Radioresistance in Chondrosarcoma using the CIRIL-ARIA biology platform

François Chevalier
Chondrosarcoma is a rare bone malignant tumor arising from cartilaginous tissues.

**CHONDROSARCOMAS**

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

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

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

Distribution:
- 7% upper humerus
- 13% ribs
- 27% pelvis
- 12% upper femur
- 6% lower femur
- 12% upper tibia

Chondrosarcoma:
- Typical
- Less typical

Occurrence: 1/200,000 between 40-70 years
CHONDROSARCOMAS TREATMENTS

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

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 direct effects of radiation on chondrosarcoma cells

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

Comparison C-ions / Protons / X-rays

- Healthy tissues: plateau
  - LET ~ 30 keV/µm

- Tumor: SOBP
  - LET ~ 90 keV/µm
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)
Biological material: chondrosarcoma cell lines

61%
Atypical cartilage tumour (Grade 1)
- 10 years survival: 83%
- metastasis: 0%

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

36%

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

3%

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>p53 Status</th>
<th>IDH1 Status</th>
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<tbody>
<tr>
<td>SW1353</td>
<td>m</td>
<td>m</td>
</tr>
<tr>
<td>CH2879</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>OUMS27</td>
<td>m p53</td>
<td>m IDH1</td>
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<tr>
<td>L835</td>
<td>WT</td>
<td>m IDH1</td>
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</table>
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

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

- 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

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

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

Reduction in cell divisions:
- X-rays < protons < C-ions
- Parpi increase the biological effect

Cell trace assay analysis

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

<table>
<thead>
<tr>
<th></th>
<th>% ALDH high cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>c</td>
</tr>
<tr>
<td>SW1353</td>
<td>0,26</td>
</tr>
<tr>
<td>Oums27</td>
<td>0,28</td>
</tr>
<tr>
<td>L835</td>
<td>0,14</td>
</tr>
</tbody>
</table>

Development of 3D models
Spheroids with chondrosarcoma cells

Role of hypoxia /CSC in radioresistance

Spheroids from chondrosarcoma cell lines
Acknowledgments

and 

beam time with iPAC committees

ARIA

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

• F. Chevalier
• S. Haghdooost
• A. Gilbert
• M. Césaire
• C. Lepleux

Platform and collaborative labs:

Funding agencies:
Thank you for your attention!

Any questions?