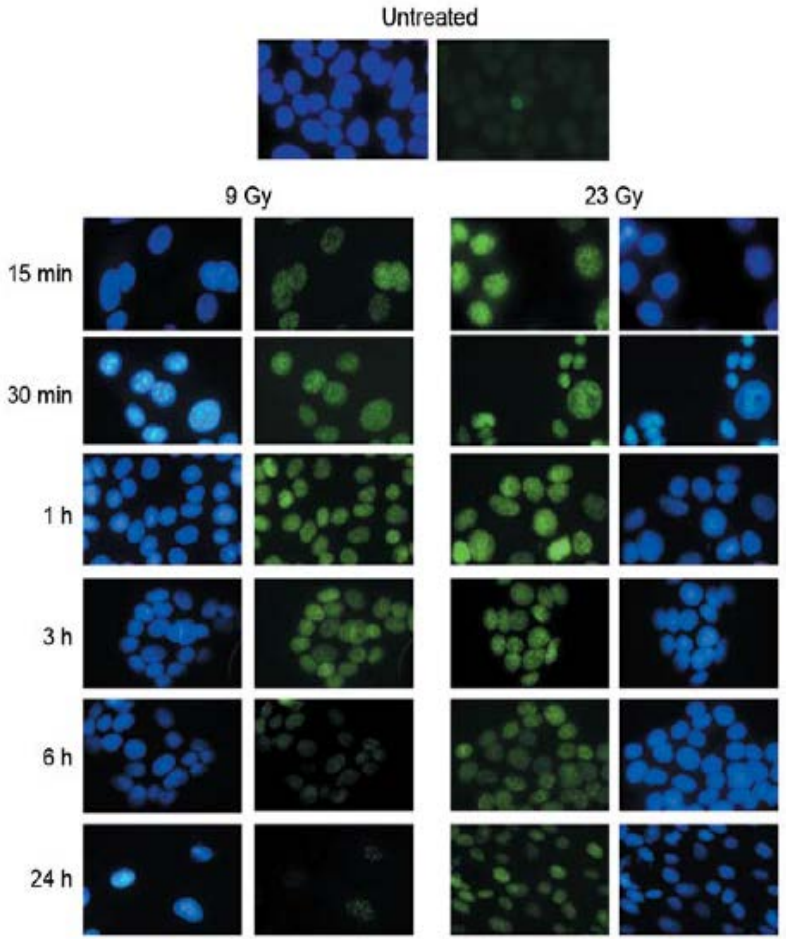
23 Gy

Anticancer Res. 2015 May;35(5):2577-91.

High-dose Ionizing Radiation Regulates Gene Expression Changes in the MCF7 Breast Cancer Cell Line.

Bravatà V[1], Minafra L[1], Russo G[2], Forte GI[2], Cammarata FP[2], Ripamonti M[2], Casarino C[2], Augello G[3], Costantini F[3], Barbieri G[3], Messa C[4], Gilardi MC[5].



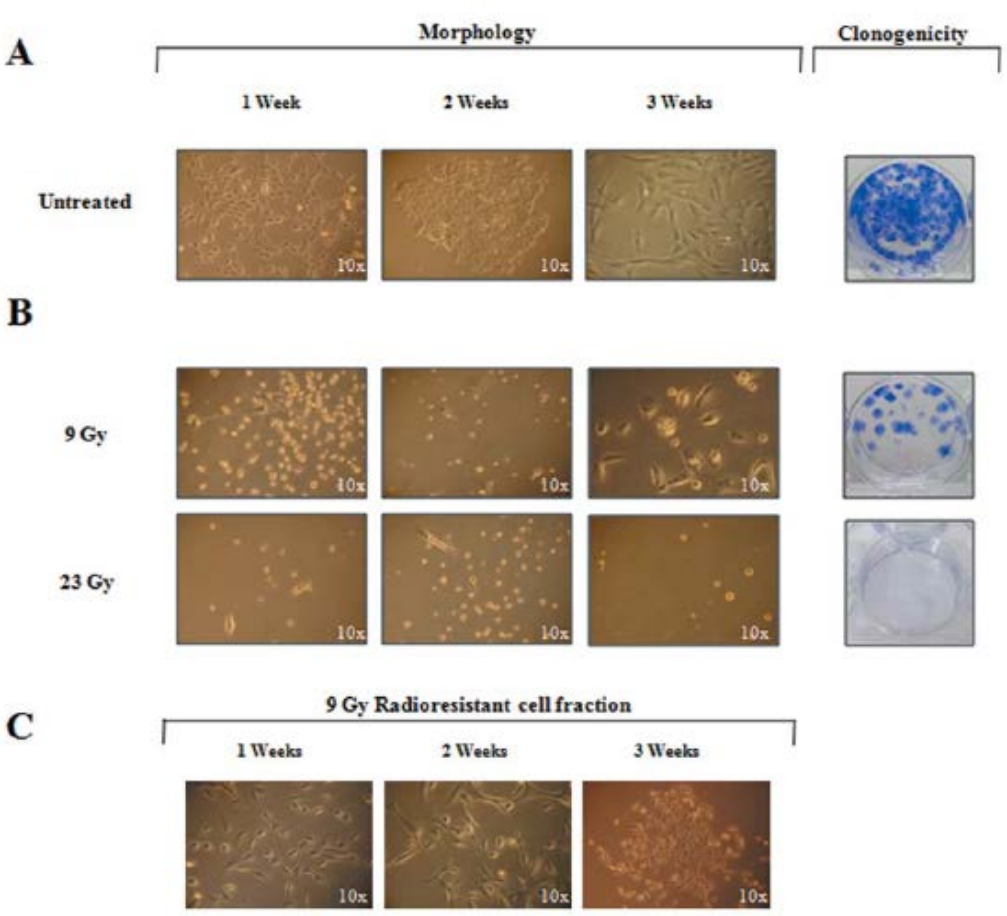


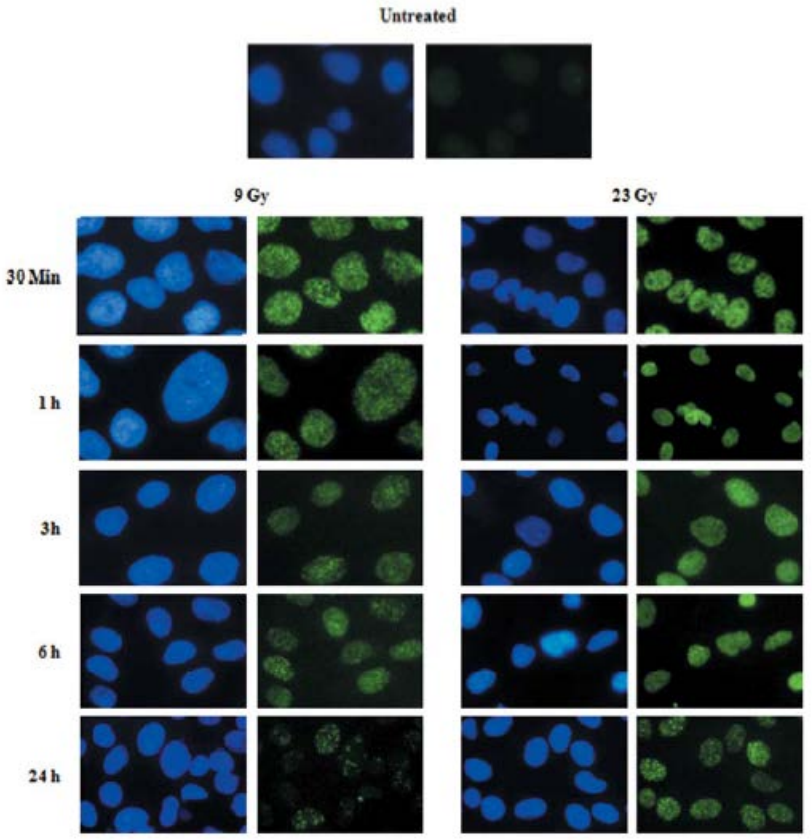
Morphology – Clonogenicity – Senescence- DNA Damage (γ-H2AX Foci)

Anticancer Res. 2015 Jun;35(6):3223-34.

Gene Expression Profiling of MCF10A Breast Epithelial Cells Exposed to IOERT.

Minafra L[1], Bravatà V[1], Russo G[2], Forte GI[2], Cammarata FP[2], Ripamonti M[2], Candiano G[2], Cervello M[3], Giallongo A[3], Perconti G[3], Messa C[4], Gilardi MC[5].

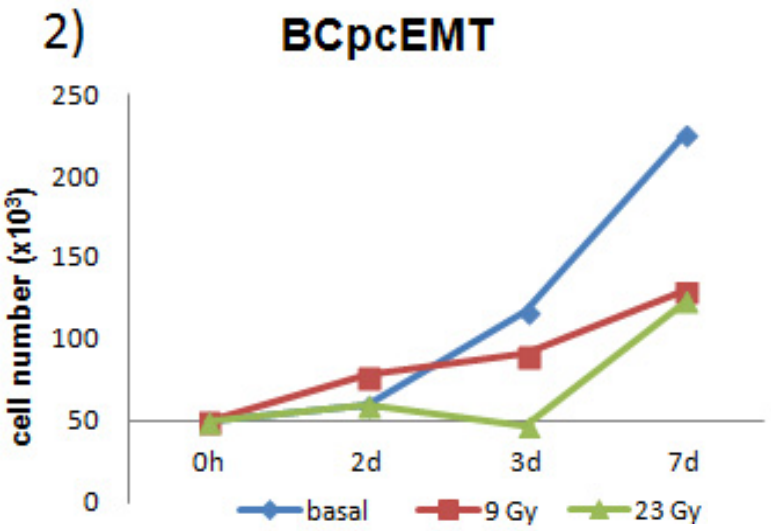




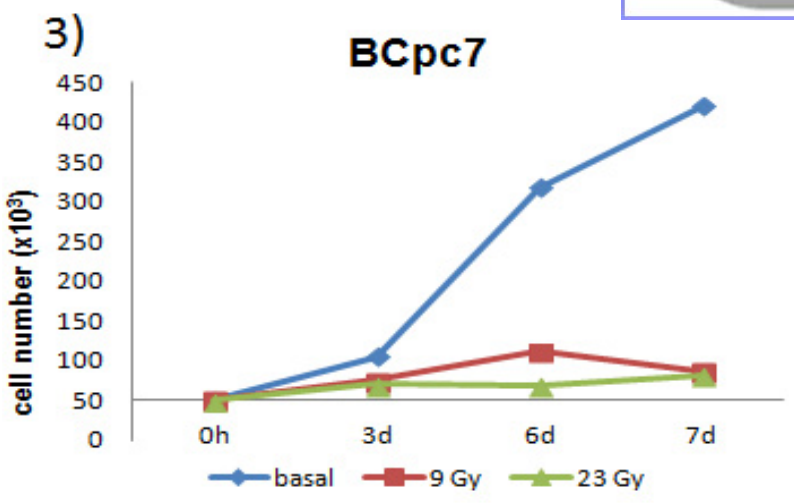
Morphology – Clonogenicity

DNA Damage (γ-H2AX Foci)

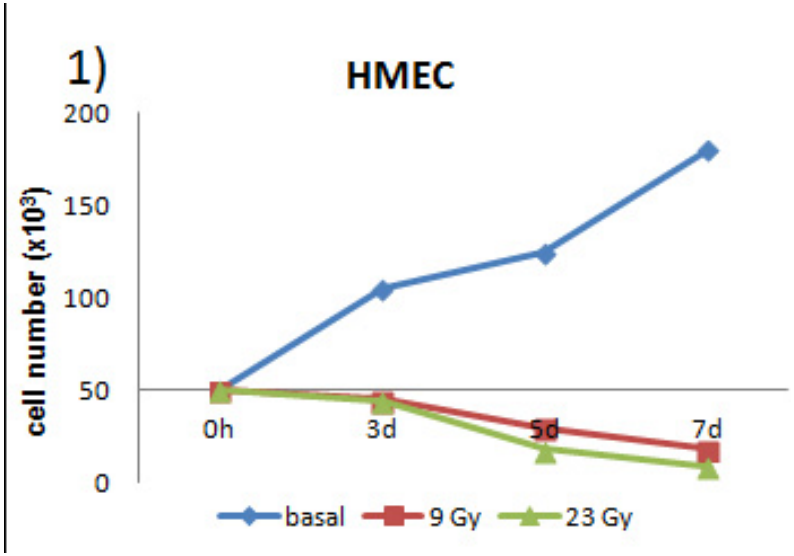
Different Radioresistence percentages to IOERT treatment in BCpc



RCF:58-55%



RCF:23-19%

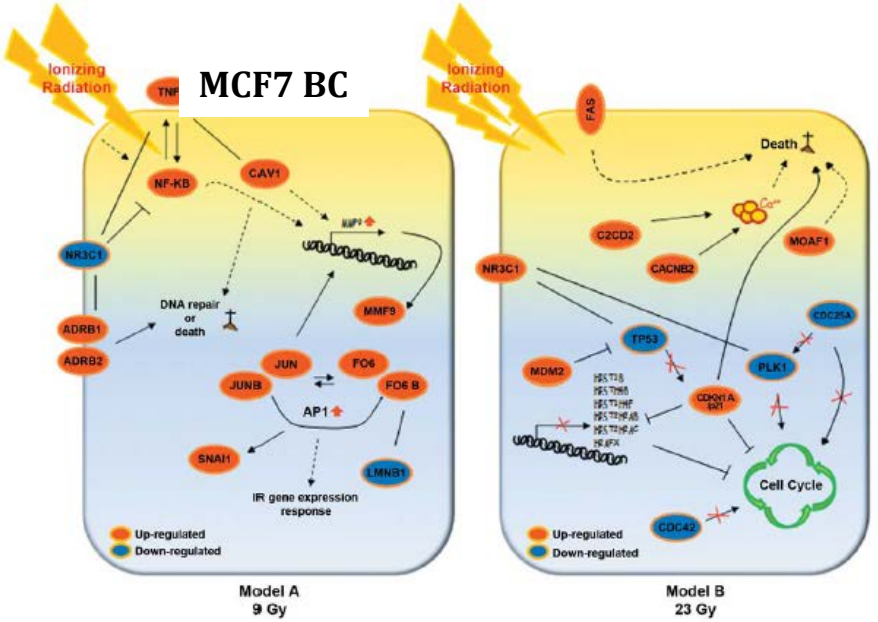


RCF:10-5%

RCF*:Radioresistant Cell Fraction

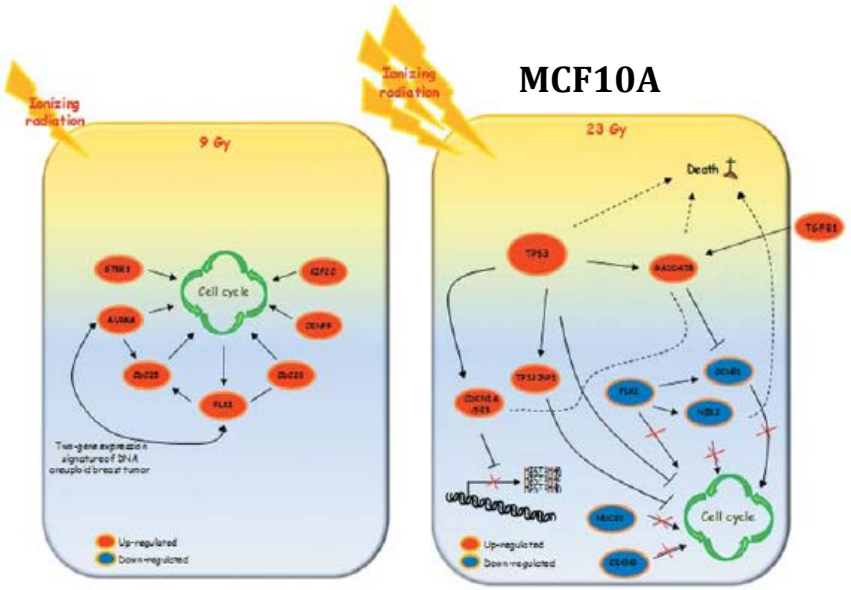
Personalized molecular models of responce to treatments

Bravatà *et al*: Gene Expression Response of MCF7 Cells Following IOERT Treatment



CANCER GENOMICS & PROTEOMICS 12: 143-152 (2015)

Minafra *et al*: Gene Expression Profiling of MCF10A Exposed to IOERT



Transl Cancer Res 2014;3(1):32–47

Biological basis of IORT

Cell and molecular response to IORT treatment

Luigi Minafra*, Valentina Bravatà*

IBFM CNR-LATO, Cefalù (PA), Italy

*These authors contributed equally to this work.

Corresponding to: Dr. Luigi Minafra. IBFM CNR-LATO, Cefalù (PA), Italy. Email: luigi.minafra@ibfm.cnr.it; Dr. Valentina Bravatà. IBFM CNR-LATO, Cefalù (PA), Italy. Email: valentina.bravata@ibfm.cnr.it.

Review

Caveolin-1, Breast Cancer and Ionizing Radiation

MARZIA PUCCI[1§], VALENTINA BRAVATÀ[1], GIUSI IRMA FORTE[1], FRANCESCO PAOLO CAMMARATA[1], CRISTINA MESSA[1,2,3], MARIA CARLA GILARDI[1,2,4] and LUIGI MINAFRA[1*]

[1]*Institute of Bioimaging and Molecular Physiology, National Research Council (IBFM-CNR) -LATO, Cefalù (PA), Italy;*
[2]*Department of Health Sciences, Tecnomed Foundation, University of Milano-Bicocca, Milan, Italy;*
[3]*Nuclear Medicine Center, San Gerardo Hospital, Monza, Italy;*
[4]*Nuclear Medicine, San Raffaele Scientific Institute, Milan, Italy*

Di Maggio *et al. Journal of Inflammation* (2015) 12:14
DOI 10.1186/s12950-015-0058-3

JOURNAL OF INFLAMMATION

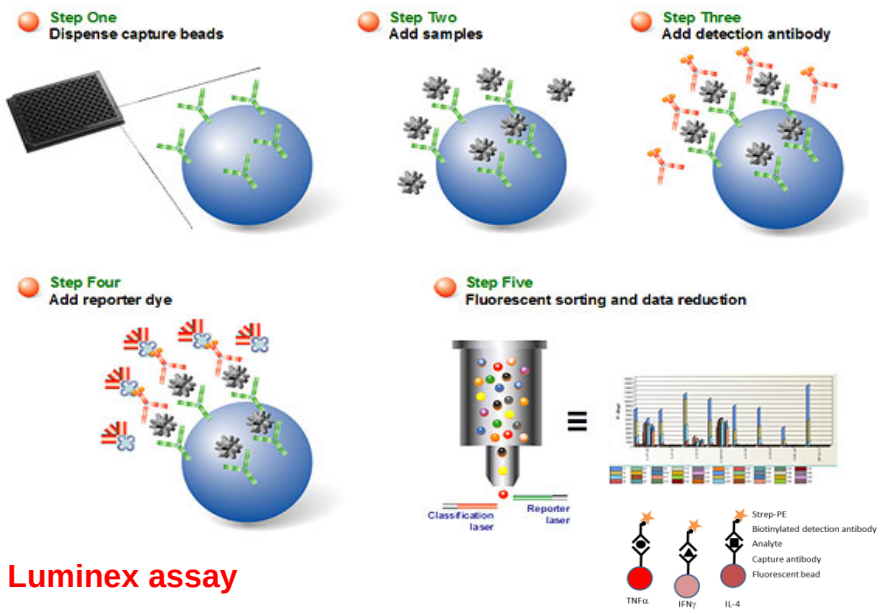
REVIEW Open Access

Portrait of inflammatory response to ionizing radiation treatment

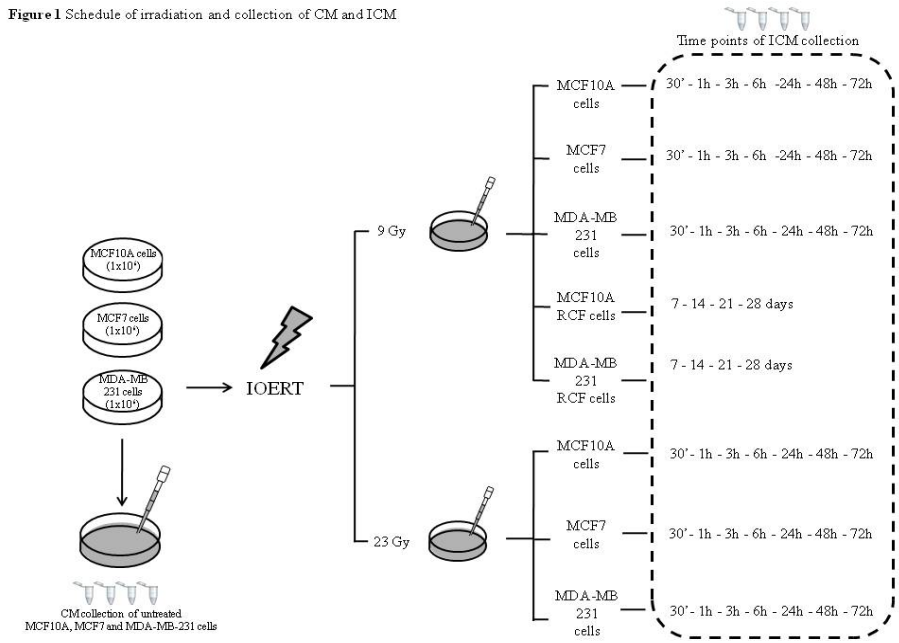
Federica Maria Di Maggio[1,2], Luigi Minafra[2], Giusi Irma Forte[2], Francesco Paolo Cammarata[2], Domenico Lio[1], Cristina Messa[2,3,4], Maria Carla Gilardi[2,3,5] and Valentina Bravatà[2*]

Inflammatory profile of BC cell lines conditioned media treated with 9 e 23 Gy

Bio-Plex Pro™ Human Th17 Cytokine Panel 15-Plex



Luminex assay

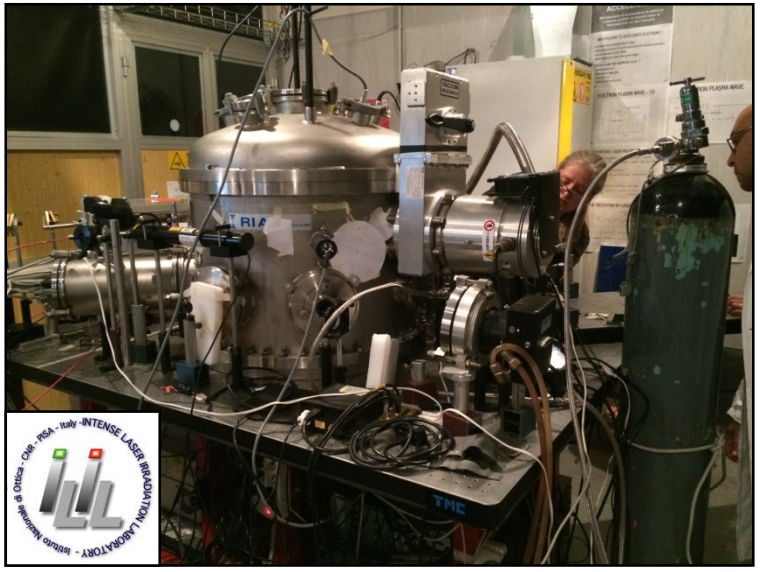


CM (conditioned medium) collection of untreated cells
ICM (irradiated conditioned medium) collection of treated cells

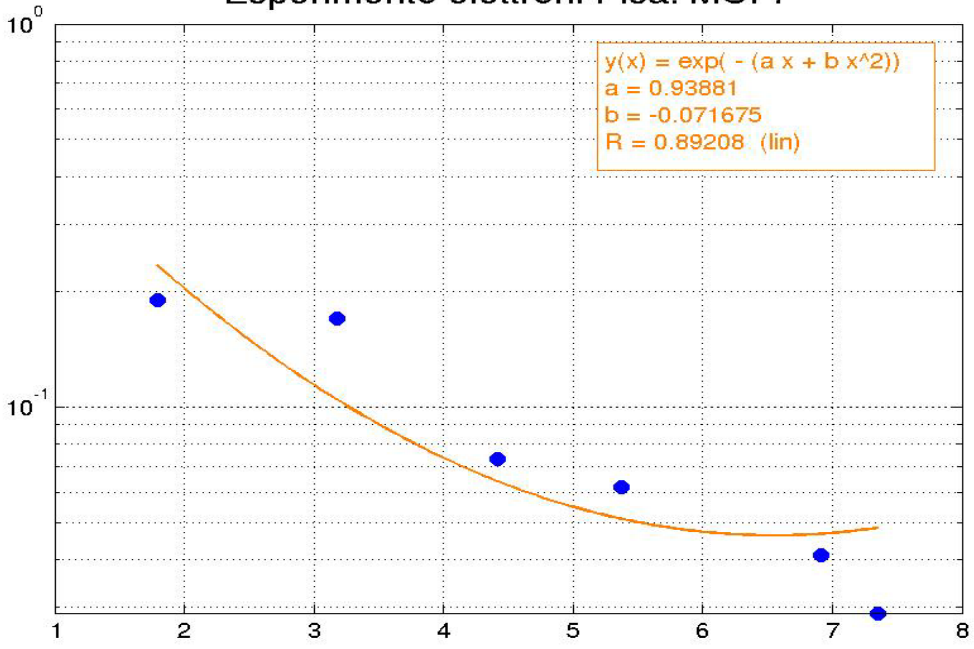
IL-1b, IL-6,TNF-α (proinflammation);
IL-12 (p70), IFN-γ (TH1);
IL-4, IL-5, IL-10, IL-13 (TH2);
IL-8, MCP-1, MIP-1b (chemokines);
IL-2, IL-7,IL-17,GM-CSF, G-CSF (immunomodulation);
TGF-β2, VEGFA (tissue modulating)

MCF7
MDA-MB-231
MCF10A
} **Cell lines**

INO
ISTITUTO NAZIONALE
DI OTTICA



Esperimento elettroni Pisa. MCF7



Machine	cGy/pulse	pulse lifetime	Gy/sec for pulse
Clinical Linac (Novac7)	10	1,2 microSec	80
ILIL Accelerator	7	1 picoSec	**$70*10^9$**

BREAST CELL LINES

Immortalised cells:

- **MCF7** (Lum.B, Er+/Pr+)
- **MDA-MB-231** (Bas.B, Er-/Pr-)
- MCF10A (Bas.B, Er-/Pr-)

Hadrotherapy (protons)

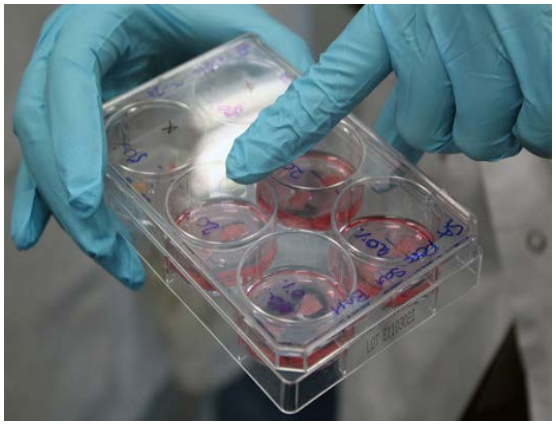
0,5 Gy, 2 Gy, 4 Gy, 6 Gy, 9 Gy

NEXT STEP:

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

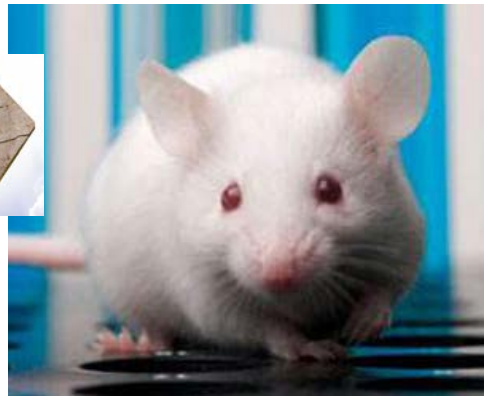
In vitro studies

Breast cancer cells



In vivo studies

Mice model



Studies on:

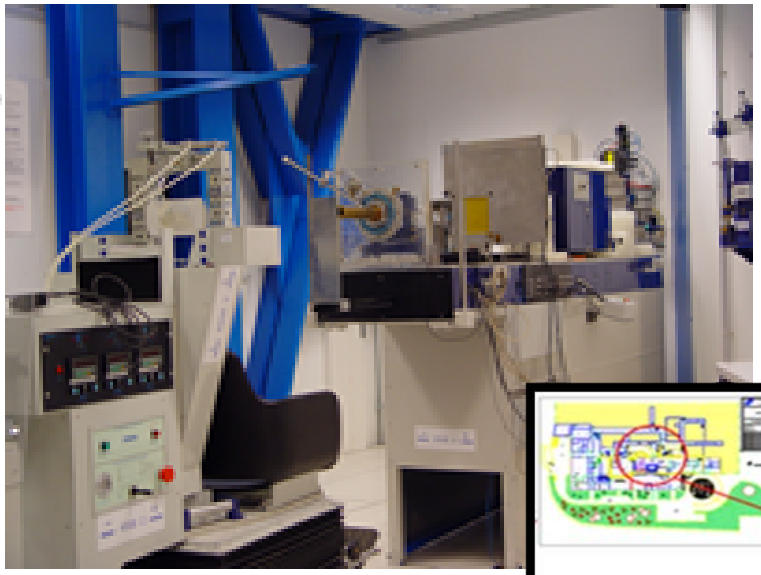
Tumour region

Health tissue

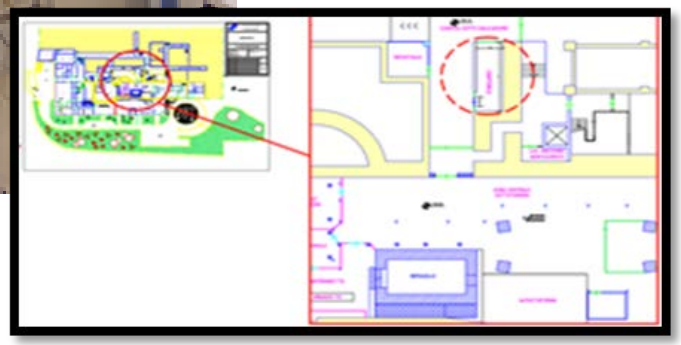
PRECLINICAL HADRONTHERAPY TREATMENT

STUDIO IN "VIVO" DI TRATTAMENTI CON ADRONI DI TUMORE MAMMARIO SU TOPI

"PETS" PRECLINICAL HADRONTHERAPY STUDIES



CATANA facility @ INFN-LNS

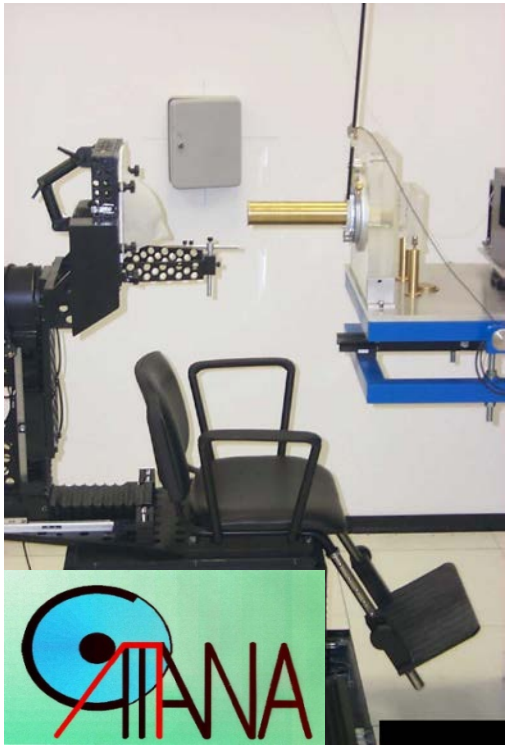


Istituto di Bioimmagini e Fisiologia Molecolare (IBFM) - CNR

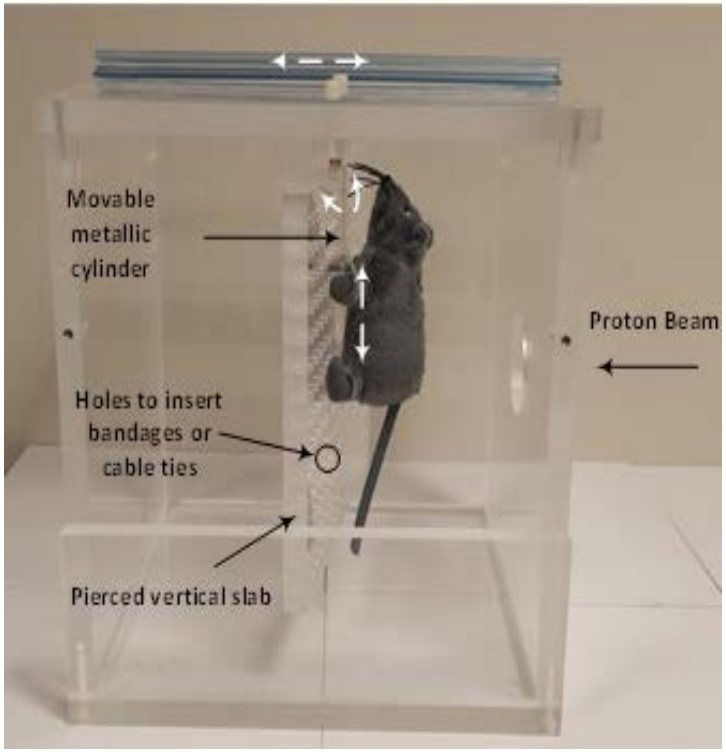
POSITIONING SYSTEM

An important part of a hadrontherapy treatment is the positioning …

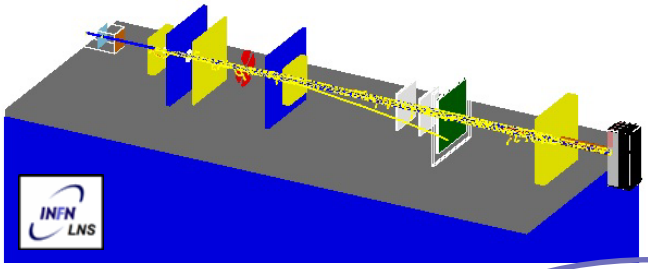
Human positioning system



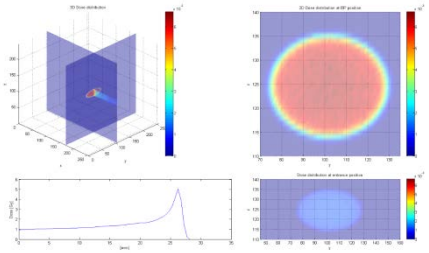
Small animal positioning system



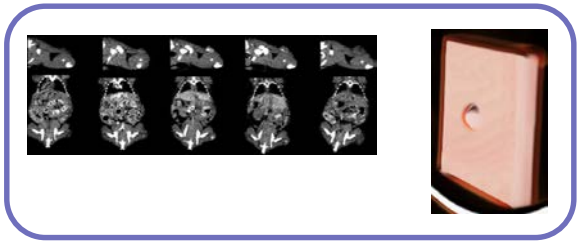
Geant 4 SIMULATION



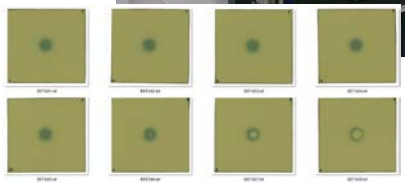
Geant4 → Our custom application → Simulation results



Validation phase needed



Experimental results



FIRST PRECLINICAL TREATMENT

PROPOSAL FOR AN INFN EXPERIMENT

2015-17

ETHICS

INFN

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

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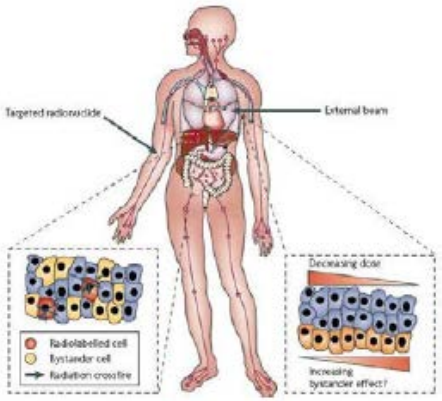

THANKS

THE RADIOBIOLOGY GROUP

IBFM-CNR

Francesco P. Cammarata
Giusi I. Forte
Luigi Minafra
Valentina Bravatà
Valentina Marchese
Giorgio Russo
Pietro Pisciotta
Debora Lamia

Maria Carla Gilardi

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Vincenzo Zimmitti
Marco Abate

Giacomo Cuttone

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Federica Baffigi
Lorenzo Fulgentini
Petra Koester
Daniele Palla

Leonida Gizzi
Antonio Giulietti