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Sensitization of chondrosarcoma cells with PARP inhibitor and high-LET radiation

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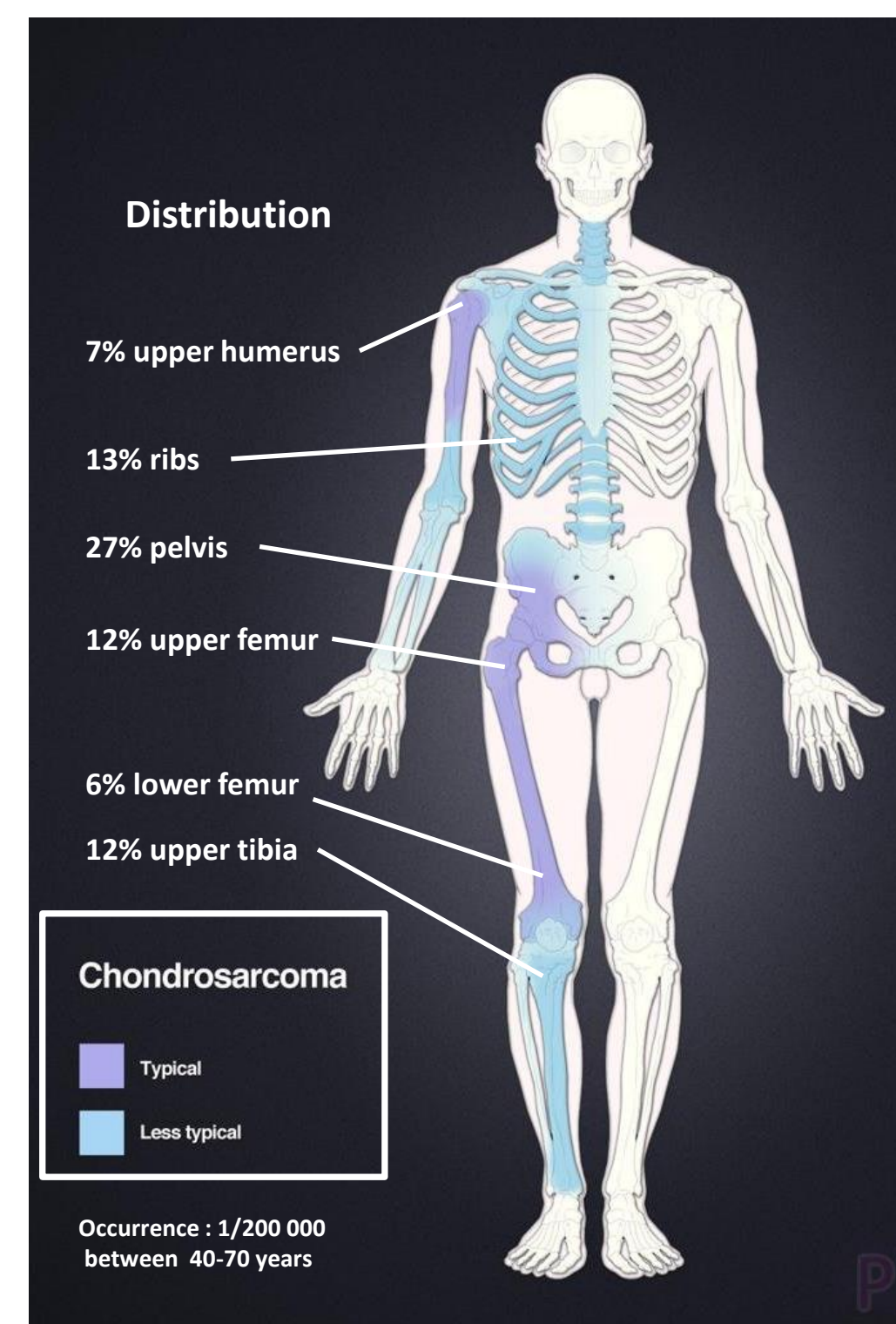
Chondrosarcoma is a malignant tumor that arises from cartilaginous tissue and is radioresistant and chemoresistant to conventional treatments. The preferred treatment consists of surgical resection, which might cause severe disabilities for the patient; in addition, this procedure might be impossible for inoperable locations, such as the skull base. Carbon ion irradiation (hadron therapy) has been proposed as an alternative treatment, primarily due to its greater biological effectiveness and improved ballistic properties compared with conventional radiotherapy with X-rays.

The goal of this study was to characterize the genetic mutations of a grade III chondrosarcoma cell line (CH2879) and examine the cellular responses to conventional radiotherapy (X-rays) and hadron therapy (proton and carbon ions) in the presence of the PARP inhibitor Olaparib.

To better understand PARP inhibition, we analyzed the formation of poly-ADP ribose chains by western blot; we observed an increase in its signal after irradiation, which disappeared on addition of the PARP inhibitor.

PARPi enhanced ratio of approximately 1.3, 1.8, and 1.5 following irradiation of cells with X-rays, protons, and C-ions, respectively, as detected by clonogenic assay. The decrease in cell survival was confirmed by proliferation assay. The radiosensitivity of CH2879 cells was associated with mutations in homologous recombination repair genes, such as RAD50, SMARCA2 and NBN.

This study demonstrates the capacity of the PARP inhibitor Olaparib to radiosensitize mutated chondrosarcoma cells to conventional photon irradiation and proton and carbon ion irradiation.



Irradiations / treatments

The human chondrosarcoma cell line CH2879 (GIII) was irradiated with :

- X-Rays (X-Rad 225 Cx)
 - Proton (INFN Catania)
 - Carbon (GANIL – NIRS)
- with / without PARP inhibitor : Olaparib

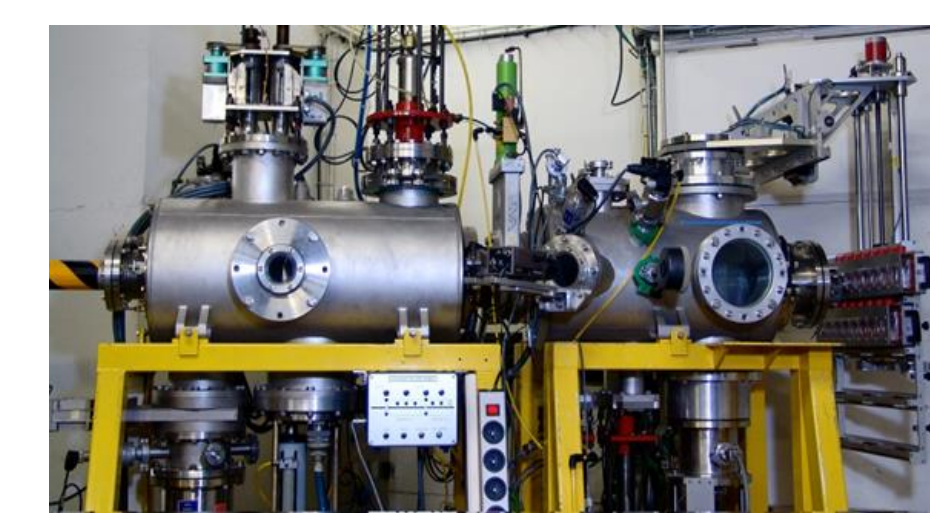
Aim of this study

- explore the differential cellular responses of chondrosarcomas to conventional radiotherapy and hadrontherapy
- understand the biological effects of carbon beams in cancer treatment
- estimate the capacity of PARP inhibitors in radiosensitizing chondrosarcoma cells

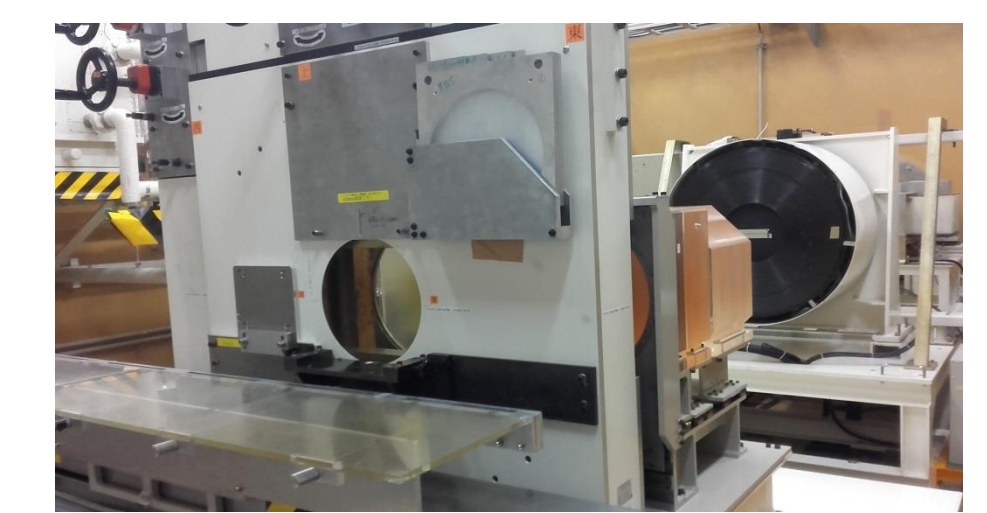
Irradiation facilities with accelerated ions



INFN, Catania, Italy
 Protons / Carbon ions



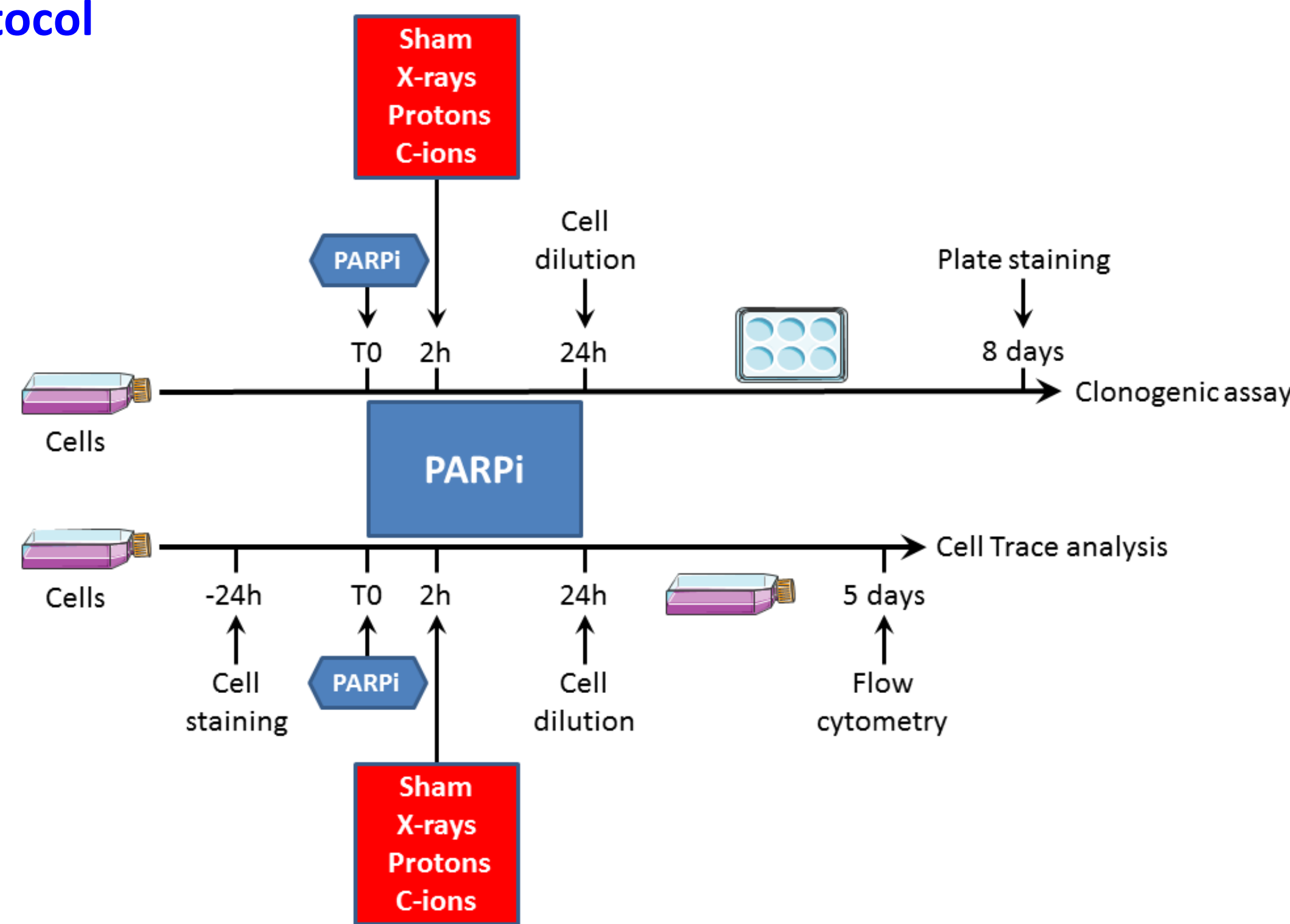
GANIL, Caen, France
 Carbon ions



NIRS, Chiba, Japan
 Carbon ions

from Césaire et al. Journal of Bone Oncology, in press - chevalier@ganil.fr

Protocol



Mutation profiles of chondrosarcoma cell lines

Gene	transcript	Description ¹	Expected consequence ²	Mutated allele frequency	Interpretation	detectable by ³
NBN	NM_002485.4	c.2249T>A	p.Leu750*	31%	probably inactive protein with premature termination codon (Nonsense mediated decay may be not active because the variant is located at the very end of the coding sequence)	HC; Lofreq; Outlyser
PTEN	NM_000314.6	c.697C>T	p.Arg233*	100%	inactive protein due to the introduction of premature termination codon	HC; Lofreq; Outlyser
RAD50	NM_005732.3	c.2165del	p.Lys722Argfs*14	54%	inactive protein due to the introduction of premature termination codon	HC; Lofreq; Outlyser
SMARCA2	N.A.	Loss of locus		100%	Loss of the entire tumor suppressor gene (probable Loss of heterozygoty)	OncoCNV

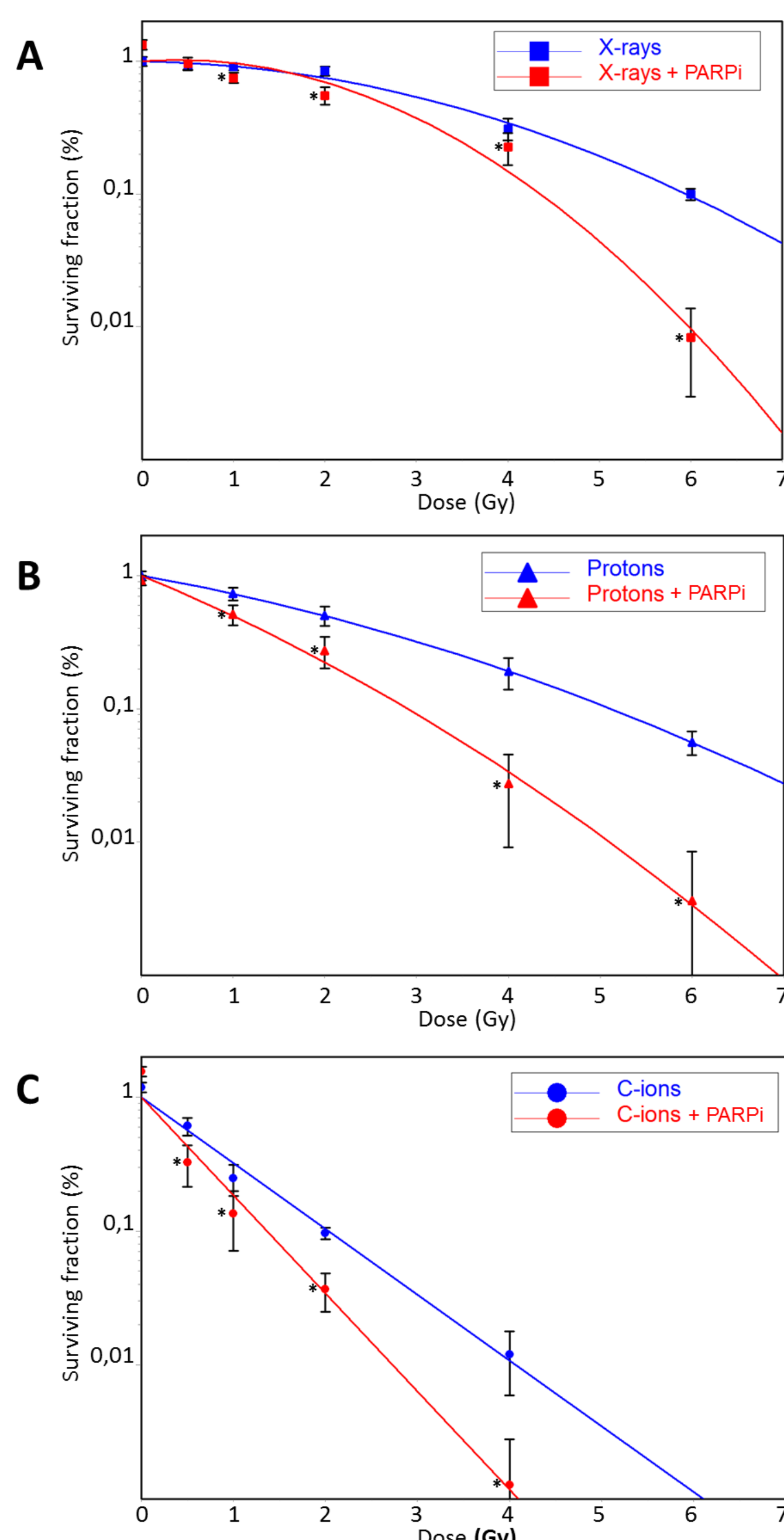
¹Mutation nomenclature per HGVS recommendations; nucleotide position +1 corresponds to the A of the ATG translation initiation codon. ²Expected consequence on protein level. ³Algorithms used for prediction: SIFT, PolyPhen, MutationTaster

A panel of genes (69), implicated in DNA repair, have been sequenced. An illumina NextSeq, 2x75pb paired-end technic was used. Bioinformatics analysis was performed HaplotypeCaller (Broad Institute).

Of the 69 genes that were analyzed, 12 were mutated by at least 2 callers: by substitution or deletion, creating nonsense, missense, or frameshift mutation or possible splice (ARID1A; ARID1B, BRIP1, FANCA, FANCG, FANCI, GATA3, HDAC3, NBN, PARP1, PTEN, and RAD50) and loss of sequence (SMARCA2). Of the 12 genes with a single mutation, 3 had a frequency of up to 10% (Table 1) : PTEN was mutated (c.697C>T; nonsense) in 100% of alleles, RAD50 was mutated (c.2165del; frameshift) at 44%, NBN was mutated (c.2249T>A; nonsense) at 31% and SMARCA2 locus was lost in 100% of alleles.

Clonogenic cell survival assays

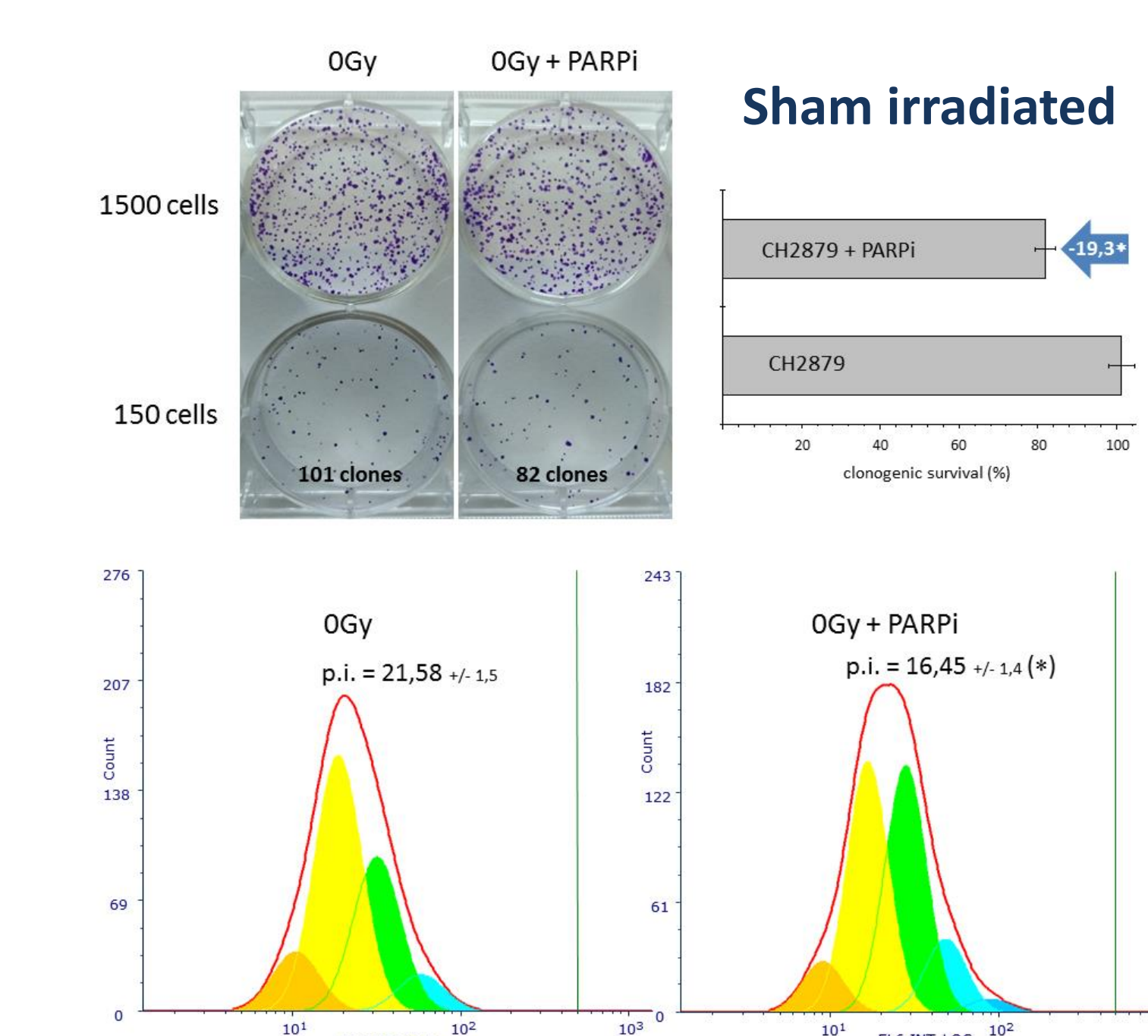
Cells were irradiated at confluency with X-Rays and ion beams at the corresponding physical doses. Following a 18h incubation time, cells were seeded at low density. Colonies were stained after 10 to 12 days and clones (>50 cells) were counted.



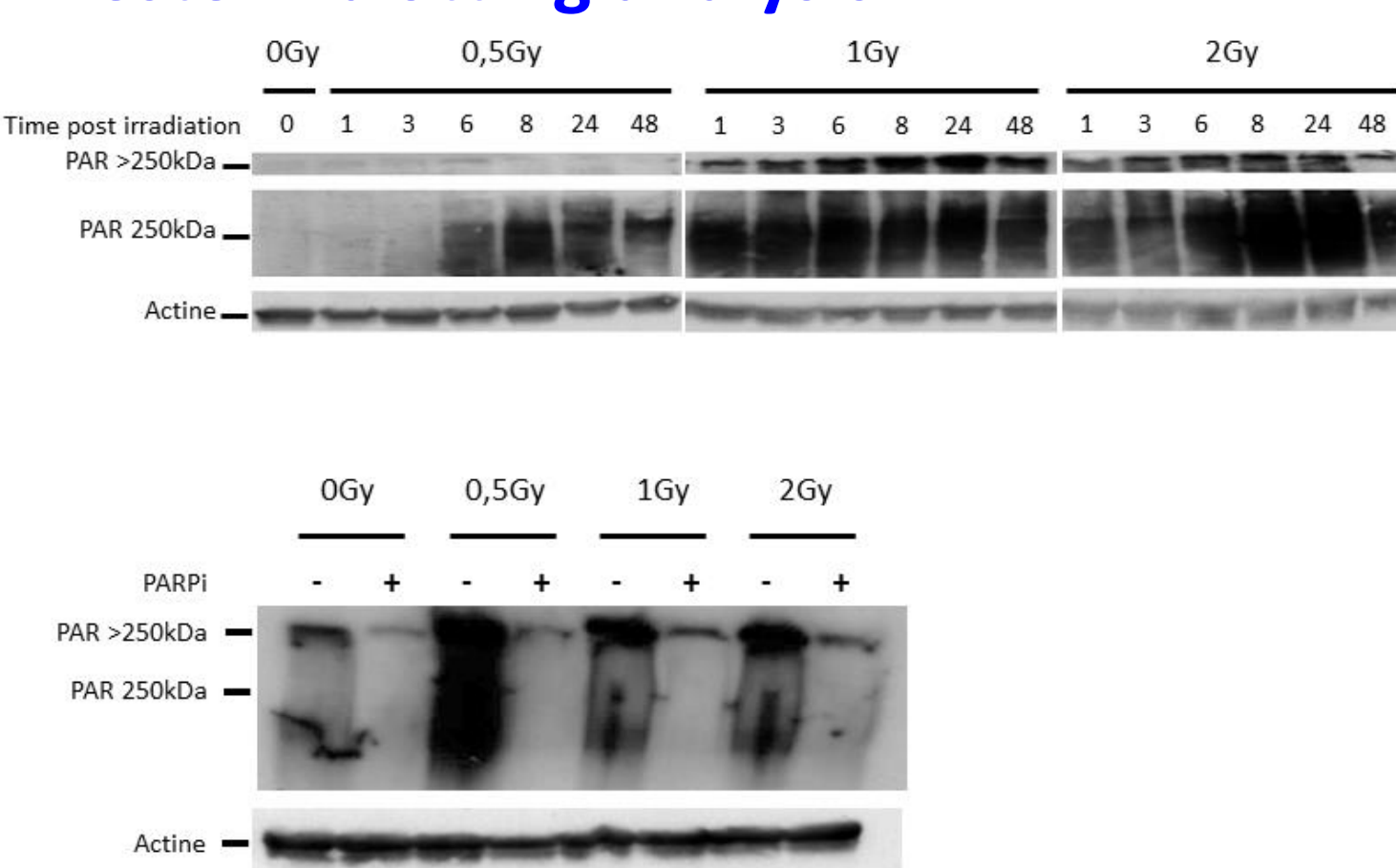
	D10	D37	SF2	ER (D10)	ER (D37)
X-rays	5.9	3.8	0.75	/	/
X-rays + PARPi	4.3	3	0.7	1.37	1.27
Protons	5.1	2.7	0.5	/	/
Protons + PARPi	2.9	1.4	0.2	1.76	1.93
C-ions	2	0.9	0.1	/	/
C-ions + PARPi	1.4	0.6	0.03	1.43	1.5

A. CH2879 cells were irradiated with X-rays (blue squares) or X-rays with PARPi (red squares).
 B. CH2879 cells were irradiated with protons (blue triangles) or protons with PARPi (red triangles).
 C. CH2879 cells were irradiated with C-ions (blue circles) or C-ions with PARPi (red circles).
 Cell survival (%) is shown as mean +/- SD of 3 independent experiments performed in triplicate for X-rays and C-ions. In the case of proton irradiation, cell survival (%) is shown as mean +/- SD of triplicate points corresponding to a single irradiation experiment. For each dose, clonogenic survival was considered to be significantly different (with and without PARPi) when $p < 0.05$ (*).

Proliferation assays: CellTrace analysis



Western blotting analysis



Analysis of PAR (Poly-ADP Ribose) chains using a specific antibody
 Top : without Parpi at different time points
 Bottom: with Parpi, at 24h

