DE LA RECHERCHE À L'INDUSTRIE

cea

ARIA

Applications in Radiobiology with Accelerated Ions
Accueil et Recherche en Radiobiologie des Ions Accélérés

CIRIL

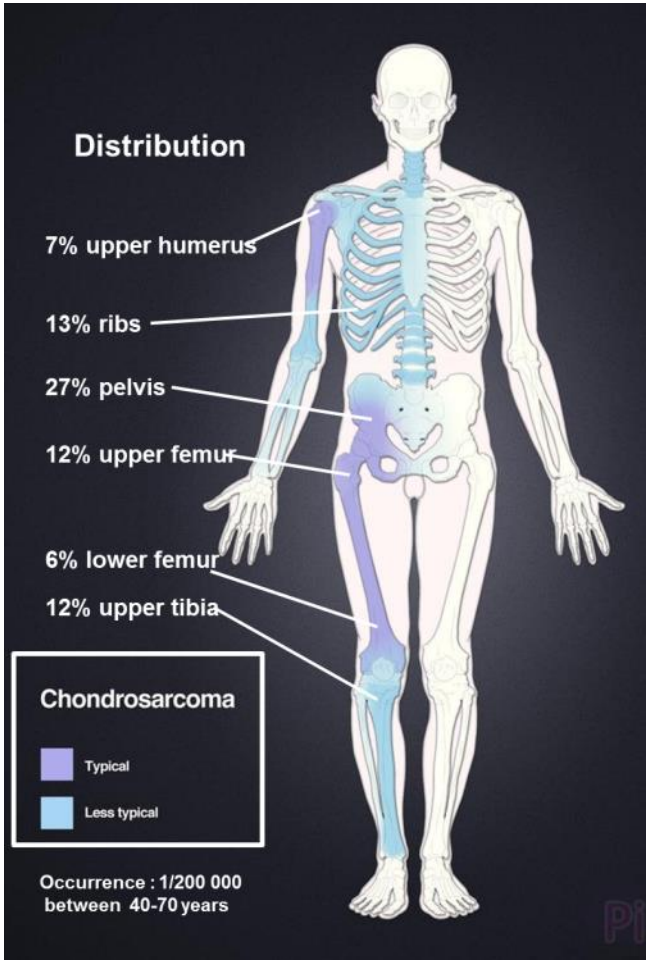
www.cea.fr

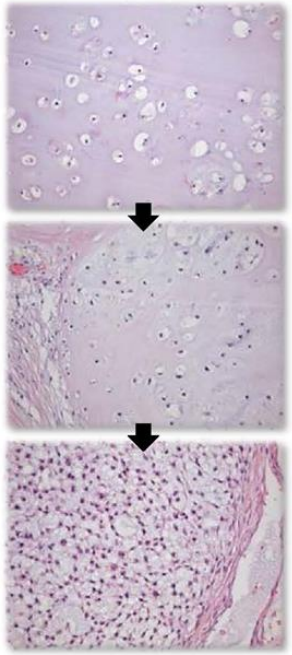
Radioresistance in Chondrosarcoma using the CIRIL-ARIA biology platform

François Chevalier

CHONDROSARCOMAS

- Chondrosarcoma is a rare bone malignant tumor arising from cartilaginous tissues





61%

Atypical cartilage tumour (Grade 1)
- 10 years survival: 83%
- metastasis: 0 %

36%

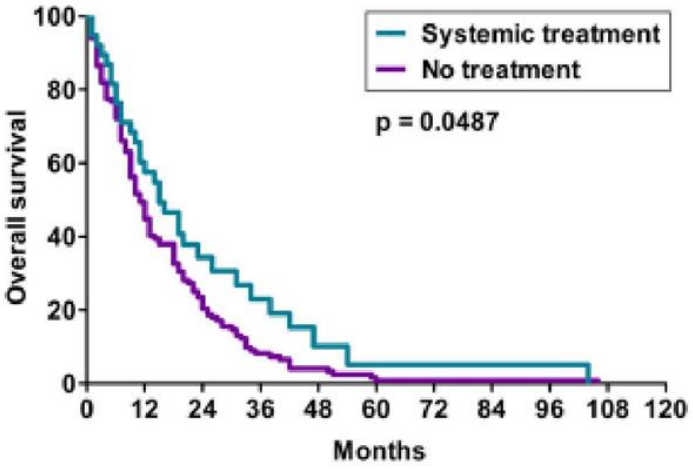
Grade 2
- 10 years survival: 64%
- metastasis: 10 %

3%

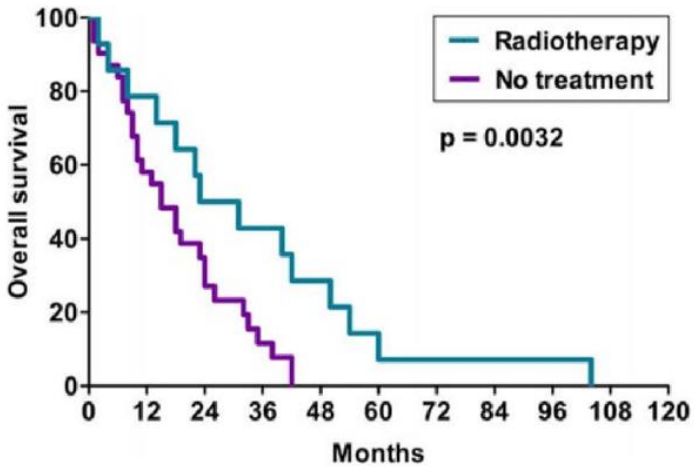
Grade 3
- 10 years survival: 29%
- metastasis: 71 %

CHONDROSARCOMAS TREATMENTS

- Surgery is the only treatment option
- Chemo and Radiotherapy is not very effective
- Patients with inoperable disease have no other treatment options



Patients with unresectable chondrosarcoma

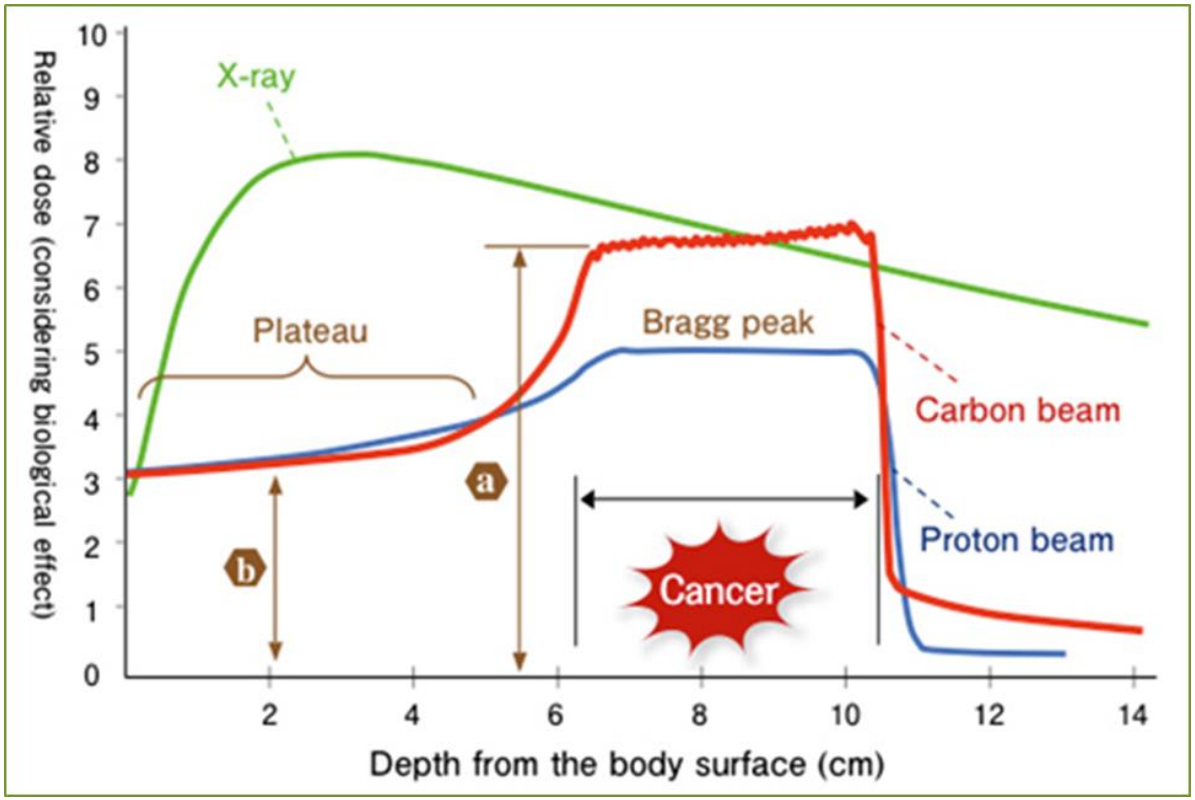


Patients with local advanced unresectable chondrosarcoma

Median survival ≈ 17 months

- ➢ Treatment are not efficient
- ➢ Need of new modalities of treatment

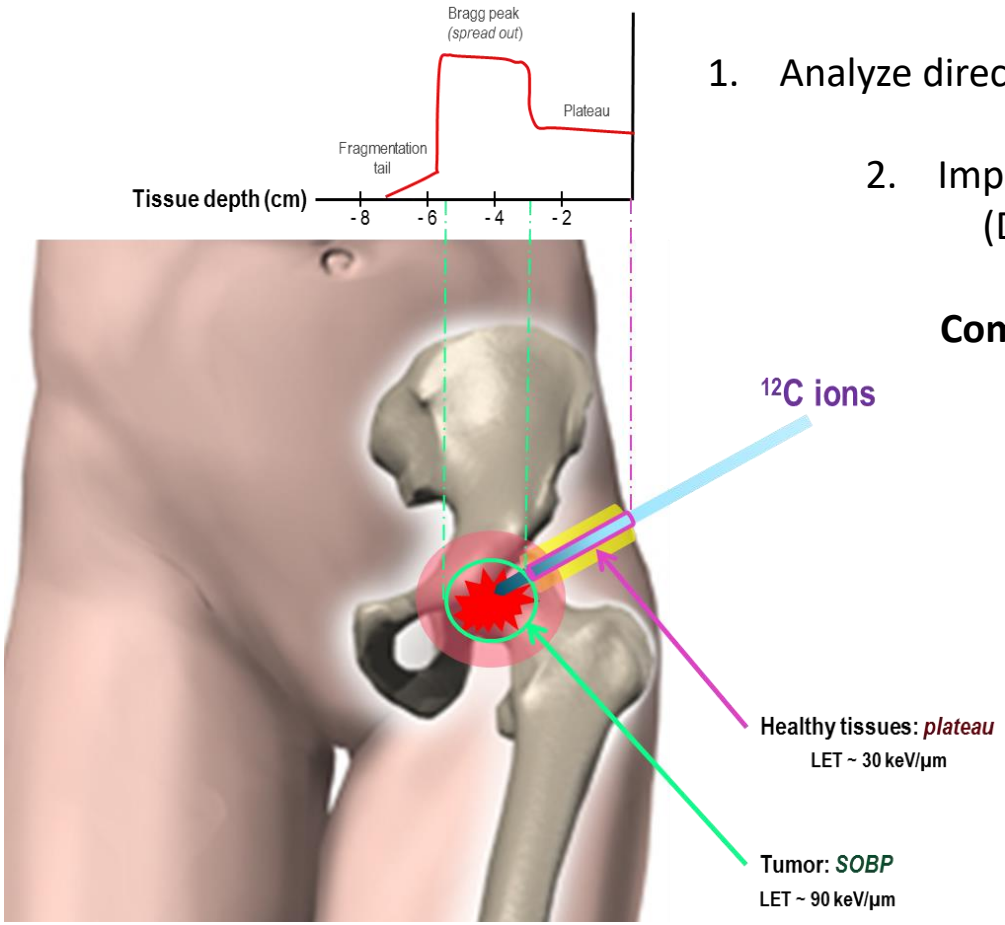
PARTICLE THERAPY
WITH C-IONS AND PROTONS



- Could be a new possibility of treatment for local advanced unresectable CH

- improved tumor control
- reduced side effects on healthy tissues

CHONDROSARCOMAS AND PARTICLE THERAPY



Objectives of our pre-clinical studies

in vitro irradiation of chondrosarcoma cell lines (different doses)

1. Analyze directs effects of radiation on chondrosarcoma cells

2. Impact of a radio-sensitizing agents: Parpi (DNA repair inhibitor = olaparib)

Comparison C-ions / Protons / X-rays

CIRIL-ARIA biology platform

for cell cultures and biochemistry experiments

Cell cultures lab

Biochemistry lab

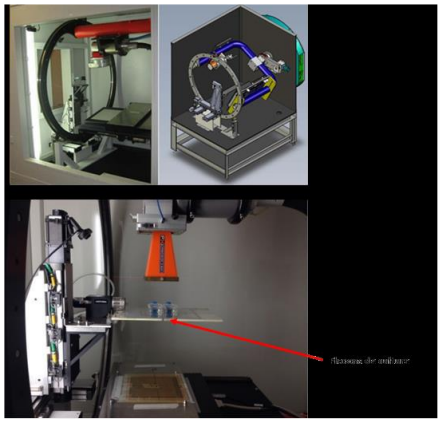
located within the INB secure area
facilitating cell transfer between irradiation room and cell culture lab

Irradiation conditions

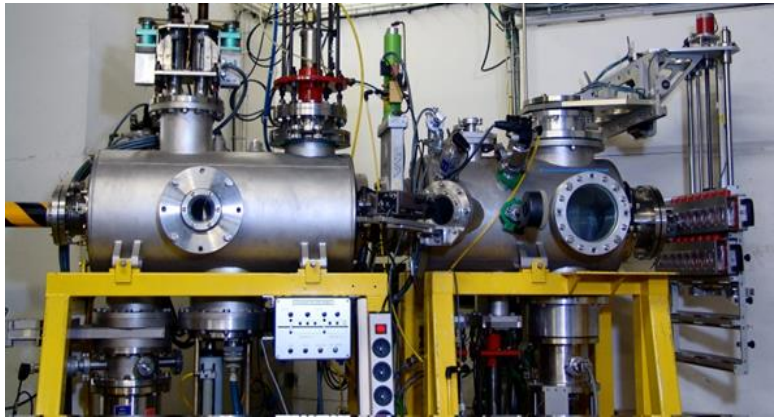
- GANIL : interdisciplinary Program Advisory Committee (iPAC) with the High Energy beam line in D1 : P1146-H and P1243-H
  - C-ions 95 MeV/A native (LET = 28 keV/µm)
  - C-ions 95 MeV/A + 16,9 mm PMMA (LET = 73 keV/µm)

- Proton SOBP (LET = 11 keV/µm)

- X-rays 225 KV (LET ~1,3 keV/µm)



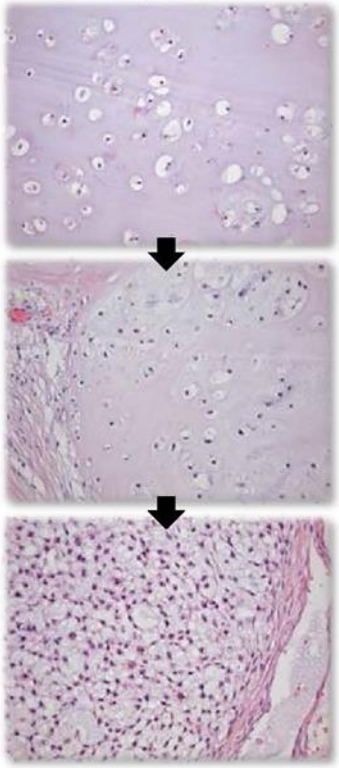
X-RAD 225 KV
CYCERON Caen



High Energy beam line in D1 GANIL

Proton (Catania, Italy)

Biological material: chondrosarcoma cell lines



61%

Atypical cartilage tumour (Grade 1)

- 10 years survival: 83%
- metastasis: 0 %

36%

Grade 2

- 10 years survival: 64%
- metastasis: 10 %

→ **SW1353** m p53 m IDH1

3%

Grade 3

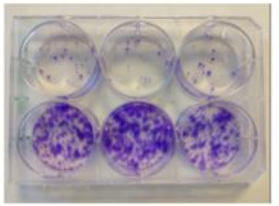
- 10 years survival: 29%
- metastasis: 71 %

→ **CH2879** WT WT
OUMS27 m p53
L835 WT m IDH1

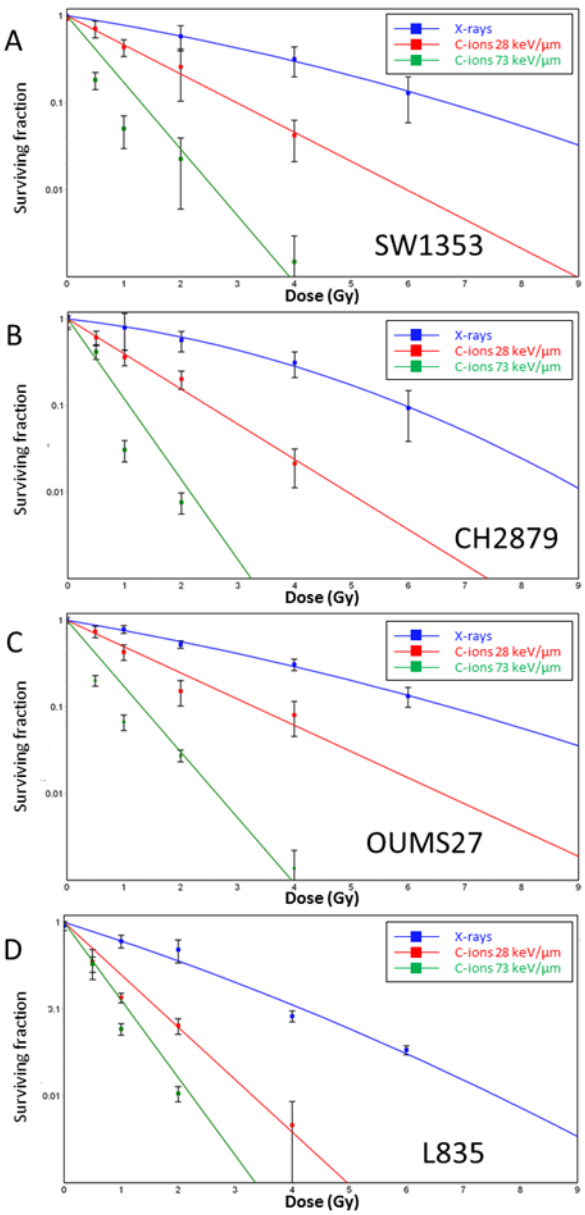
Main experiments :

- Cell survival : clonogenic assays
  - Different LET
  - With PARPi
- DNA damages : micro-nuclei assays
  - X-Rays vs C-ions
- Cell-cycle effects : flow cytometry
  - X-Rays vs C-ions
- growing effects : cell Trace assays
  - X-rays vs Protons vs C-ions + Parpi

Cell survival with X-rays vs C-ions (diff LET)



Clonogenic survival



radioresistance

+++

++

+++

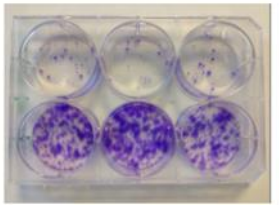
+

	D10[a]	D37[b]	SF2[c]	RBE (D10)[d]	RBE (D37)[e]
SW1353					
X-Rays 225 KV	6,696	3,413	0,584	/	/
Carbon 28 keV/µm	2,989	1,291	0,214	2,240	2,645
Carbon 73 keV/µm	1,306	0,564	0,029	5,126	6,051
CH2879					
X-Rays 225 KV	5,947	3,400	0,618	/	/
Carbon 28 keV/µm	2,467	1,065	0,155	2,410	3,191
Carbon 73 keV/µm	1,079	0,466	0,014	5,513	7,300
OUMS27					
X-Rays 225 KV	6,737	3,333	0,570	/	/
Carbon 28 keV/µm	3,303	1,426	0,248	2,040	2,337
Carbon 73 keV/µm	1,319	0,570	0,030	5,106	5,849
L835					
X-Rays 225 KV	4,182	1,938	0,358	/	/
Carbon 28 keV/µm	1,657	0,715	0,062	2,524	2,708
Carbon 73 keV/µm	1,119	0,483	0,016	3,738	4,012

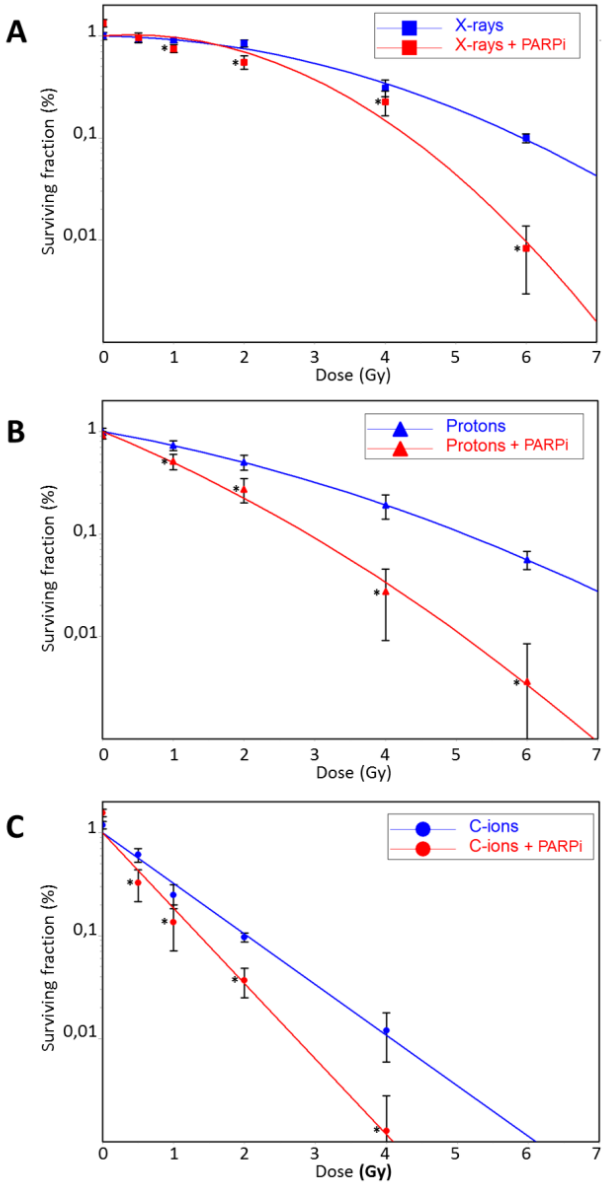
- differential radioresistance
- increased effect of C-ions (vs X-rays)
- increased effect with increasing the LET

from Chevalier et al. TCRP 2019

Cell survival with X-rays vs Protons vs C-ions + PARPi



CH2879



Clonogenic survival

	D10[a]	D37[b]	SF2[c]	ER (D10)[d]	ER (D37)[e]
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] the D10 dose gives a surviving fraction of 0.1.
[b] the D37 dose gives a surviving fraction of 0.37.
[c] the SF2 fraction is observed at 2 Gy irradiation.
[d] ER (D10) values are calculated as: D10 (with PARPi) / D10 (without PARPi) for each irradiation quality.
[e] ER (D37) values are calculated as: D37 (with PARPi) / D37 (without PARPi) for each irradiation quality.

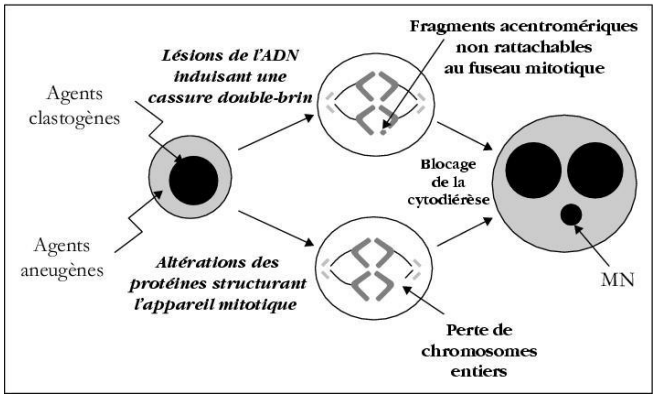
- Biological effect : X-rays < protons < C-ions
- PARPi effective as radio-sensitizing agents with both irradiations

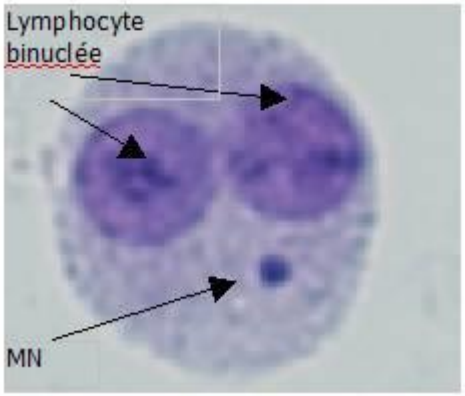
from Césaire et al. JBO 2019

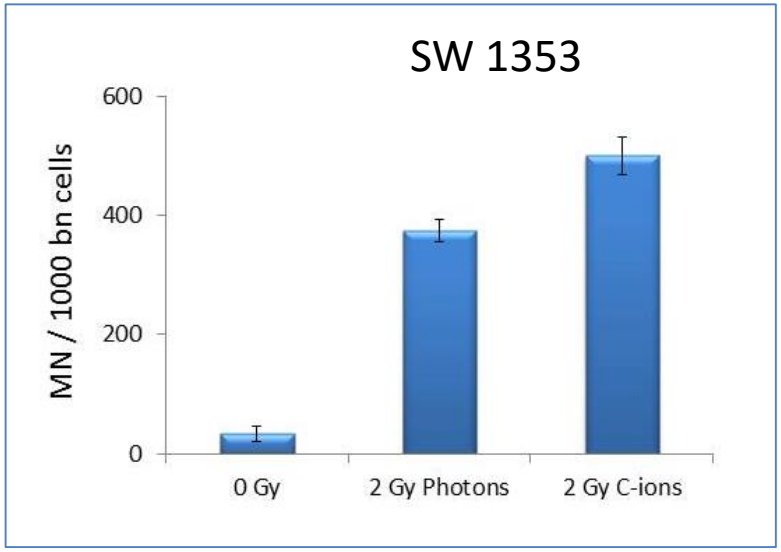
DNA DAMAGES: X-RAYS vs C-iONS

Micro-nuclei assays

Observation of MN after cell division = DNA damages



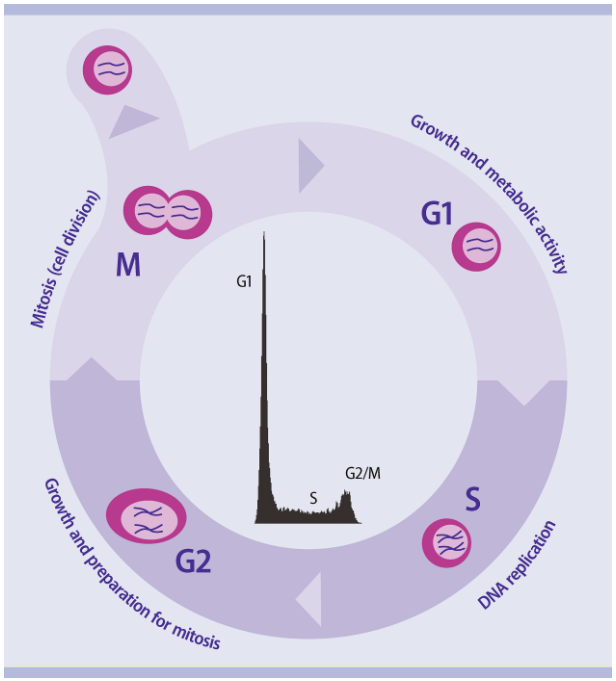


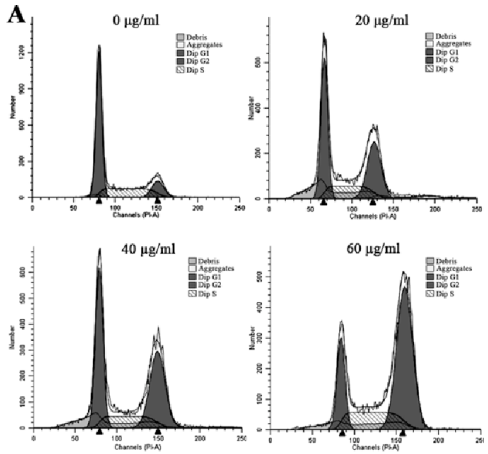


- More DNA damages with same dose
  X-rays < C-ions

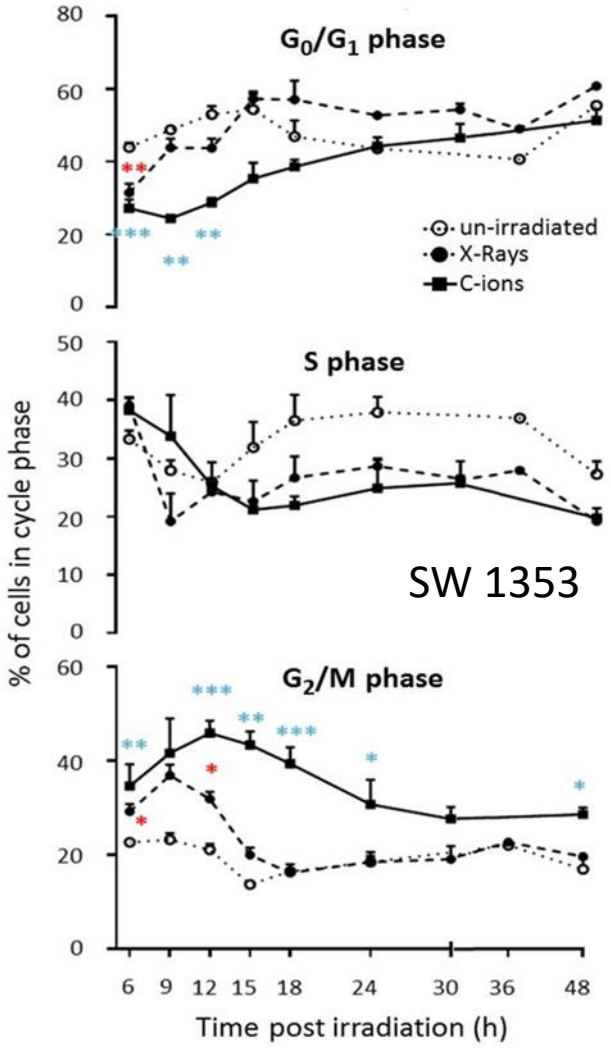
from Chevalier et al. TCRP 2019

CELL-CYCLE EFFECTS : X-RAYS vs C-iONS





Cell cycle (Flow cytometry)



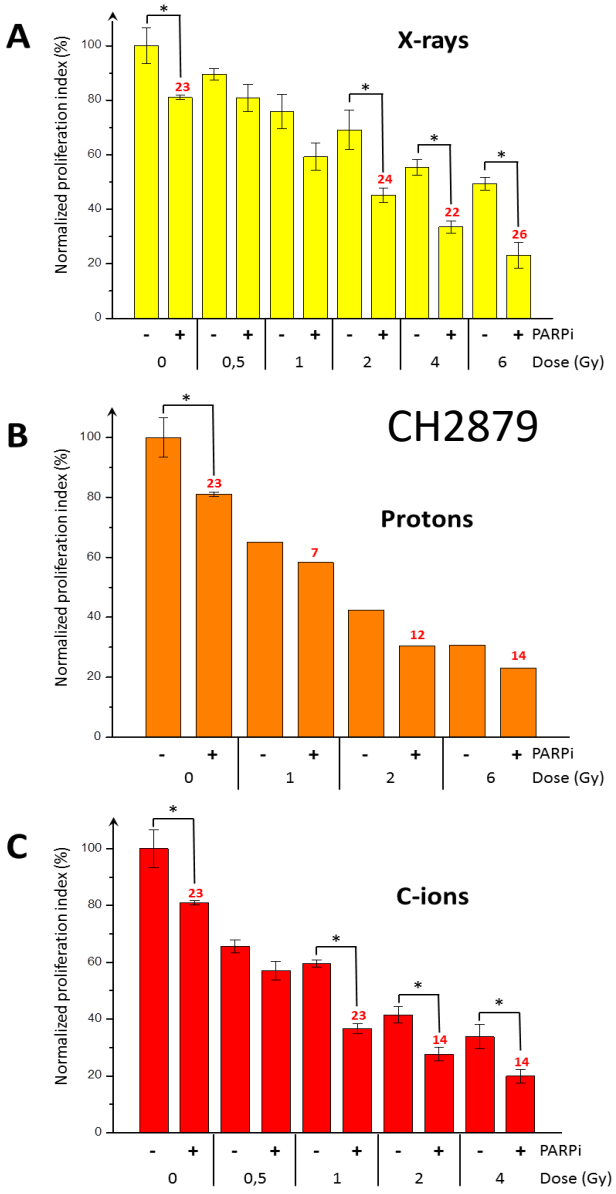
- Increase in S and G2/M phases with C-ions
- Cell cycle blockage in G2/M phase

➢ **More DNA damages**
➢ **DNA damages more difficult to repair**

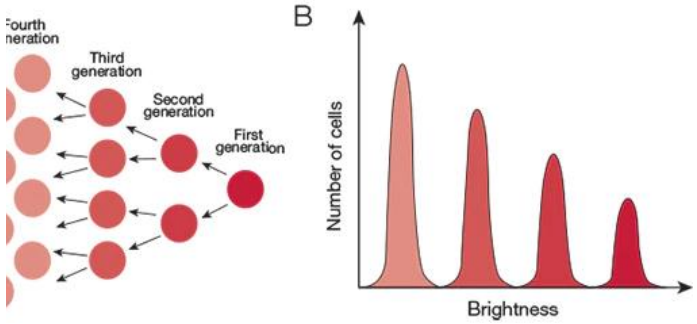
X-rays < C-ions

from Chevalier et al. TCRP 2019

GROWING EFFECTS : WITH X-RAYS VS PROTONS VS C-IONS + PARPI



Cell trace assay analysis



proliferation index → cell divisions

- Reduced cell divisions
- X-rays < protons < C-ions
- Parpi increase the biological effect

from Césaire et al. JBO 2019

Main conclusions :

- Chondrosarcoma cell lines are radioresistant

- Differences between cell lines
  L835 > CH2879 > OUMS27 – SW1353

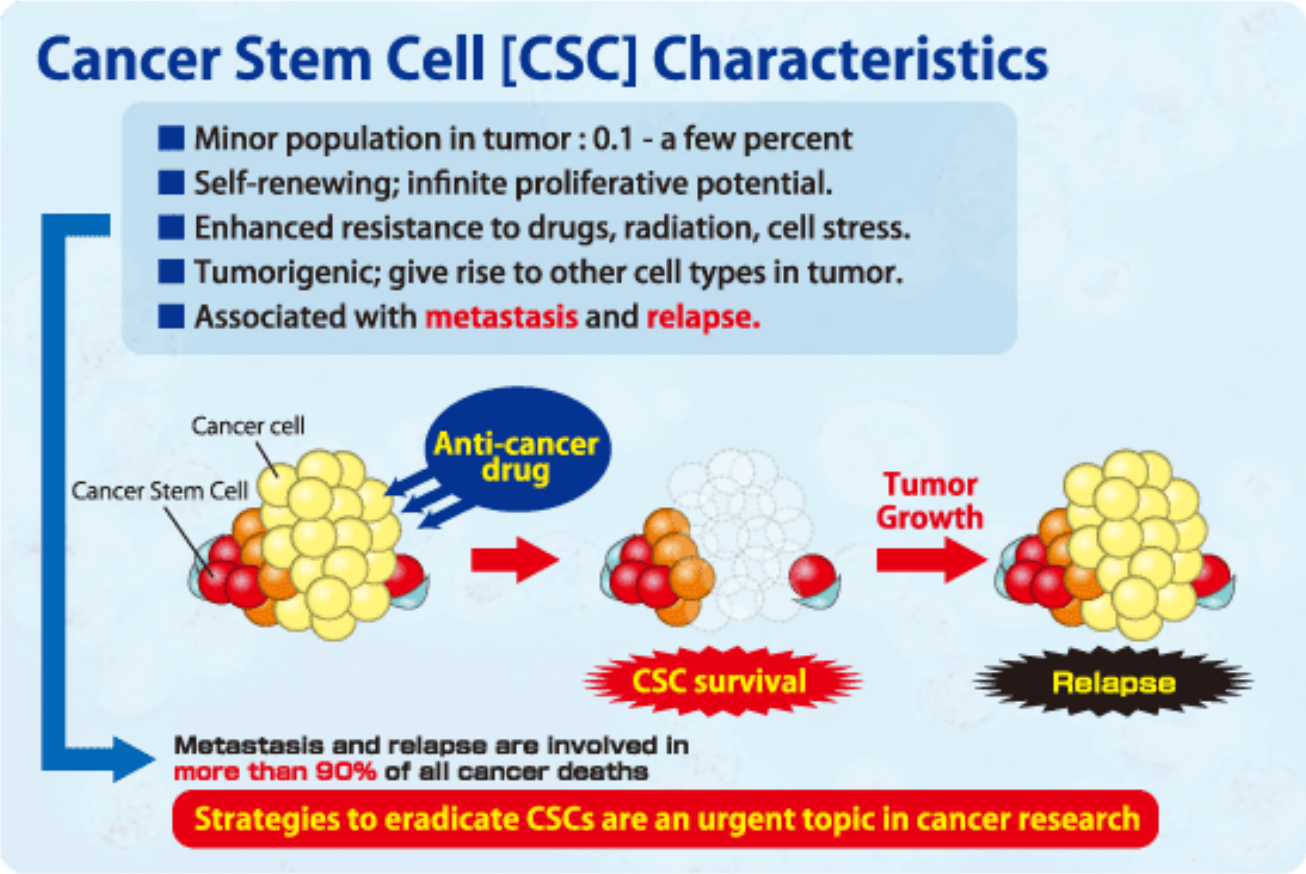
- carbon ions decrease survival compared to X-rays
- carbon ions increase micro-nuclei
- carbon ions increase blockage in G2 / M
- carbon ions induce damages more difficult to repair

- PARPi effective as radio-sensitizing agents
- With X-rays / protons / C-ions

Ongoing projects (2020 – 2024)

Chondrosarcoma and radiation-resistance

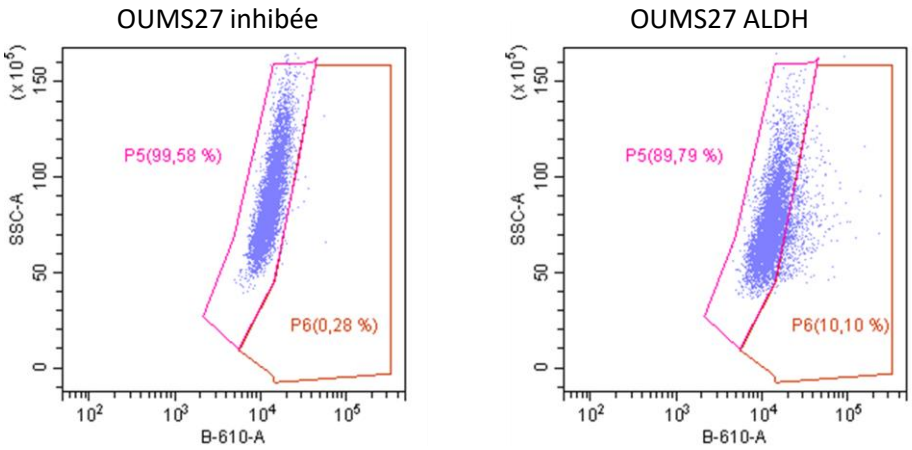
- status of cancer stem cells (CSCs) in tumor radioresistance?
- Can we overcome this radioresistance with hadrontherapy in combined treatments?

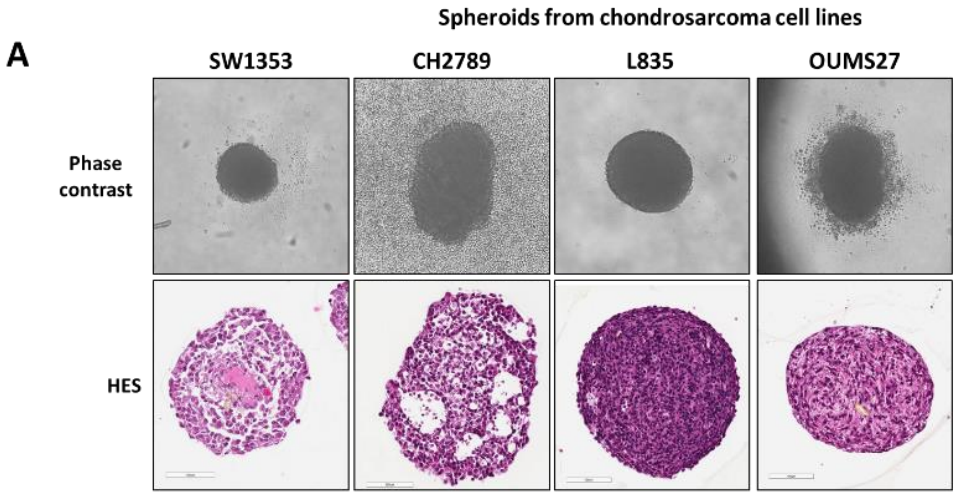


Ongoing projects (2020 – 2024)

CSC Biomarkers analysis

	% ALDH high cells		
	c	t	diff
SW1353	0,26	1,17	0,91
Oums27	0,28	10,1	9,82
L835	0,14	1,69	1,55





Development of 3D models
Spheroids with chondrosarcoma cells

Role of hypoxia /CSC in radioresistance

Acknowledgments

GANIL laboratoire commun CEA/DRF spiral2 CNRS/IN2P3 and CiMap beam time with iPAC committees

ARIA
Applications in Radiobiology with Accelerated Ions
Accueil et Recherche en Radiobiologie des Ions Accélérés

- F. Chevalier
- S. Haghdoost
- A. Gilbert
- M. Césaire
- C. Lepleux

Platform and collaborative labs:

IMPEDANCELL REAL TIME CELLULAR ACTIVITY
ORGAPRED TUMOROID PLATFORM
IFIN-HH
PPC Plateforme de Protéomique Clinique
IST2

Funding agencies:

Liberté • Égalité • Fraternité RÉPUBLIQUE FRANÇAISE
Partenariat Hubert Curien (PHC) Sakura Sakura プログラム
CAMPUS FRANCE campusfrance.org
Vallée de la Seine
RÉGION NORMANDIE
EDF
ENSAR2
Fonds Européen de Développement Régional
FONDATION ARC POUR LA RECHERCHE SUR LE CANCER

Thank you for your attention !

Any questions?