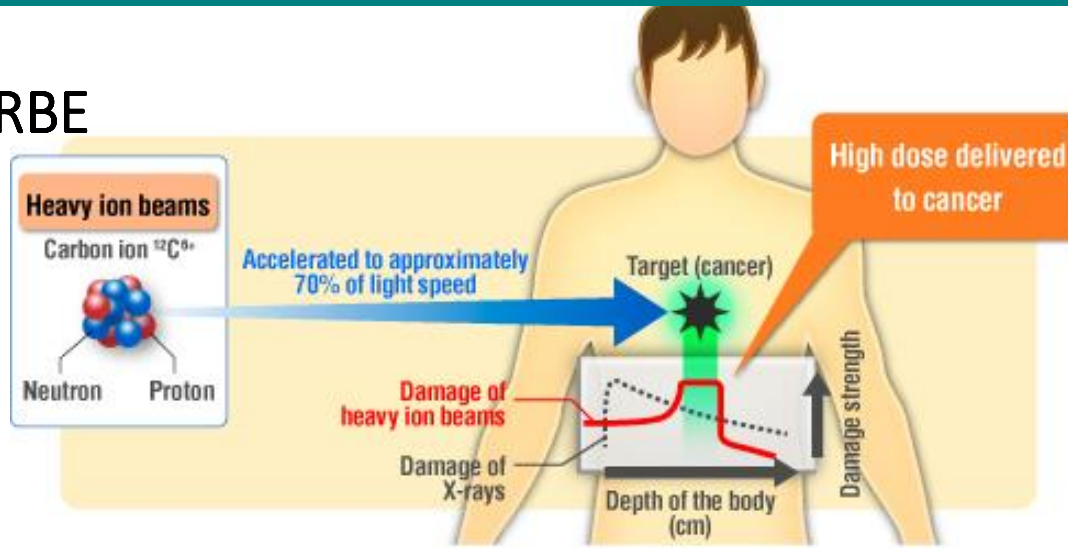
Central role of spatial ROS distribution at the nanometric scale in the molecular response to carbon ion irradiation

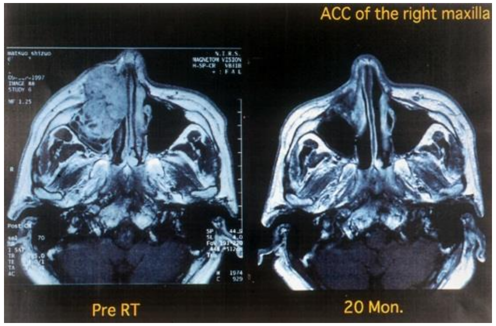
Cellular and Molecular Radiobiology Lab
UMR/CNRS 5822 - IP2I
Lyon-Sud Medical School
Claire Rodriguez-Lafrasse' Lab

Hadrontherapy with carbon ions

- Precise ballistics and high RBE



- Treatment of deep-seated and radioresistant cancers



Which molecular mechanisms are specifically involved in the tumor response to carbon ions?
How to explain RBE?

Hadrontherapy with carbon ions

Irradiation since 2003 iPAC, FranceHadron

- ^{13}C 75 MeV/n and ^{12}C 95 MeV/n
- More than 100 UTs / About 4 to 6 UTs per years
- For biology experiments : 2/3 UTs max - 1 entrance/hour

Possible thanks to :

- Beam for biology (energy, homogeneity, size, dosimetry, motorised sample holder…)
- ARIA Laboratory

Paradigm of the stealth bomber

To explain the tumor cell response to carbon ions

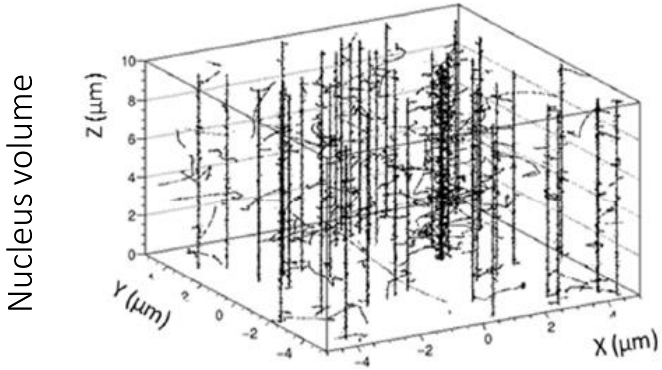
Bomber

Stealth

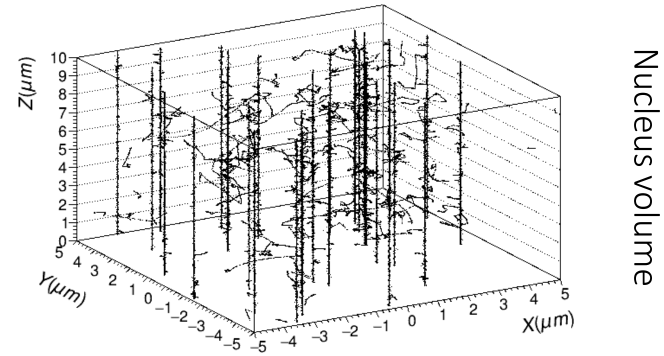
Relies on the spatial distribution of Reactive Oxygen Species (ROS) at the nanometric scale

Monte Carlo simulations of OH° radicals

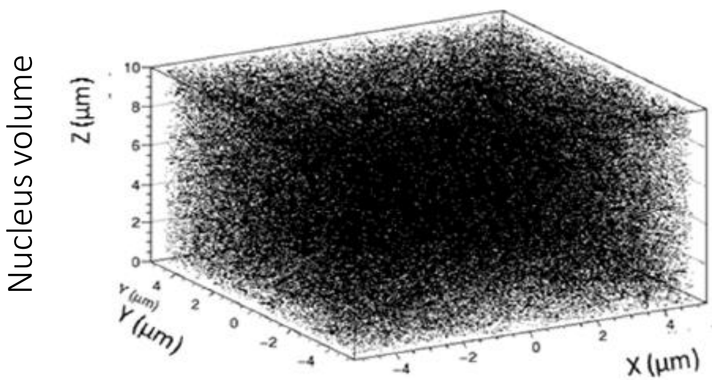
2 Gy C-ions (physical equivalent dose)



1 Gy C-ions (biological equivalent dose)



2 Gy photons

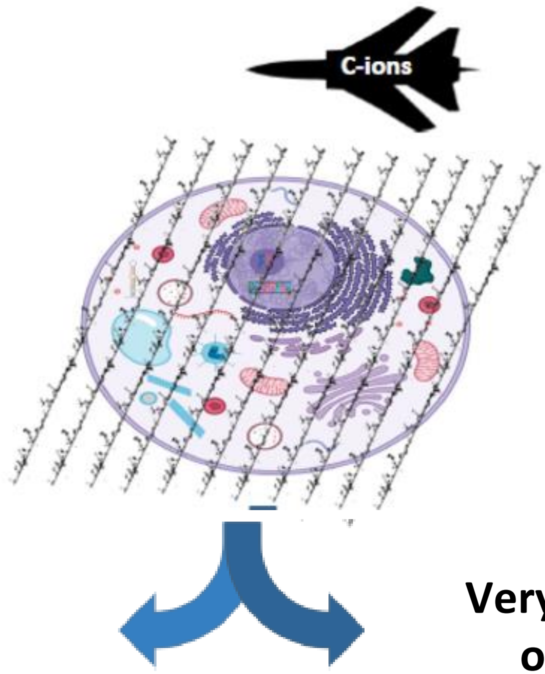


- Local distribution at the nanometric scale:
  - Clusters around tracks **(C-ions)**
  - Dense and homogeneous distribution **(photons)**

Very different consequences at the molecular / cellular level!

Wozny et al. Cancers 2019

Paradigm of the stealth bomber



Subcellular targets are on the trajectory of C-ion tracks

↓

ROS-induced complex damage at the DNA and cellular levels

↓

Bomber effect

Very low production of ROS out of the C-ion tracks

↓

Absence of triggering of cellular alarms and defense mechanisms outside areas hit by ROS

↓

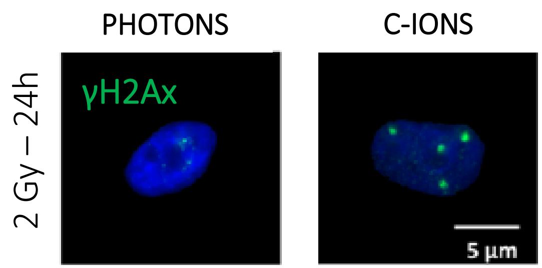
Stealth effect

Experimental data supporting both effects

The bomber effect

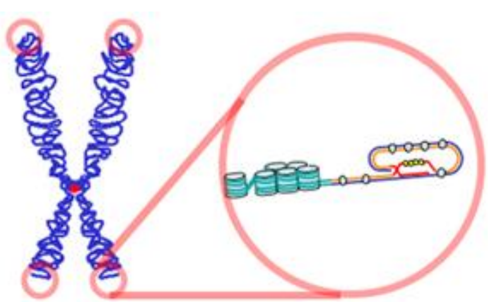
At the DNA level

- Complex DNA lesions, Clusters of un-repairable DNA lesions (DSBs)



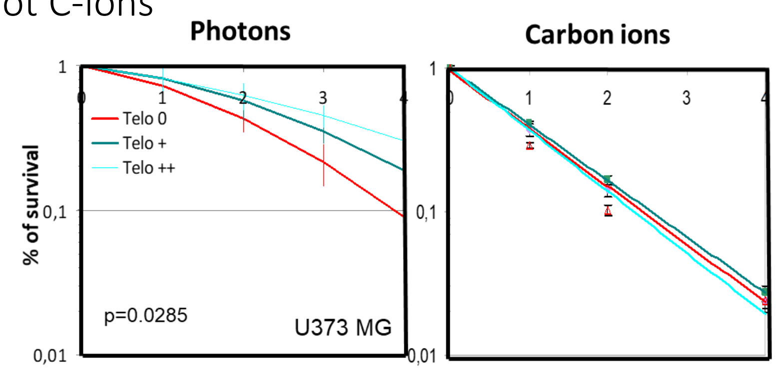
iPAC p866 3UT / FranceHadron
Wozny *et al.*, Scientific Reports, 2020
Wozny *et al.*, Cancers, 2021

- No influence of telomeres' length on cell killing



- Correlation between telomeres' length and radioresistance in 12 glioblastoma cell lines after photons exposure BUT not C-ions

- No relationship between telomeres' length and the response to C- ions in cells transfected with telomerase (artificial increase of telomere' length)



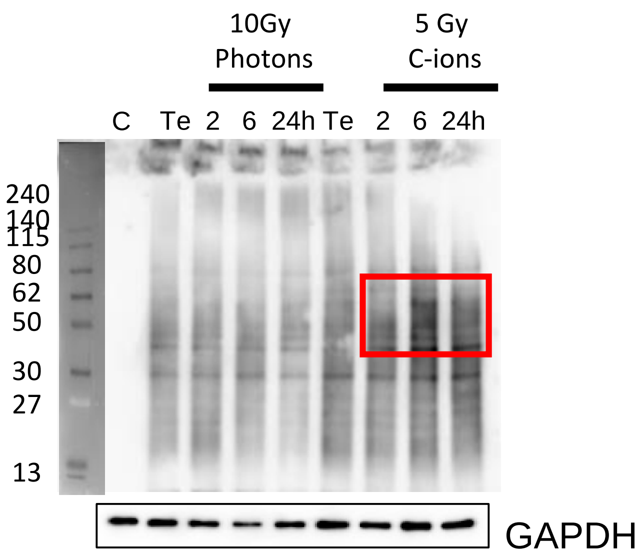
iPAC p813 5UT
Ferrandon et al. Mol Neurobiol, 2013

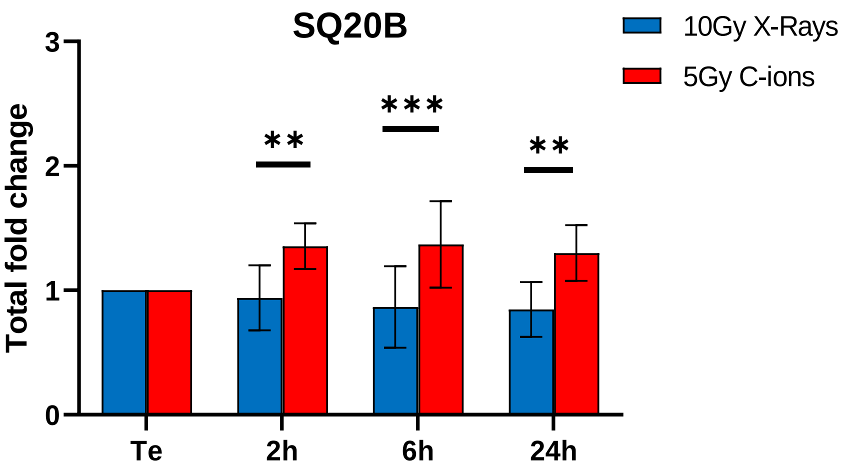
The bomber effect

At the cellular level

- Protein homeostasis:

Increase of damaged proteins





More oxidized proteins miscleared by the proteasome

iPAC p1166H 4 UT/ p1299H 8UT (on going)

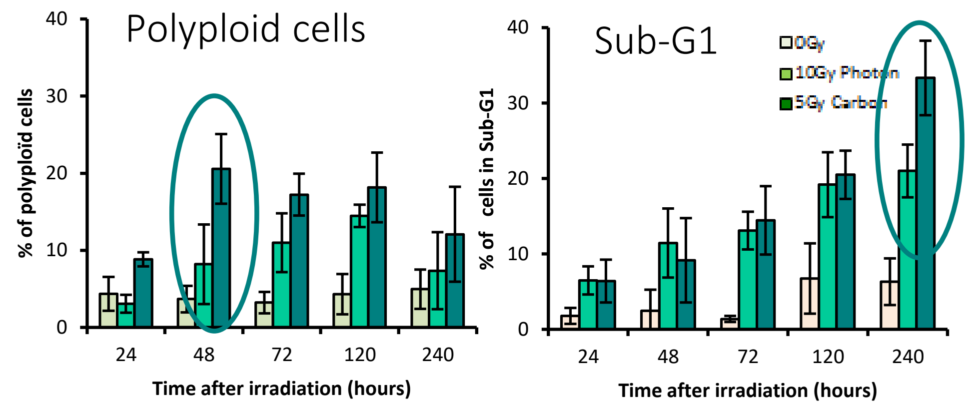
Consequences of the bomber effect

Cell death

- Earlier and more important compared with photons

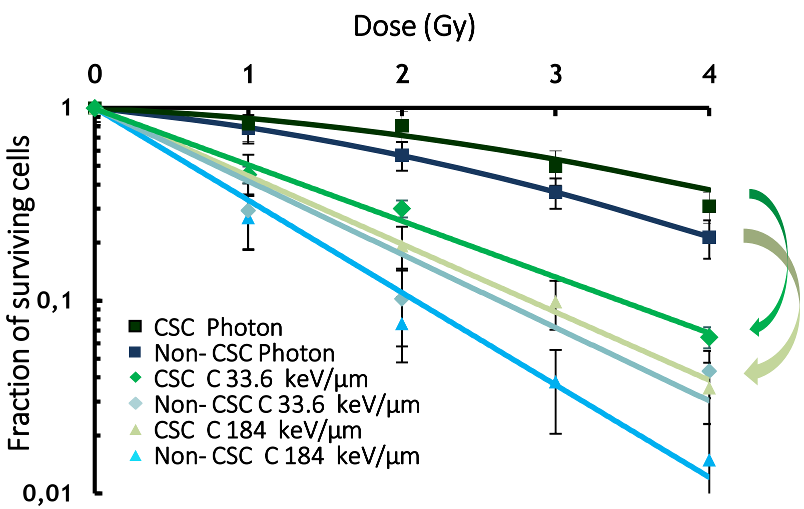
- No specific mechanism involved
  - early apoptosis or mitotic death
  - p53-independent ceramide-dependent apoptosis

iPAC p744H 4UT / p790H 18UT
Maalouf et al., IJROBP 2009
Alphonse et al., BMC Cancer, 2013
Ferrandon et al. Cancer Letter, 2015



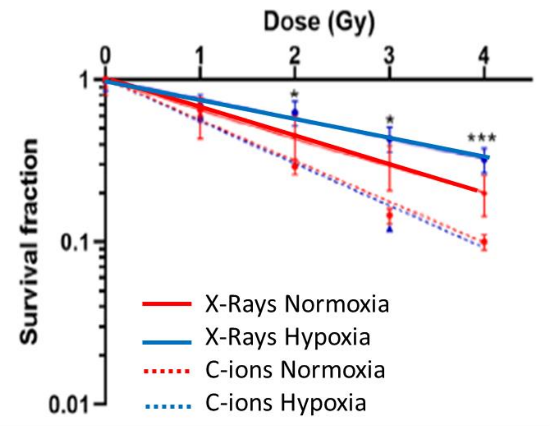
- More efficient on cancer stem cell killing

FranceHadron
Bertrand et al., Stem Cell, 2014
Moncharmont et al. Oncotarget, 2016



Consequences of the bomber effect

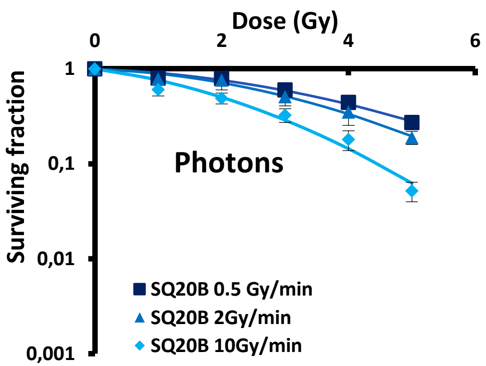
- Cell killing is independent of :
  - the O_2 concentration

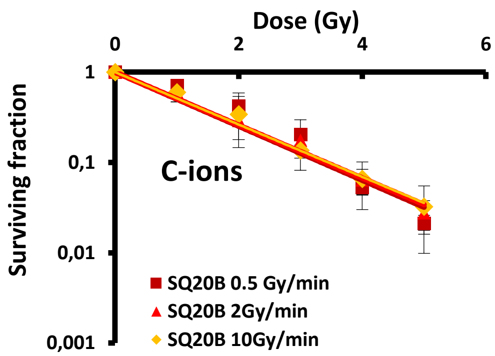


Interest in the treatment of hypoxic tumors

FranceHadron / iPAC p1166H 4UT
Wozny et al., British Journal of Cancer, 2017
Wozny et al. Scientific Reports, 2020

  - the radiation dose-rate

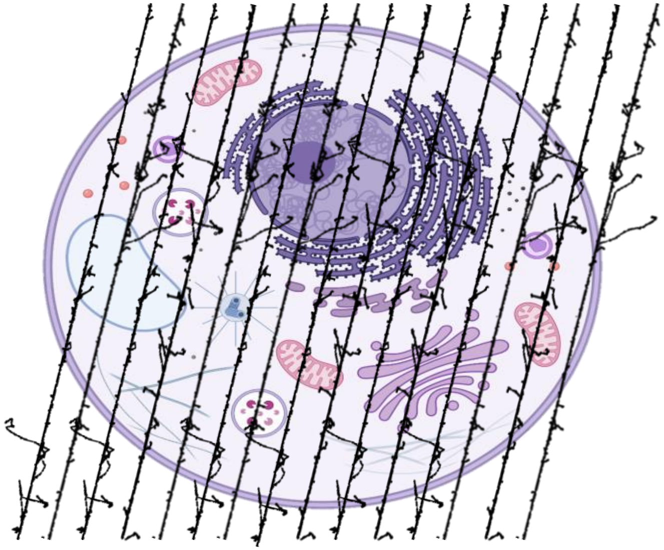




Interest in the planification of treatment

iPAC p737H 9UT
Wozny et al., Frontiers in Oncology, 2016

The stealth effect

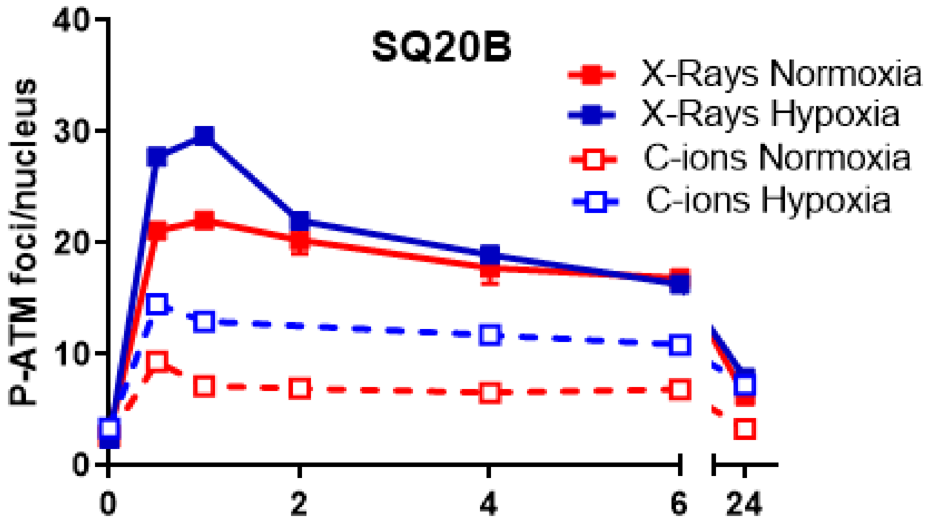


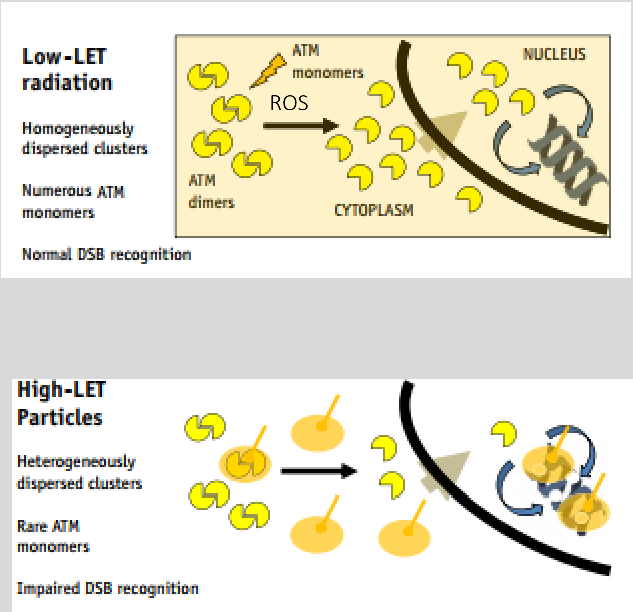
A large proportion of cell volume is not hitten by C-ions:

- thresholds of ROS necessary to trigger survival and defense mechanisms not reached

The stealth effect

- Less DNA Damage detection (nucleoshuttling of ATM) under normoxia or hypoxia





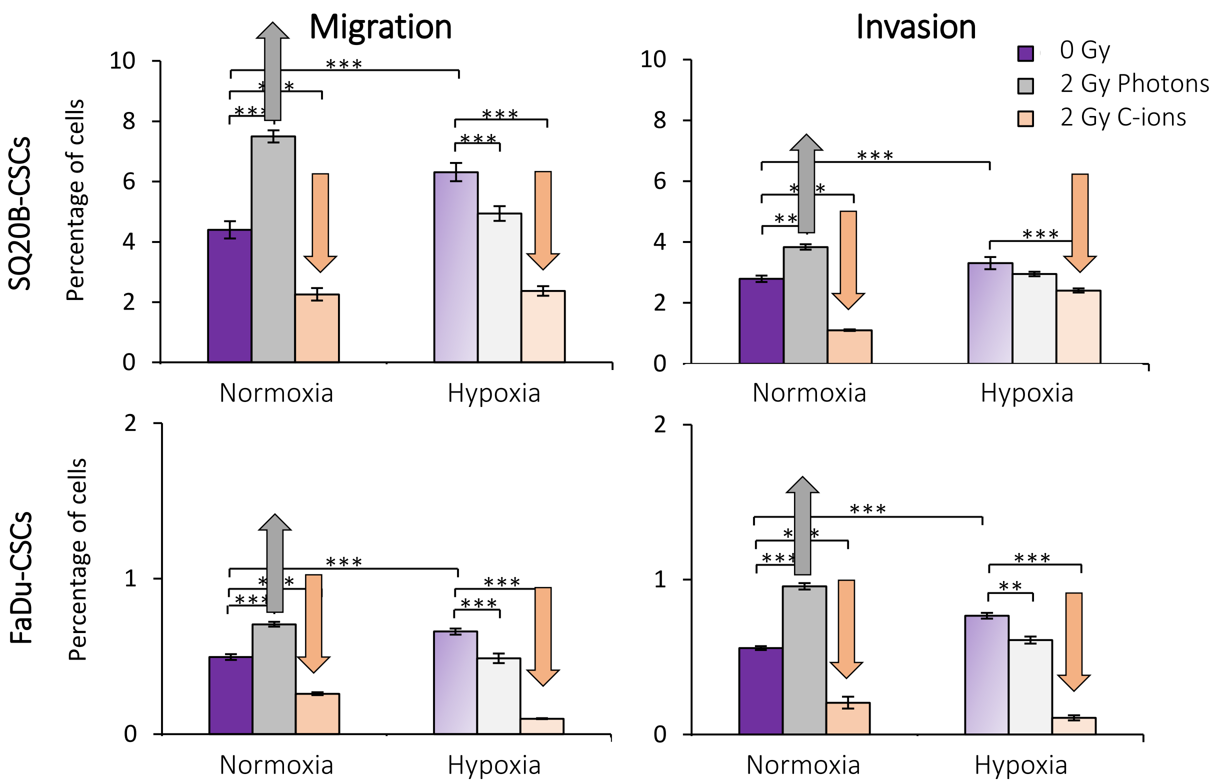
Maalouf et al. IJROBP 2019

- Lower DNA damage signalling and repair (NHEJ/HR) under normoxia or hypoxia

Wozny et al., Scientific Reports, 2020
Wozny et al., Cancers, 2021

The stealth effect

- No invasion-migration of CSCs



Less metastases under normoxia and hypoxia

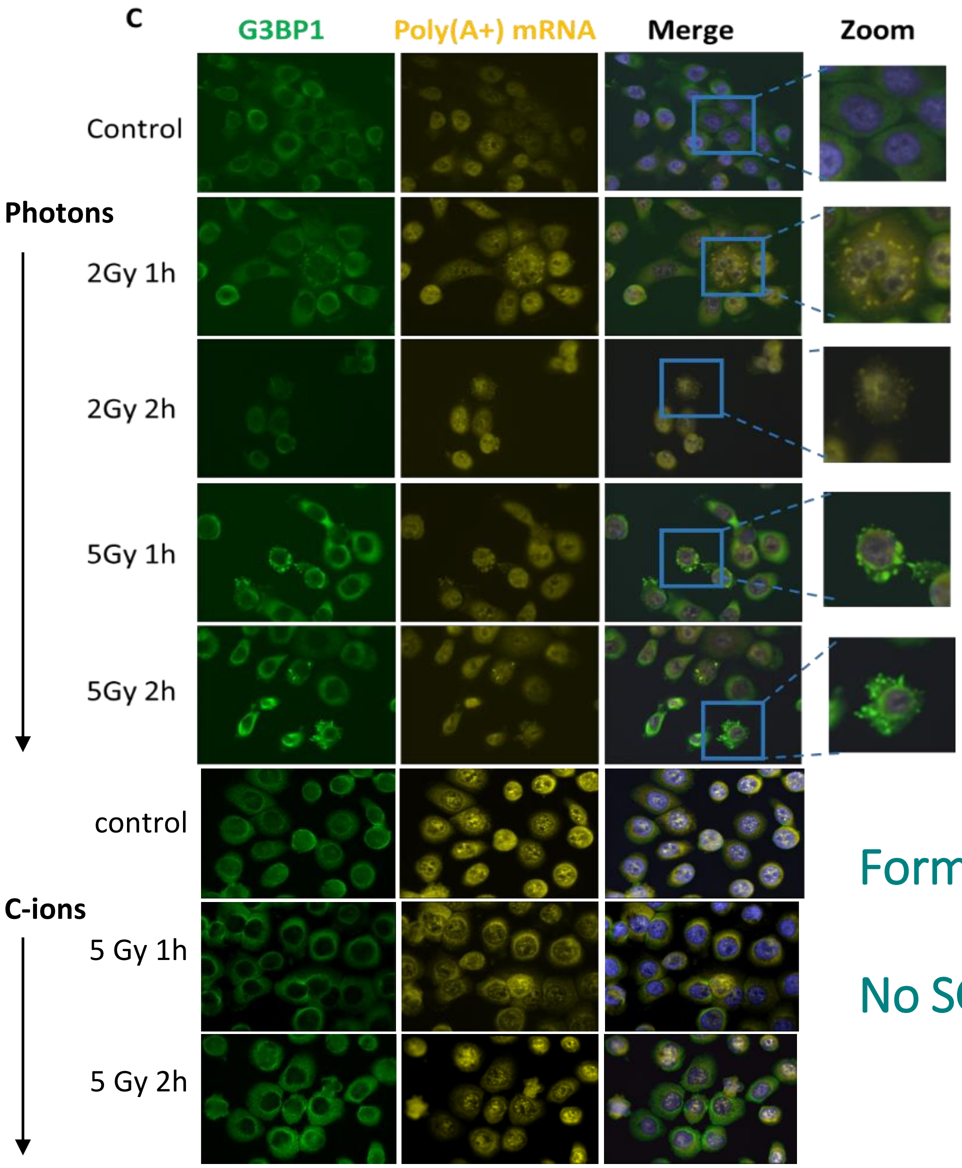
Few/no activation of invasion/migration signalling pathways

FranceHadron

Montcharmont et al. Oncotarget 2016

Wozny et al. Cancers 2019

The stealth effect



Stress granules (SG):

- non-membrane cytoplasmic aggregates
- regulate gene expression to protect cells

Formation of SG following photon irradiation

No SG in response to C-ions

iPAC P1251-H 1UT

Louati et al in redaction

Conclusions

Carbon ions better cure radioresistant cancers

The Bomber effect

- Complex DNA damage
- Independent of the telomere length
- More oxidized proteins and less induction of proteasomal activity
- More cell death
- No oxygen effect / Independent of Dose-rate

The Stealth effect

- Lower DNA damage detection and repair
- No invasion/migration
- No/lower activation of cell survival pathways
- No stress granules formation

Maalouf *et al.* 2009, IJROBP
Hanot *et al.* 2012 Plos One
Alphonse *et al.* 2013 BMC cancer
Ferrandon *et al.* 2013 Mol Neurobiol
Bertrand *et al.* 2014 Stem Cell Rev

Ferrandon *et al.* 2015 Mol Cancer
Ferrandon *et al.* 2015 Cancer Letter
Moncharmont *et al.* 2016 Oncotarget
Wozny *et al.* 2016 Frontiers in Oncology
Wozny *et al.* 2017 British J. Cancer

Wozny *et al.* 2019 Cancers
Wozny *et al.* 2020 Scientific Reports
Wozny *et al.* 2021 Cancers
Averbeck and Rodriguez-Lafrasse 2022 IJMS

Perspectives

- Oxygen, Proton, and Helium ions for the irradiation of cells in 2D or 3D culture
- Possibility to have a vertical beam (3D culture)
- Irradiation at lower energies + at different positions SOBP
- Animal facility for *in vivo* experiments
- Flash irradiation at very high dose rate with Protons, Helium and Carbon ions