UNIVERSITÀ DEGLI STUDI FIRENZE

Tecniche di irradiazione sperimentali in vivo e in vitro

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Brescia, 8/10/2015

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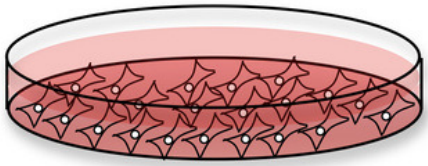
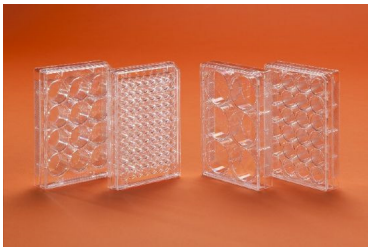
Irradiazioni sperimentali in vitro





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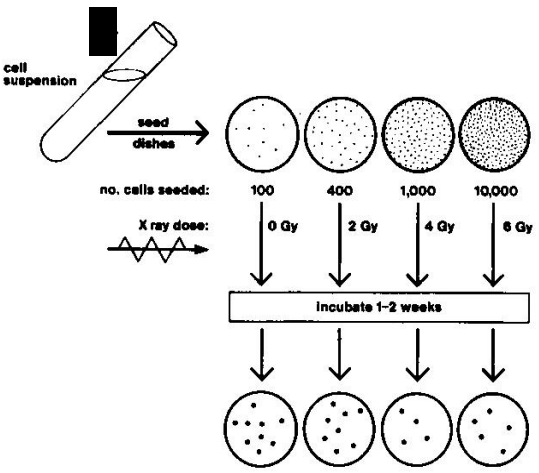
clonogenic survival assay

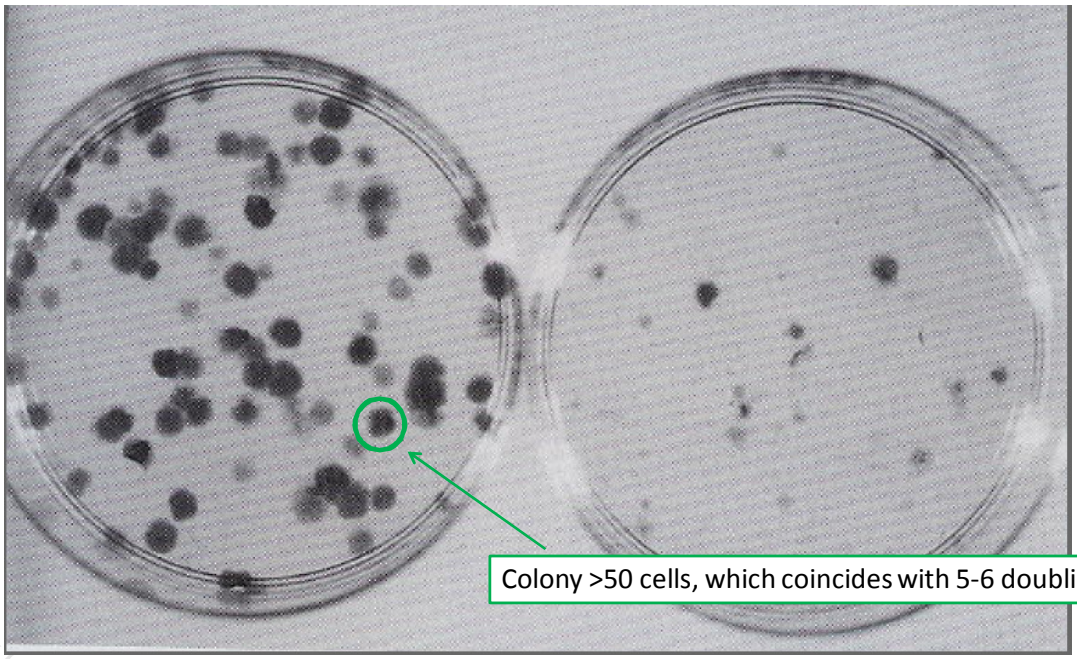
- Measures the proliferative (clonogenic) capacity of individual cells

- Single cell suspensions are seeded into tissue culture plates, allowed to attach and irradiated; colonies are then counted after 10-21 days, depending on the specific cell line

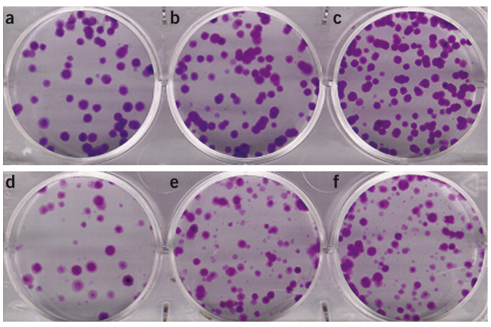
- The minimum colony size is typically set at 50 cells, which coincides with 5-6 doublings.



clonogenic survival assay

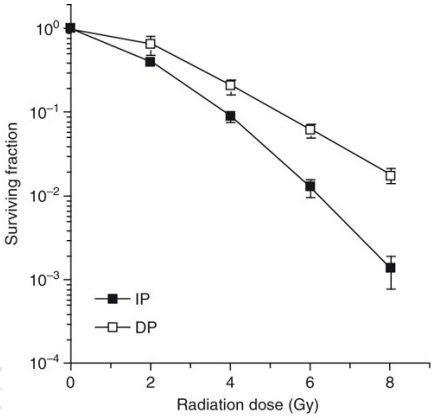


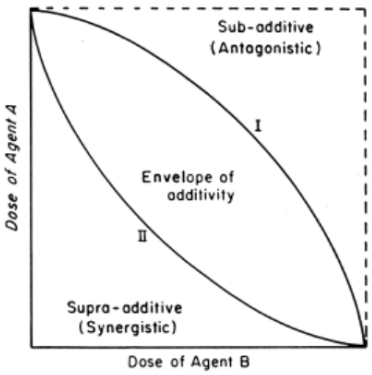
clonogenic survival assay



PE (plating efficiency)= number of colonies / number of cells seeded

SF (surviving fraction)= colonies counted / cells seeded x (PE/100)

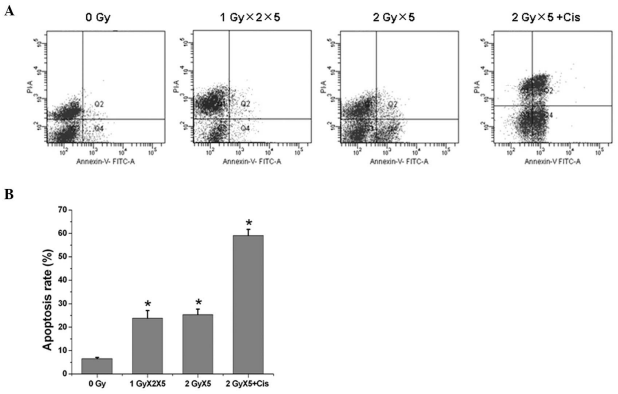




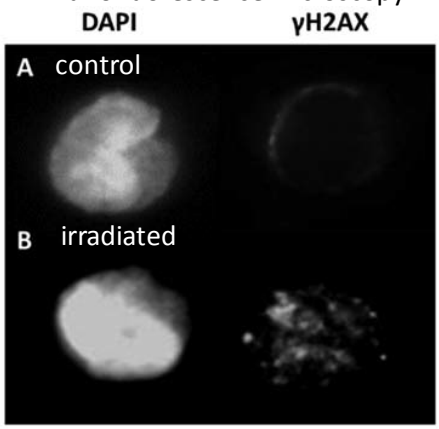
Isobologram

Non-clonogenic assays

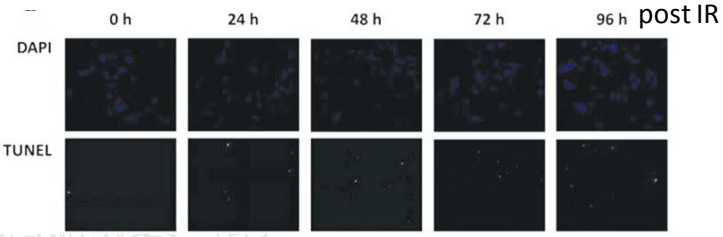
Apoptosis-flow cytometry



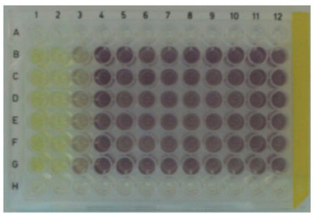
Immunofluorescence microscopy



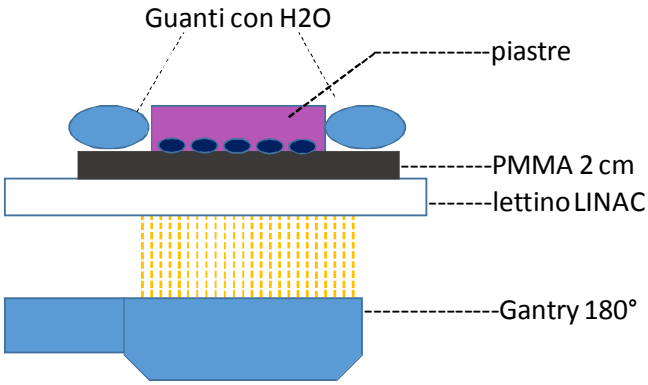
Test TUNEL



MTT-tetrazolium based colorimetric assay



Modello di irradiazione con LINAC di cell in coltura



- Customized phantom was made for cellular radiation using Perspex
- The plate was placed in a Perspex slab with an inbuilt hole of the size of the plate
- Perspex sheets of 10 cm thickness were kept below the plate to generate maximum backscatter
- A build-up of 1.2 cm perspex (equivalent thickness in water for 1.5 cm) was used for 15 MV photon and build up was not used for 6 MV photons as dmax was less than or equal to 1.5cm
- The treatment time calculation was done for SSD technique for a field size of 20 x20 cm2 at dmax
- Calculation was done using available treatment planning system (TPS).

Saikat Das,
Journal of Clinical and Diagnostic Research. 2015 Jun, Vol-9(6): XC05-XC08

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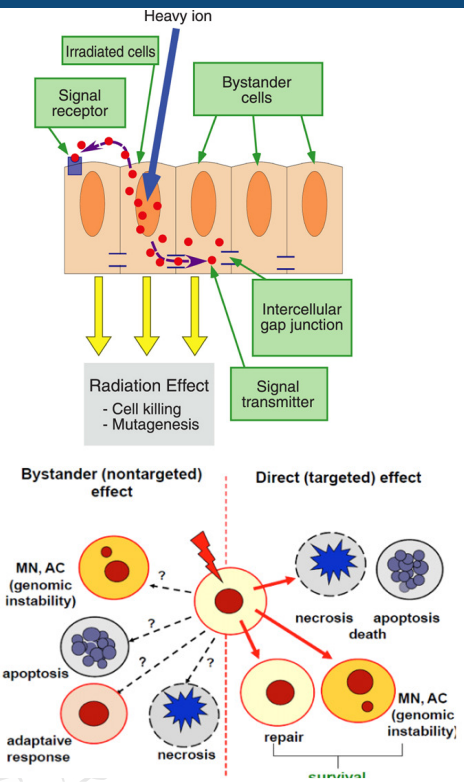
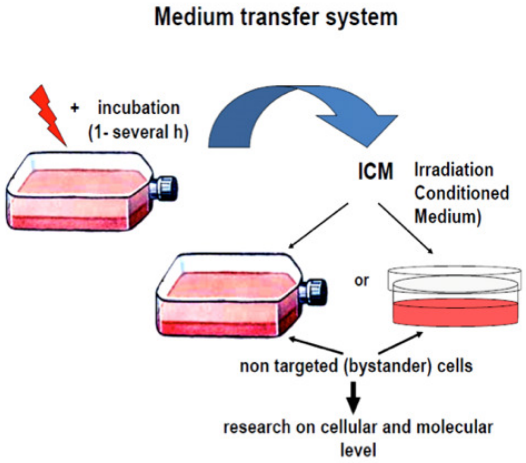
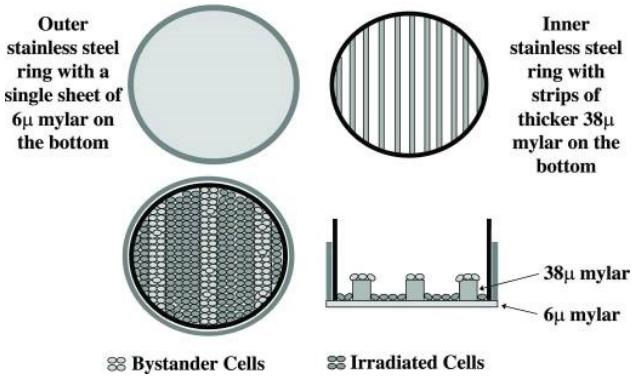
Bystander effect





Bystander effect

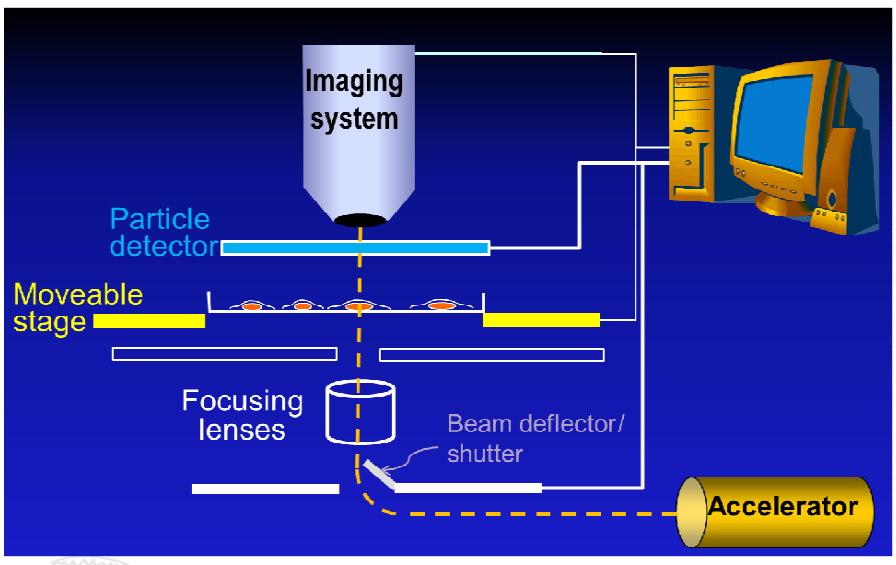
A 50-cGy dose of ^{4}He ions (120 keV/µm) was delivered by using the 4-MV van de Graff accelerator at the Radiological Research Accelerator Facilities of Columbia University



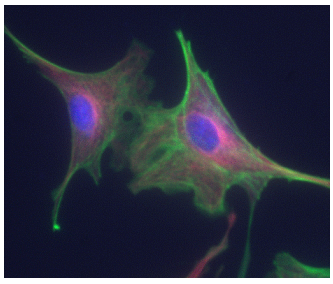
A schematic diagram of the stripped dish used in the present study. A6-µm-thick mylar sheet was epoxied to the outer stainless ring to provide a bottom for the dish. The mylar of the inner rings (38 µm thick) was cut as strips. Exponentially growing NHLF cells were plated into the concentric rings 2 days before irradiation at a density to ensure a confluent state.

Zhou H,
Proc Natl Acad Sci U S A. 2005 Oct 11;102(41):14641-6.

Bystander effect- microbeams

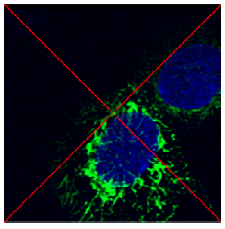


Fluorescent Imaging



Cells with multiple parts labeled with separate fluorophores. DNA (blue), mitochondria (red) and Actin fibers (green)

Multi-Photon Imaging



Multiphoton microscope image acquired during an irradiation experiment using mitochondria sites (green) as targets. The cross-hairs (red) mark the center of the image, which coincides with the position of the ion beam.

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Irradiazioni sperimentali in vivo

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Tumour models

- Several types of immune deficient mice:
  - athymic nude mice
  - mice with severe combined immune deficiencies
- **Heterotopic vs. orthotopic transplantation**

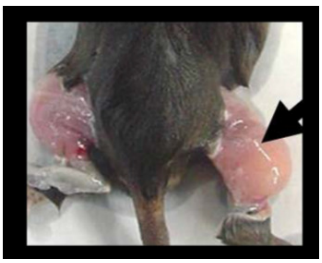
Heterotopic:

-Preferentially on the hind leg

-The transplantation site allows for standardised irradiation and repeatability of the experimental results + easy tumour growth measurements with callipers without additional imaging

-Therapeutically relevant doses (i.e. potentially curative) can be applied without irradiating dose-limiting normal tissue

-Local tumour control as an endpoint



Orthotopic:

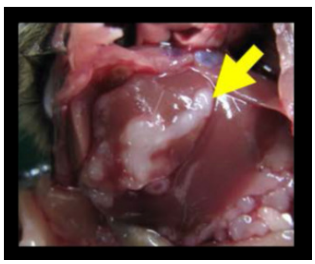
- Account for the potential influence of tumor specific microenvironments

-Experiments are much more complex

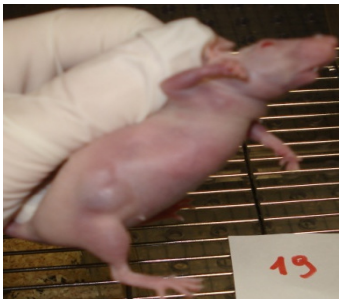
-Modern imaging techniques are required for monitoring tumour growth or normal tissue alterations

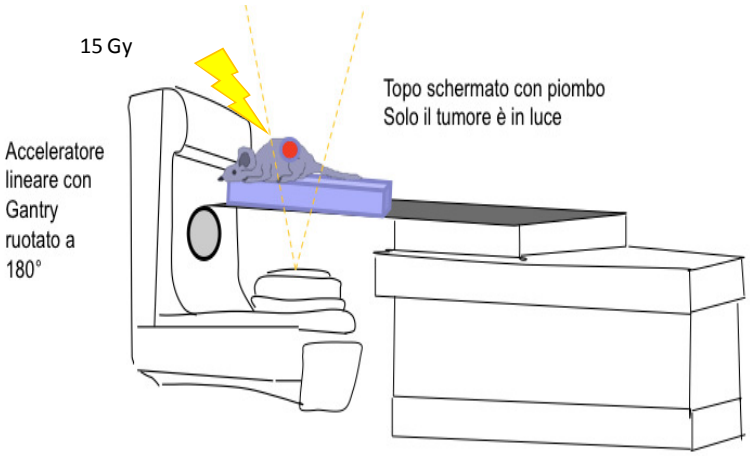
-Irradiation includes exact pretherapeutic image-based tumour localisation and reproducible positioning of the animal

-+++radiation-induced toxicity to at risk organs



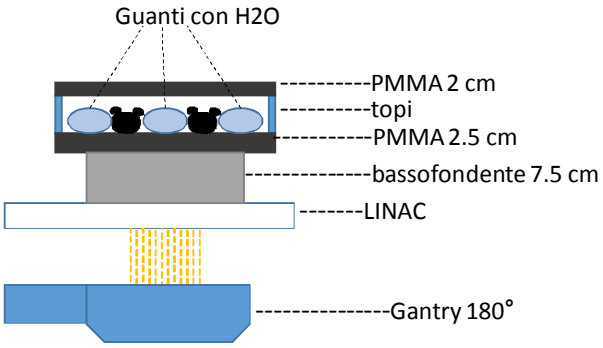
Heterotopic tumour xenograft irradiation

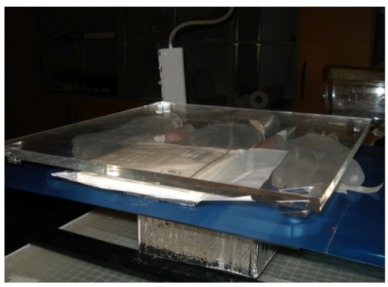




2 Gy/5 fr
3,5 Gy/5 fr
4,5 Gy/5 fr
6 Gy/3 fr
5 Gy/5 fr

Heterotopic tumour xenograft irradiation

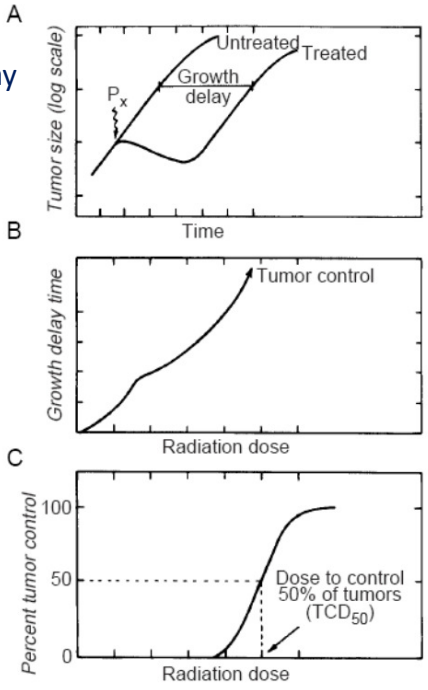


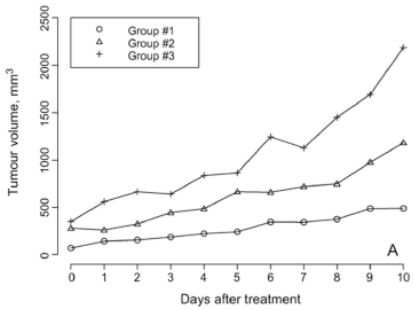


Heterotopic tumour xenograft irradiation

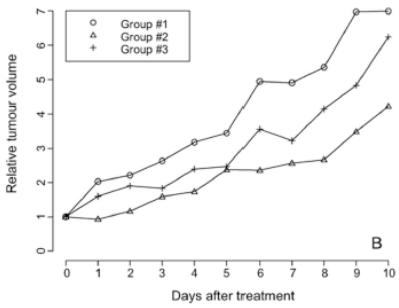
Tumor Volume= D (mm) x d ^{2}(mm^2) /2

Tumor growth delay (TGD) assay





Tumor volume

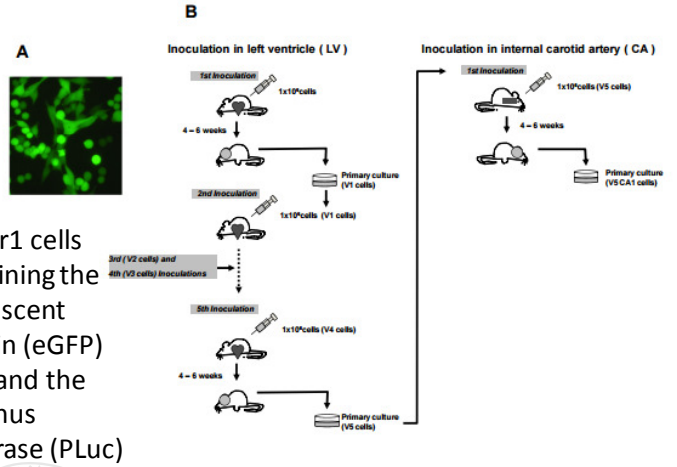


Relative tumor volume

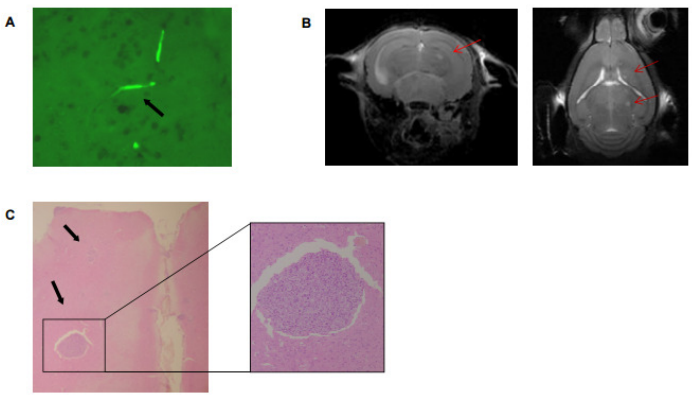
Orthotopic tumour xenograft irradiation

Antonio Martínez-Aranda. Int. J. Mol. Sci. 2013, 14, 8306-8327

Experimental brain metastasis study

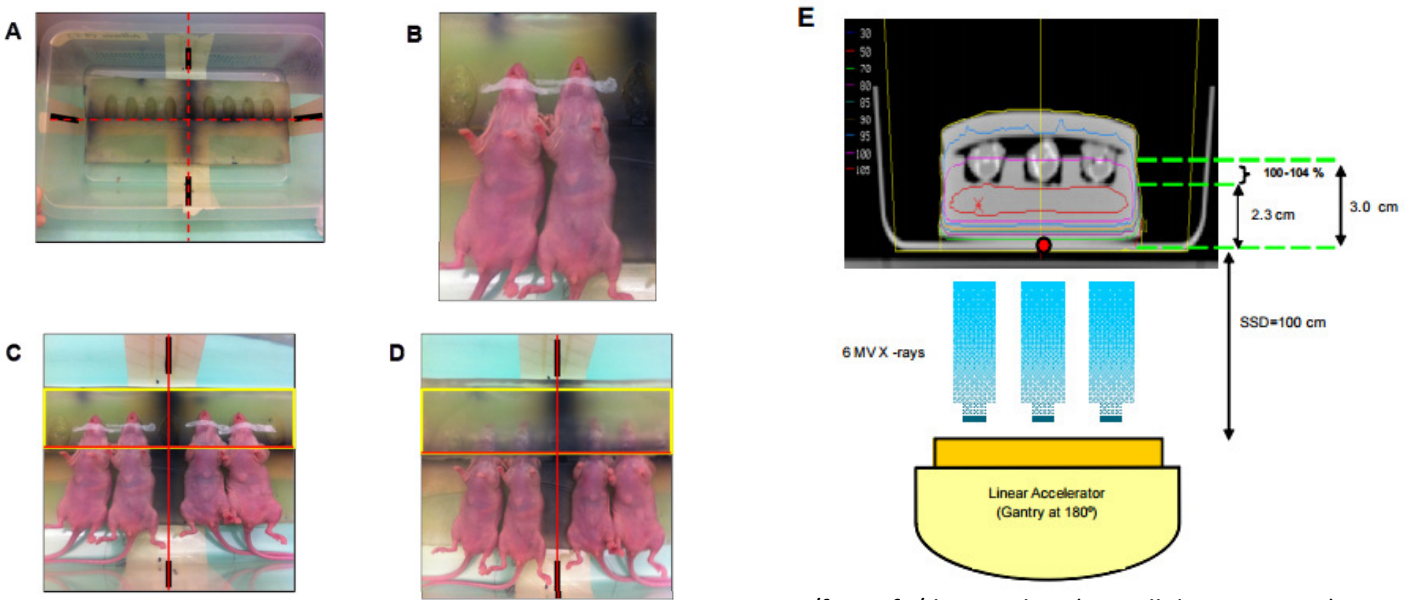


435-Br1 cells containing the fluorescent protein (eGFP) gene and the photinus luciferase (PLuc) gene



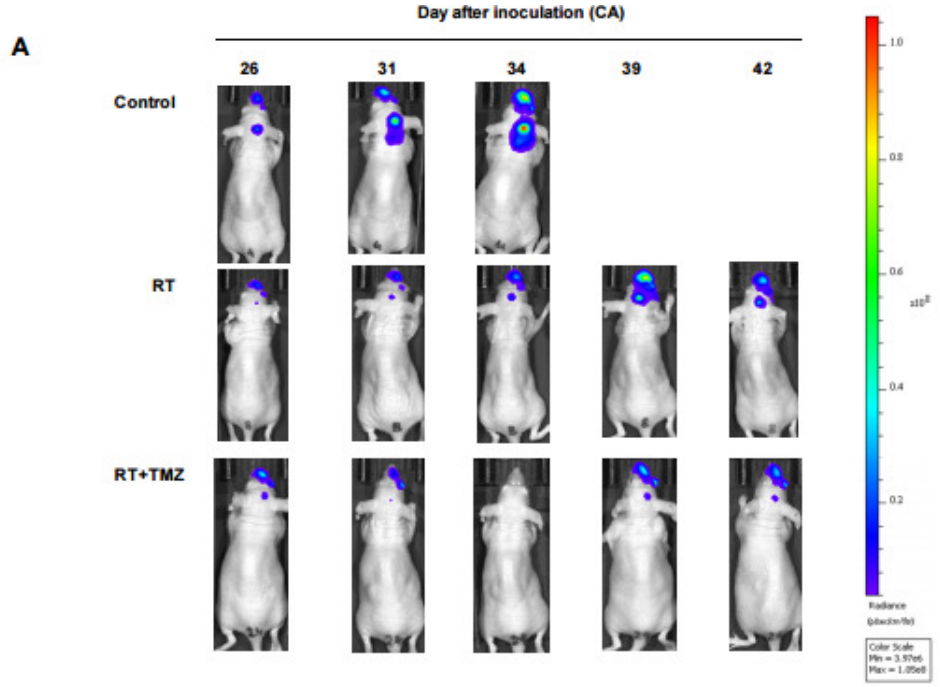
29 days post-injection of BR-eGFP-CMV/Luc (V5CA1) cells

Orthotopic tumour xenograft irradiation



5.5 Gy/fx × 1 fx/day × 3 days (overall dose: 16.5 Gy)

Antonio Martínez-Aranda. Int. J. Mol. Sci. 2013, 14, 8306-8327



Radiation-induced toxicity

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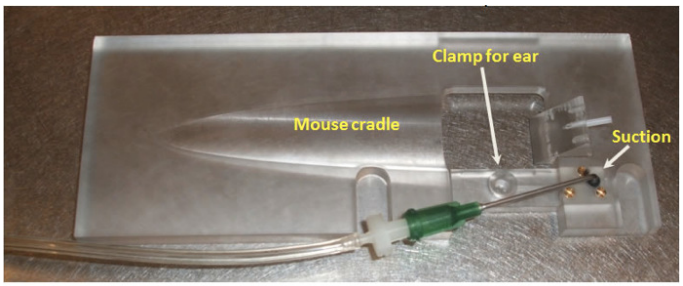
Mouse Ear Irradiation Model

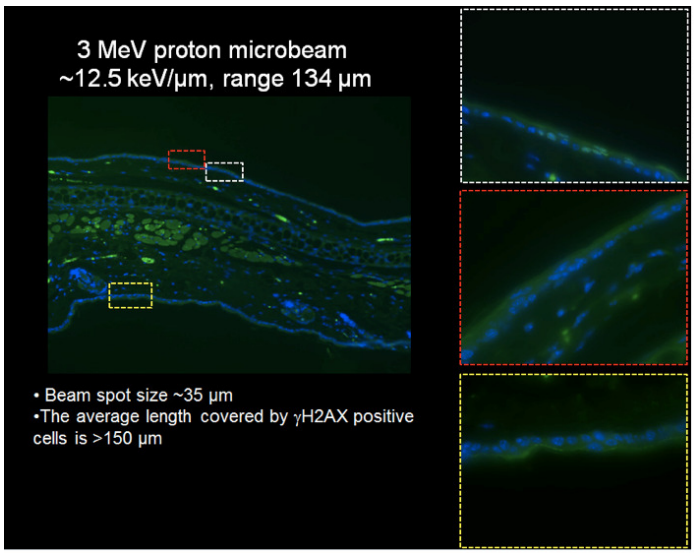
Buonanno M. Radiat Res. 2015 Aug;184(2):219-25



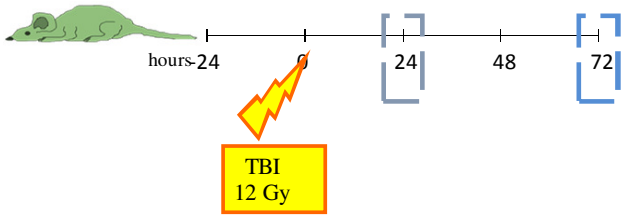
gammaH2AX foci formation

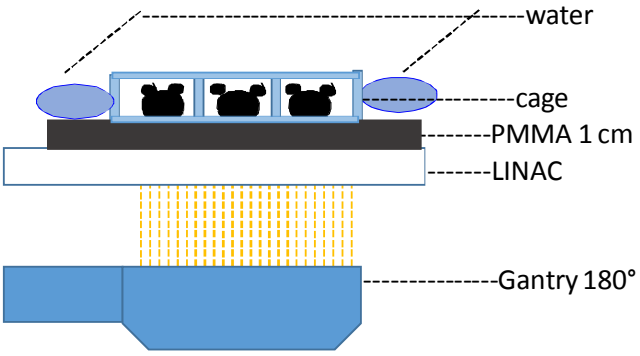
bystander studies in vivo

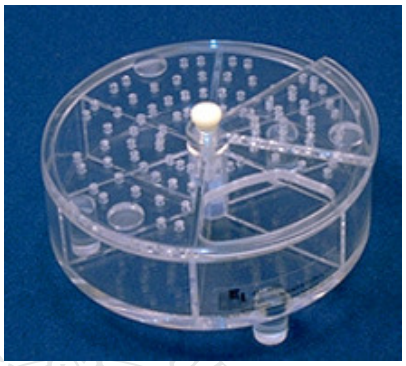
cells along a 6 mm line irradiated with 3-MeV proton microbeam (range of 134 µm) with a diameter of ~ 35 µm

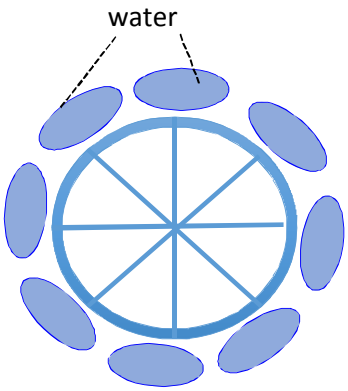


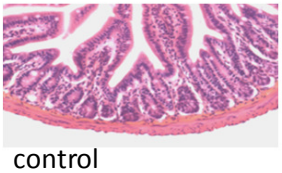
Acute intestinal damage

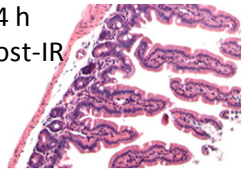


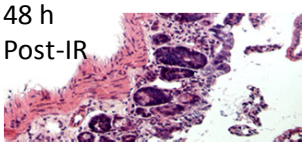






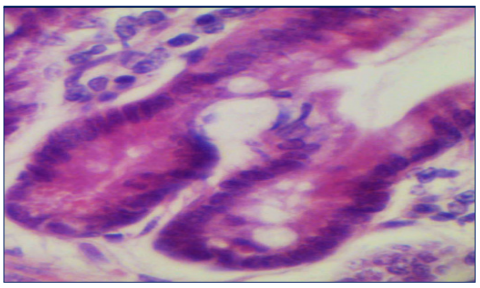

control



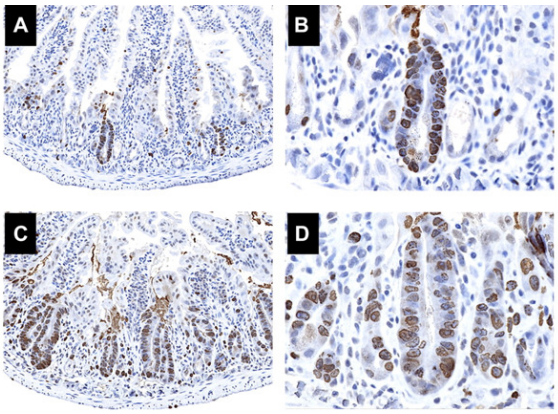


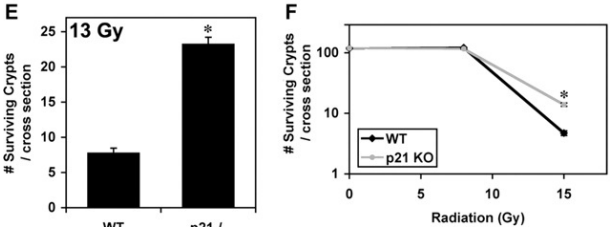
Microcolony assay

Withers and Elkind, 1970



Regenerating crypt > than 10 viable cells



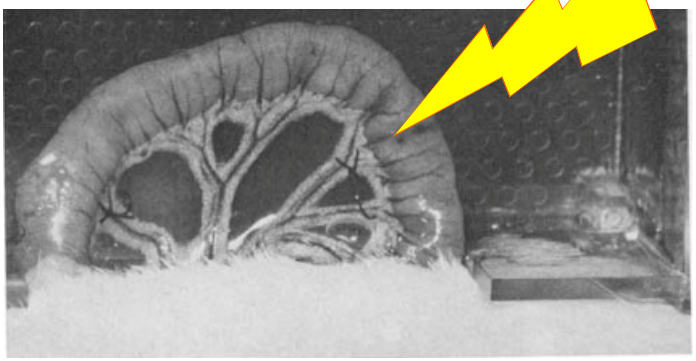


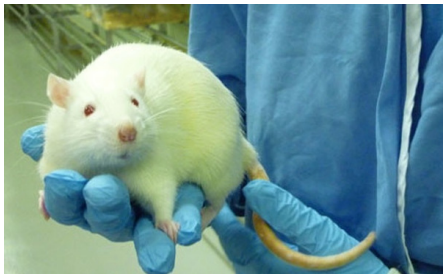
A surviving crypt was defined as containing five or more adjacent, BrdU-positive nuclei (3,5 days after IR)

delayed intestinal radiation injury

Ileum irradiation

19 Gy to 6 cm area

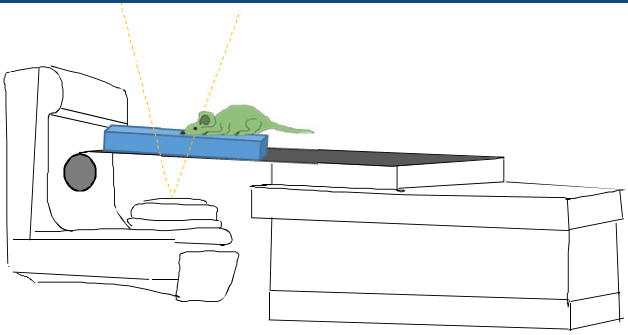




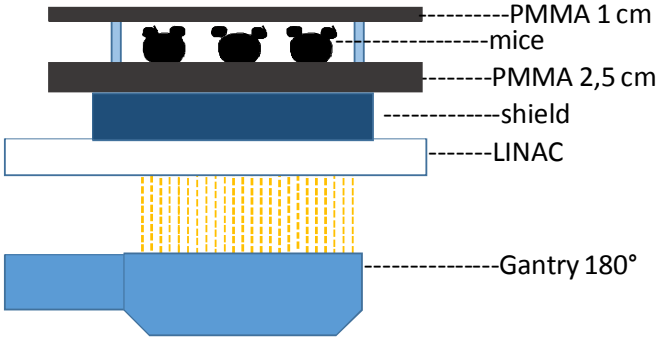
J. O. Forsberg, B. Jung & B. Larsson (1978) : Acta Radiologica: Oncology, Radiation, Physics, Biology, 17:6, 485-496

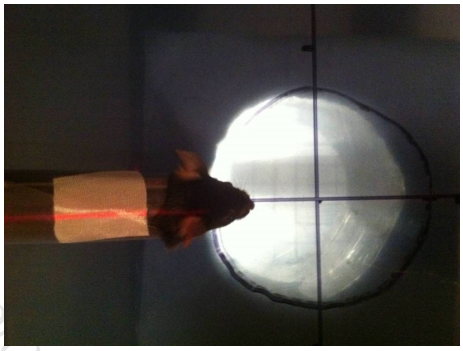
Haydont V, Int J Radiat Oncol Biol Phys. 2007 Aug 1; 68(5):1471-82.

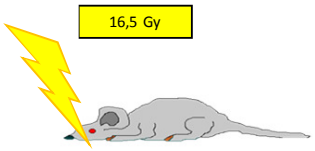
Oral mucositis



Acceleratore lineare con Gantry ruotato a 180°

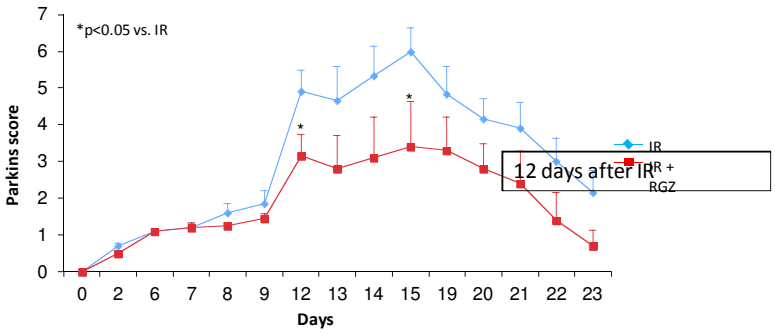


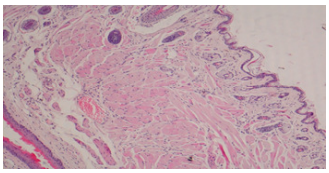


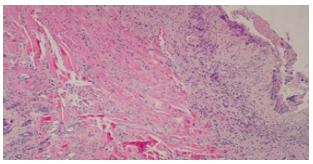


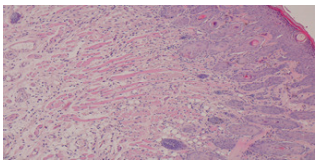
Oedema score	
0,5	50-50 doubtful if any swelling
1	Slight but definite swelling
2	Severe swelling
Erythema scores	
0,5	50-50 doubtful if abnormally pink
1	Slight but definite reddening
2	Severe reddening
3	Focal desquamation
4	Exudate or crusting involving about ½ lip area
5	Exudate or crusting involving more than ½ lip area

(Parkins, Radiother Oncol 1983)




control


12 days after IR


23 days after IR

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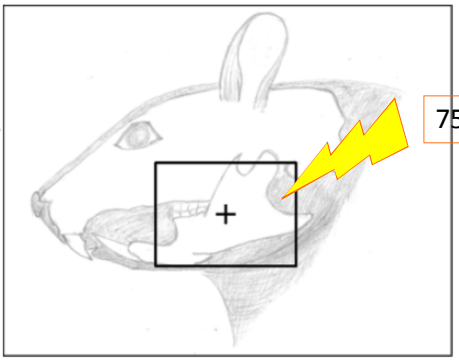
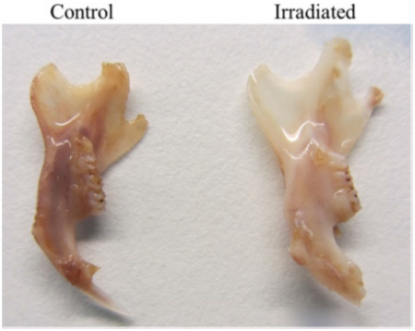
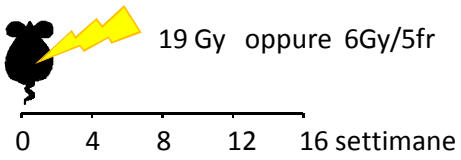
A rat model of radiation injury in the mandibular area
Tonje Sønstevold1, Anne Christine Johannes and Linda Stuhr1
Radiation Oncology (2015) 10:129

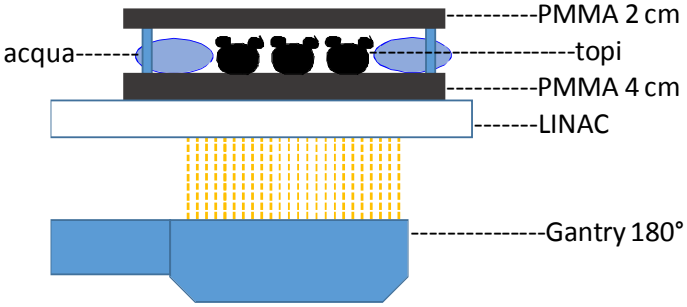


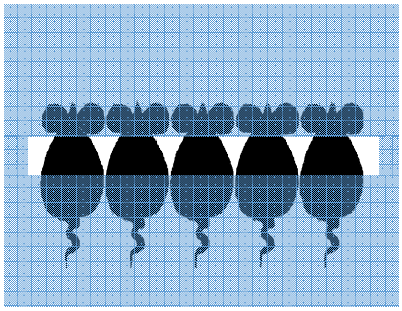
isocenter: the mandibular body between the angle process and the molars
radiation field of 2.6 × 3.5 cm , SSD 75 cm
10 mm bolus was applied above the mandible
lead blocks positioned at the edge of the radiation field



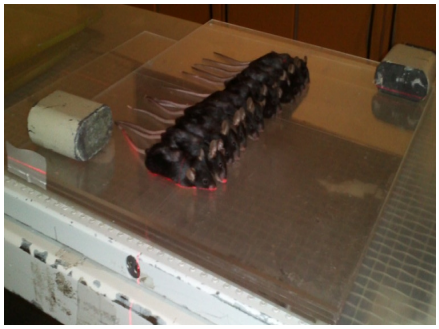
Lung toxicity







Polmoni irradiati conformando il fascio al solo volume d'interesse



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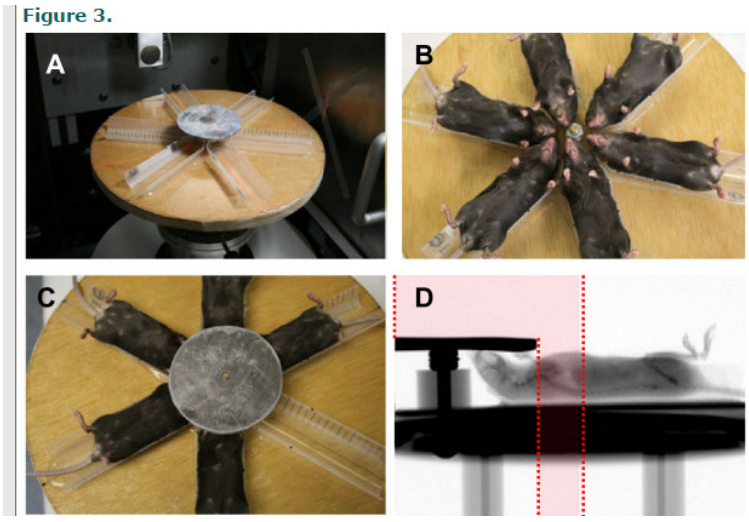
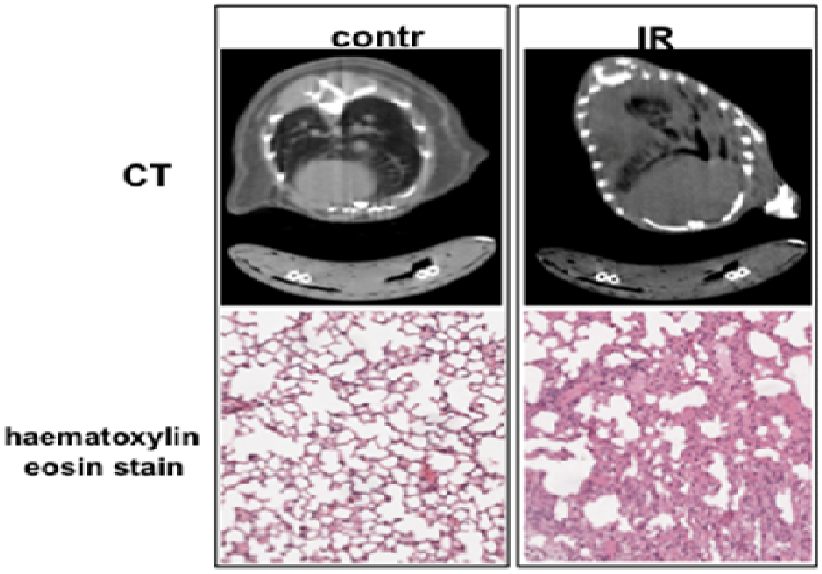


13,5 Gy

Pietrofesa R, BMC 2013, 13: 179



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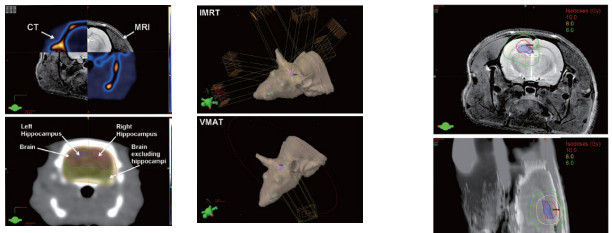
Linear accelerator

- The majority of investigations on small animal radiation research uses linear accelerators
- Different positioning procedures are described to place animals on the treatment table
- In-house built animal jigs are presented to similarly position several mice or rats for simultaneous irradiation
- The jigs are mostly made from acryl to minimise the influence on the radiation field and to permit proper disinfection
- Some of this equipment is also commercially available (e.g. from Braintree Scientific, Inc., Braintree, MA, USA)
- Bolus material or acrylic plates may be used to assure proper dose build up or to shift the depth dose curve of an electron beam
- Sufficient radiation shielding is obtained by lead blocks integrated into the animal jigs [55] or by beam colli- mation
- No image guidance is usually applied for superficial targets; for internal targets the animal positions may be verified
- The field shape is simple and not conformal (e.g. rectangular, circular or trapezoidal)

translation of stereotactic methods to small animal radiation research

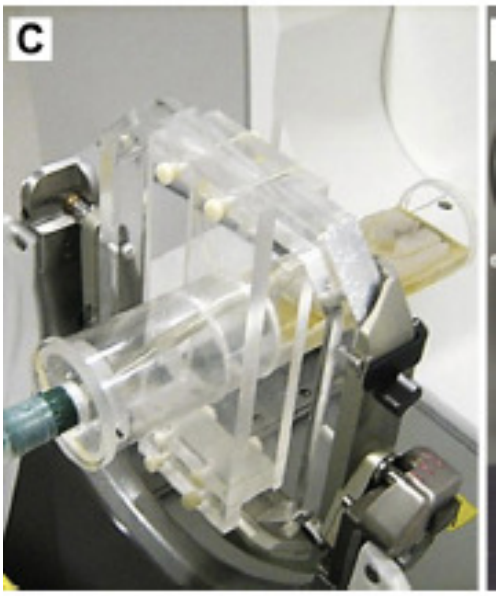
LINAC



IMRT and VMAT to irradiate one hippocampus or both hippocampi, respectively
total doses of 10 Gy to one or both hemispheres of the rat brain.

Vipan K. Parihar , Translational Cancer Res, Vol 3, No 2 (April 2014)

GAMMA- KNIFE



TOMOTHERAPY
+++ rats

CYBERKNIFE
Few studies

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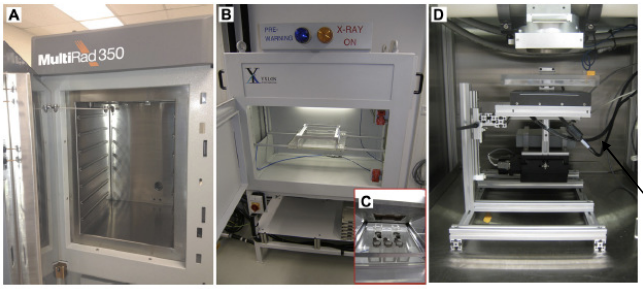
Table 2
Typical properties of research devices used for small animal irradiation.

Research device	Beam quality [MeV] [a]	Dose rate [Gy/min] [b]	Irradiation technique	Collimator	Image guidance	Targeting accuracy
X-ray cabinet systems	X-rays: 0.01–0.35	1–5	fixed beam (vertical)	—	depends on device: radiography	< 1 mm–few mm
^{192}Ir MicroRT [115,116]	γ (^{192}Ir): 0.35	1–3 (300 GBq)	fixed beam (orthogonal)	collimator assembly with conical inserts	—	< 0.2 mm
SARRP Research Platform [117–121]	X-rays: 0.005–0.225	1–4	fixed beam, conformal arc	nozzle	radiography, CBCT,	0.2 mm
X-RAD 225Cx [6,122–125]	X-rays: 0.005–0.225	1–4	fixed beam, conformal arc	nozzle	radiography, CBCT	0.2 mm
MicroCT/RT system [126–128]	X-rays: 0.07–0.12	1–2	fixed beam, conformal arc	variable iris aperture	CBCT	0.1 mm
SAIGRT system [129–131]	X-rays: 0.01–0.225	1–4	fixed beam, conformal arc	flat aperture	radiography, CBCT	0.1 mm

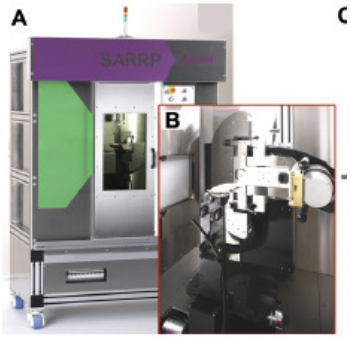
[a] maximum energy for X-rays, mean γ and X-ray photon energy for nuclides [49].
[b] at a typical dose reference point.

F. Tillner et al. / Z. Med. Phys. 24 (2014) 335–351

X-ray cabinet systems



Imaged-guided stereotactic small animals irradiator



-computer-controlled 360◦ isocentric gantry carrying an X-ray tube
-3D robotic stage carries a 360◦ turn table stage enabling CBCT imaging and non-coplanar irradiation
-3D TPS developed

SARRP Research Platform

192Ir MicroRT

MicroCT/RT system

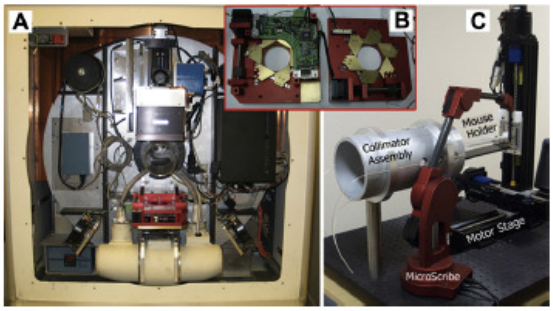


Figure 3. Usage of specially designed small animal irradiators (I): A–B: MicroCT/RT system [126–128] (A: MicroCT gantry; B: disassembled variable aperture collimator). Image courtesy of Edward Graves; C: ^{192}Ir MicroRT system [115,116]. Reproduced with permission.