



# CELL RADIOBIOLOGY FOR BNCT STUDIES

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

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#### Hepatic metastases from colon adenocarcinoma

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Lung metastases Mesothelioma

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#### RESEARCH

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

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

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# PRE-CLINICAL TESTS

- 1. Evaluation of boronated carrier toxicity
- 2. Intracellular <sup>10</sup>B <u>uptake</u> varying <u>concentration</u> and <u>time of incubation</u>
- 3. <u>Effectiveness</u> of the treatement

- 1. Evaluation of boronated carrier <u>toxicity</u>
- 2. Tissue biodistribution
- 3. <u>Effectiveness</u> of the treatement

in-vivo





# **IN-VITRO MODELS**



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





### 1. Intracellular <sup>10</sup>B uptake varying concentration and incubation time



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

BPA enriched medium at different <sup>10</sup>B concentrations is delived to cells.

At the end of the time of contact the <sup>10</sup>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 <sup>10</sup>B enriched medium, PBS, surnatant and cells are deep-frozen in liquid nitrogen for <sup>10</sup>B concentration analyses by inductively coupled plasma mass spectrometry (ICP-MS).

Cells are layered on mylar disks for neutron autoradiography













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



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

BPA enriched medium at <sup>10</sup>B concentrations from 10 to 160  $\mu$ g/ml is delived to cells for 4 h and 18 h.

At the end of the time of contact the <sup>10</sup>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**







# **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



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

Cells are submitted to neutron irradiation at the concentration of  $5 \times 10^6$ /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**





# 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





**BNCT** treated cells (15 days post seeding)



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Gracias!

