UNIVERSITÀ DI PAVIA

Istituto Nazionale di Fisica Nucleare

MEASUREMENT OF CELL SURVIVAL AS A FUNCTION OF THE DOSE TO ASSESS BNCT EFFECTIVENESS

Nicoletta Protti

For the BNCT group - Pavia

Kick-off meeting Neptune – LNS – 14 December 2018

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Clinical lessons from the first applications of BNCT on unresectable liver metastases.

A Zonta, U Prati, L Roveda, C Ferrari, S Zonta, AM Clerici, C Zonta, T Pinelli[1], F Fossati[1], S Altieri[1], S Bortolussi[1], P Bruschi[1], R Nano[2], S Barni[2], P Chiari[2] and G Mazzini[3]
Department of Surgery, University of Pavia and IRCCS San Matteo, Pavia
[1]Department of Nuclear and Theoretical Physics, University of Pavia and I.N.F.N., Pavia
[2]Department of Animal Biology, University of Pavia
[3]IGM CNR Histochemistry and Cytometry Section, University of Pavia, Italy

Hepatic metastases from colon adenocarcinoma

Lung metastases
Mesothelioma

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Boron uptake measurements in a rat model for Boron Neutron Capture Therapy of lung tumours

S. Bortolussi [a,b,*], J.G. Bakeine [a], F. Ballarini [a,b], P. Bruschi [a], M.A. Gadan [a,c], N. Protti [a,b], S. Stella [a,b], A. Clerici [d], C. Ferrari [d], L. Cansolino [d], C. Zonta [d], A. Zonta [d], R. Nano [e], S. Altieri [a,b]

Bortolussi *et al. Radiation Oncology* (2017) 12:130
DOI 10.1186/s13014-017-0860-6

Radiation Oncology

RESEARCH Open Access

Understanding the potentiality of accelerator based-boron neutron capture therapy for osteosarcoma: dosimetry assessment based on the reported clinical experience

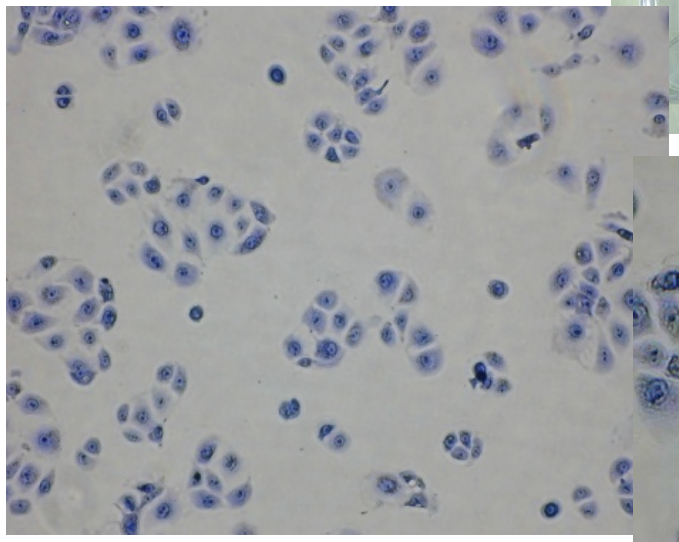
Silva Bortolussi[1,2*], Ian Postuma[2], Nicoletta Protti[2], Lucas Provenzano[3,4], Cinzia Ferrari[5,2], Laura Cansolino[5,6], Paolo Dionigi[5,6], Olimpio Galasso[7], Giorgio Gasparini[7], Saverio Altieri[1,2], Shin-Ichi Miyatake[8] and Sara J. González[3,4]

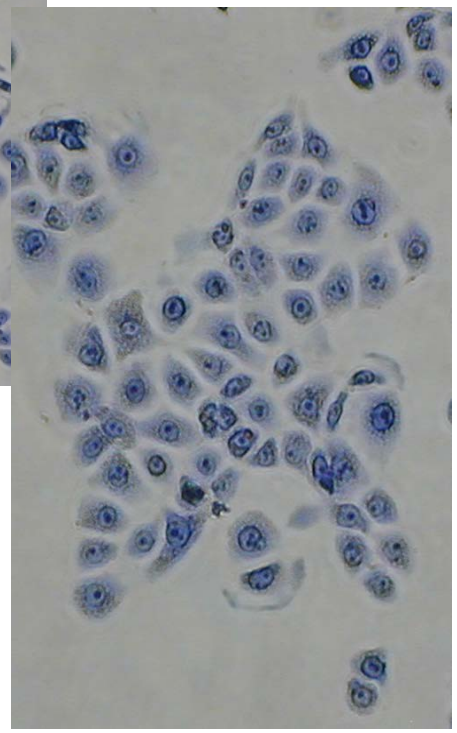
Limb osteosarcoma

PRE-CLINICAL IN-VITRO TESTS

1. Evaluation of boronated carrier toxicity
2. Intracellular ^{10}B uptake varying concentration and time of incubation
3. Effectiveness of the treatement

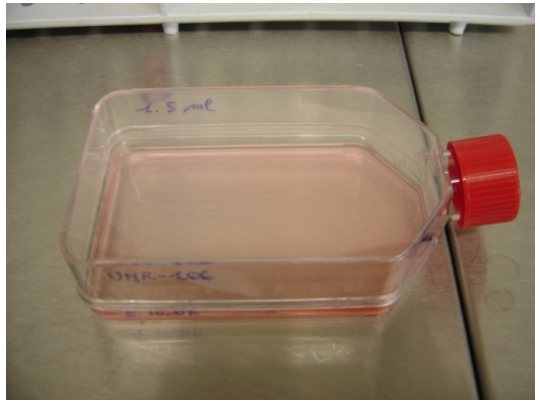
IN-VITRO MODELS

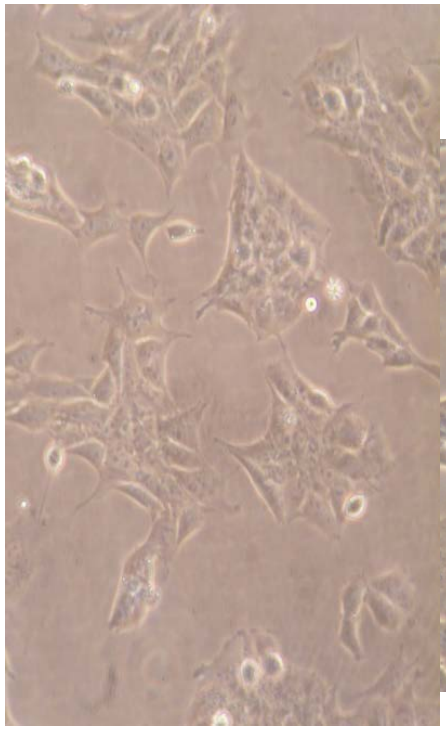


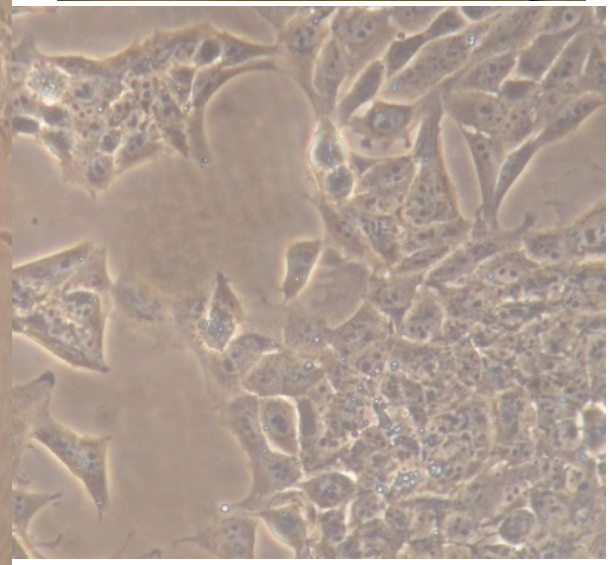


DHD/K12/TRb (DHD)
rat coloncarcinoma cell line

UMR-106 (UMR)
rat osteosarcoma cell line



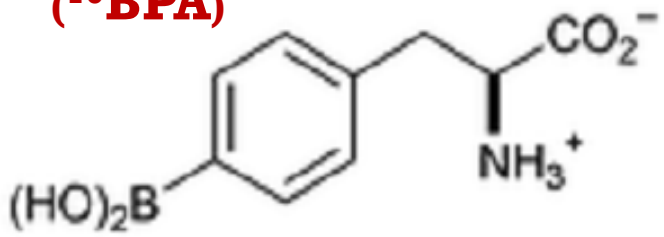


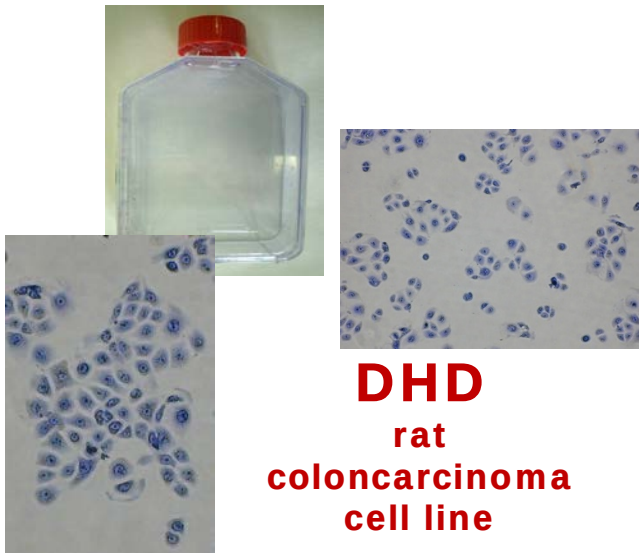


1. Intracellular ^{10}B uptake varying concentration and incubation time

Methods

D,L- ^{10}boronophenylalanine (^{10}BPA)





DHD
rat coloncarcinoma cell line

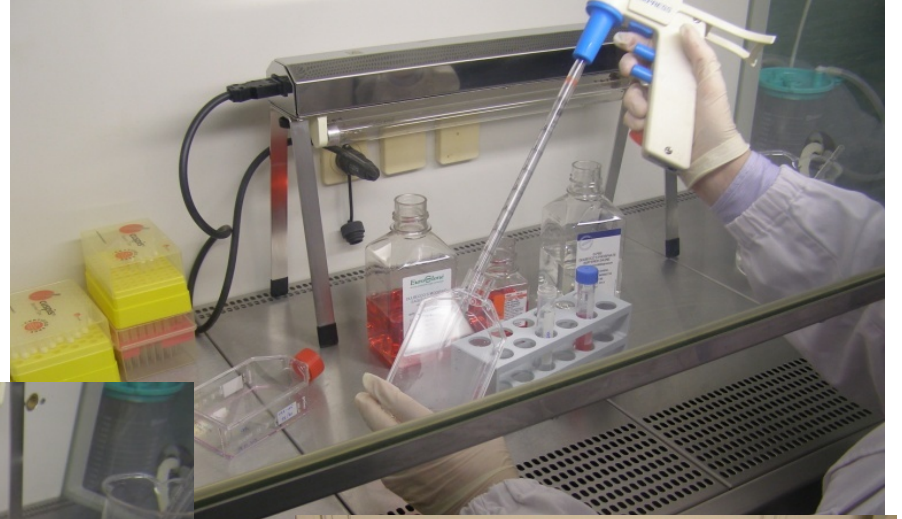
Cells are seeded and allowed growing for 24 h in DMEM/HAM'S F10 medium.

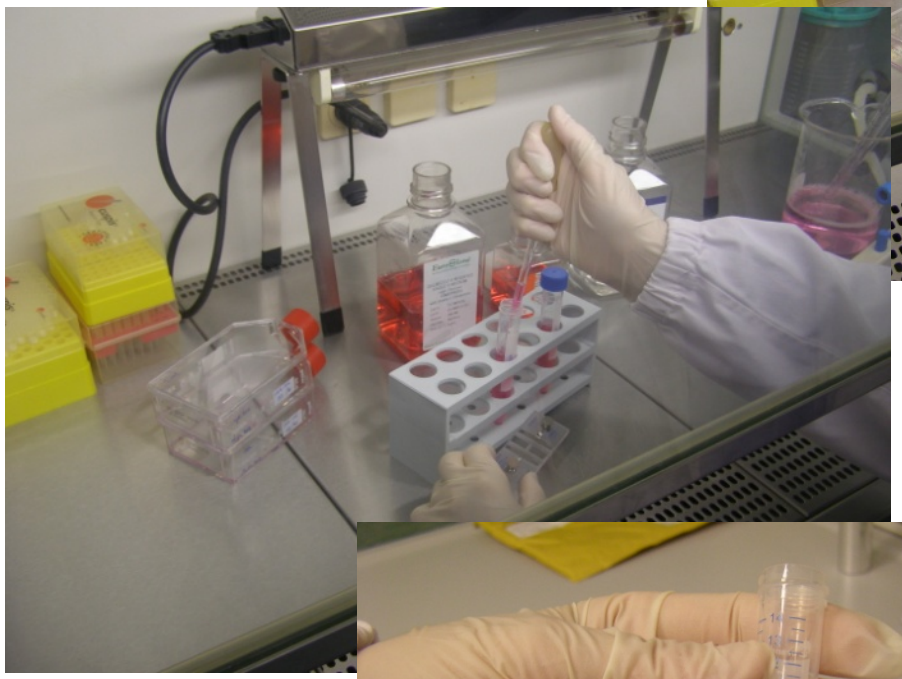
BPA enriched medium at different ^{10}B concentrations is delived to cells.

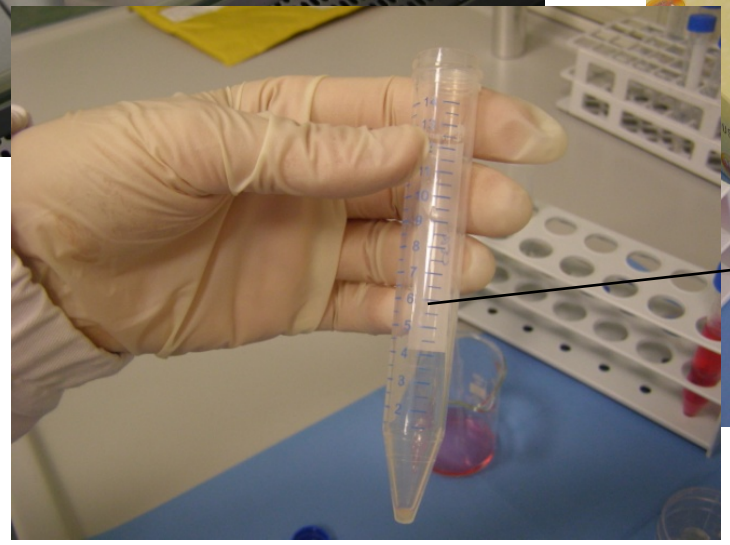
At the end of the time of contact the ^{10}B containg medium is replaced and cells are washed three times in PBS, trypsinized, harvested and centrifuged in boron deprived medium and counted.

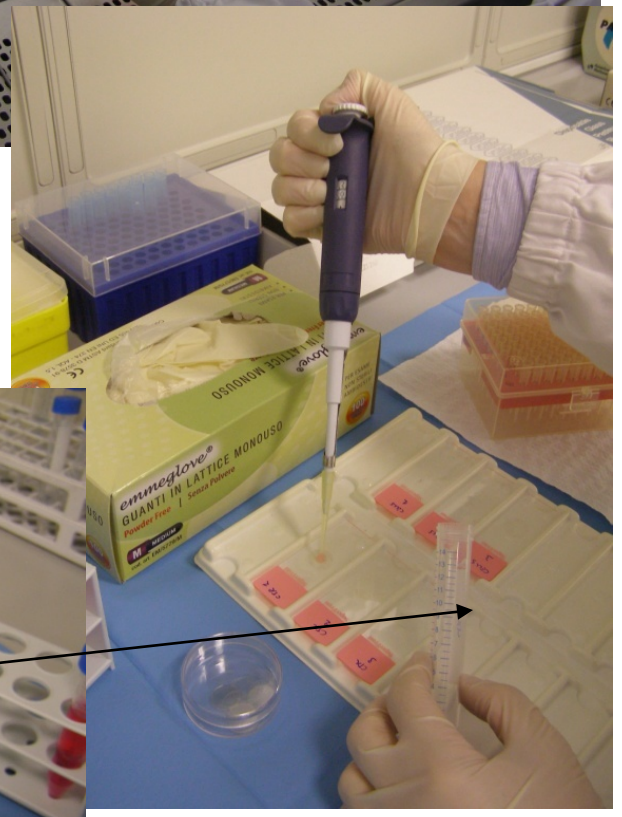
Samples of ^{10}B enriched medium, PBS, surnatant and cells are deep-frozen in liquid nitrogen for ^{10}B concentration analyses by inductively coupled plasma mass spectrometry (ICP-MS).

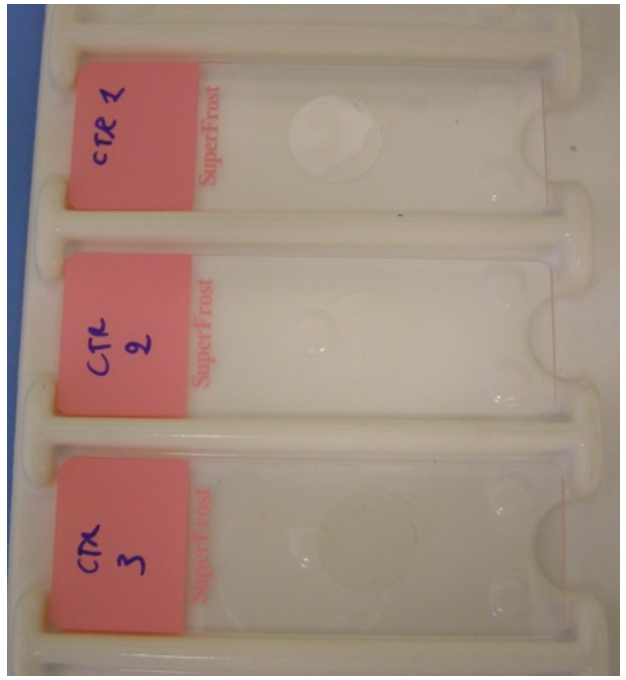
Cells are layered on mylar disks for neutron autoradiography







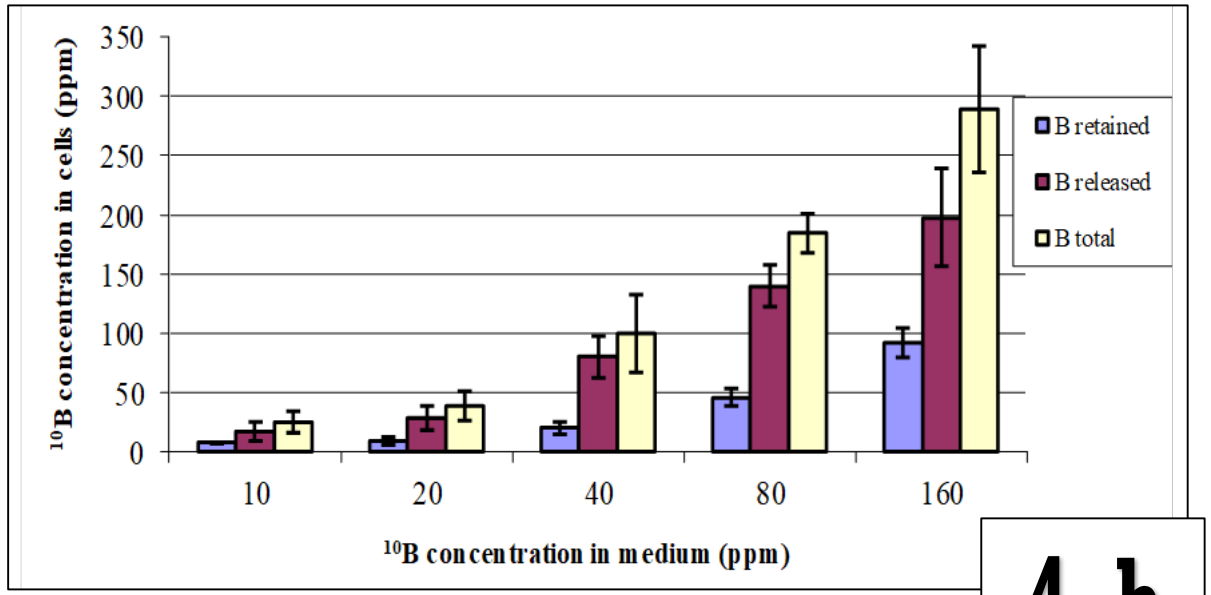

emmeglove®
GUANTI IN LATTICE MONOUSO


CTR 1
SuperFrost
CTR 2
SuperFrost
CTR 3
SuperFrost

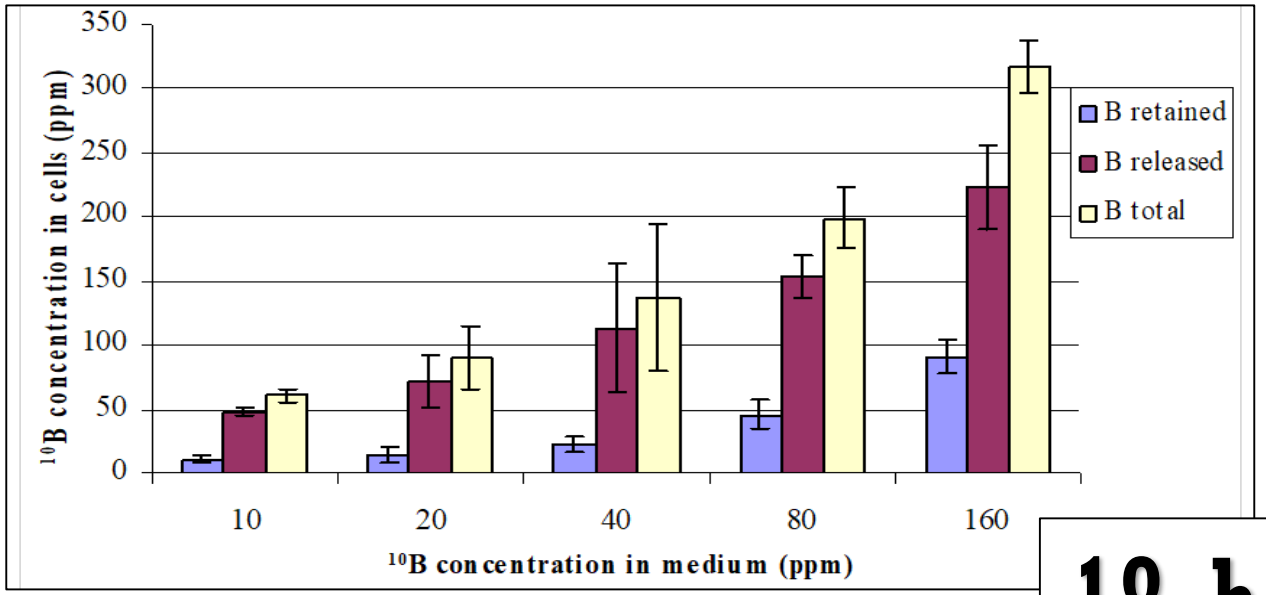

CTR 1
SuperFrost


CTR 1
SuperFrost

Results



4 h



18 h

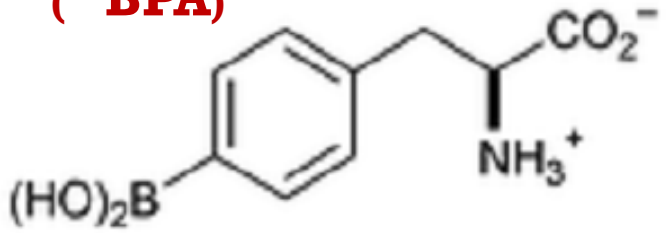
despite the boron release, 80 ppm treatments result always in a retained boron fraction of more than 40 ppm which is about half the concentration supplemented in medium and sufficient to deliver an adequate radiation dose to cells when studying effectiveness

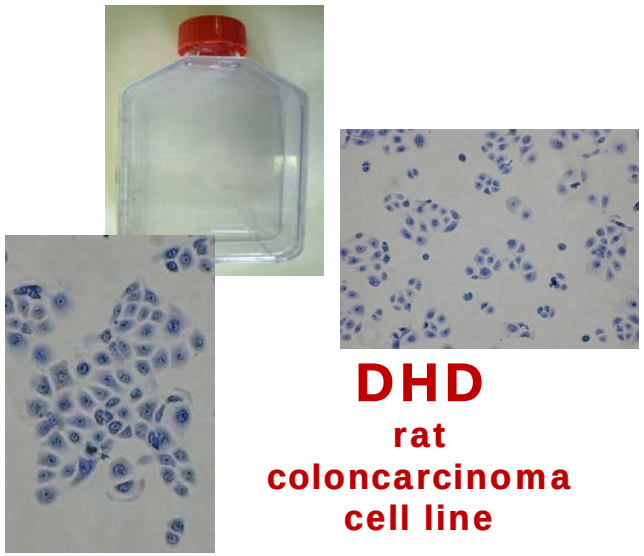
2. Toxicity

Methods

D,L- ^{10}boronophenylalanine

(^{10}BPA)





DHD
rat coloncarcinoma cell line

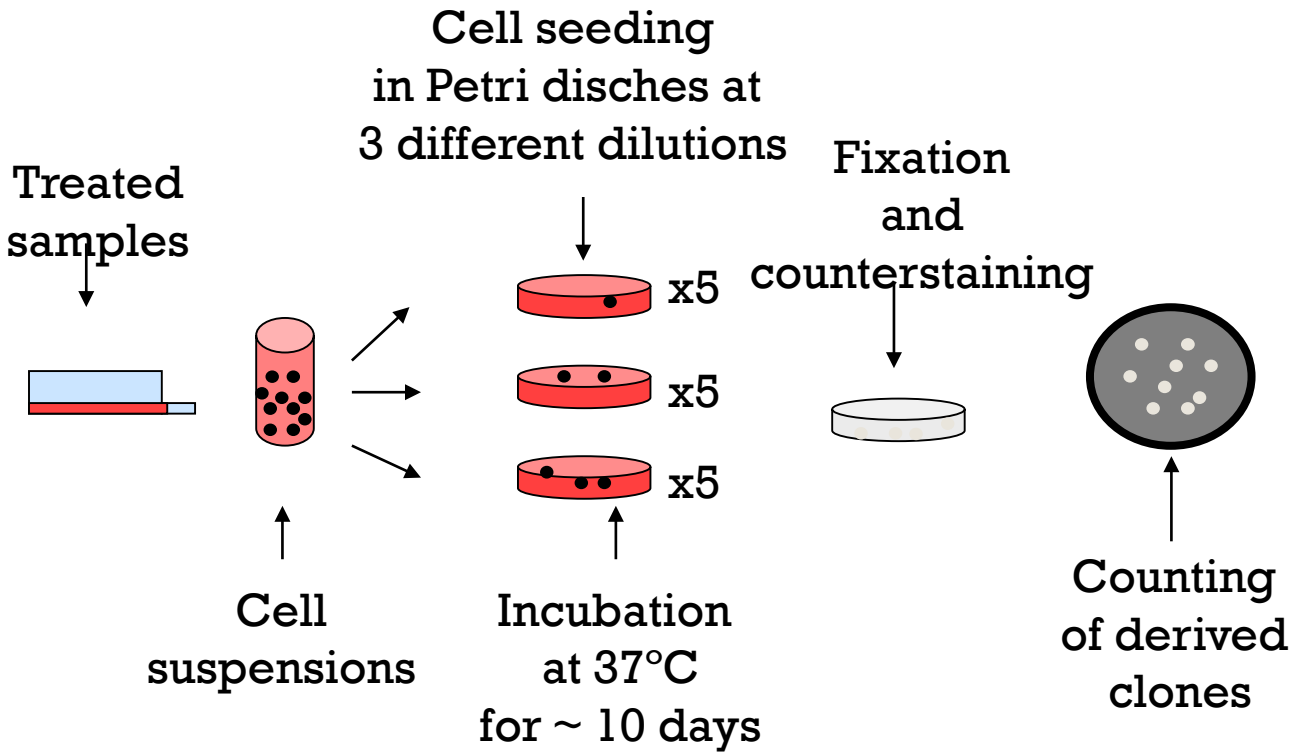
Cells are seeded and allowed growing for 24 h in DMEM/HAM'S F10 medium.

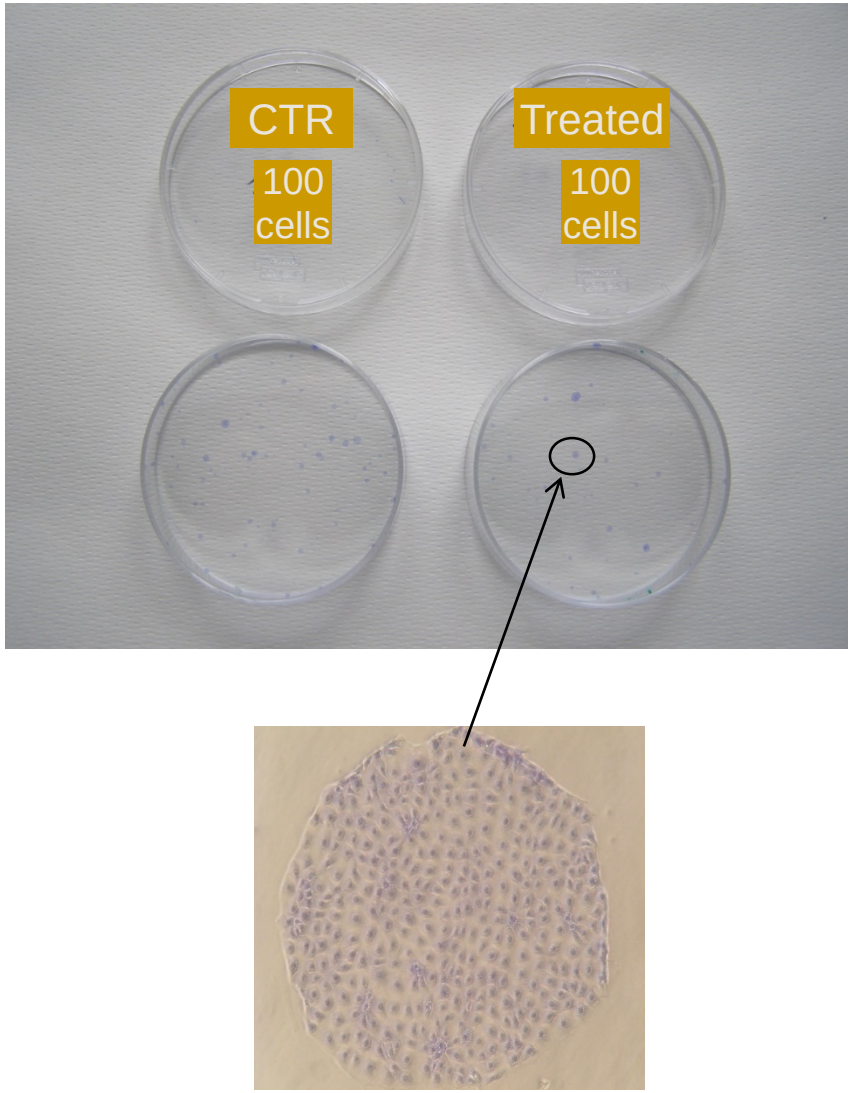
BPA enriched medium at ^{10}B concentrations from 10 to 160 μg/ml is delived to cells for 4 h and 18 h.

At the end of the time of contact the ^{10}B containg medium is replaced and cells are washed three times in PBS, trypsinized, harvested and centrifuged in boron deprived medium and counted;

Cells, diluted at three different concentrations are plated in five different Petri plates for each of them and allowed to grow for about 10 days for the plating efficiency test.

Plating Efficiency Test

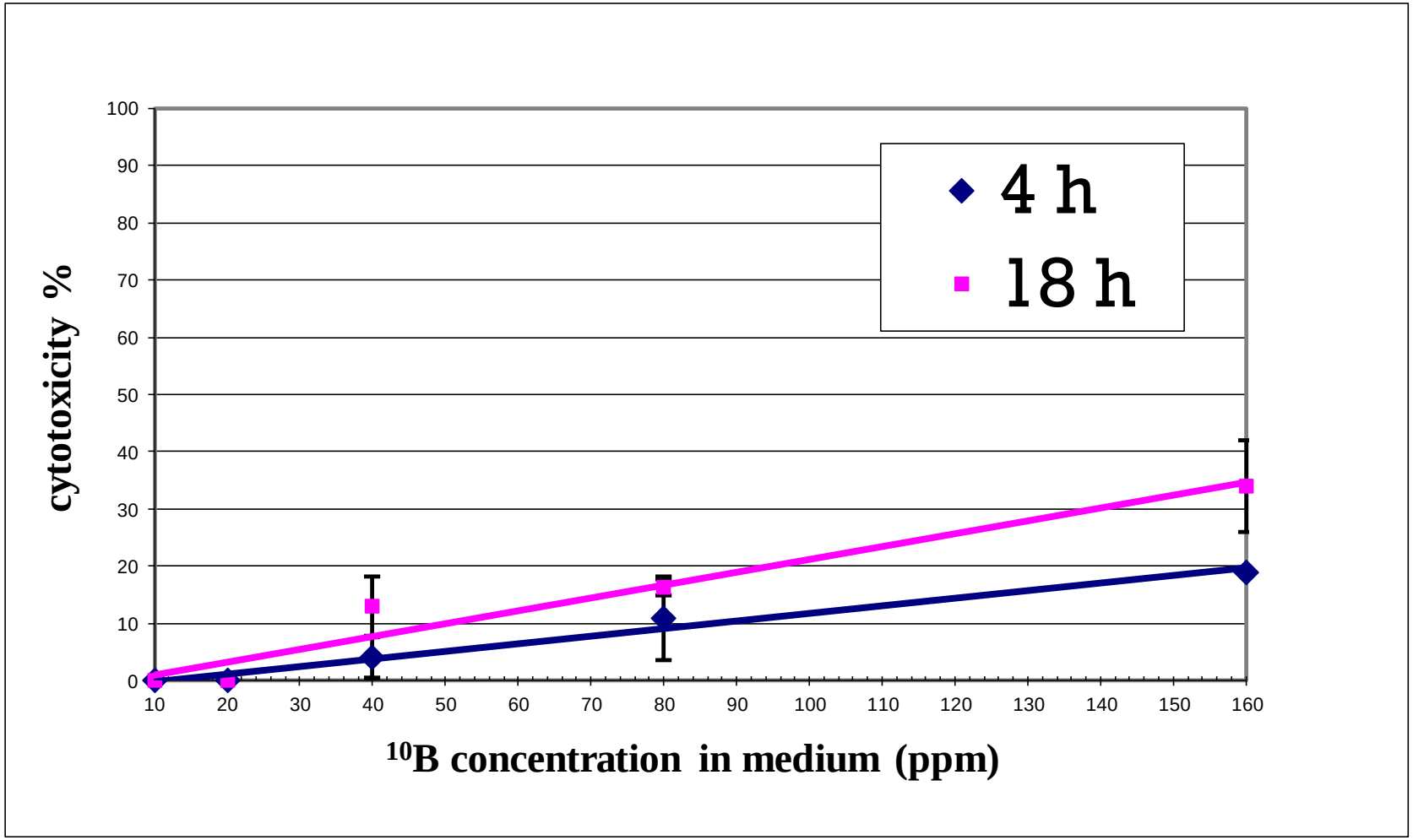




$$\text{Plating Efficiency (EP) (\%)} = \frac{\text{n° of colonies}}{\text{n° of seeded cells}}$$

$$\text{Cell survival (\%)} = \frac{\text{EP treated sample}}{\text{EP ctr cells}}$$

Results



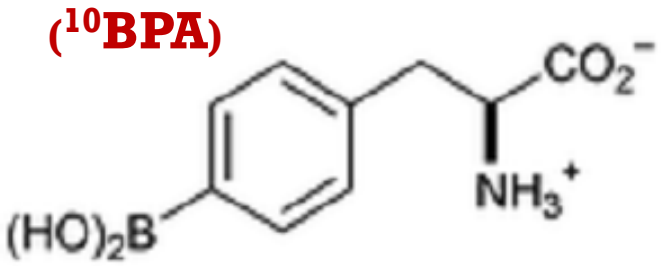
Starting from 40 ppm, BPA shows a time and concentration dependent cytotoxic effect on DHD cells that reaches 20% in case of 4 h treatment and 30% in case of 18 h treatment at the highest concentration.

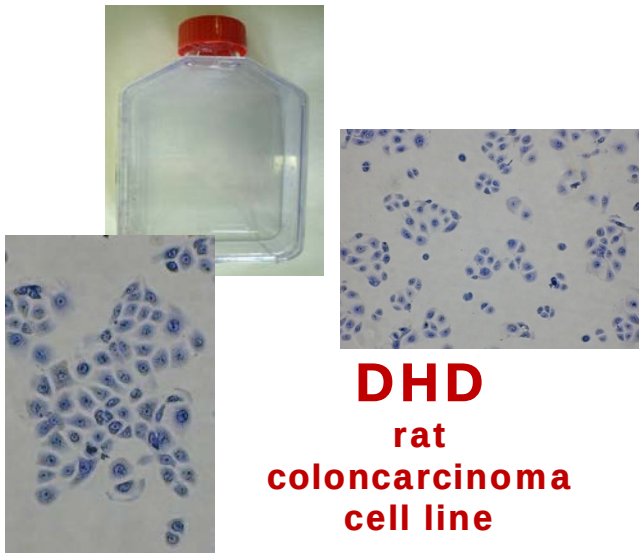
3. Effectiveness of irradiation

Methods

D,L- ^{10}boronophenylalanine

(^{10}BPA)





DHD

rat coloncarcinoma cell line

Cells preincubated for 4h with 80 ppm ^{10}B enriched medium and untreated cells are harvested, centrifuged and mantained in boron-free medium at 4 °C

Cells are submitted to neutron irradiation at the concentration of $5x10^6$/ml within one hour, replacing medium when transferred into the irradiation tubes

Irradiation of boron enriched and control cells is performed in the thermal column of the TRIGA Mark II reactor (University of Pavia)

After neutron exposure cells are diluted in B-free medium for subsequent clonogenic assay. Non irradiated boron enriched and boron lacking cells are treated as the irradiated samples;

B^-R^- B^+R^-

B^-R^+ B^+R^+

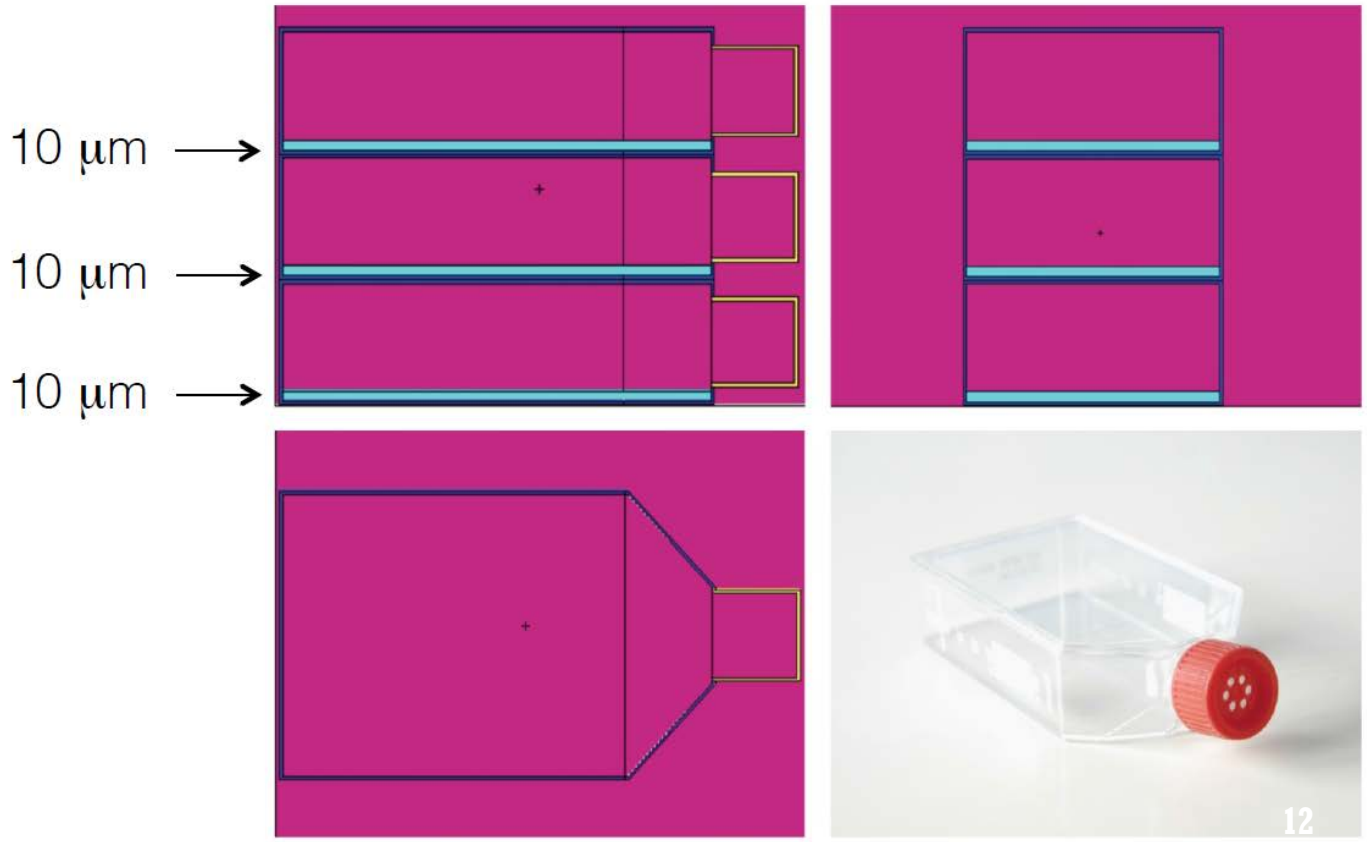
Boron intracellular concentration is measured FOR EACH experiment.

Dose is escalated by increasing reactor power, at a fixed irradiation time of 10 minutes.

Neutron flux and photon dose is well characterized in the irradiation position, by previous experimental measurements and by MCNP calculations.

Flasks have been simulated in the irradiation position.

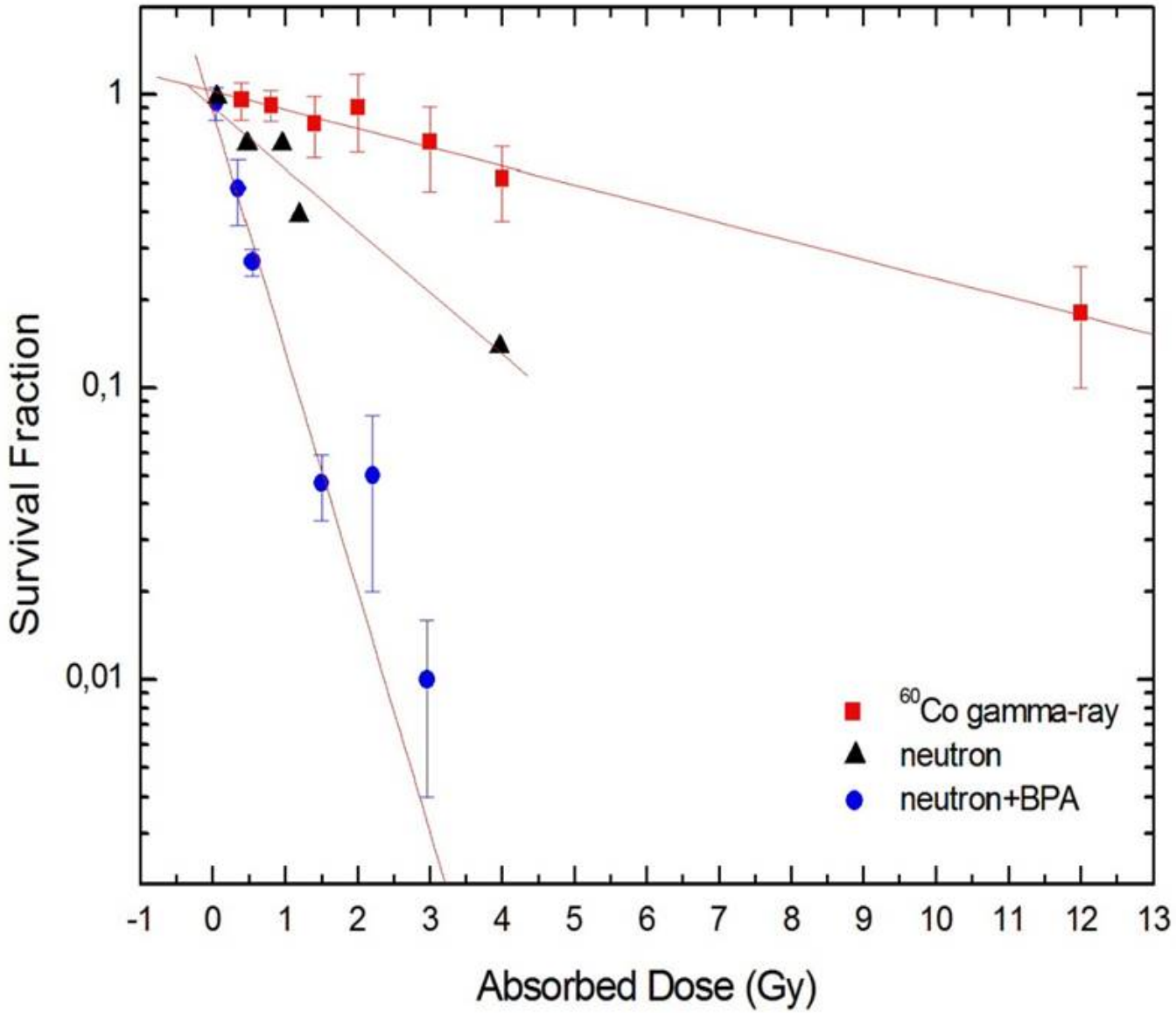
Dose calculations



Production rate was calculated in cells, then transport

• $^{10}B(n,\alpha)^{7}Li$	selective contribution
• $^{14}N(n,p)^{14}C$ • H(n, n')H • 2.2 MeV γ from $^{1}H(n,\gamma)^{2}H$ • γ from background	non-specific background

Results

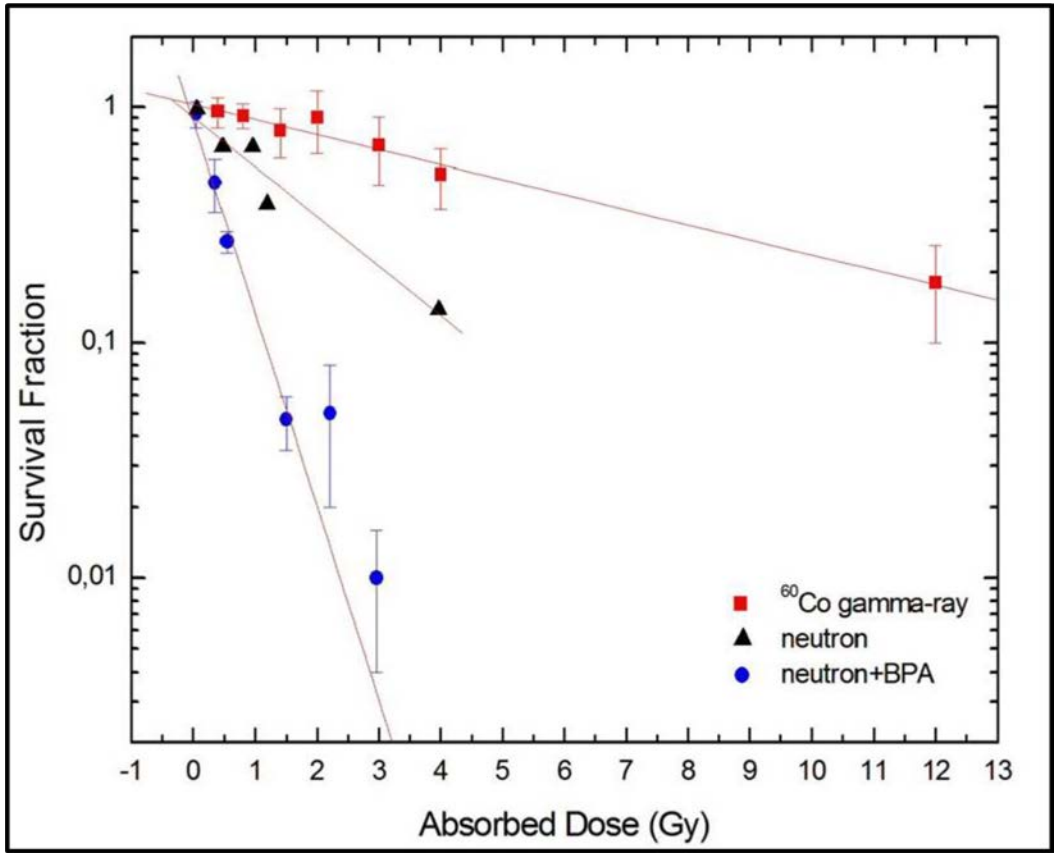


Survival curves of DHD cells:

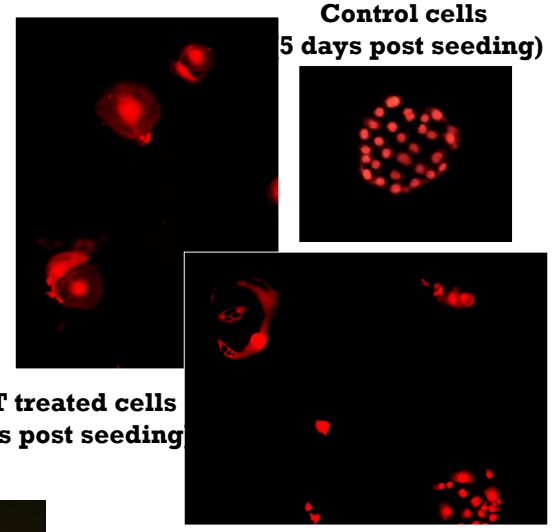
- Incubated for 4h in 80 ppm BPA enriched medium and exposed at different reactor power.
- Irradiated with neutrons only at different reactor power
- Irradiated with photons

Used to calculate RBE/CBE and parameters for isoeffective dose

Cell survival

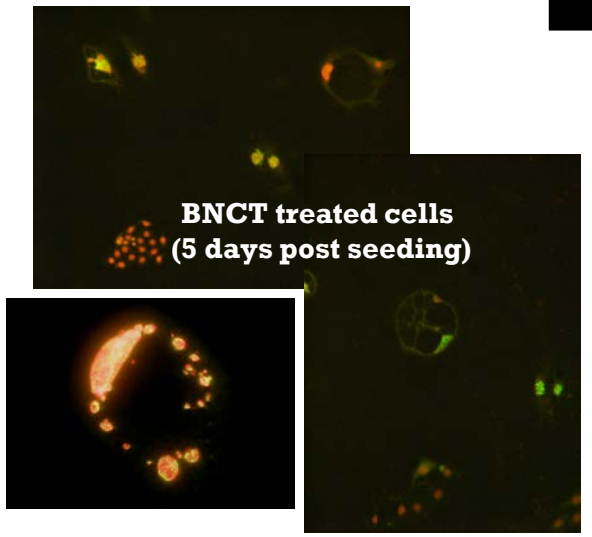


Nuclei of DHD cells stained with Propidium Iodide



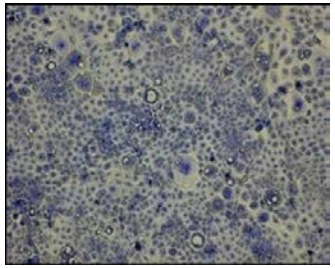
Control cells (5 days post seeding)

BNCT treated cells (5 days post seeding)

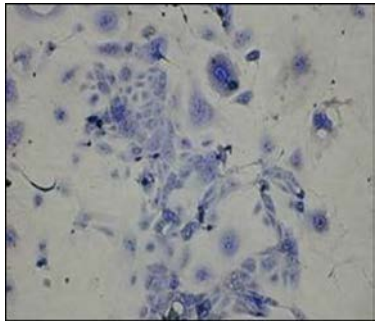


BNCT treated cells (5 days post seeding)

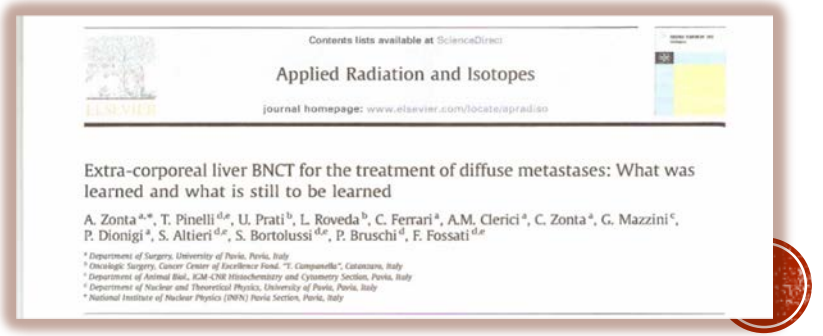
Cellular damages induced by BNCT: apoptotic and giant cells



Control cells (5 days post seeding)



BNCT treated cells (15 days post seeding)



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Extra-corporeal liver BNCT for the treatment of diffuse metastases: What was learned and what is still to be learned

A. Zonta[a,*], T. Pinelli[d,e], U. Prati[b], L. Roveda[b], C. Ferrari[a], A.M. Clerici[a], C. Zonta[a], G. Mazzini[c], P. Dionigi[a], S. Altieri[d,e], S. Bortolussi[d,e], P. Bruschi[d], F. Fossati[d,e]

[a] Department of Surgery, University of Pavia, Pavia, Italy
[b] Oncologic Surgery, Cancer Center of Excellence Fond. "T. Campanella", Catanzaro, Italy
[c] Department of Animal Biol., IGM-CNR Histochemistry and Cytometry Section, Pavia, Italy
[d] Department of Nuclear and Theoretical Physics, University of Pavia, Pavia, Italy
[e] National Institute of Nuclear Physics (INFN) Pavia Section, Pavia, Italy