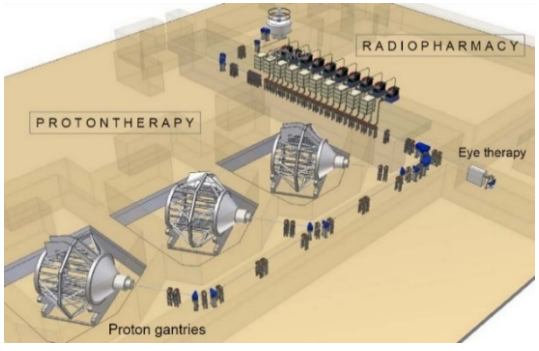
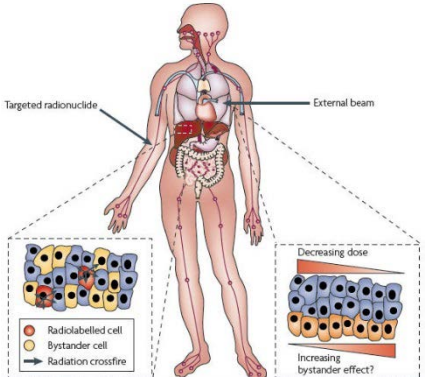
Pre-clinical experimental and theoretical studies to improve treatment and protection by charged particles

ETHICS

(2015-17)

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





Collaborazione

Sezioni *(Responsabili)*	FTE
NA *(L.Manti) Responsabile nazionale*	3.5
Roma1-Gr.coll. Sanità *(M.A. Tabocchini)*	3.0
PV *(F. Ballarini)*	3.1
LNL *(R. Cherubini)*	2.0
AQ *(L. Palladino)*	2.0
Totali	13.6

Collaborazioni esterne: Queen's University, Belfast, UK (K. Prise & co.); Biophysics Department, GSI, Helmholtzzentrum für Schwerionenforschung, Germany (M. Durante & co.); Strathclyde University of Glasgow, UK (M. Boyd & co); Experimental Medicine Department, Seconda Università degli Studi di Napoli (SUN), Napoli (U. Galderisi & co.)

Infrastrutture e tipologie di fasci impiegati:

- esposizioni acute (LNL, LNS, CNAO): p, He, ^{12}C
- esposizioni protratte (ISS, LNL): He

Unità di Pavia

- Francesca Ballarini, RU 60%
- Mario P. Carante, dottorando 100%
- Nicoletta Protti, assegnista 30%
- Elio Giroletti, RU 40%
- Angelica Facoetti, CNAO 60%
- Mario Ciocca, CNAO 20%

- TOTALE : 3.1

Razionale e scopo

- **Ambito adroterapia:** applicazione terapeutica di fasci esterni di particelle cariche si è dimostrata molto efficace nel trattamento tumorale, grazie alla loro migliore precisione balistica e, specialmente per il carbonio, alla più elevata efficacia biologica rispetto ai fotoni.
- **Ambito "targeted therapy":** stesse proprietà radiobiologiche sfruttate dalla radioterapia con radionuclidi, basata su particelle cariche a brevissimo range rilasciate da radioisotopi incorporati grazie ad anticorpi
- ***Ambito radioprotezione:*** *necessità di accurate stime di rischio essenziale anche per la popolazione generale, esposta in varie situazioni a dosi croniche (tipicamente nel caso dell'inalazione di gas radon indoor)*

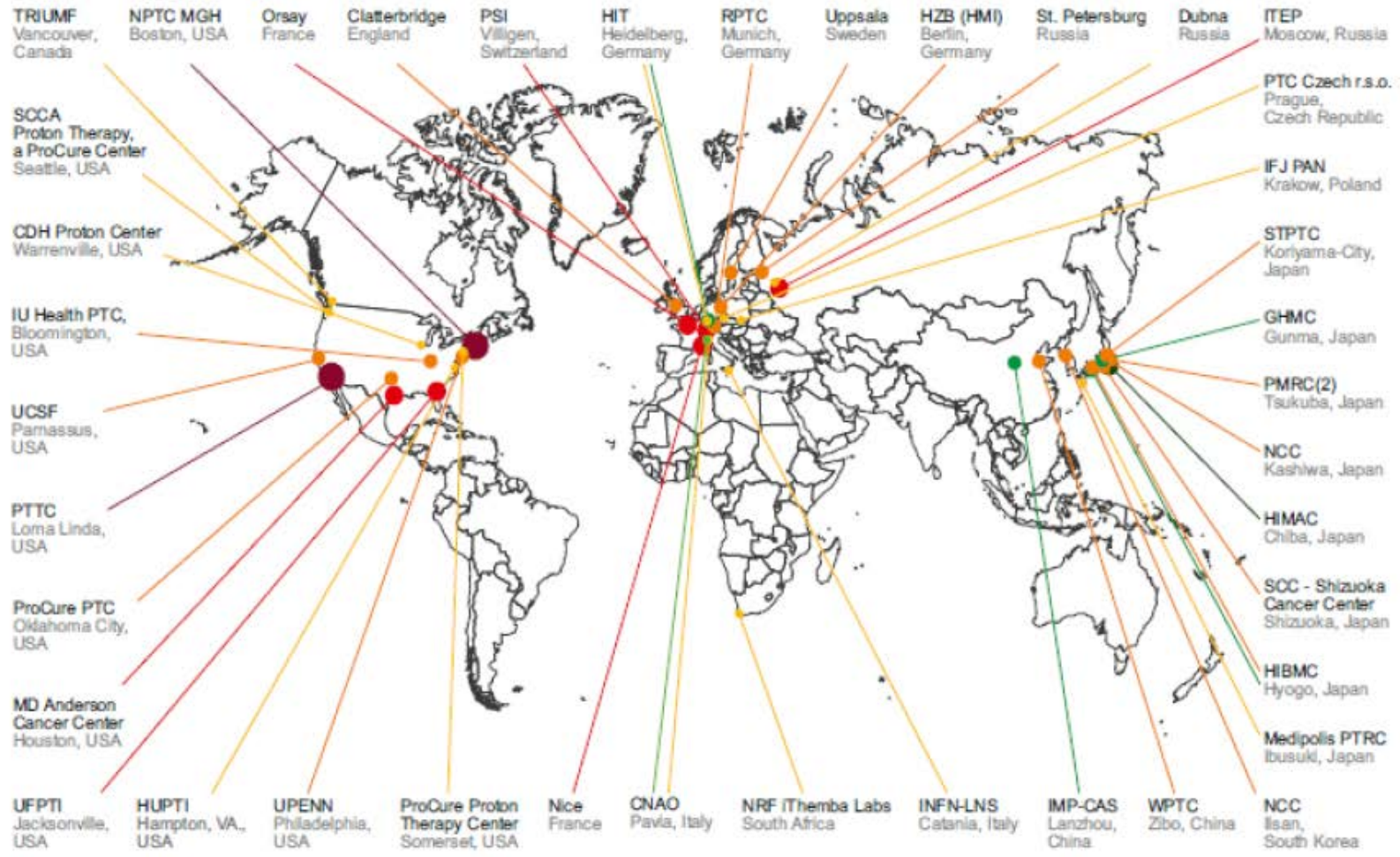
Tuttavia……..notevoli **incertezze** sugli effetti di **particelle cariche** nei **tessuti sani**

⇒ **Scopo generale del progetto:** caratterizzare a livello di **meccanismi biofisici** l'azione delle particelle cariche d'interesse (p, C, He…) in cellule **sane**, per sviluppare strategie finalizzate a **minimizzare i rischi per la salute**

Targeted therapy with alpha particles

- Deliver radiation to the tumour in a **highly localized** manner, from the **inside** of the body
- **Alpha-emitting radionuclides** attached to antibodies via linking molecules. **Antibodies** bind with specific tumour antigens on the target cell surface
- Advantages: **high biological effectiveness** and **small range** (50-100 μm)
- Radionuclides
  - ^{213}Bismuth ($t_{1/2}$ = 45.6 min; E_α = 8 MeV)
  - ^{225}Actinium ($t_{1/2}$ = 10 d; E_α = 5.9 MeV)
  - **^{223}Radium** ($t_{1/2}$ = 11.4 d; E_α = 5.7 MeV)
- Tumours
  - **Leukemia**
  - Solid tumours (e.g. breast, prostate)
  - **Skeletal metastasis**
- Many pre-clinical and some clinical **trials** with **encouraging results,** in particular for the treatment of skeletal metastasis with ^{223}Ra
- Necessary to invesigate the damage to **normal cells**

Hadron therapy



March 2014: 44 proton/7 heavy ion centers

Under construction: 25 proton/ 3 heavy ion centers

Only in USA, 27 new centers expected by 2017

NuPECC report „Nuclear Physics in Medicine“, 2014

New challenges in charged-particle radiobiology *(M. Durante, Br. J. Radiol. 2013)*

I) Late effects:

Second cancers *(especially important for paediatric patients):* epidemiological data are very limited ⇒ "*in vitro* and *in vivo* experiments remain mandatory". In particular, **chromosome aberrations** are related to the risk of leukemias, whereas **inflammation/cellular communication** are related to the risk of solid tumors

Non-cancer effects, *especially* ***cardiovascular diseases:*** charged-particle radiobiology for these effects is scarcely known ⇒ need of further studies, e.g. on **endothelial cells**, which are the most likely target of cardiovascular damage (also considering that breast cancer is becoming a major application of protontherapy); in particular, **premature cellular senescence** is related to non-cancer effects

II) Uncertainties in RBE (Relative Biological Effectiveness):

in particular for **proton** therapy, which applies a constant RBE of 1.1, although slow protons present in the distal region of therapeutic Bragg peaks have higher effectiveness, causing a **shift of the biological range** of some mm ⇒ *"Modelling will always be necessary….there is room for improvements switching from phenomenological to* ***mechanistic models****"*

III) Hypofractionation *(1-3 fractions, with up to 25-30 Gy/fraction)*

⇒ particle radiobiology research at 'high' doses is also needed

IV) other particles than p or C for hadrontherapy:

ions heavier than C, such as ^{16}O, may be beneficial for hypofractionation, thanks to the increased effectiveness under hypoxic conditions

Piano generale *(tutte le Unità)*

*caratterizzazione dell'azione di diversi ioni in cellule umane **sane** esposte a dosi **moderate/bass**e; gli studi in vitro saranno seguiti da studi **in vivo***

Particelle: **p**, He, C, O

Principali cellule bersaglio: fibroblasti umani normali AG01522 e **cellule endoteliali** (⇒rischio cardiovascolare)

Principali effetti: **aberrazioni cromosomiche** (⇒ rischio di leucemie), **senescenza cellulare prematura** (⇒ non-cancer effects), **comunicazione tra cellule sane e cellule tumorali** (⇒ tumori solidi)….

Modalità di esposizione: dosi non solo basse ma anche moderate (⇒ **ipofrazionamento**) ; anche basso dose-rate (⇒ target therapy, radon)

3 Work Packages (external exposure, internal exposure, in vivo studies)

Unità di Pavia – attività modellistica

modelization/simulation of ***chromosome aberrations*** *and* ***cell death*** *in* ***normal*** *cells exposed to* ***charged*** *particles, applying and extending an existing model/code ("BIANCA", BIophisical ANalysis of Cell death and chromosome Aberrations)*

particles: p, C, O(?) *(⇒ hadron therapy)* and α *(⇒ targeted therapy, radon)*

energies: focus on energies of **~MeV/n**, which are present in the distal region of therapeutic Bragg peaks and thus represent a risk for normal tissues beyond the tumor, due to the higher biological effectiveness of these particles (not only C but also p!); these energies are also of interest for Radon and for targeted therapy

doses: from single traversals up to a few Gy *(⇐ hypofractionation)*

target cells: whenever possible, the same cells used by the experimental partners, e.g. AG01522 normal human fibroblasts *(=reference cell line)* and endothelial cells *(=main target for cardiovascular damage)*

irradiation modalities: both external *(⇒ hadrontherapy)* and from the cell surface *(⇒ targeted therapy)*

damage types: both cell death, related to normal-tissue damage, and non-lethal damage such as specific chromosome aberrations (typically, translocations), which are related to cell conversion to malignancy

Unità di Pavia – attività sperimentale

Evaluation of the effects of different radiation types (p and C, plus photons) on the cellular mechanisms that govern inflammation, cell adhesion and migration

The tumor microenvironment consists of a variety of normal cells, extracellular matrix proteins and extracellular molecules, whose functionality/concentration is altered by radiation, also at low doses

In particular, fibroblasts release many different proteins (growth factors, chemochines and proteins remodelling the extracellular matrix) that can promote inflammation, angiogenesis and tumor progression/metastasis

$\Rightarrow$ *evaluation of the effects of cellular communication between irradiated normal cells and tumor cells (with focus on the effects of paracrine factors on adhesion, proliferation and migration, and on the effects of proteins involved in extracellular matrix remodelling)*

$\Rightarrow$ *evaluation of the migration capacity of irradiated tumor cells*

Unità di Pavia – milestones

1 st year

- june 2015: characterization of the **DNA cluster damage** that is critical for chromosome aberrations/cell death (e.g., by comparisons with data on DNA fragmentation)
- *december 2015: evaluation of the effects of* ***paracrine factors*** *secreted by irradiated normal human fibroblasts on adhesion, proliferation and migration of tumor cells*

2 nd year

- june 2016: evaluation of the implications of taking into account the higher **biological effectiveness of low-energy protons**
- *december 2016: evaluation of the release by irradiated cells of proteins involved in* ***extracellular matrix remodelling*** *(MMPs, Matrix metalloproteinases)*

3 rd year

- june 2017: implementation of conditions typical of **targeted therapy** (e.g., alpha particles starting from the cell surface)
- *december 2017: evaluation of the* ***migration*** *ability of irradiated tumor cells*

Richieste finanziarie

(richiesta finanziaria totale: dell'ordine di ~100 + 20 k€/anno)

Richiesta finanziaria PV per il I anno (2015): 20 keuro, di cui:

Materiale di consumo (14 keuro):

- reagenti per il mantenimento delle cellule in coltura (terreni, sieri, fattori di crescita, supplementi): 3.3 keuro
- acquisto di cellule: 1.2 keuro
- plastiche per il mantenimento in coltura delle cellule e supporti per lo studio di motilità/migrazione cellulare: 3.5 keuro
- pellicole per dosimetria fascio (5 confezioni): 3.0 keuro
- kit ELISA e anticorpi primari e secondari: 3.0 keuro

Missioni interne (4.0 keuro):

- 4 missioni x 2 persone a Legnaro per turni presso i LNL (2.0 keuro)
- 2 missioni x 2 persone a Roma per turni all'ISS (2.0 keuro)

Missioni estere (2.0 keuro):

- 1 missione x 2 persone a Darmstadt, Germania, per attività congiunta con il gruppo di biofisica del GSI (2.0 keuro)

Backup slides

attività modellistica – work plan

2015:

- evaluation of the implications of taking into account the higher **biological effectiveness of low-energy protons**
- modelization of cell death for normal **human** cells (typically, the AG01522 fibroblasts used as a reference by the experimental partners) exposed to p, He and C *(the current version of the model has been validated for alphas in hamster cells but not human cells)*
- characterization of the DNA cluster damage that is critical for chromosome aberrations/cell death *(e.g., by comparisons with data on DNA fragmentation)*

2016:

- quantification of the effects by **single traversals** of different charged-particle types
- inclusion in the model of other lethal **chromosome aberrations** *(typically, non-transmissible complex exchanges)*
- extension of the model to other cells used by the experimental partners, e.g. **endothelial cells** *(main target for cardiovascular damage)*

2017:

- implementation of conditions typical of **targeted therapy** *(e.g., alpha particles starting from the cell surface)*
- quantification of specific chromosome aberrations (typically, translocations) that are associated to specific **(second) cancers**
- possible extension of the model to **^{16}O**
- feasibility study for including **low dose-rate** *(important for Radon and targeted therapy)*