A PARADIGM TO EXPLAIN THE DIFFERENTIAL EFFECTS OF CARBON ION AND X-RAY IRRADIATION ON TUMOR CELLS

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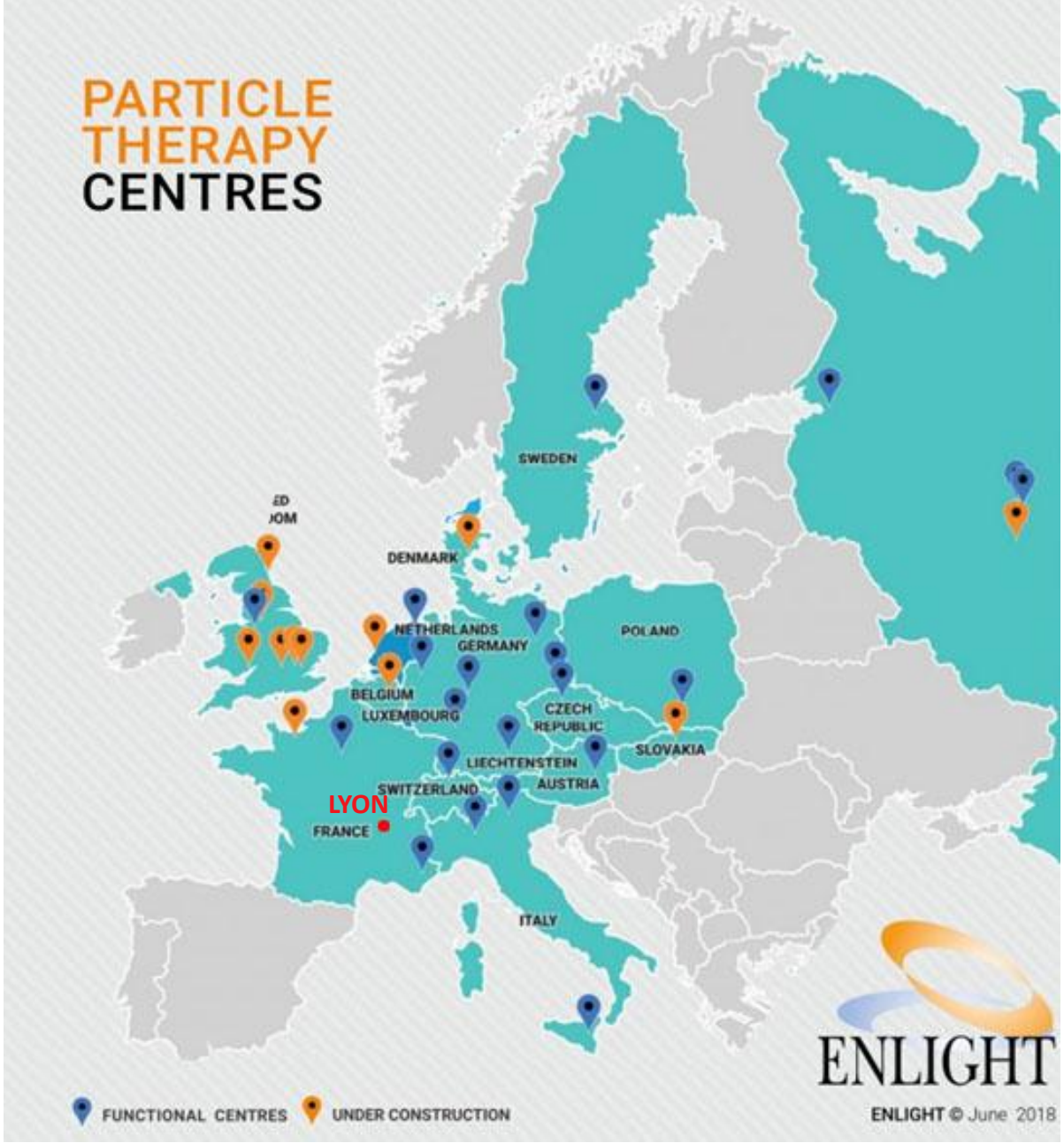
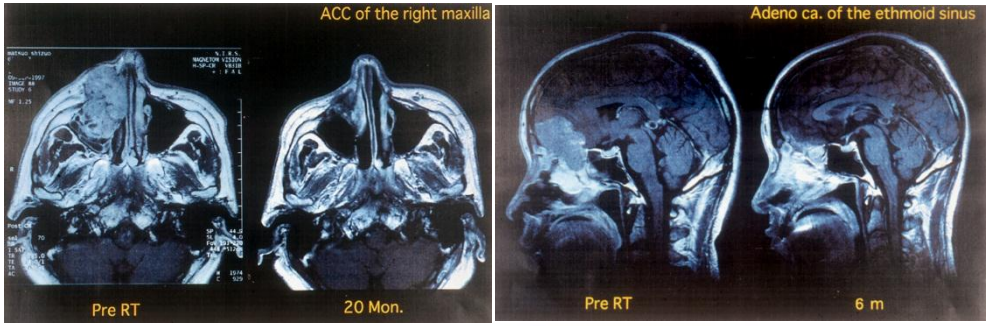
Caen, July 2019

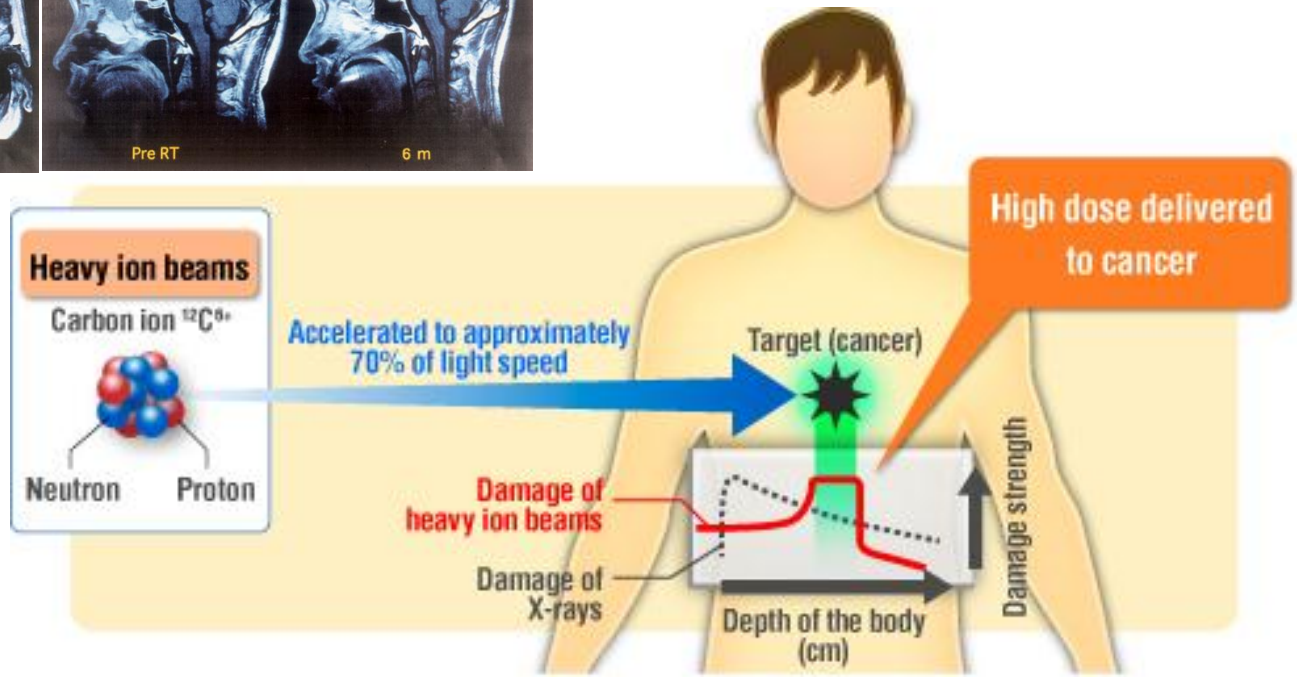


C. Rodriguez-Lafrasse ENLIGHT 2019

✓ Promising clinical results of Pr Tsujii (2003): ballistics and high RBE of carbon ions



Pr Tsujii, NIRS, Japan

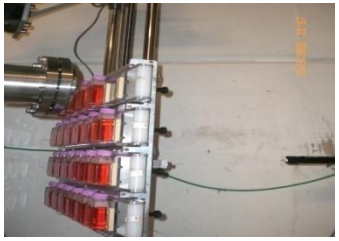


Specific molecular mechanisms involved in response to carbon ions that could explain the clinical response and the high RBE?

Irradiation facilities

Carbon ions:

- **LET 33,6 keV/µm (75 MeV/n)**
  GANIL, Caen (2003 -…)

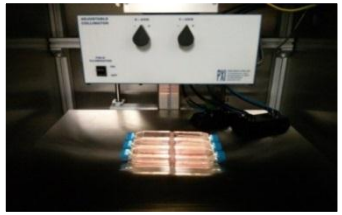


- **LET 184 keV/µm (11,4 MeV/n)**
  GSI, Darmstadt (2004-2012)

- **LET 13 keV/µm (SOBP 290 MeV/n)**
  HIMAC, Chiba (2016-…)

Photons:

- **250 kV**
  Lyon-Sud irradiator (X-Rad 320)



Pardigm of the stealth bomber to explain the tumor cell response to carbon ions

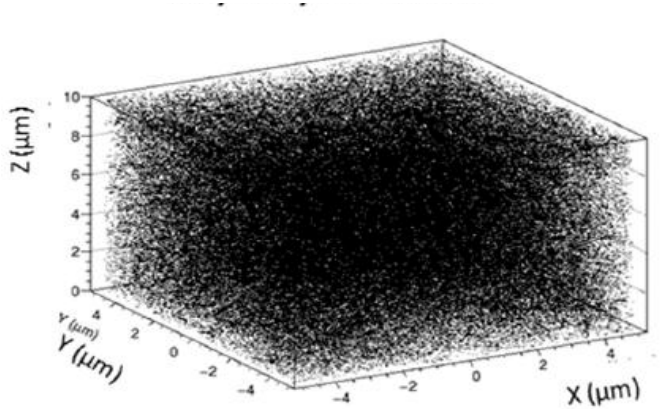
Bomber

Stealth

relies on the spatial distribution of reactive oxygen species (ROS) at the nanometric scale

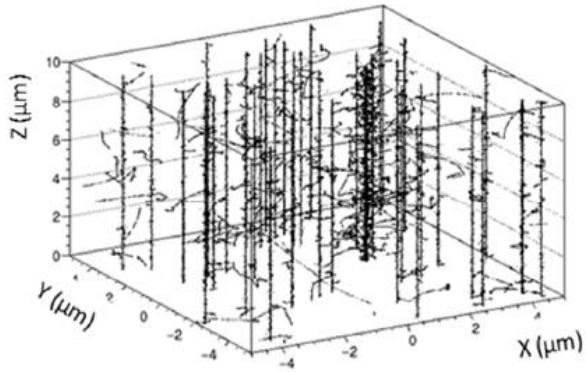
Monte Carlo simulations of OH° radicals

2 Gy photons



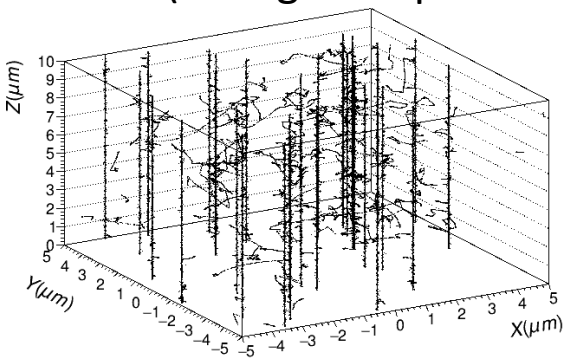
Nucleus volume

2 Gy carbon ions (physical equivalent dose)



Nucleus volume

1 Gy carbon ions (biological equivalent dose)

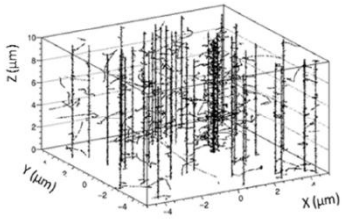


averaged-dose LET of SOBP: 13 keV/µm
(NIRS irradiation)

✓ Very different local distribution at the nanometric scale:
- dense and homogenous (photons)
- clusters around tracks (carbon ions)

➡ Very different consequences at the cellular level

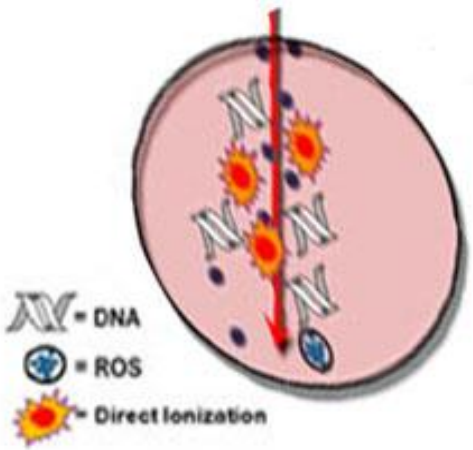
C. Monini & M. Beuve

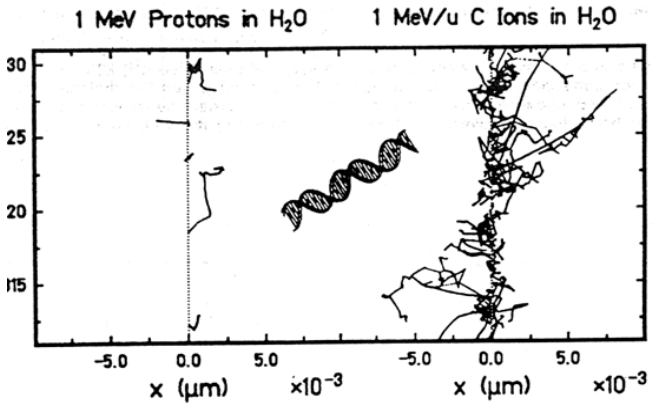


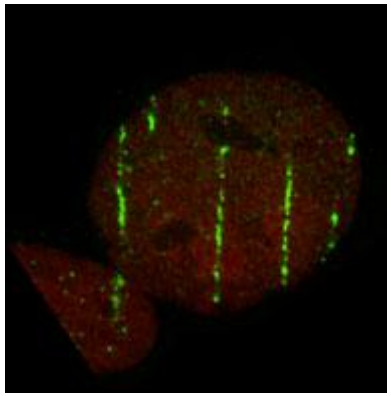
The bomber effect at the DNA level

✓ Locally Multiply Damaged Sites (LMDS)

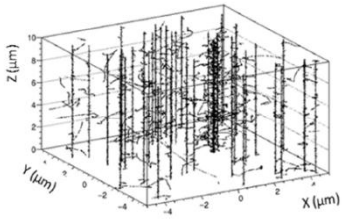
- Direct and indirect effects
- Clusters of irreparable DNA lesions







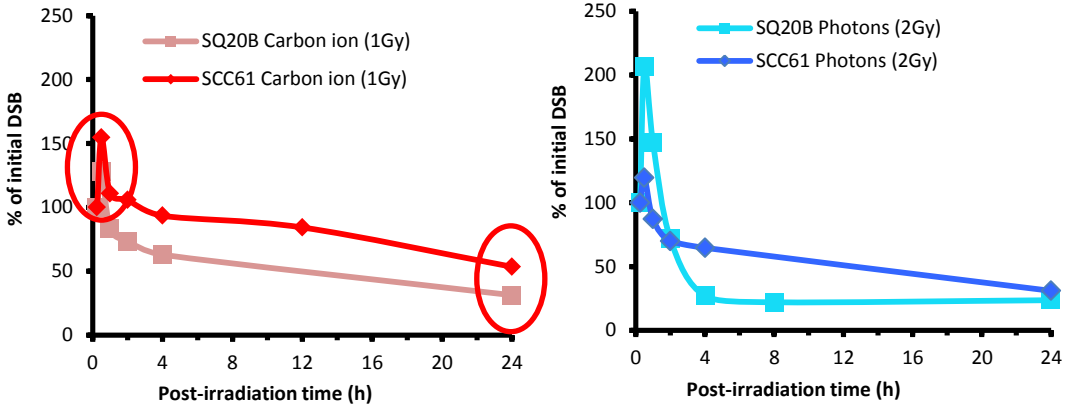
Courtesy C. Fournier GSI



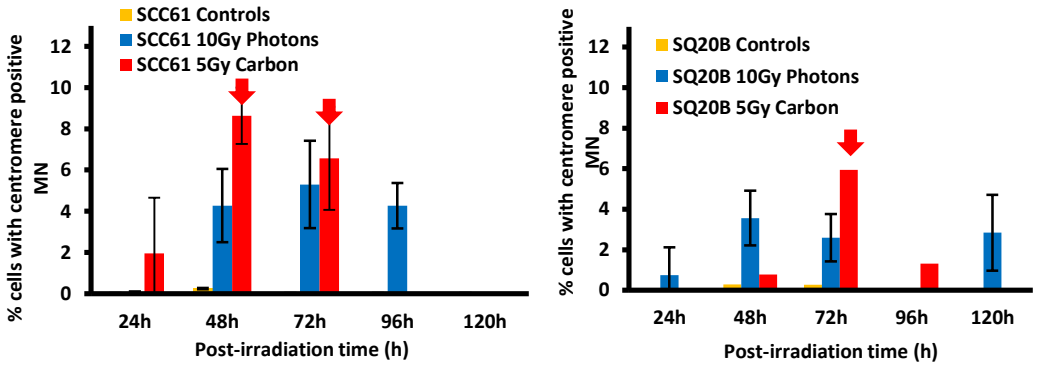
The bomber effect at the DNA level

- ✓ More non-repaired double strand breaks

γH2AX foci



- ✓ Chromosome loss: specific signature

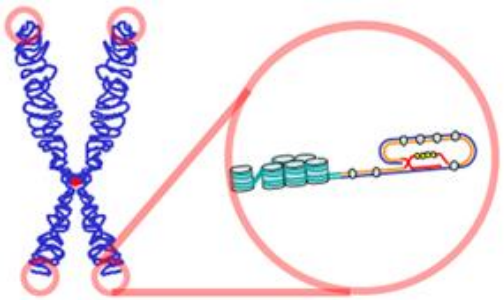


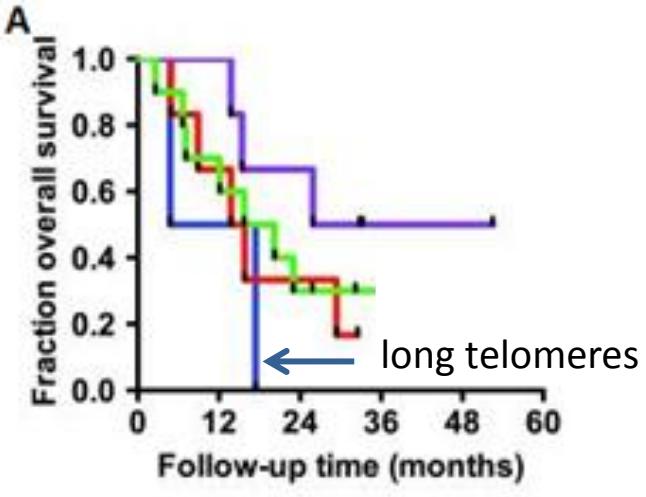
Hanot, Plos One 2012

At the telomeres' level ?

CG repeats are highly sensitive to oxidative stress: increase of telomere's lenght in tumor cells by telomerase to protect chromosomes' end

Glioblastoma patients with long telomeres = resistance to radiotherapy = lower survival

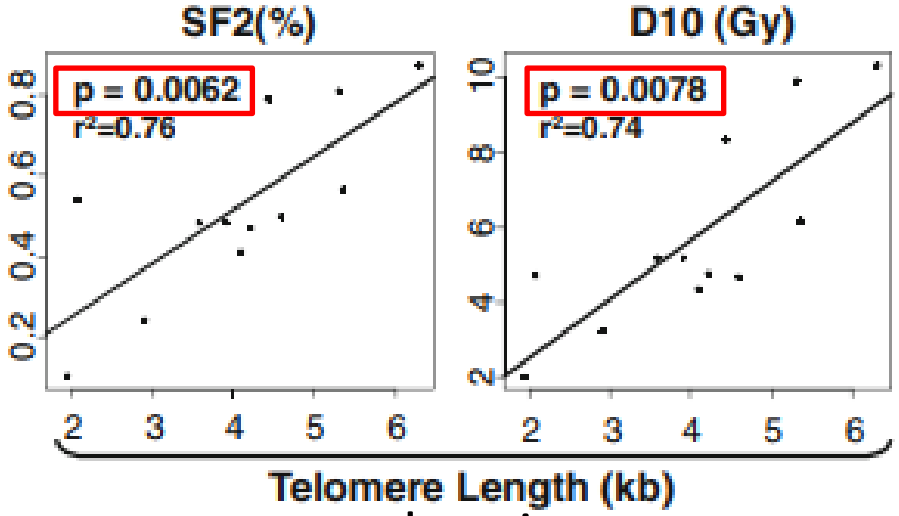




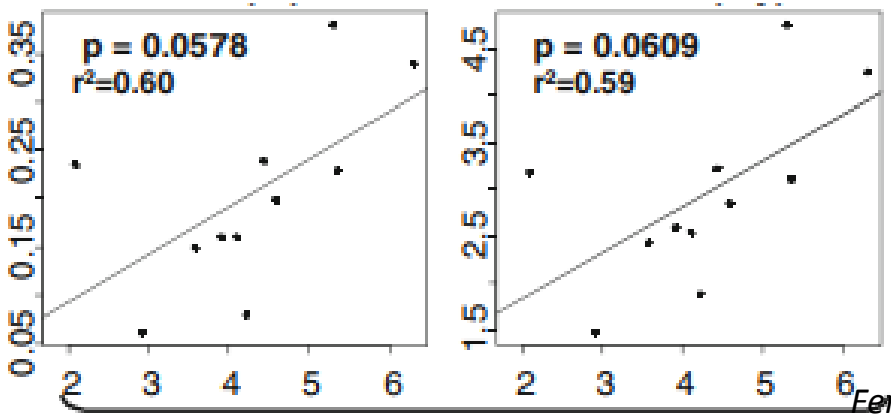
Sampl et al., 2012

The bomber effect at the DNA level

Correlation between telomeres' length and radioresistance in 12 glioblastoma cell lines irradiated with photons



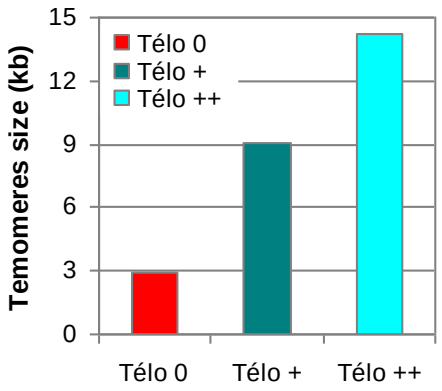
but not in response to carbon ions

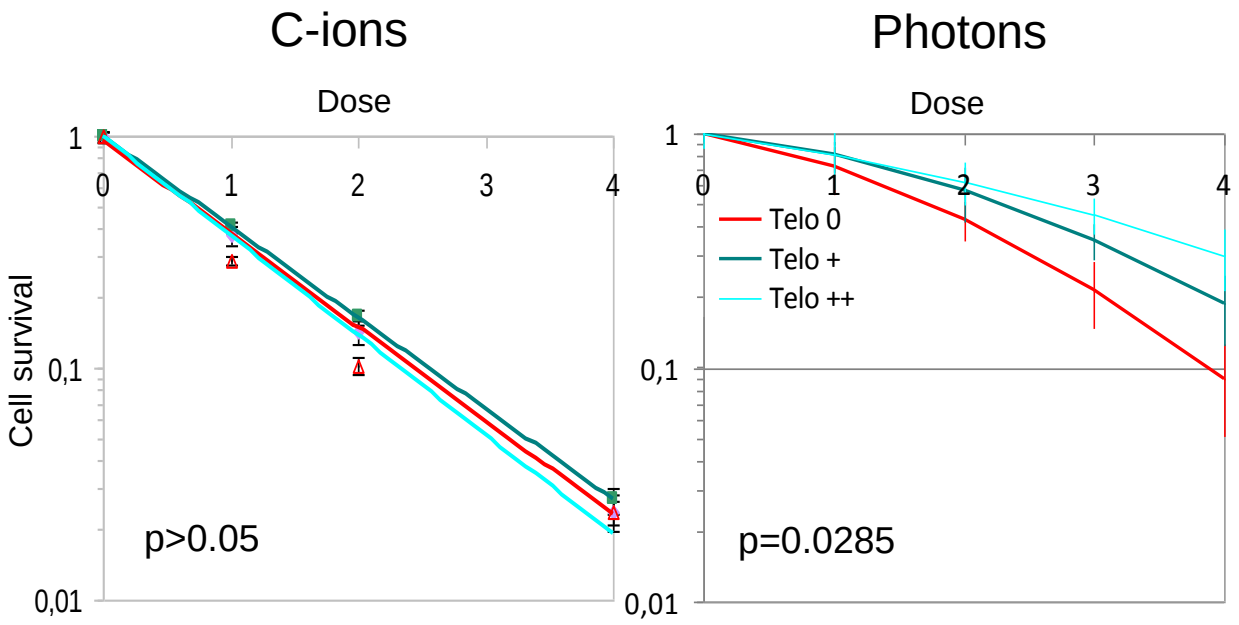


Ferrandon et al., Mol Neurobiol, 2013

The bomber effect at the DNA level: independent of telomeres' lenght

Transfection of U373MG glioblastoma cell line with telomerase: three clones with increased length of telomeres



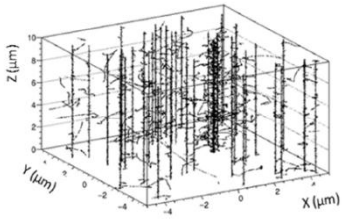


No link between telomere status and response to carbon ions

Increase in telomerase activity and/or telomere size induces radioresistance

Ferrandon et al., Mol Neurobiol, 2013

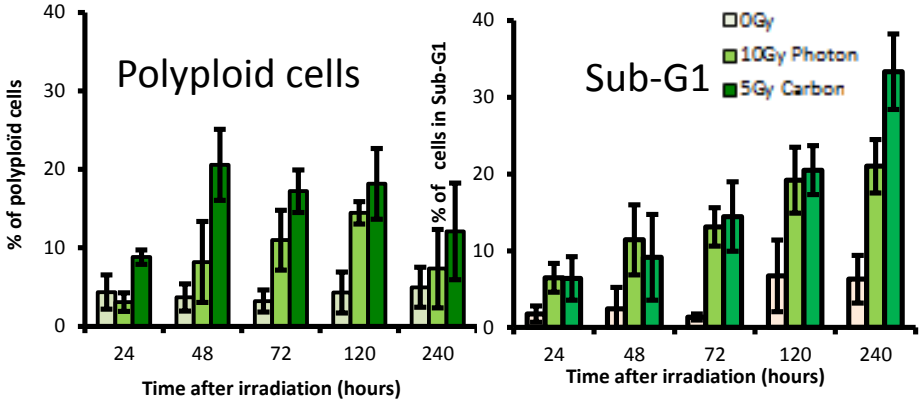
- *Glioblastoma patients with long telomeres can advantageously benefit from a carbontherapy*

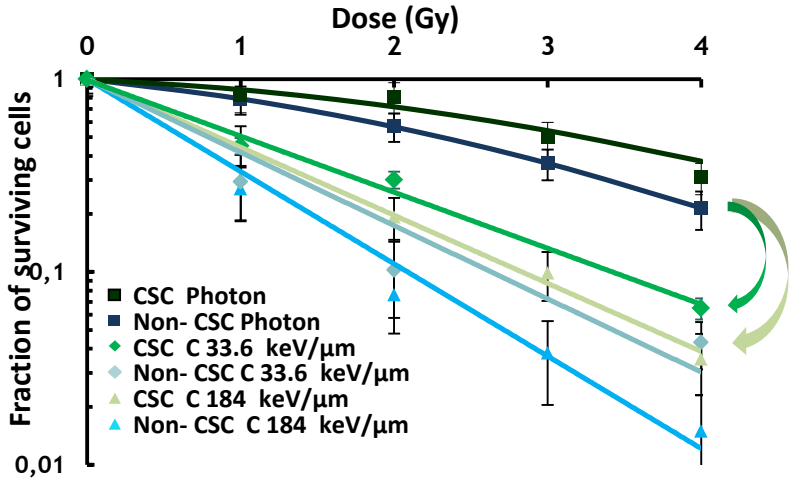


Consequence of the bomber effect: cell death

- ✓ **No specific mechanism:** early apoptosis or mitotic death + p53-independent ceramide-dependent apoptosis *(Alphonse et al. BMC Cancer 2013; Ferrandon et al Cancer Letter 2015)*

- ✓ **More efficient on cancer stem cell killing** *(Bertrand et al. Stem Cell 2014; Moncharmont et al. 2016)*





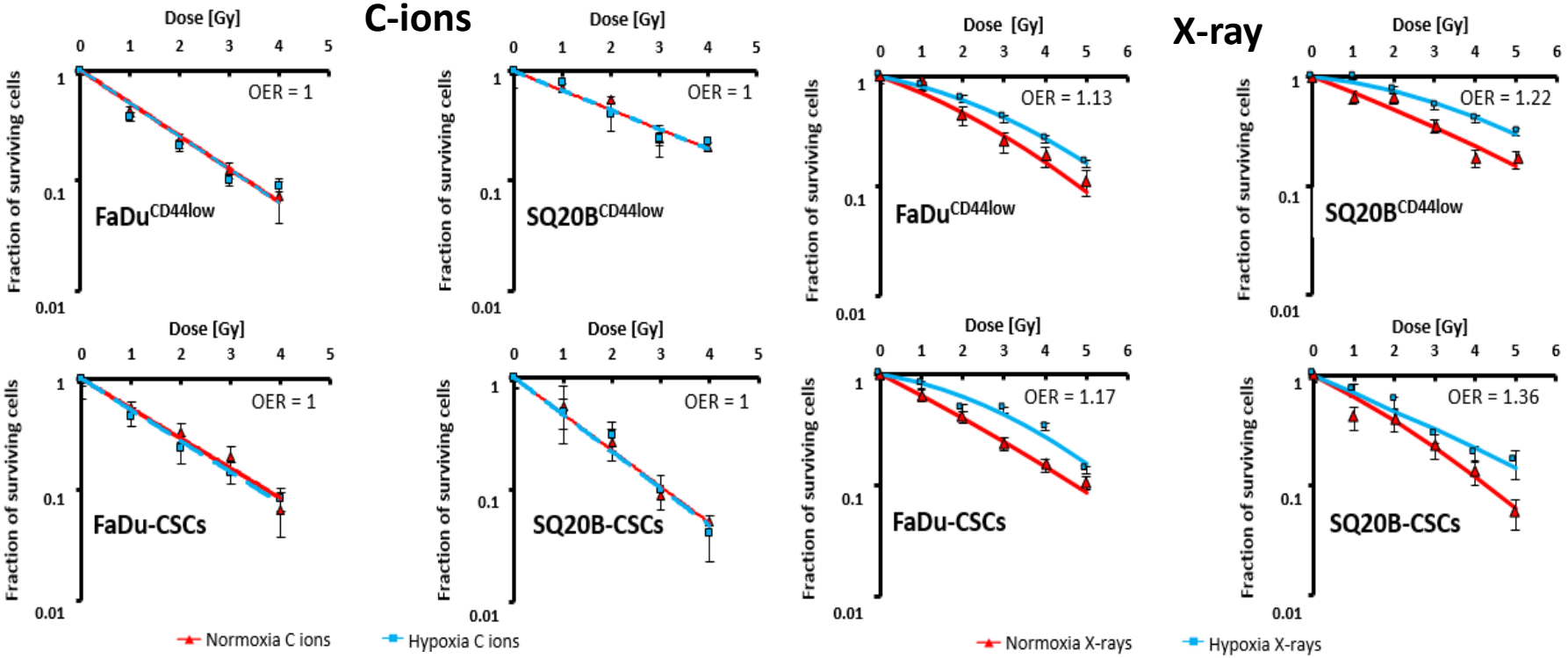
Consequence of the bomber effect:

✓ Cell killing independent of the O_2 concentration

Interest in the treatment of hypoxic tumors

No oxygen effect OER=1	Radioresistance under hypoxia OER>1

HNSCC stem-cells and non-stem cells

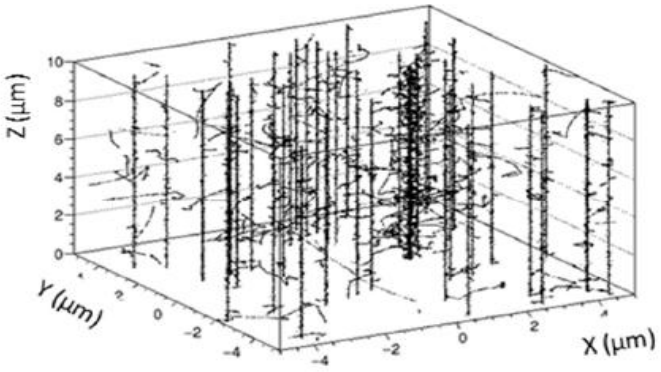


Wozny et al., Br J Cancer 2017

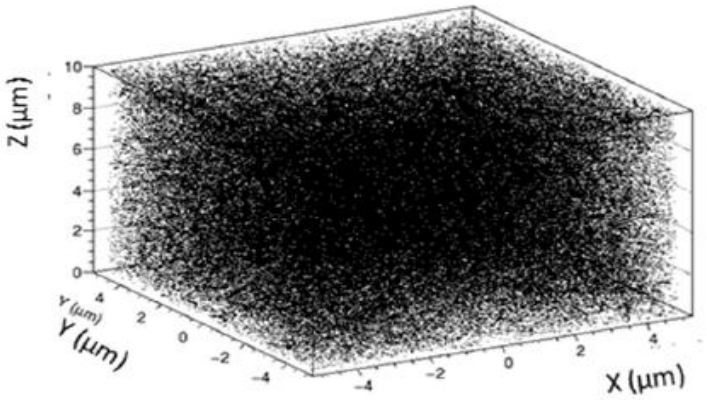
The stealth effect

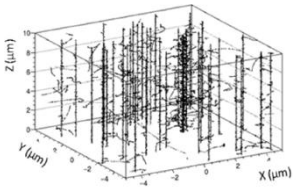
Local dose distribution of ROS → few/no activation of cell survival and defense pathways

2 Gy carbon ions



2 Gy photons

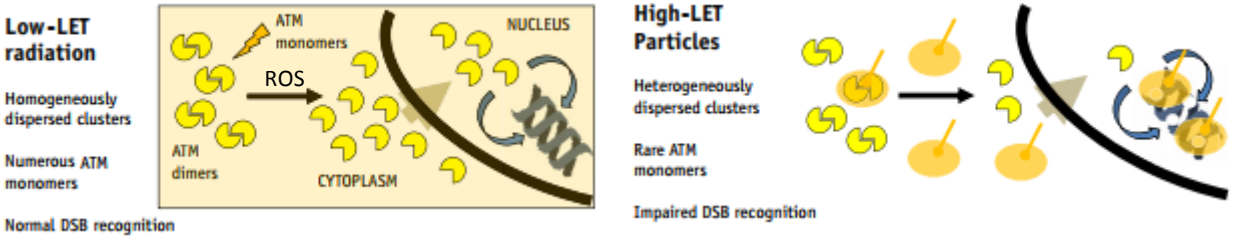




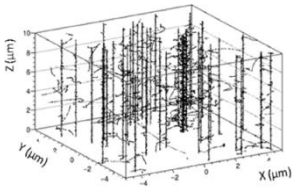
The stealth effect

Wozny *et al*. In preparation

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

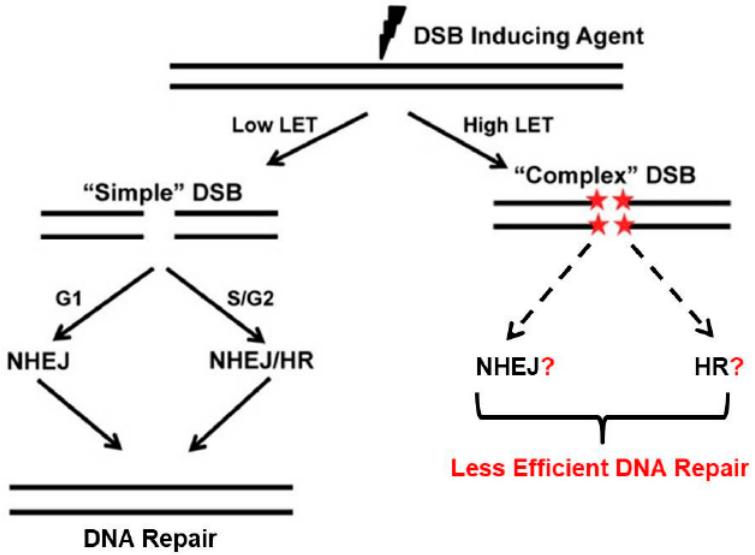


Maalouf et al. IJROBP 2019

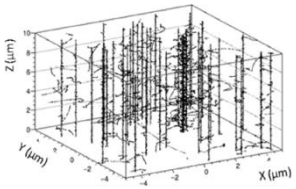


The stealth effect

- ✓ **Lower DNA damage repair (NHEJ/HR) under normoxia or hypoxia**



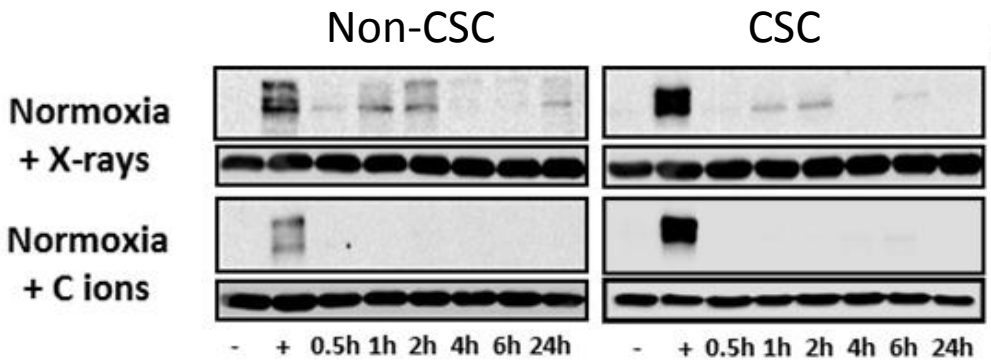
Wozny *et al*. In preparation



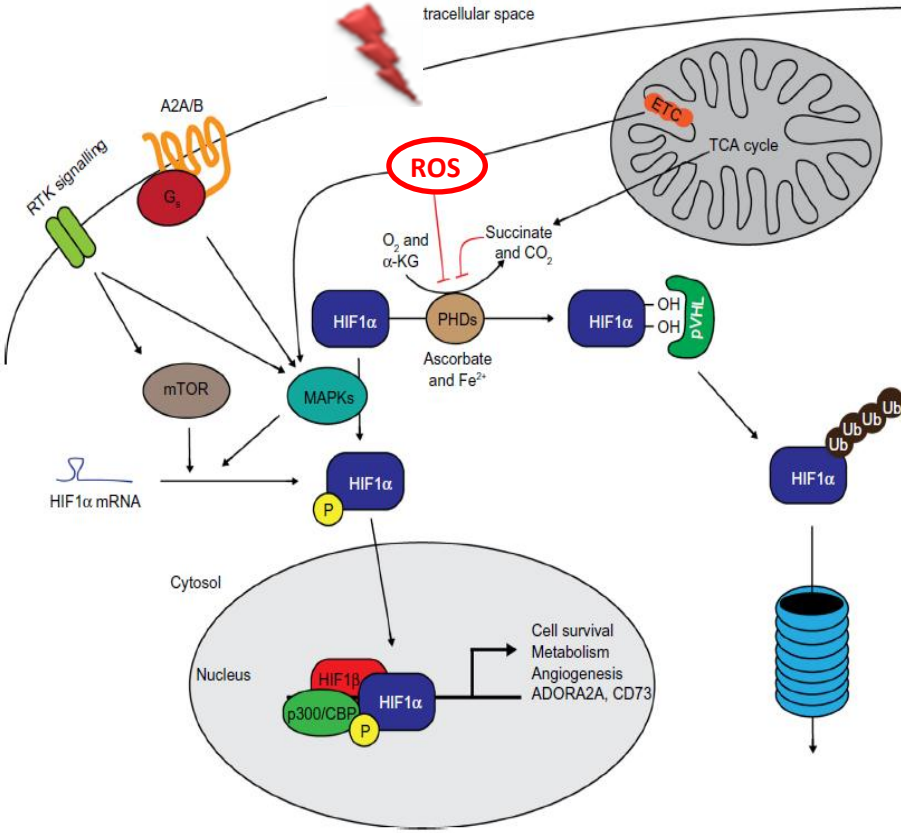
The stealth effect

✓ No HIF1-α stabilisation:

Major transcription factor involved in the response to hypoxia

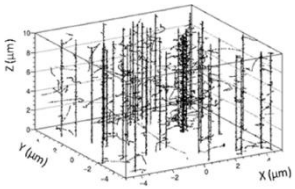


Wozny et al. Br J cancer 2017

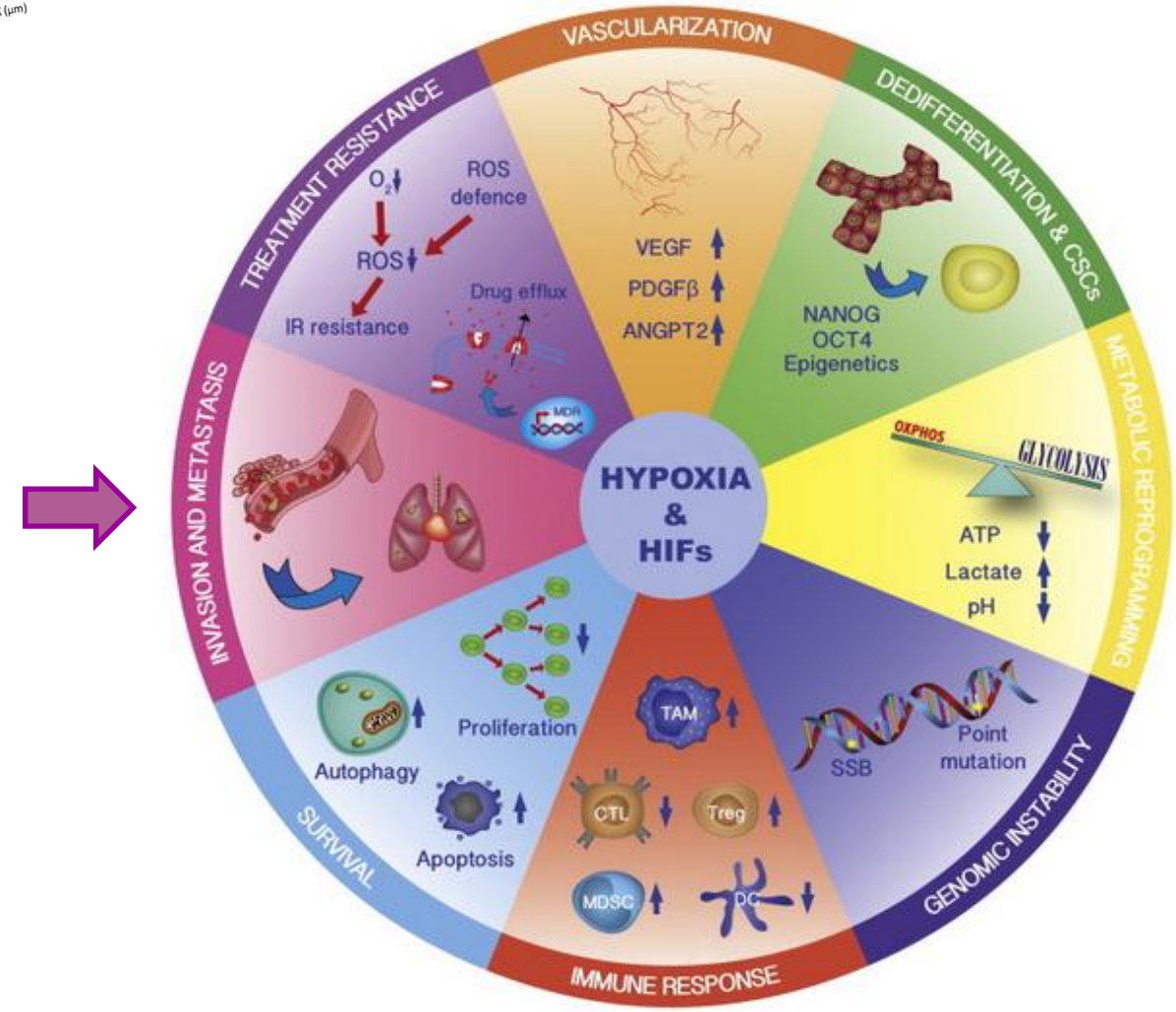


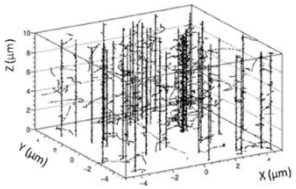
Adapted from Howell and Tennant, 2014

participate to the absence of oxygen effect



The stealth effect





The stealth effect

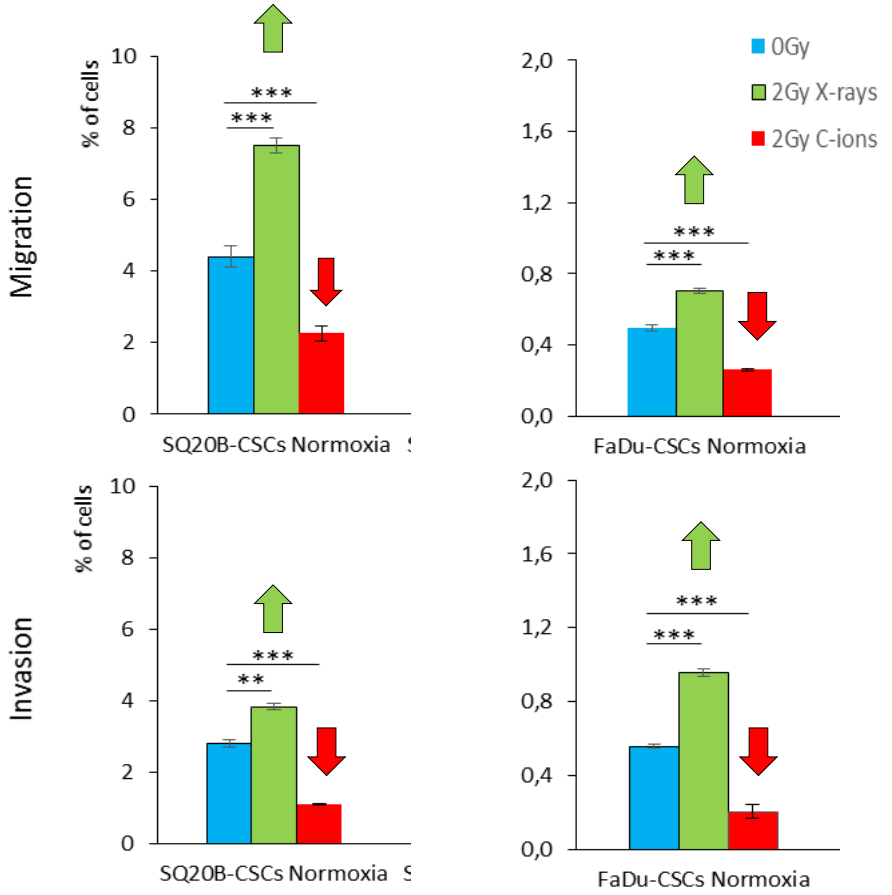
✓ No invasion-migration of CSCs

(Montcharmont et al. Oncotarget 2016 ; Wozny et al. Cancers 2019)

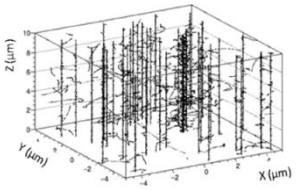
Less metastases

Photons
Normoxia: ↗ EMT*

C-ions
Normoxia: ↘ EMT*

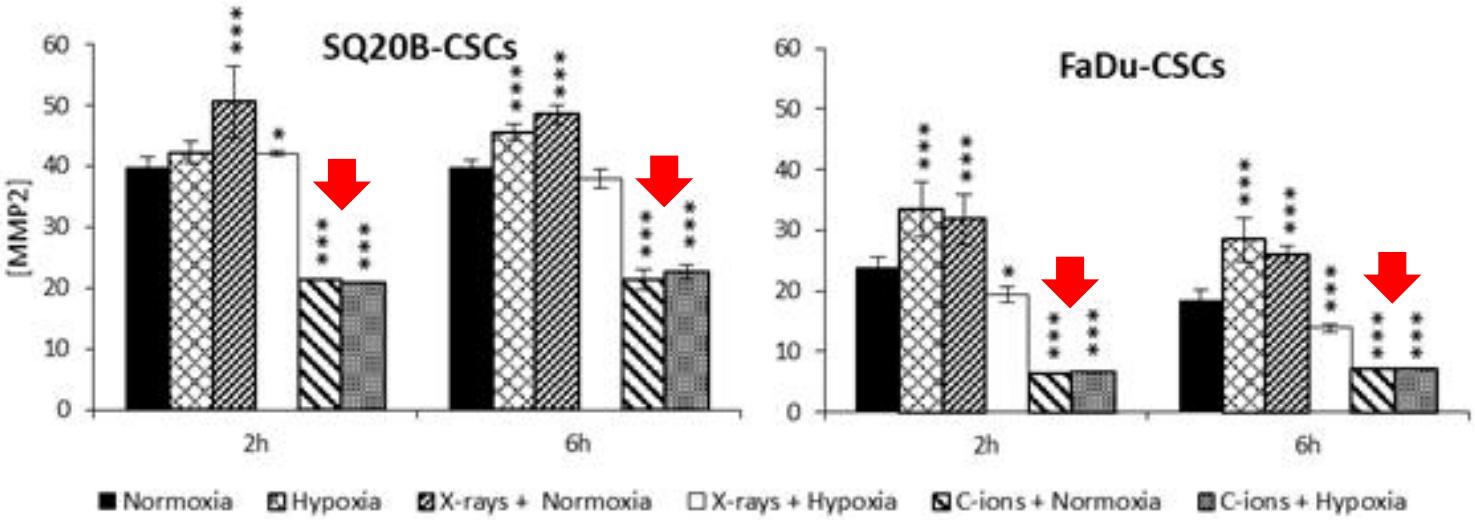


* Epithelial-to-mesenchymal transition

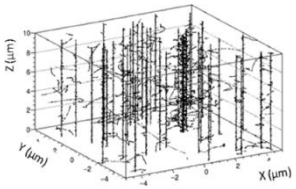


The stealth effect

- ✓ **Significant decrease of MMP-2 concentrations**: a major metalloprotease involved in the degradation of the extracellular matrix

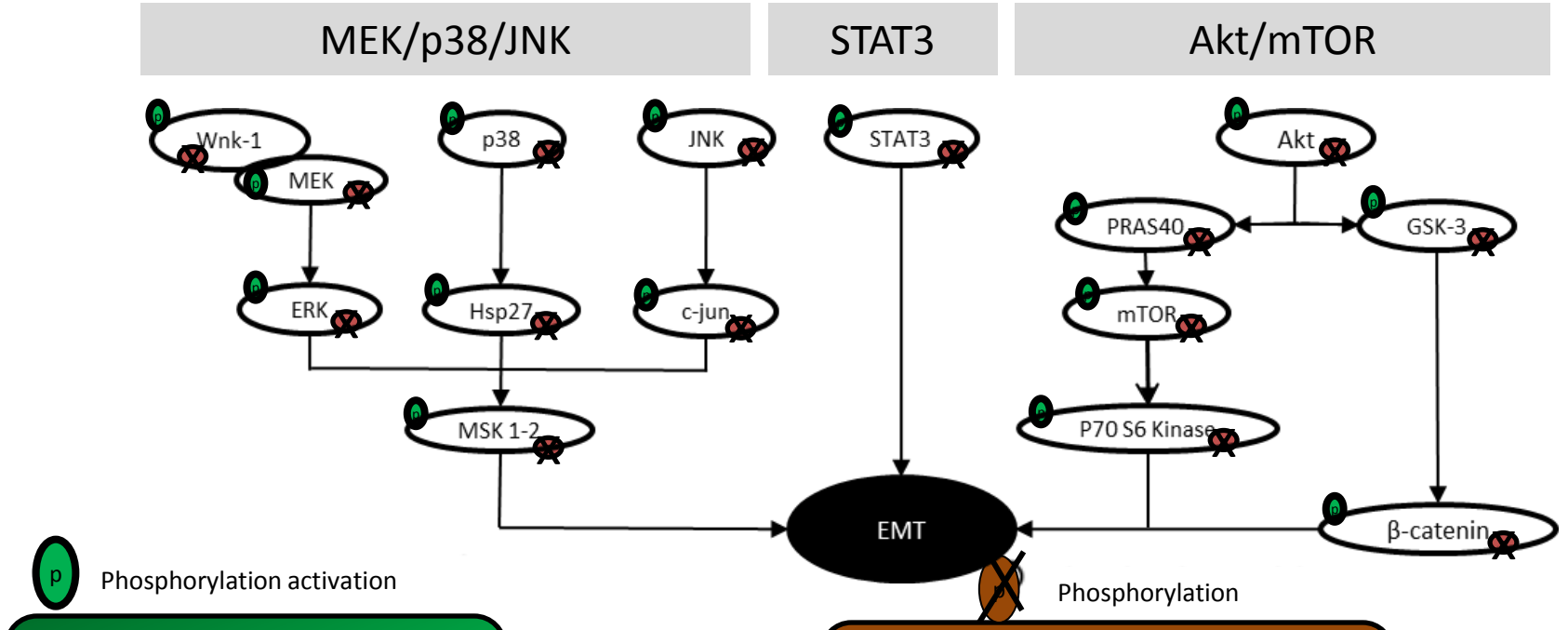


Wozny et al. Cancers 2019



The stealth effect

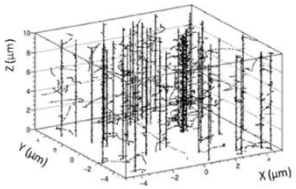
✓ **Few/no activation of invasion/migration signaling pathways**



Photons + Normoxie
Kinase phosphorylation

Ions C + Normoxia
No phosphorylation

Wozny et al. Cancers 2019



Conclusions

✓ Answer to the initial question: **Molecular mechanisms specifically involved in the tumor response to carbon ions!**

✓ **Stealth bomber pardigm:**

Bomber effect

- Increased DNA damage
- No oxygen effect
- More cell death

Stealth effect

- Lower DNA damage detection and repair
- No HIF1α stabilization
- No invasion/migration
- No/lower activation of cell survival pathways

✓ New question: **Sequence of treatment associating carbon ions and photons?**

Acknowledgement

UMR CNRS 5822

LRCM : C. Rodriguez-Lafrasse

- M-T Aloy
- **G. Alphonse**
- D. Ardail
- P. Ceruse
- A. Gauthier
- J-B. Guy
- R. Ladjohounlou
- **P. Lalle**
- A. Lauret
- **S. Louati**
- N. Magné
- **C. Malesys**
- **V. Varoclier**
- **A.S. Wozny**

PHABIO: Pr M. Beuve

- E. Testa
- C. Monini

Collaborations

Y. Saintigny (IRCM-DSV-CEA)
C. Fournier (GSI)
G. Vares (OIST)
T. Nakajima (NIRS)
O. Tillement (ILM)
M Janier (HCL)
JL Ravanat (CEA)
JC Monboisse (CNRS 7369)
C Tomasetto (IGBMC)
MJ Stazia (CDiReC)

…

Thank you for your attention !