Combining Hadron Therapy with Magnetic Hyperthermia: a New Tool for Pancreatic Cancer Treatment

(HADROCOMBI) – 1 year feasibility study

Partecipanti - Unità di Milano, di Roma3 e di Pavia	FTE
Alessandro Lascialfari – PO - UNIMI – responsabile	0.5
Daniela Bettega – PA – UNIMI	0.75
Flavia Groppi – PA – UNIMI	0.2
Cristina Lenardi – PA – UNIMI	0.2
Ivan Veronese – RI – UNIMI	0.6
Paola Calzolari – Tecnico – UNIMI	0.75
Miriam Lafiandra – PhD – UNIMI	0.75
Francesco Orsini – Tecnico – UNIMI	0.4
Giovanni Savini – PhD – UNIMI	0.2
Antonio Antoccia – PA – Roma 3	0.75
Antonella Sgura – RI – Roma 3	0.75
Marco Cobianchi – PhD – UNIPV	0.3
Maurizio Corti – PA – UNIPV	0.4
Manuel Mariani – RI – UNIPV	0.3
Totale	6.85

Partecipanti esterni :

Fondazione CNAO – Pavia
Istituto Nazionale Tumori - Milano
C. Sangregorio (CNR-ICCOM-FI), C. Innocenti (UNIFI+INSTM), A. Guerrini (UNIFI-INSTM)
P. Arosio, S. Manenti, E. Sabbioni, UNIMI

Istituto Naz. dei Tumori– letter of support

Sistema Socio Sanitario

IRCCS Istituto Nazionale dei Tumori — Regione Lombardia

Gent.mo Professore
Alessandro Lascialfari
Dipartimento di Fisica
Università degli Studi di Milano
Via Celoria, 16
20133 Milano

Milano, 18 luglio 2016

Oggetto: progetto HADROCOMBI "Combining Hadron Therapy with magnetic Hyperthermia: a new tool for pancreatic cancer treatment".

Gent.mo prof. Lascialfari,

Con la presente sono a manifestare l'apprezzamento e l'interesse del nostro Istituto per il progetto di HADROCOMBI.
L'Istituto è quindi interessato a collaborare a tale proposta mettendo a disposizione l'accesso ai fasci di fotoni dei nostri acceleratori lineari, per eseguire l'irraggiamento delle cellule e promuovere l'expertise nel trattamento del tumore del pancreas con la combinazione di fasci di fotoni di alta energia e ipertermia. Le condizioni che regoleranno l'accesso ai nostri acceleratori saranno stabilite dall'Istituto al fine di non interferire con i trattamenti dei nostri pazienti.

Restando in attesa di conoscere gli sviluppi e le risultanze, invio i più cordiali saluti.

Dott. Giovanni Apolone
Direttore Scientifico

Fondazione CNAO – letter of support

fondazioneCNAO
Centro Nazionale di Adroterapia Oncologica

Il Direttore Generale

Pavia, 08.07.2016

SR/sm

Gent.mo Professore
Alessandro Lascialfari
Dipartimento di Fisica
Universita' degli studi di Milano
Via Celoria 16
20133 Milano

Comunicazione inviata via mail a: alessandro.lascialfari@unimi.it

Oggetto: progetto HADROCOMBI "Combining Hadron Therapy with Magnetic Hyperthermia : a new tool for pancreatic cancer treatment".

Gent.mo Prof. Lascialfari,

con la presente sono a manifestare l'apprezzamento e l'interesse della Fondazione CNAO per il progetto HADROCOMBI "Combining Hadron Therapy with Magnetic Hyperthermia : a new tool for pancreatic cancer treatment".

Il CNAO è quindi interessato a collaborare a tale proposta mettendo a disposizione l'accesso ai fasci di particelle generati nel centro per eseguire l'irraggiamento delle cellule e promuovere expertise nel trattamento del tumore al pancreas con l'adroterapia . Le condizioni che regolano tale accesso saranno stabilite dalla Fondazione CNAO.

Restando in attesa di conoscere gli sviluppi e le risultanze, invio i più distinti saluti.

Sandro Rossi

Sede legale: via Caminadella, 16 – 20123 Milano - Sede operativa: strada Campeggi, 53 – 27100 Pavia Tel. +39 0382 0701 - P. IVA 03491760965
info@cnao.it - www.cnao.it

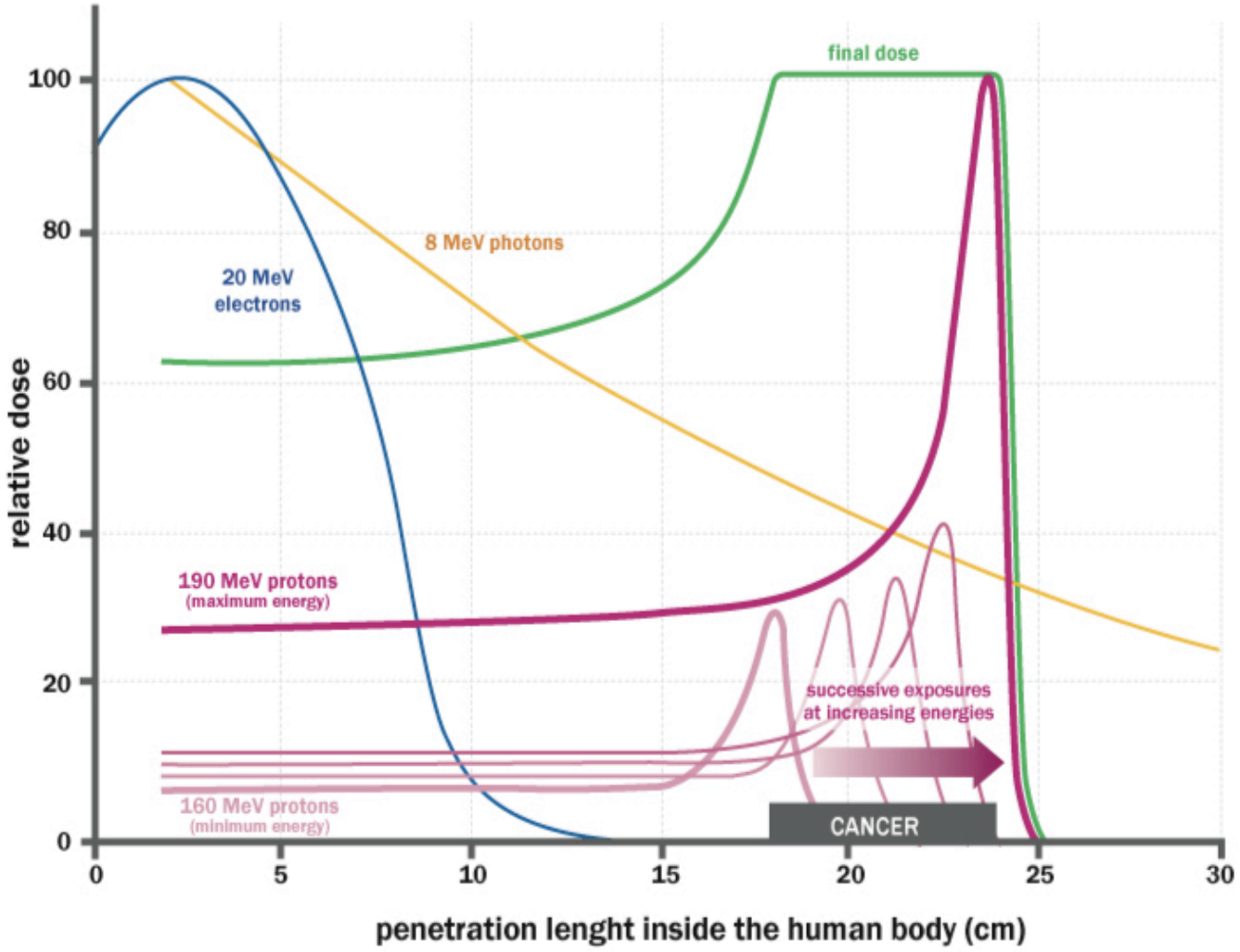
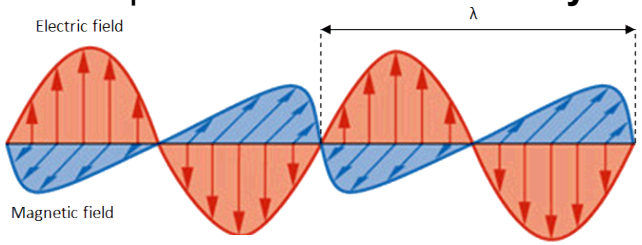


Figura 3.4: Lo Spread Out Bragg Peak (SOBP) è una distribuzione costante di dose (in verde) ottenuta dalla sovrapposizione di molti picchi di Bragg corrispondenti a diverse energie e intensità.

Ratiation types used

a) Low Linear Energy Transfer (photons)

Widespread ionizations → **mainly SSBs**

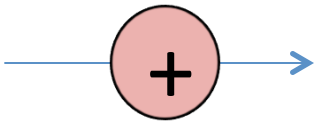


X-rays (LET ~1 KeV/μm)

High- Linear Energy Transfer (particle beams)

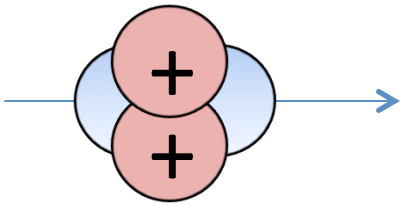
Ionizations clustered in tracks → **mainly DSBs**

b)

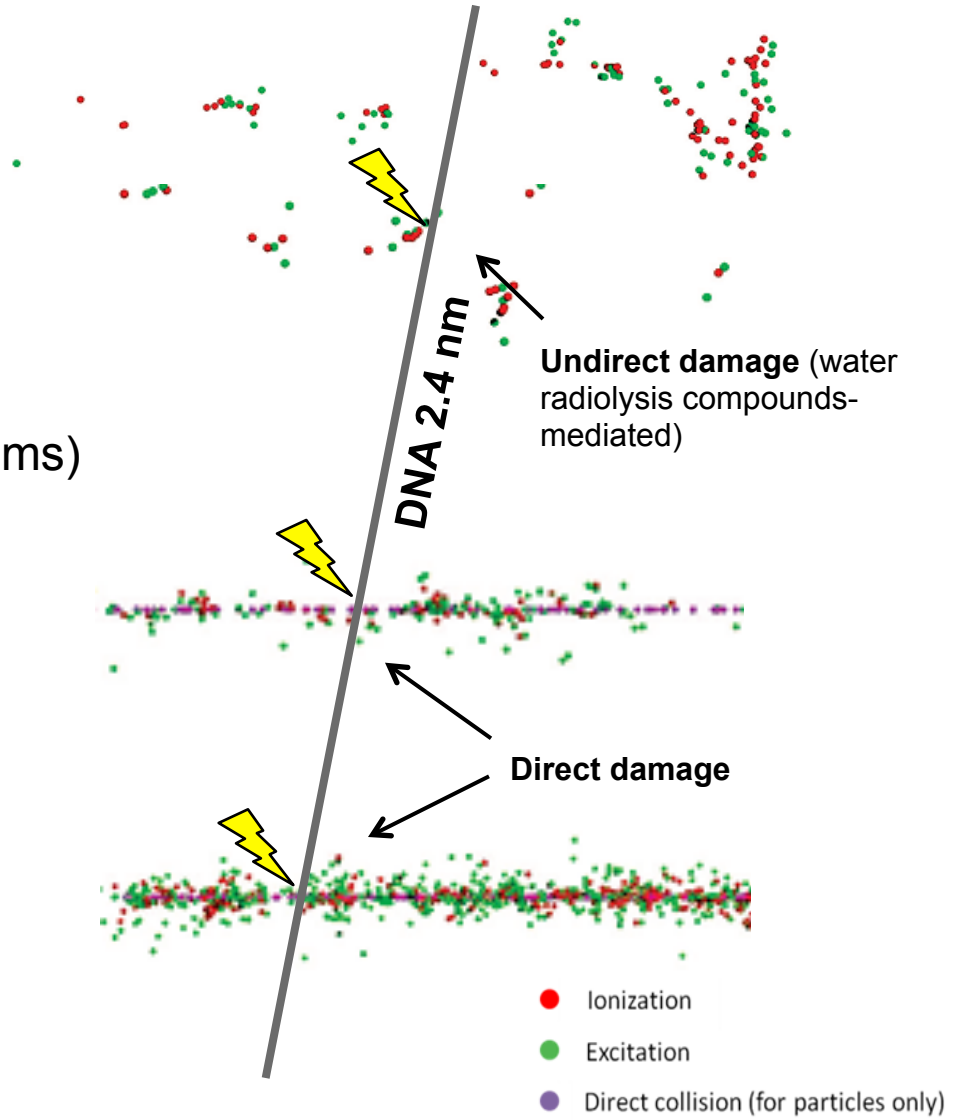


H^+ protons (LET 28.5 KeV/μm)

c)



$4He^{2+}$ α-particles (LET 63 KeV/μm)



a) Low-LET radiations (photons) produce spread ionization, the radiation dose is delivered homogeniously as a lamp delivers the light

b, c) High-LET particles beams produce clustered ionizations and the radiation dose is delivered in tracks

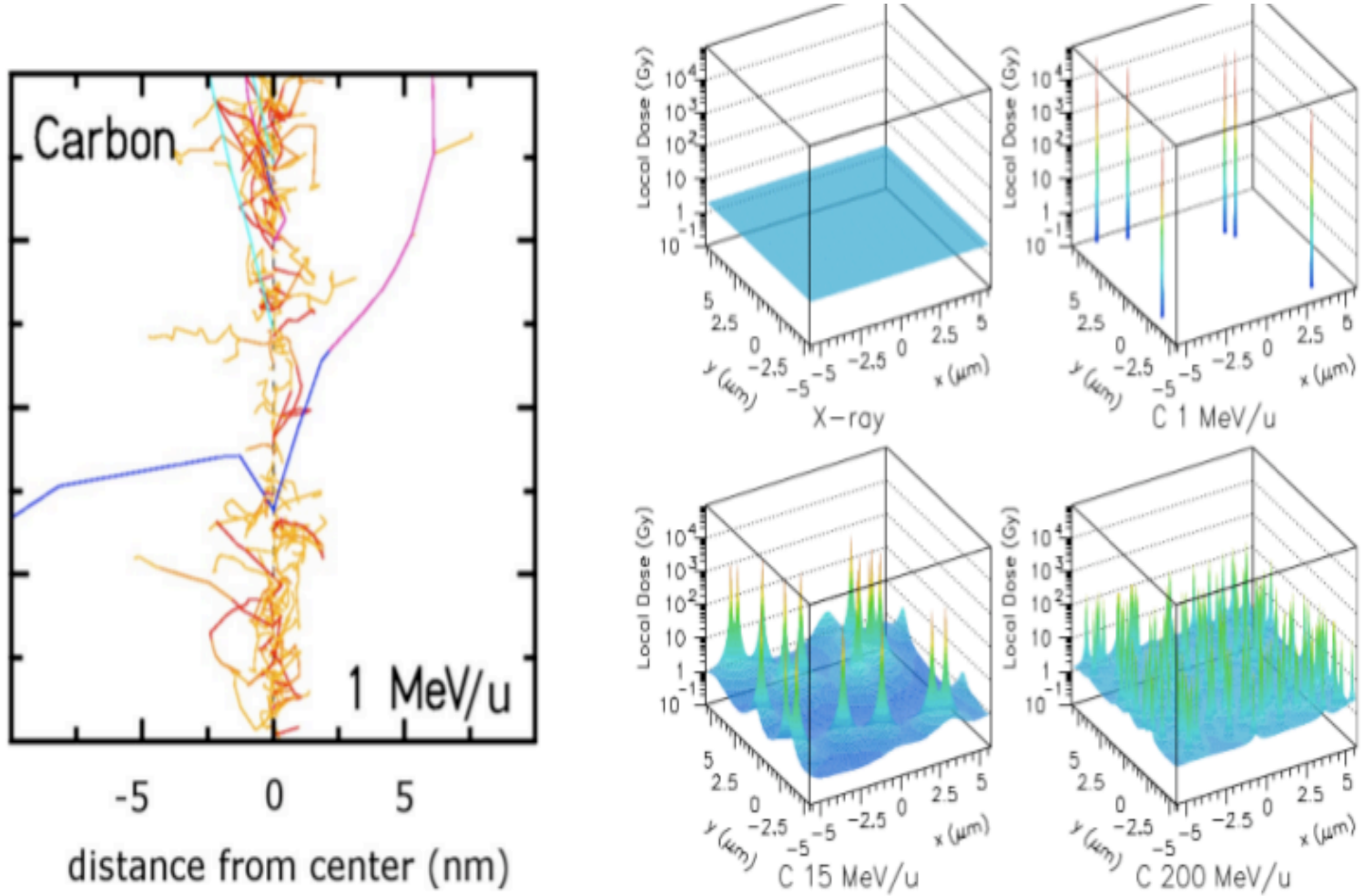
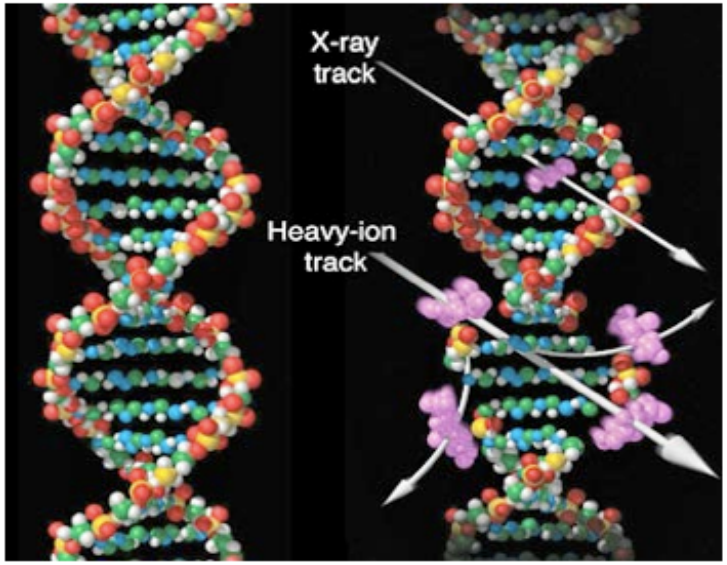
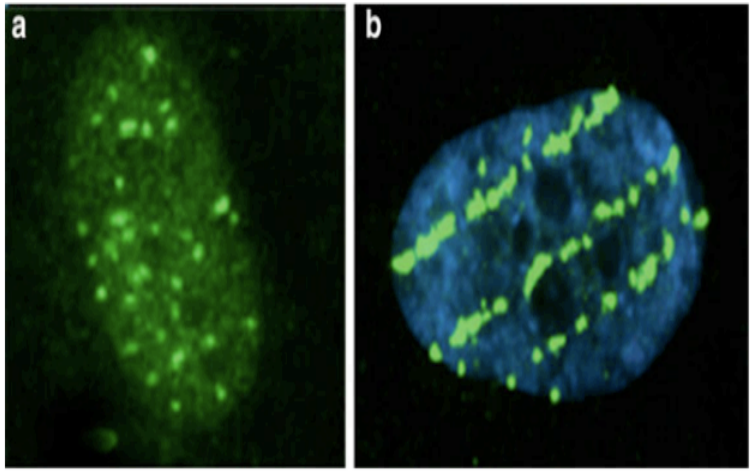


Figura 3.5: A sinistra: la traccia di uno ione carbonio di energia $1\,\mathrm{MeV}/u$ simulata con un codice Monte Carlo; le linee rappresentano il percorso dei singoli elettroni secondari. A destra: Differenti distribuzioni microscopiche di dose con diverse energie; in tutti i casi la dose media è di $2\,Gy$ [28].



+ ENLARGE Different types of radiation treatment cause different kinds of damage to the DNA in a tumor cell. X-ray photons (top arrow) cause fairly simple damage (purple area) that cancer cells can sometimes repair between treatments. Charged particles—particularly ions heavier than protons (bottom arrow)—cause more and more complex forms of damage, resulting in less repair and a more lethal effect on the tumor. (Credit: NASA)



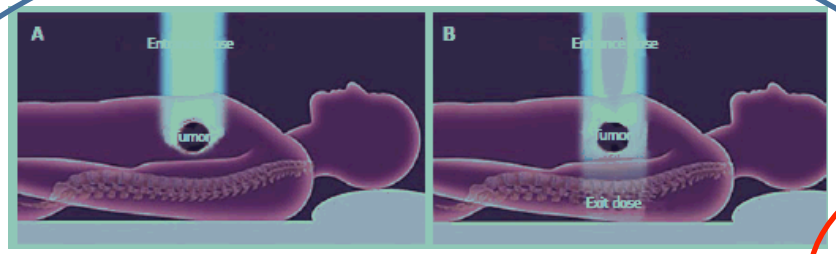
ura 3.9: Nuclei di fibroblasti, cellule del tessuto connettivo, irradiati da raggi X e ioni
ɔonio. I punti verdi individuano i DSB (sono in realtà proteine di riparo). Con i raggi X
ɨinistra) i danni sono uniformemente distribuiti nel nucleo, con il carbonio (a destra) sono
ɛce visibili tre tracce ben definite [2].

ADROTERAPIA IN ITALIA: LNS-INFN, CNAO, TIFPA

Figura 4.6: Il ciclotrone Proteus^{TM}235 dell'azienda belga IBA. Energia: 230 MeV, peso: 220 t, diametro: 4.34 m. Esso è presente in molti centri europei, tra cui al Centro di Protonterapia a Trento [12].

Combining Hadron Therapy with Magnetic Hyperthermia: a New Tool , for Pancreatic Cancer Treatment

(HADROCOMBI)



(A) targeted proton therapy deposits most energy on target;
(B) conventional radiation therapy deposits

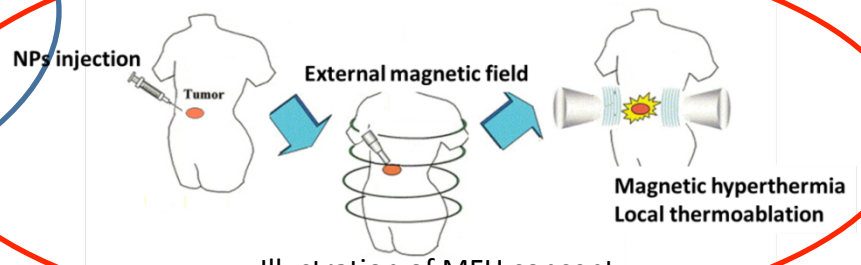


Illustration of MFH concept

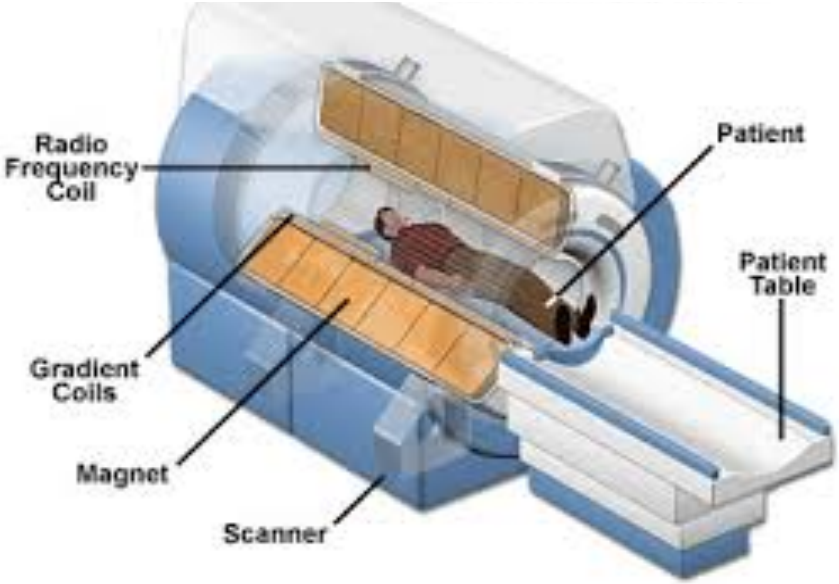
Hadron Therapy and Magnetic Hyperthermia are new and interesting treatments for cancers **where the "classical" therapies fail**.

The **goal of the project** is the investigation of the **possible combined action of the two therapeutic techniques**, for going one step beyond the state of art of pancreatic cancer therapy. **X-rays irradiation will be used as control and comparison technique**

Magnetic Fluid Hyperthermia (MFH)

* It refers to **local temperature rises**, usually to 40–45°C, rendering the cells susceptible to various forms of damage including apoptosis, leading to subsequent cell death.
* Other effects: activation of immunological responses, enhancement of tumor blood flow and oxygenation via greater vascular perfusion and permeability.
* A number of therapeutic benefits in producing **localized heating** (delivering toxic doses of thermal energy to tumors, or increasing the efficacy of anti-cancer drugs).

Magnetic Fluid Hyperthermia allows to strictly controlling the region under treatment by using Magnetic Nanoparticles (MNPs) as heating elements.



Used in clinics (Germany, USA)

Web-site : http://www.magforce.de/en/home.html

- Heating through application of AC magnetic field via activation of MNPs directly injected in the tumour mass at high doses (ca. 50 mg/cm^3).
- Typically: f ~ 100 kHz, amplitude 10 kA/m.
- Minor side-effects

magforce®

THE NANOMEDICINE COMPANY

- About Us
- Vision
- Management Board
- Supervisory Board
- Careers

Using advanced nanomedicine for innovative therapies

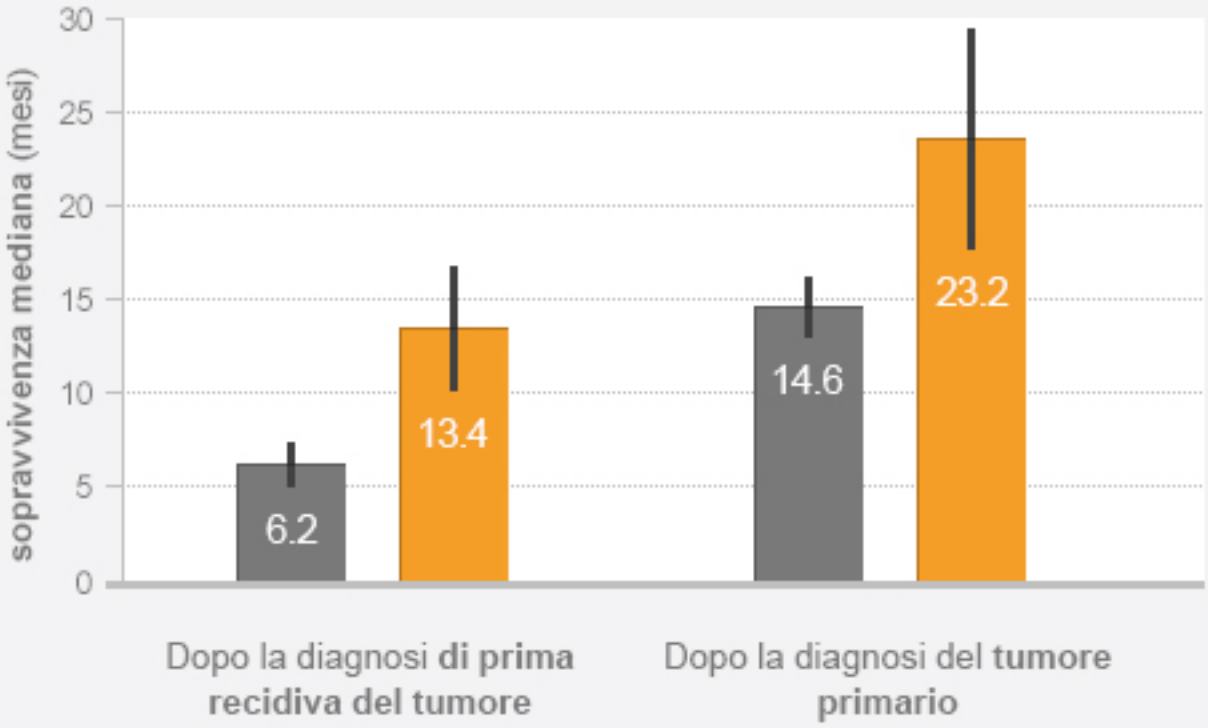
AT A GLANCE

- Founded: 1997, head office in Berlin, Germany
- Management Board: Dr. Ben J. Lipps (CEO), Prof. Hoda Tawfik (Chief Medical Officer), Christian von Volkmann (CFO)
- Employees: 25 employees plus a production company with 14 employees

MED CERT

Compliant with European Standard

Vantaggio di sopravvivenza della terapia NanoTherm®
in combinatione con la radioterapia stereotassica in 59 pazienti con glioblastoma recidivo

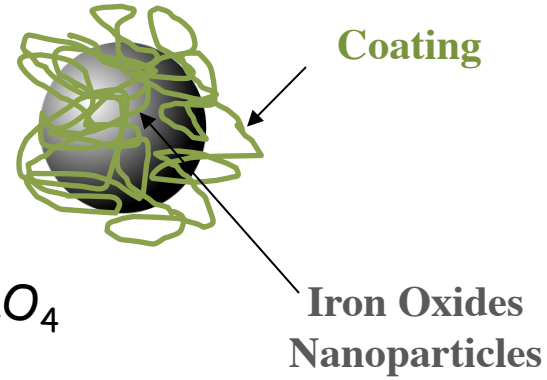


Workpackages

- ***WP1 Nanoparticles synthesis and characterization (Months:1-9; participants: CNR-ICCOM, Milano, Pavia)***
- ***WP2 Irradiation experiments with X-rays and Hadrons (Months:1-12; participants: Milano, CNAO-PV, INT-MI)***
- ***WP3 Magnetic Fluid Hyperthermia experiments (Months:2-12; participants: Milano, Pavia)***
- ***WP4 Responses of tumor pancreatic cells (Months:1-12; participants: Milano, Roma3)***
- ***WP5 Imaging Techniques (Months:4-12; participants: Milano)***

Firenze: CNR-ICCOM, UNIFI, INSTM

Synthesis of Magnetic Nanoparticles and their characterization



- Synthesis: thermal decomposition of the metallo-organic precursors with surfactant:

-> Magnetic cores of MNPs: γ -Fe_2O_3, Fe_3O_4 or $Co_xFe_{3-x}O_4$

-> Size of cores of MNPs: 15-20nm

-> coatings: PAA (polyacrylic acid); PEG (polyethylen glycol); Citrate; Folic acid; etc.

- characterization: XRD, DLS, TEM, ICP, SQUID-AC and EPR

The control of structural properties of MNPs is crucial to obtain the optimal material for MFH. The coating will be chosen for favoring the uptake of MNP inside the cells, that is a crucial parameter to maximize the therapeutic effect

Unità di Pavia

Magnetic characterization of magnetic nanoparticles via DC SQUID

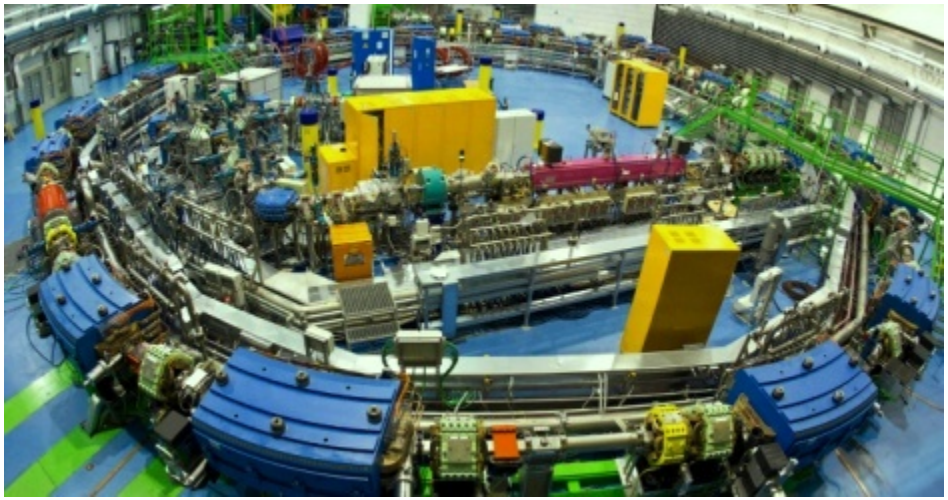
- hysteresis curves at T=2, 300 and 310 K (physiological temperature)
- ZFC – FC curves

The magnetic characterization will be used to determine the saturation magnetization , the remanent magnetization, the coercive field and the relaxation time of the magnetization . All these quantities enter in the expression of the SAR, the heat release efficiency of MNPs.

Irradiation with Hadrons and X-rays

Fondazione CNAO, Pavia

(0.5 - 4 Gy for Carbon ions;
0.5 - 5 Gy for protons)



Istituto Nazionale dei Tumori, Milano

(0.5 – 7 Gy for photons)

Unità di Milano

Magnetic Hyperthermia protocol

- Nowadays the clinical protocol is not unambigous!
- Investigation of the optimal combination of H_{AC} and *f* to gain the best SAR values (e.g. 30 mins at 110 kHz and 10 kA/m)
- Treatments of the cells line with the optimal concentration of MNPs.

The H_{AC} and *f* values, the time duration of treatment, the MNPs concentration are crucial parameters to evaluate the heat release efficiency of MNPs and as a consequence the efficacy of the hyperthermic treatment.

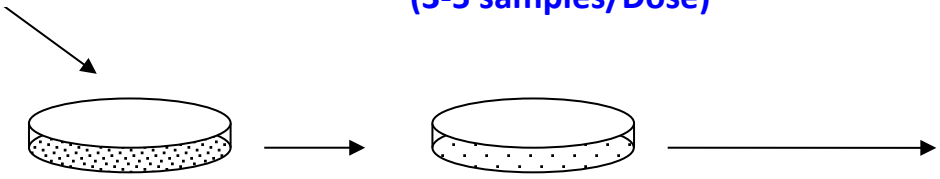
Unità di Milano

Responses of pancreatic tumor cells: clonogenic survival

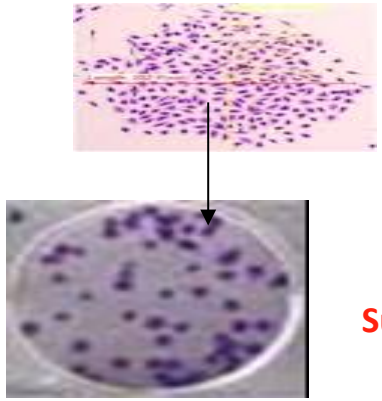
Cell clonogenic survival after (X or HT) +/- (NPs +MFH)

(Radiation, DoseD)
+/- (NPs+MFH)

Cells seeded at low density
(to get 2-3 colonies / cm^2)
Seeded cells/sample = No
(3-5 samples/Dose)



Incubation at 37° for
2-3 weeks

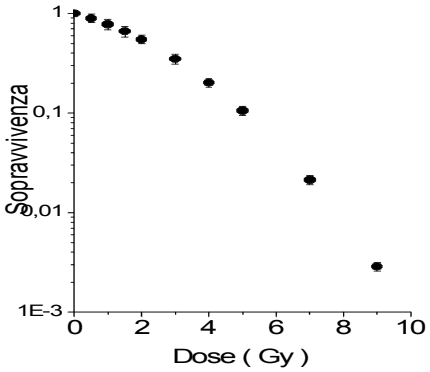


Colony from a survived cell: more than 50cells

Survived cells = N

Control samples are treated in the same way with the exception of irradiation

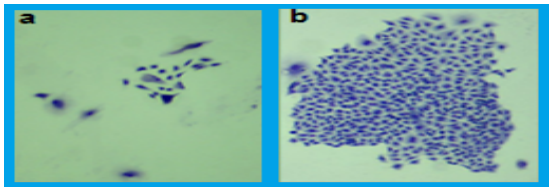
Survival vs Dose



Plating Efficiency at Dose D,
(PE(D)) : PE (D) = N / No

Survival at Dose D. S(D):
S(D) = PE(D) / PE(0)
(PE(0) = Control samples Plating Efficiency).

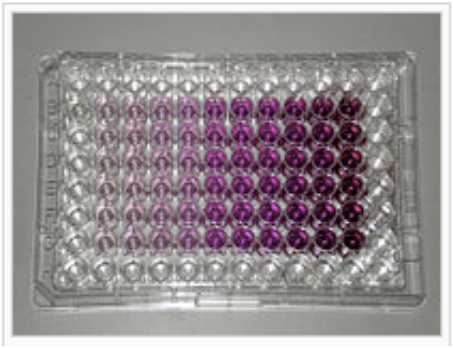
Examples of colonies from survived (b) and not survived (a) cells



Unità di Roma3

Endpoints to be investigated in tumor pancreatic cells

(magnetic particles alone or in combination with radiations)

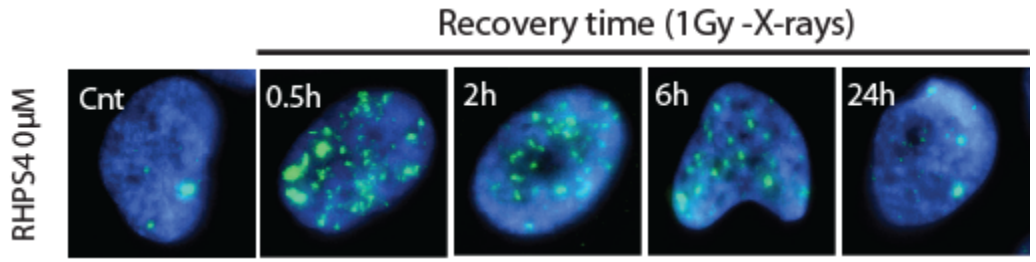


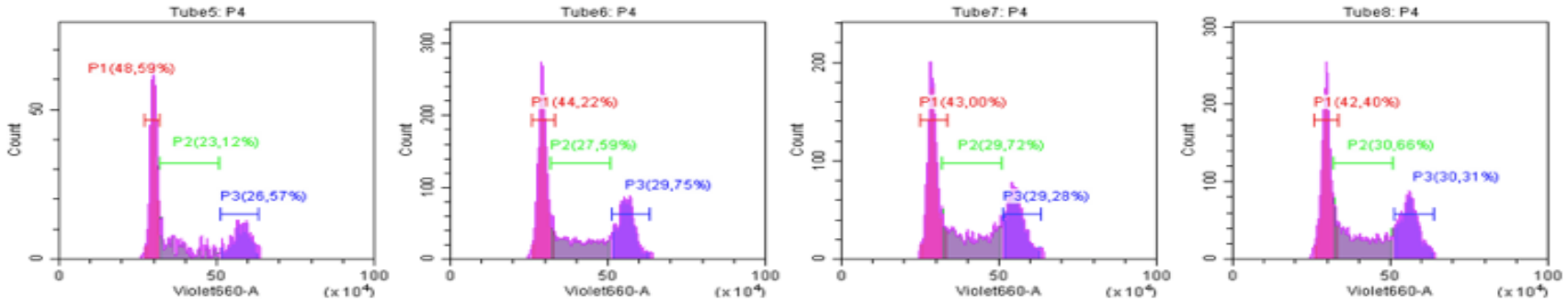
- Cytotoxicity with MTT assay (*)

- DSB repair kinetics (γ-H2AX and 53BP1 foci)

-Induction of Apoptosis (Cytofluorimetric analysis)

* For particles alone





Unità di Milano

Radiotracers protocol

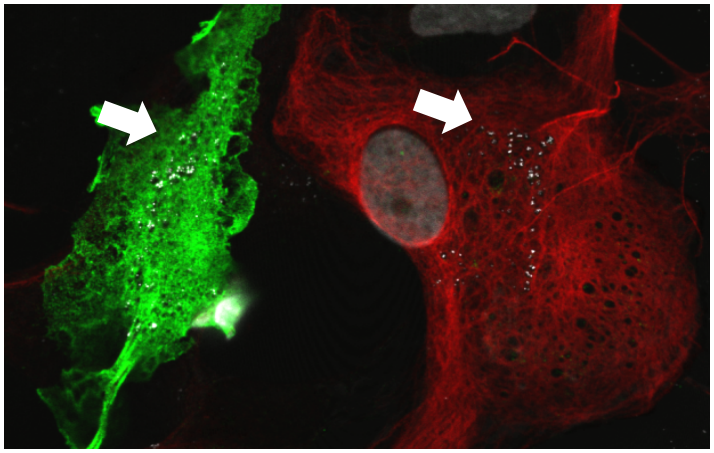
- isotopic tracing of the Fe_2O_3 nanoparticles (FeONPs) through the radiotracer technique with the use of the gamma emitting isotope of Fe (^{59}Fe) as tracer;
- the study of variations of the natural abundance of stable isotopes.

Both these techniques compared with other analytical methods, not only have a high sensitivity, good accuracy and are time saving, but **can distinguish the endogenous and exogenous iron** after exposure of the *in vivo* animal models (body fluids and tissues) as well as *in vitro* toxicity testing models (cell culture media , whole cells and subcellular fractions).

Unità di Milano

Uptake quantification & Imaging

- Quantitative cellular uptake of MNPs with inductively coupled plasma mass spectrometry (ICP-MS).
- Localization of MNPs in intracellular structures with high spatial resolution with Scanning Electron Microscopy (SEM).
- Analysis of distribution of MNPs in cells with Confocal Laser Scanning Microscopy (CLSM).



Reflected light in CLSM:
distribution of Ag-NPs (100 nm)
in primary cells (**Astrocytes and Microglia**)

Milestones

* First synthesis of MNPs constituted by magnetic core (maghemite, magnetite or cobalt-ferrite) and coated with organic biocompatible moiety. (M2)
* First synthesis of radiotracers starting from ^{59}FeCl and radioiodination of proteins or from commercially available ^{57}Fe or ^{58}Fe – enriched salts. (M2)
* Determination of the main magnetic (physical) properties of first synthesized MNPs (M_s, H_c, M_r, etc). (M3)
* Determine the best methods to establish qualitatively and quantitatively the MNPs cellular uptake. Verify correlation between uptake and MNPs administrated doses. (M4)
* First cells irradiation with photons and hadrons. (M4)
* First results of viability and clonogenic survival on the treated tumour pancreatic cells (M6)
* Results on the kinetics of DSB-rejoining and Apoptosis in tumor pancreatic cells exposed to Photon, after single and combined treatments. (M6)
* Choice of the best MNPs and the best protocol for MFH cell treatments after irradiation. The duration, the number of treatments and the correct concentration of MNPs will be optimized for the hyperthermic effect on cells. (M8)
* Imaging of cells (SEM, CLSM and radiotracers) before and after irradiation with different (combined or not) irradiation techniques. (M8)
* Results on the kinetics of dissolutions/release of iron ions in the aqueous and biological media studies. (M12)
* For single and combined treatment determine the kinetics of DSB-rejoining and Apoptosis in tumor pancreatic cells exposed to Proton and C-ions. (M12)
* For single and combined treatment clonogenic survival experiments in tumor pancreatic cells exposed to Proton and C-ions. (M12)
* First conclusions about the effects of combined HT (XRT) and MFH techniques on cellular damage (M12)

HADROCOMBI - Spese previste

- Materiale di consumo per coltura cellule e determinazione della sopravvivenza (linea cellule tumorali del pancreas da acquistare presso banca (US), terreni di coltura , siero fetale, reagenti , filtri per produzione acqua ultrapura, plastiche monouso per coltura cellulare (flasks , pipette), puntali per determinazione densità cellulare alla semina

  10 k €

- Per esperimenti MFH : Materiale criogenico, gas (azoto, elio), materiale elettronico, schede/ moduli elettronici, vetreria, materiale elettrico (bobine, codensatori)

  5 k €

- Reagenti chimici per cell staining e essiccamento (viability test, functional tests, etc.), materiale per microscopia confocale e SEM, target metallici per SEM (4), analisi ICP-MS (1)

  5 k €

- Irraggiamenti presso il LENA (Reattore di PV) per la parte dei radiotraccianti (1), preparazione di nanoparticelle FeO arricchite isotopicamente in ^{57}Fe o ^{58}Fe (0.5), Trasporti radioattivi Pv-LASA (0.5)

  2 k €

- Reagenti chimici, liquidi e gas criogenici, per preparazione nanoparticelle magnetiche (in collaborazione con UNIFI e CNR-ICCOM-Firenze)

  6 k €

- Unità Roma3 : Materiale di consumo per colture cellulari, terreni, sieri, plasticheria, anticorpi per danno al DNA in immunofluorescenza: gamma-H2AX, 53BP1, reagenti per apoptosi e citotossicità MTT

  5 k€

- Unità Pavia : liquidi criogenici, gas criogenici, schede/moduli elettronici, elettronica, vetreria, plasticheria, per misure SQUID

  3 k €

- Missioni Unità MI

  6 k €

- Missioni Unità Roma3

  3 k €

- Missioni Unità Pavia

  2 k €

•Unità Roma3 : Materiale di consumo per colture cellulari, terreni, sieri, plasticheria, anticorpi
•per danno al DNA in immunofluorescenza: gamma-H2AX, 53BP1, reagenti per apoptosi e
•citotossicità MTT 5 k€
• Unità Pavia : liquidi criogenici, gas criogenici, schede/moduli elettronici, elettronica, vetreria,
•plasticheria, per misure SQUID 3 k €
• Missioni Unità MI 6 k €
• Missioni Unità Roma3 3 k €
• Missioni Unità Pavia 2 k €

TOTALE (34 Unità MI + 8 Unità Roma3 + 5 Unità Pavia) 47 kEuro

Roma Tre 5 + 3 k€

WORKPLAN

Materials:

* The pancreatic tumor cells lines: PANC-1 and/or BxPC3
* MNPs for MFH: maghemite (γ-Fe_2O_3) MNP of 16-20 nm, coated with a biocompatible organic moiety.

Three different modalities for each experiment:

- Irradiation alone;
- MNPs + irradiation;
- MNPs + irradiation + MFH.

Protocol for the in-vitro experiments:

- Incubation period of the cells with MNPS
- Irradiation treatment with Carbon Ions or protons at CNAO foundation (Pavia, Italy) and, for comparison, with photons at IRCCS - Istituto Nazionale dei Tumori (Milano), with the following dose-ranges: 0.5 - 4 Gy for Carbon ions; 0.5 - 5 Gy for protons; 0.5 – 7 Gy for photons.
- Following exposure to radiation, cells treatment with MFH (30 mins at 110 kHz, 10 kA/m)
- The treated cells will be followed as concerns viability and imaged. When possible, MNPs distribution and cellular uptake will be determined.
- The application of radiotracer techniques by using ^{59}Fe – radiolabelled FeONPs starting from $^{59}FeCl$, that will allow biokinetic and mechanistic studies to optimize :

a) the uptake, the bioavailability of MNPs in tumor target cells, the fate in the biological system;
b) the MNPs surface effects and the influence of the protein corona.
c) the determination of the variation of natural abundance of stable isotopes.